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Burnet's Rickettsia Disease (Q Fever)
by
Prof. G. Valdolei

Few problems have retained as much attention and interest and have had such rapid success in clinical, epidemiological and experimental research as during the last few years, the investigation of Burnet's rickettsia disease, (Q fever). In May of 1948 a series of papers were presented to the 54th Congress of the Society of Internal Medicine meeting at Karlsruhe. In October of 1949 on the topic of the relationship of pulmonary disease of virus and rickettsia, a paper was presented by Frugoni, Kagrassi and Giunchi at the 50th Congress of the Italian Society of Internal Medicine meeting in Rome. On the 6th of May 1950 it was the subject of the Swiss Society of Internal Medicine meeting at Neuchatel; for the biological section the paper was presented by Hoosor, for the clinical section by Gaell. Today our Society is meeting to consider this same question, especially because in these last few years, a great number of contributions in the field of clinical, epidemiological and experimental work have been made, not only in America and Australia, but also mainly in Europe; in view of these contributions it must be concluded today that this disease which up to some time ago was considered an exotic disease, also occurred during war.
periods for causes strictly due to the war, especially it occurs following the movement of troops from one continent to another and now is being considered as a disease which is common in all the countries in Europe and occurs frequently in Italy, especially in some regions, in others it exists some of the time, especially during the time prior to the last war and exists in epidemic or endemic form.

The story of Q fever is short and recent. It was isolated and described for the first time in Australia by Derrick in 1935, as a special clinical form, the existence of which had however been noted among cattle workers in Queensland and was named fever of Mattatoi at the end of 1933, and finally was established in 1937 by Burnet and Freecman as a disease caused by a particular rickettsia, the name of which it now bears.

In 1938 Davis and Cox, studying a filterable virus, which was pathogenic to the mouse and which, towards the end of 1926, Negouki had isolated from a tick, the Dermacentor Andersoni, identified in this pseudo virus a certain rickettsia, which besides being pathogenic for some animals in the laboratory, according to the observations of Dyer, demonstrated itself capable of provoking in man, a laboratory infection, the symptoms of which are similar to those of Q fever. Studied accurately in its morphological and biological characteristics, this particular rickettsia which had been described by Cox as diapherical rickettsia. The following year, however, Burnet and Freecman, Derrick and Dyer were able to ascertain
that no great difference from the immunological point of view
existed between the diaporical rickettsia of Cox and the rickettsia
described in Australia by Burnet, as an etiological agent of Q fever.
This was demonstrated by Cox in 1940 in a brilliant manner, demonstrat-
ing the diaporical rickettsia was the etiological agent of the Q fever,
the existence of which in North America was found out through the
demonstration of a specific antibody of the circulating blood of the
individuals located in various states, Montana, Idaho, Wyoming,
Nebraska, Nevada, Arizona and Washington.

It was only during the last World War that the Q fever was
first noted in Europe during the period from Nov. 1944 to July, 1945
among the American troops which were on the Italian front. In general,
during this short lapse of time, were observed three small epidemic
explosions, perfectly well geographically limited entirely to the
military personnel. The first was observed among the troops which
were on the Gothic line (Appenines, Tosco-Emilian, Puglino, Bolvedere,
Sassoleone), with a light continuation among certain spread out
leave takers, successively, along the Lake of Garda (Malcesine) and
in Corsica (Rossius and Coll). The second epidemic center was born
among the troops of the American Air Force, concentrated in the
airport of Frottaglie in Puglia (Brumpt) and followed them during
their home journey, both on a steamer in the Atlantic Ocean (Schultze),
and successively, a short time after the landing in Camp Patrick Henry.
The last center was represented by a laboratory epidemic, which was
comprised of twenty cases of infection among the personnel in the
Since in all three cases it was question of centers of
origin and geographical distribution which were very well described,
without any diffusion of the disease through the civilian population
which was in direct and immediate contact with the military, it was
thought that there was a possibility that the virus had been
carried into Italy by the military from America or Australia, and
that the infection had not spread among the civilian population or
among the other troops because the imported disease had not found
a favorable habitat in our country. Effectively on termination
of the war, for a few years, no clue of any sort could be found in
Italy of the existence of Q fever.

In this respect one must note that during the years 1939
(Kalk and Froesellen; Stehr) 1941-1942 (Häuser; Hirt and Bauer;
Wielö; Böling; Böning) in the Balkan Peninsula and especially in
Bulgaria and eventually in meridional and eastern Greece, there
occurred in epidemic form, a disease of rapidly diffusive character,
characterized with high fever, with splenomegaly and with pulmonary
infiltration with little clinical symptoms and identified only
through radiological examination. This disease extensively affected
the German occupation troops but it did in no way affect the native
population, which proved itself non-receptive, almost as if it was
protected by an acquired immunity. The etiological problem of this form troubled Bieiring as well as Brochberg who believed to have solved it by calling it a virus disease, "Balkan Grippe"; this dissection was questioned by clinics and especially by Imhauer who felt that it was not justified to identify as a common grippe, a disease which had such little diffusability.

The strain of this "Balkan Grippe" isolated in Greece by Caminopetros during an epidemic in 1943 and transported to America, were studied by the Commission for Acute Respiratory diseases at Fort Bragg and identified as a strain of rickettsia Burnotii (Balkan strain). The identification of this was immediately elucidated and resulted in a large laboratory infection, which attacked the personnel of the institute for study of this disease, and was characterized by a symptomatology, clinical and serological, typical of that of Q fever.

It was only in 1946, that Gsell described the first cases of Q fever, definitely autochthonous in Europe, in Switzerland and in France (Strasbourg). The Swiss cases were rapidly noted worthy by the works of Gsell and Engel, Gutscher and Nufer, Heymann, Nießmann, Wachter, Koch, Alphonso and Loeffler, Koch. The disease appeared in the cantons (St. Gall, Argau, Graubünden, Ticino, Geneva, Valais). In 1947 our first observations were made in Sicily and successively in Germany, in Stuttenberg, (Heidi and Germer), in Baden (Bieiring), simultaneously to a small
epidemic among American soldiers in Monaco (Downing) and lastly in 1946 in Asia (Inhauser). In 1948 the disease was described in Greece (Caminopetros) and Turkey (Payzin and Golem) in Italy in 1949, we had the small epidemics of Calcutta (Muncik) of Ravarino (Coppe, Bertoli and Zanussi), of Chiaravalle (Zabudieri, Bevero and Simonetti) and numerous cases in Sardinia (Tagrassi, DeRitis and Scalfi) in Alto Adige (Forconi and Salvato), in Liguria (Bevero and Guerra). In the Abruzzi (Capurale and Camisa) in the Marche (Potignani and Bacchiocco; Marconi, Cristofinotti and Balico), in the Eolie Islands (Lopez), in the Republic of San Marino (Sassi-Valli).

The observations by Do Prada, Parker, Do Prada and Bell in Spain; by Funesca, Pinto Gauder, azaveda and Lacorva, in Portugal; by Combiesco and Cull in Romania; and finally by McCallum and Coll, by Henderson, by Raman, by Stoker, by Turner, by Adams and Coll, by Caugnet and Dudgeon in England.

From all this, it must be concluded that Q fever is a well diffused morbus entity throughout all Europe with the exception of the Scandinavian countries and Bonelus and that in some of these countries it is quite frequent. Its presence has also been found in other places outside Europe and North America; in Morocco (Blanc, Martin and Maurice); in Algeria (LeGrand); in French Equatorial Africa (Giroud and Gaud); in the Congo (Jadin); in India and Palestine (Rosenkranz); in Central America it has been observed in Panama (Chenev and Geib, De Rodaniche).
In North America, Q fever is a spontaneous disease and frequent in many regions, whether they be agricultural in nature (Texas, Illinois, California, North Carolina, South Carolina) or industrial (Michigan, Minnesota) and often appears in Connecticut with certain trades. The official statistics of the USA point out thousands of cases of observed Q fever; but these numbers cannot express the actual real statistics of the disease since in many cases the symptoms pass unobserved and evade the statistics.

In an experiment of Beck and Cole made in California with 10,000 individuals, who appeared to be healthy, there showed about 5% of cases of rickettsia Burnetii and a certain number of individuals showed an evidence of a state of disease or reminders of disease which might be referred to as Q fever.

Therefore, in epidemiological study of this form of the disease one must consider the possibility which is not infrequent, of cases of infection which are not at all apparent.

With respect to Italy, the attention has been attracted by the paper of Frugoni and our paper at the 50th Congress of Internal Medicine and has shown that the observation of the cases has increased rapidly in the last six months. Actually no region of Italy can consider itself completely immune: Sicily, Marche, Lazio, Tuscany, Umbria seem to be actually the zones most susceptible right now.

Q fever can present itself at any moment or in any region.
to the practicing doctor; thus he must be able to know it perfectly, not only from the clinical point of view but also from the epidemiological point of view so that he may be in a better position of recognizing it and diagnosing it rapidly, with assurance and decision.

**Etiology.**

The pathogenic agent of Q fever is that particular rickettsia isolated in Australia in 1937 by F.M. Burnet and in his honor named rickettsia Burnetii. Cox has proposed the name rickettsia diaporica, to remind us of the singular capacity of property that differs from that of other pathogenic rickettsia, known to date, in that it is filterable through cendela; and Bergey has named it Coxiella Burnetii with the wish of separating in a particular group (coxiello) the rickettsia which have this property of filterability.

It is a very small organism of variable form and dimensions. It is observed in the cytoplasm of the cells under a form of micro colonies, which are distributed either uniformly or in masses at the periphery next to the cellular membranes in characteristic way from the center around the nucleus. It has the aspect of very small sticks of lanceolate shape or of form linked to diplococci. It is visible in chains of 2-3 elements; the dimensions vary from .25 microns by .4 microns in the lanceolate form, to .25 microns by 1 micron in the bipolar forms. It is immobile; it passes easily through the Bersonfeld 25 filter impermeable to the other common bacteria and to the other rickettsia, it passes through colloidal membranes with...
of 400 millimicrons, impartable to other rickettsia. It does not pass through a single disk of Saltz (Cox).

The Burnet rickettsia, as opposed to all the other rickettsia, presents a major resistance to the common physical agents.

While the common rickettsia are easily inactivated by heat, drying, chemical disinfectants and ultra violet radiations, the Burnet resists to a heat of 60° for 1 hour and 30 minutes, to formalin in solution of 5%, to phenol in solution up to 1% and to 1 hour of ultra violet rays (Abadieri).

While the other rickettsia in organic material maintain themselves virulent for only a few days, the Burnet strain can make itself virulent for nine months and 18 days in blood, maintained at environmental temperature (normal) (Combiesco and Dimitresco). According to Phillips, Burnet rickettsia maintained at ambient temperature proved to be infectious after 586 days; after six years they were still visible under the microscope and had conserved their antigenic power for immunization of the guinea pigs and to provoke the formation of antibodies noticeable during the deviation of the complement.

According to Jollison and Coll, rickettsia Burneti remains infectious for a long time, both in non-pasturized milk and in its derivatives, not obtained through boiling; for example, butter produced with infected milk, remains infectious after 3 months. Infected guinea pig blood, dried and left at ambient temperature,
may still be infectious after 162 days; urine after 45 days.

According to Sandel, Inudici and Bondiurelli the structure of Burnet's rickettsia does not appear any different than that of any other rickettsia under the electronic microscope.

In serological examination of Burnet's rickettsia, following spontaneous infection, does not give cross-immunity to any other type of rickettsia. The various strains of Burnet's rickettsia, known to date, give complete cross-immunity among themselves for experimental infection in the guinea pig; however, at the deviation of a complement, the various strains may demonstrate diverse quantitative behavior, especially with reference to the sensibility towards spontaneous infection of man. Sandel states that the antigen "nine mile" only gives a positive deviation of the complement and too late, meaning even 2-3 months after the beginning of the disease, while the antigen "Henzlerling" gives a positive showing during the first days. The "Palermo" antigen, established by us starting with strains isolated from patients of endemic form, has shown itself even more precocious and in the beginning more sensitive. For such reasons the American department of Research has only used for suspected cases of Q fever, the antigen "Henzlerling".

Experimental Burnet Rickettsia

A. In the cow.

The disease hardly ever presents any fever; the animal has an aspect of good health. The more frequently effects are those of mastitis, cheilitis and in some cases, of bronchial pneumonia or pericarditis (Jellison and Coll).
D. In the guinea pig.

The subcutaneous, the intramuscular, the intraperitoneal methods have been used as avenues of inoculation in the guinea pig. With experimental infections of Burnet's rickettsia, most of the time, causes a non-serious disease and of low mortality, it does increase considerably in cases where powerful infecting material is used, taken from a culture of rickettsia in chicken embryo, or from spot tumor from endometrous broncho pneumonia cases. The disease appears after a certain period of incubation, which varies according to the way in which it was introduced; from 3-6 days for subcutaneous infection, 4-7 days for intramuscular or intraperitoneal infection (Battitta). The disease is always feverish (excepting certain subcutaneous cases) and with a clinical curve typically difasis, as it is usual in diseases of virus and rickettsia.

The formation of antibodies can always be determined or found in the infected guinea pig. If the subcutaneous inoculation was used, deviating antibodies can be noted after the 5th day, and agglutinated antibodies after the 15th day. If the subcutaneous or intraperitoneal injection has been used, the deviating antibodies occur after the 5th day and the agglutinated after the 10th (Battitta). There is established a solid immunity which can be interpreted in a sense of a pre-immunization, given that, according to the observation of Parker and Steinhaus, the Burnet rickettsia in the experimentally infected guinea pig, persists in the organs for a long time after
the passing of the fever. It has, as a matter of fact been found in the liver after 60 days; in the testicles after 50 days; in the kidney cells and in the urine after 100 days; in the spleen 120 days from passing of the fever.

The anatomical, pathological alterations are represented as:

1. Constant index - has been noted an increase in the volume of the spleen; very rich in blood, lesions of the lymphatic glands, of small false myocardial, which are all evident in the 5th day of the disease (Lillie).

2. Frequent index - in the lung it creates bronchitis and endoavalvular exudations in the acofalo, circulatory alterations, which creates meningitis of the pharynx especially at the level of the bulb.

3. In the case of inoculations which have been done subcutaneously there is an inflammatory lesion at the point of ingress.

The histological alterations or symptoms were studied by Lillie and DeScavo. They are in general represented by:

Exudation, perivascular foci of the type lymphocytal, less frequently lymphoblasts or monocytes, with vascular endoteliosis of the heart, lungs, the fatty tissue of the intestines, of the omentum, of the peritoneum, of the intestinal submucosa, of the epidermis, of the pelvis; less frequently in other regions (Lillie and Coll).

In the lungs there exists focal infections, with predominantly
monocytoid cells, while in the intravascular exudate, the epitaloid cells predominate. Similar follicular infections are encountered in the spleen and the liver where there are degenerative lesions of the parenchymal and regressive processes of the vascular type of the hepatic cells (Savo).

In the brain is observed constantly a slight istiocid movement around the lepto-meninges with evident lymphocyte infiltrate and proliferation of the vascular endothelium. In respect to the nervous system there are signs of cerebral edema and presence of small foci of infiltration for the cellular way, irregularly spaced in the encephalitic region (Savo).

Ways of Infection.

Under experimental conditions, rickettsia Burneti has been determined as a general infection in the animal or in man.

1. Through intradomnic or subcutaneous inoculation. According to this method Blanc and Coll have observed infections in man.

   a. A characteristic local lesion of the disease of diverse seriousness, it appears in the form of a boil or blister on the skin which persists for 10 to 12 days, or a scab which disappears in a few days.

   b. The average or median of fever which appears in 24 hours and generally does not last more than 1 day, it can even be lacking.
c. Deviation of the positive complement, resulting in weakening.

2. Through subcutaneous injection (Camnipetros).

3. Through intramuscular injections in man, Manc and Coll have observed following intradeltoid injection:
   a. Locally a marked edema, persisting for almost a week and which does not tend to liquify and is accompanied by reaction in the region of the lymphatic glands or by leukocytosis.
   b. High fever, up to 40°C, with a typically biphasic march. It is of diverse length of time and gravity in proportion to the quantity of injected virus, but it is well tolerated by the patient who does not need to be kept in bed.

4. thru deviation of the complement, sera agglutination.

4. Through intramammary injection. Camnipetros with the cow, the sheep and the goat has observed localized infections when he made the injection in the mammary lobes. In such cases the serological reactions (deviation of the complement, sera agglutination) are constantly positive according to Battits and Galle.

5. Peritoneal injection, (blood, urine, infected sputum).
There results a typical feverish reaction with the relative formation of specific antibodies.

6. Through percutaneous absorption. Injection of suspension
of Rickettsia through the intact skin in the guinea pig (Blanc and Coll). In man, Liebman describes an infection as a thing in the lung or going through the mucus membrane. Infections in the laboratory are known to have occurred through handling highly infectious material.

7. Through direct contact with the mucus membranes. Conjunctivitis nasally or rectally, infections are known that have been produced in the guinea pig by introduction of a thermometer covered with infectious material in the rectum.

8. Direct contact with the vaginal mucus membrane especially in copulation. Parker and Steinhaus have made copulation of virgin guinea pigs, non-infected, with infected males and Rickettsia was observed in the seminal vessel. The females were infected up to 50% they aborted during fever, the fetus was infected. One of the three guinea pigs had an infection of such gravity that it died.

9. Direct inoculation of infected material through the excretory canals of the mammary glands, limited mastitis was determined in the corresponding mammary lobe.

10. Direct contact of the bronchial mucus or the alveolar epithelium of the lung, as is verified by the inhalation of finely powdered suspensions containing Rickettsia powder (infectious material) (Blanc and Coll). In these cases of inhalation in addition to the fever there was also a typical pulmonary infiltrate of bronchial
pneumonia type. The cases of infection especially in the labora-
tory are numerous where the pathogenic character of this nature,
that was verified in the laboratory of the OIE at Ft. Bragg, 1945,
where all were infected with the exception of some who had the
custom of wearing masks while working.

II. Through injection of infected material or foods.
This possibility is still controversial. Cunicopetres has not
seen such cases with sheep, goats or cows. Jullien and Eack
only have succeeded in infecting a bull calf, born of non-infected
mother and feeding him with milk from an infected animal. They had
not observed infection in the calf fed from a diseased cow which was
suffering from a form of edematos bronchial pulmonitis, with
observed presence of rickettsia in the sputum. Goll says that he
has experimentally infected three dentated persons by feeding them
with infected milk. There exists in the literature on the part of
some, the tendency, to try to explain the pathogenesis of certain
epidemics by means of alimentary infections; mainly by injection of
milk from infected animals, or from nourishment imbibed with
excrements from animals carrying the disease, although they do not
show the disease themselves. In the epidemic at Galliata, Giunchi
proposes the hypothesis of the injection of susceptible greens or
other foods which were infected with excrements of infected animals.
In the majority of cases these have been contradicted rather
strongly or put into doubt.

12. The clinical experience has not yet securely demonstrated the possibility of direct infections through contagion of touch from the diseased to the healthy (persons to animals) except in cases of handling of highly infectious materials, or excretions, as in diseases of pulmonary, bronchitis, etc. (Moldelesi). Duran and Terzani affirm having seen doctors or nurses who had assisted, especially, such dear people who have been diseased with pulmonary localizations of Q fever. These cases, however, of direct contact of man to man and which are in opposition to our knowledge of the epidemiology of rickettsia in general, eventually will be found to have had a definite reason. From what has been previously presented Q fever appears to be a disease of multiple means of infections; it must, however, be observed generally all the ways of infection, from the percutaneous to the intra-peritoneal and even to the ways of inhalation, always to lead to a general infection even in a latent form as is demonstrated from the rapid formation of antibodies from the sera reaction AP. Only through the inhalation, the most frequent, is there always a result with the formation of characteristic pulmonary infiltrate.

Vectors of Q fever which are Skin Ectoparasites.

As for all rickettsial diseases the problem of the existence of the vector of this disease of Q fever presents itself as to the eventual vector of the virus. For the endemic form has been indicated that ticks, in Morocco, Blanc and Coll speak of the
It has not been systematically examined whether they are infected specific antibodies in the blood have not been demonstrated or revealed through sero-agglutination or deviation of the complement. Autopsy has presented splenomegaly or other characteristic alterations of the moribund form. In any case we had injected in the peritoneum of the guinea pig "pappe di nilza" and other organic elements taken up from suspected rats; in these cases we concluded that there was an infection of Burnet's rickettsia. Similar result was obtained recently by Wiesmann in systemic ways as well as serologically and microbiologically.

It appears impossible, considering this, to prove a rickettsia burneti deposit in the Sicilian rat, comparable to that of the Australian bandicoot and the maroccan gerboa, a deposit which as with other rickettsia close the epidemiological link from virus to man.

As for the vector, it has been identified as the ticks for the Austrian strain, which can carry the infection from the bandicoot to man.

A systematic investigation carried out in Sicily with some infectious demonstrations has led to the consternation that in the "regruppativi animals" the infection of ticks was not demonstrated as being of a notable importance. Specimens of these ticks on sheep, bovines or infected donkeys and put from these onto guinea pigs, have not
been able to create the typical febrile reaction, nor to demonstrate the formation of specific antibodies, revealed through sero reaction, agglutination, and fixation of complement. These research works would exclude the possibility of the presence of Jacto's infection in those groups of animals that would have to be necessarily conditioned through the intervention of ticks or other arthropod intermediate agents, functioning as the vector of the infection. Since then, Buck and Coll, Jollison and Coll, have arrived at an analogous conclusion through examining systematically the ticks which have been introduced deeply into the Grecchio of the cow (Oxalis legum) did not find infection above a certain minimal percentage, less than 2%; thus they could not attribute disease, the importance of the cause or factor, efficient or direct of the mechanism of the infection.

Even though the role of the parasite, especially of ticks, has a factor contributing to infection through which Q fever could be spread, represents maybe high coefficient of major importance and should not be excluded at first.

That the ticks can contain and maintain activity, the virus of Q fever has been demonstrated through the first experiment of Dave and Cox and of Parker and Davis, who have isolated for the first time in America, the disporic rickettsia credited to the tick: Dermatocenter Anderson. Furthermore, similar infections may
occur spontaneously in the tick, even though in small proportions
and this has been demonstrated by the systematic investigations of
Bock and Coll and P. Parker and Coll, but in the majority of the
common epidemics it must be recalled that their reserve of virus
is partly constituted by the live ticks, but we must consider the
efficiency factor.

On the other hand we must not disbelieve that because of the
fact that some of our investigations on ticks in Sicily have had
negative results, we are not authorized to exclude the fact that
extending to a larger scale our investigations, it would not be
possible to demonstrate that in Sicily a certain number of the
ticks, even though not numerous, can be found infected with
Burnet's rickettsia and we must not refuse at first the possibility
that this eventually may not be very probable. In such a case, in the
more common epidemics, the tick must function as a reservoir of
virus and not as much as vector for propagating the infection, this
is certain as to when it feeds its vectors it must necessarily
become infected. So then the tick and the major part of the species not
only conserves the rickettsia but can also transmit it according
to hereditary mechanisms and thus results a closed circuit where
there is stabilization being established, where the virus becomes
securely conserved in a practically indefinite way.

Having been demonstrated that the virus remains active, even
in a dead tick, (Blanc and Coll) or even in powder of ticks (Blanc and
Cell) and that experimentally it is sufficient to know that they transact the infection to men, that there be direct contact with an infected tick through a non-altered skin (Lichau. and Cell) and furthermore the inhalation of powder, which has been ground (Blanc and Cell) it cannot be concluded that the possibility that powder can result in infection through the presence of residual of infected ticks. Many species of ticks have the habit of leaving the animals after having sucked the infected blood (in the Hyalomma Savigyi, which is common in Sicily in animals, throughout their life cycle), and to return to straw, or to straw where they can deposit eggs and can make them infected. Generally the regions that can be easily infected can be recognized through the presence of dead ticks in sawdust, in straw or in general in the stable. Through these infectious media (powder, straw, sawdust, etc) it is possible that the virus can be transported at long distances and establish epidemics, according to the type which was found in the carpentry shop of Acoru (Goell), during the working of the wood, which contained certain residues of ticks, through dispersion in the environment of infectious material.

It has been determined, even though only in the experimental way, that the ticks can infect and create diseased animals, even though resulting in forms that are not readily apparent; that once infected this constitutes a reserve of the virus which can not only not be lost, but can further continually result in infections.
through means of the eggs and the feces and above all, can be transmitted indefinitely through hereditary means. It must be concluded that the ticks, even though it does not constitute the proper vector of the infection and of the disease to man, represents still the vector of the virus in so far as it can maintain infection in dust, wood, sawdust, straw and other materials, other than the feces and the eggs, even in the dry state. Considering the great resistance of Burnet's rickettsia to the common physical agents and especially to drying, we must conclude that the mechanism represents one of the factors which in practice, conditions the possibility of infection through inhalatory means, which represents the way most common to man and probably for animals.

In this respect one must not forget to mention the experiments of Shu-Hsian-Chao, who recently has demonstrated with respect to Prowazeki rickettsia, that the resistance of the rickettsia is conserved or maintained in organic materials (feces, organic remains, etc.) especially the resistance of the virulent strain, very notably in respect to particular factors, especially heat, humidity, etc.

In view of these factors, which can be modified today by climate or location, the activity of the virus conserved in powder form or in dust can be influenced in the case of Q fever, modifying
either in a negative or positive sense, its activity, and it seems
that in most cases the dry state is the most favorable for the
conservation of this virus.

Of the other factors, especially the factor tick, which
has not been sufficiently illuminated through observations and
experiments; there must be still considered that, in so far as this
constitutes one of the important mechanisms by which the individual
infections can be stabilized, for example in the laboratory, as well
as the epidemic infections (through dust inhalation of particles)
and finally the factor "secca" through which the factor tick
which maintains the epidemic in a determined region.

Epidemiology.

The Burnet rickettsiosis is an infectious disease common as well
to man as to animal so that it really constitutes a true and proper
"zoonosis".

In man it can present itself in diverse forms:

1. An epidemic form, which is the one that has been most
often described until now, especially by Anglo-Saxon AU, and is
easier to find in the city and in country towns.

2. An endemic form with a predominantly rural character; its
existence was first ascertained in Sicily and has been successively
demonstrated by Beck and Call in California. Its presence has been
described in Greece by Gairopetrou. With respect to the remainder
of Italy, the presence of the local infection of long duration
has been established in the Republic of St. Marino, described by
Suzzi-Valli. From the information brought out in the meetings and discussions (DeCastro,uzzi-Valli, Fontana) during the discussion that followed my presentation on Q fever in March, 1950 to the Medical Surgical Society of Romagna, it must be concluded that there exists a large endemic zone in Romagna, which extends deeply into the Tuscan-Eilian Appennines and which was securely established prior to the focus of military epidemics which appeared during the last war in 1944-45. It is not difficult to think that there exists other endemic foci of infection in other regions whether to the north or the south, in the mountains as in the valleys. It must exist in the animals, in the milk and in the meat, also in the local industries as well as in the saw mills and the carpentry shops. In this respect we must mention the disease with non-epidemic character which was described in Alto Adige by Forconi and Salvato.

A. The Endemic Q Fever.

According to our research in Sibilly, the endemic Q fever is extensive in many parts of the island and the countryside, where it occurs as isolated, where were not demonstrated direct or visible bonds or contact either through simultaneous cases or though cases occurring previous to the disease. They are diversely scattered throughout the different seasons of the year, without any apparent variations of climate or temperature, without prevalence for either one of the sexes or being determined by either one. Contrary to the American cases, our cases have been observed having a preference for the female sex, with the exception of children and young girls. Those cases have constantly demonstrated characteristics of affecting almost exclusively and always to
be in a determined zone where there existed a stall or a barn and
the animals of which, even though they belong to different species,
were demonstrated to be infected with this rickettsia. There thus
exists for most cases of endemic Q fever, a constant relationship either
topographical or due to work conditions, between man and animal,
either endemic or zoonotic.

Our experience demonstrates that:

1. Wherever a new case of Q fever is encountered in the
countryside, the zoological examination always indicates that in
the immediate vicinity or in immediate conjunction with the case, there
exists a barn or congregation of diseased animals, even if these are
in non-apparent or non-active form.

2. If in a grange or a barn a diseased animal is found:
   a. A systematic investigation demonstrates easily
      that for other animals, even of different species, there are present
      infections of these forms, even of non-apparent forms, even of the
      fact that they are in the same barn or stall.
   b. Around the central nucleus of this barn or
      grange at different times and different cases, it is possible to
      demonstrate from time to time a certain number of cases of these
diseases to man. In our patients of rural endemic cases, are in
general, herdsmen, employees of the barn, or housewives. One of
our female patients was the wife of a man who carried the milk
from the barn; the man did not show any disease even in the last

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year that could be called Q fever, but he did show the constant deviation of complement, an analogous case has been described by Blein. In our cases there also existed some who had no direct contact with barn or fumes, but who for their travels through the countryside, used horses, mules or donkeys as transportation and who were thus affected by the inapparent form of Q fever.

In view of these veterinarian and clinical reports and taking into the contemporaneous approach then in a rural zone, there occur cases of absolutely sporadic infection of Q fever, with a single outbreak, whether it be numerous animals affected by the active form or whether they be affected by the inapparent disease, in view of all these, we initiated in 1947, a diligent and methodical work of research based on systematic serological examination and when there were any doubts, went into the biological tests, using guinea pigs. Our work received impulse and help in 1949, when we were able to benefit by the precious collaboration of Prof. Mirri and the large veterinarian material offered by the Sicilian Zooprophylactic Institute that he directs. It was then possible to study individually a large number of focal endemic cases and of "endozootics" which comprised a large part of Sicily and the province of Palermo, Caltanissetta, Enna and sections of Messina, Catania, Trapani, Agrigento, Ragusa. In all this large agricultural region, whether it be in the valleys or in the mountains, this endemic form demonstrable in run and appearing in
close conjunction with an endoecotic of domestic animals, the knowledge of clinical, epidemiological matters, as well as the geographical extent of the disease are of great importance for knowing the general epidemiology of Q fever.

The results obtained which will be presented later in this discussion have been successively confirmed by other authors, whether they be European or American.

Another method has been followed by Beck and Coll in their investigation in California. Instead of our clinical method, they have followed one which is characteristically statistical with research with "Tappeto" carpet (blanket?). In the first series of investigation these other authors have been able to establish the existence of approximately 300 cases of Q fever throughout all of southern California, but it was almost impossible in cases to establish any epidemic connection. Only evident was a prevalence for the male sex and for the individuals connected with the milk industry, the meat products, the canned meat and the skin products. It must be said that these occupations were those that obliged a direct and continuous contact with the animals or with the products of direct derivation and working with them in the rough stage.

We shall go now to the investigation of large numbers that these authors examined as to their reaction of deviation of complement in the blood of approximately 10,000 individuals who were apparently healthy but resided in places where cases of Q fever
would have been observed. A positive result was obtained in only 5% of the cases examined; but the positive cases were almost exclusively found in individuals who had lived for a long period of time in the areas suspected of endemic and prevalently in individuals who were close to the occupational work previously mentioned. Among the cases that gave positive results, a certain number was made up of individuals without objective signs and without information leading to the conclusion that there was an active Q fever infection or that there had been for some time. It is of value to say that they were probably carriers of an infection of the disease in an inapparent stage. Granting that very frequently the cases appeared to exist in relationship with animals or in professions dealing with animals or with their raw products, these authors thought that endemic could be established as related with this Burnet zoonosis, which previously that had demonstrated in that area and putting thus in evidence, cases of spontaneous Q fever in many species of animal. These authors than went on to examine systematically the bovines of that area, and they found that from 10% to 20% were infected, the majority of them with the inapparent form of the disease. The milk of these infected animals and the butter produced from it were demonstrated to be capable of resulting in a typical Burnet rickettsia disease in guinea pigs when injected in the peritoneum. These authors concluded by affirming that the animals were the source of the endemic in respect to the transfer
of the infection, ingestion of milk or butter has some importance, but the most important, was the direct contact with the animals and with the raw products derived from them (working with milk, meat products, skin or hides, and earning meat products).

B. The Burnet zoonosis.

Analogous results to those obtained presented in the publication of Beck and Coll in March, 1950, were obtained in our research which was initiated in 1947, and communicated in 1947 to the 50th Congress of the Italian Society of Internal Medicine and latter on, to the Society of Experimental Biology, section of Palermo on the 7 January, 1950, and in the presentation to the Sicilian Regional Congress of our Society in February, 1950.

Our investigations have demonstrated that Burnet's rickettsia presents itself in domestic animals in a disease characterized by high diffusion and which, originating in a barn or herd, can extend rapidly and massively to all the animals which are stabled in that barn or belong to that herd without any distinction to the species. In Sicily, it is a spontaneous disease of bovines, equines, sheep and dogs. In all these animals it almost always appears as an inapparent infection.

In the goat, Burnet's rickettsia, other than being an inapparent infection, can also give rise to a disease with a course very similar to brucellosis, resulting in abortion (Kuldoled and Kirri, Kilchesperger and Wiesmann, Caminopetros).
We have demonstrated the presence of Burnet's rickettsia in the milk of infected goats through the biological test but in contrast with the statement of Asimopoulos, the animal demonstrated at the same time a positive deviation of the complement for the specific agglutination. Not all the goats with deviation of the complement of a positive sera agglutination, had infected milk; this confirms the experience of Parker and Coll, who, injecting directly into the mammary tissue some infecting material, could not demonstrate later rickettsia in the circulating blood, but have found in the sera specific revealing deviation of the complement and always to comparison with the rickettsia in the milk.

In Sicily Burnet's rickettsia is very widespread in goats, entire herds are struck. Systematic investigations by Kerr in Palermo county have demonstrated up 45% of infected goats among those examined. Lennette and Coll gave 43% infection for California.

In Sicily sheep have shown behavior similar to that of goats in respect to spontaneous infections of Q fever, whether it be with respect to the frequency of infection or of infected milk or through characteristics of the disease in these places. Also all the infected sheep showed deviation of the complement and positive sera agglutination and also they did not produce infected milk. But we have observed, as did Asimopoulos, three cases forms of acute bronchial pneumonitis.

Also the sheep presented in Sicily of infected cases, according
to vary 47% in California, Lutetia and Coll found 37.9% infection.

Among the equines, horses, asses and mules, the infection in Sicily is frequent, usually completely inapparent. In no case did we find an equine with positive serological reaction where the objective examination furnished data for a previous or actual disease in apparent form. For the equines in respect to Q fever there does not exist now other data in the literature except for Caminopetros, the animals have only received the experimental infection.

In the bovines, rickettsia in Sicily is frequent but only in inapparent form. Besides ourselves, Wiesmann has succeeded in demonstrating rickettsia in the urine of apparently healthy cows.

Miri, with his systematic investigation in the city and county of Palermo, has put into evidence a deviation of the positive complement of 33% in cows that were milking. Derrick and Coll in Australia have found positive results in 13 animals among 879 examined. In California, Shepard and Huebner have found positive 41 cases out of 150. Bock and Coll in 60% of the cases.

Huebner and Coll, having observed by means of biological tests in guinea pigs that the milk distribution by four dairies was infected with Q fever examined all the cows in that area and found that there was from 10% to 20% infection.

Jollison and Coll, having observed that a pregnant cow in apparent optimum physical condition, was eliminating infected milk,
kept her under observation for four months, during which time the
elimination of infected milk continued uninterrupted; after which
they kill her. Microscopic examination evidenced no splenomegaly;
large bodies through leucocytes, pleomorphic adhesions through a
recent bronchial pulmonitis, a mastitis. Subject to the biological
test through different organisms, the results were negative by
means of the fetus, the antibiotic liquid and all the organs, with
the exception of the mammary glands and the supramammary lymphnodes.
With respect to those last, the presence of rickettsia was securely
demonstrated. When the spleen, by injection unto guinea pigs, did
not result in feverish reaction, but in one of four animals injected
there was later a positive deviation of the complement. It was just
possible to give a positive proof through the use of the spleen even
though there was a lack of increase of volume.

In all the infected herds, the herd dogs give us a positive
deviation of the complement. By second investigation, anterior to
that of Mirri, showed a deviation of the positive complement in 50% of
the dogs which had been captured by the municipal dog catchers
of the city of Palermo. In one of these foci of acute broncho-
pulmonitis was found, from which was isolated Burnet rickettsia in
pure culture.

There does not exist any other information with respect to this
animal in the literature; only Caminopoulos, Elano and Coll have
written that these animals have received the experimental infection

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of Rickettsia burnetii.

With respect to other domestic animals which might show this spontaneous disease, they are not known at this time. Only one animal has shown a positive reaction to the positive complement. With respect to the wild animals, other than the Australian bandicoot, and the Karakorum gerbilles, there is record of some rats in Australia, and in the case of the tricosa vespigul (Burnet and Freeman), in America, the porcupine, Rocky Mountain laboratory. To those animals which have received experimental infection can be added; several species of rat and mice of house and country, the rabbit, domestic and wild, the sparrow, pigeon, cock, parrot, and the "scalptolo" according to Cunicotro.

Of all the animals where we have observed positive reaction of the sera complement, that is to say, signs of spontaneous Burnet's rickettsia whether accompanied or not with apparent disease, we have systematically attempted to carry the rickettsia into the guinea pig, the biological proof in the animal species according to inoculating once during;

a. During the feverish disease, the blood.

b. At the peak of the feverish disease, milk or urine.

In the case of the urine it can be demonstrated infective also for animals and it may take a long time to arrive from the apparent disease to a clinical recovery.

The fact that we have demonstrated that infected animals also continually eliminate rickettsia through the milk or the urine; the demonstrated resistance of Burnet's rickettsia to all the
common physical agents (heat, exposure for a certain amount of time to ultraviolet radiation) seem to make it lose its infecting activity; all these can easily explain why the rural endemics once established go on maintaining their presence in infected animals; and also on the other hand the rural endemics is, in its turn maintained in the presence of these infected animals also can affect forms completely inapparent of infection and always in condition to eliminate as well for the animals as for man rickettsia through the urine and to contribute thus to maintain infected the soil, house, etc.

Once a location is infected, whoever lives there can be infected, by inhalation of the virus or directly through the intermediary of dust, the virus can also be acquired through the means of ingestion of milk or foods.

The mechanism as well of the established as of the persistence, whether it be the endoosiosis or the endemic of Q fever becomes better understood through reciprocal interchange of the endoosotic phenomena, infection of ticks and of the direct consequence of all these phenomena upon the conditions of the soil, the houses or the water supply, etc.

Endemic Q fever.

The study of Q fever epidemic is much more complex due to the large variety of the clinical framework and epidemiologic mechanisms.
The varieties of the epidemiologic mechanisms are related to:

1. The resistance of the virus reaction to time or to common physical agents and to its maintenance in liquid and in general in organic material.

2. The large number of the means of infection through percutaneous and perimucosa way, through means of inhalation and finally through means of ingestion and the possibility of excluding direct contact with diseased people cannot be omitted.

3. The dissemination, especially in the endemic form, of the infection through animals to man with easy interchange and interdependence between endemics and zoonosis and the eventual development of epidemics.

Due to these variable interfering factors, in practice four types of epidemic mechanisms are possible:

1. The simplest and most evident mechanism is represented by the laboratory epidemics or surgical room epidemics, where the epidemic is obtained through encounter between the virus in its native state and the receptive individual; this mechanism is very, very simple.

There is no laboratory in which work has been conducted with Q fever which is immune to infection, regardless of the most diligent prophylactic measures.

We have had 7 infection cases among research and personnel of the microbiology laboratory of the Institute.

Laboratory infection cases have been reported with certain
In addition to those cases of infection which can be traced back directly to the function of the laboratories, must also be considered as laboratory infections those cases which are observed among occasional visitors, as has happened in our Institute on two subjects who were infected through short visits to our laboratory of microbiology. Cases of this type are not rare in the literature. Cases of this type go back to 1938 (Dyer) who had shown the first practical demonstration of the virus isolated from the tick of Mogouild and was infected not only by mouse but also by man and was identified to be a disease identical to Q fever. After the case of Dyer, the following cases were observed:

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Researcher</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naples</td>
<td>1945</td>
<td>Robbins &amp; Rustigan</td>
<td>7 cases</td>
</tr>
<tr>
<td>Montana</td>
<td>1948</td>
<td>Oliphant &amp; Parker</td>
<td>1 case</td>
</tr>
</tbody>
</table>

In this exposition we must also point out that in addition to the occasional visitor, there exists a third group of infection which in the literal sense could not be considered as laboratory
infections. In the question of this disease induced in two medical men who were attached to the cardiological laboratory of the Institute and four sick persons who were taken to the electro-cardiograph department for examination, but in no case did they stay there longer than one hour. The infection was produced through the mistake of an orderly who cleaned the room with a broom and dust cloth which was supposed to be used only in the microbiology laboratory. The epidemic was stopped only when the electrocardiograph was taken to another location and the room was subjected to an energetic disinfection.

All those laboratory infections took place even though the most minute precautions (prophylactic) were taken. When research ticks determined experimental direction, it must be said that when the virus goes even to the embryonic egg or tick and thus come to make large amounts of cultivated virus or in the passage to the tick can develop particular properties that increase the virulence.

In respect to the pathogenesis of such laboratory infections it it can be ascribed to the inhalation phenomenon; in fact, in the Ft. Bragg Laboratory in 1945, all the experimental workers that were working with the volcanic source (strain) were infected with the exception of two, who, when working, were masks (CARD).

Among our cases of laboratory infection there were some that were due to virus research ( 7 cases), some among occasional visitors (2 cases), some among frequent visitors, medical doctors (6 cases) and patients who had visited the laboratory of electrocardiography, and
all with the exception of two bacteriologists had normal disease with pseudo-influenza syndrome, with typical pulmonary infiltrate which was observed radiologically. The two bacteriologists did not have any forms of the evident disease, but in a week the virus developed in embryonic chicken egg culture and for a few days they did not feel too well, had some fever in the evening, without any other subjective disturbance or objective fact, but still could not carry on their research work. The infection was determined through the deviation of the complement which was carried through all the personnel in a systematic manner for several months during the whole period of research and eventually found that later on the two subjects obtained a constantly negative report.

The serological examination maintains itself in the majority of the cases positive for six consecutive months. Numerous radiographic controls are repeated systematically and the series have not put into evidence, with the exception of these two subjects, the presence of a pulmonary infiltrate.

With respect to the other 13 cases of infection in our laboratory, we can easily go back to the common pathogenesis of inhalation whether through the means by which the virus is stabilized or whether through the characteristics of the same disease (feverish disease, evident, typical pulmonary infiltration) in these two bacteriologists the infection was typically inapparent in development
without fever or with minimal fever, especially the absence of the pulmonary infiltrate that was characteristic of the form of the disease of inflammatory origin. We believe that in the bacteriologists the infection was derived from another means of infection and probably through the percutaneous way which have demonstrated Blank and Coll, if there is no pulmonary reaction, causing inapparent disease. The infection obtained through brushing of the virus with the tip of the fingers. In fact, at that time it was common to put the virus into the embryonic egg. It was made with out sterile cap and without sterile gloves. Thus it was possible that the skin of the hands could come into constant contact with a large concentration of the virus, facilitating virus infection.

As a second group of infection originating in the laboratories we can consider the epidemics which result following autopsies of men or animals who died of Q fever.

The first group of these is represented by an explosive epidemic which hit the personnel of the anatomy room from 14 to 38 days after the autopsies of animals or men, dead of Q fever, these times corresponding to the incubation time of (Q fever) spontaneous infection of Burnet's rickettsia.

The first episode of this kind is described by Torzani at the Anatomy Department, University of Florence, from 20 to 58 days after the autopsy of a dead patient, who was diagnosed as having pulmonary virus and the autopsy took place on 21 January 1947.
The first of the following March, five doctors and ten students that assisted as spectators to the examination without taking part directly in the operation and without having touched the cadaver or the instruments. Q fever was diagnosed in these cases only several months later after their recovery by the means of the deviation of the complement (Babudori) instead it was not ascertained that Q fever was the cause of the cadaver, which according to anatomy had only atypical bronchial pulmonitis.

The second epidemic happened in London at the Royal Cancer Hospital following the autopsy of a cancerous old man, dead through pulmonary form which was not diagnosed as infective and according to post-mortem serological examination, it was successively ascertained as Q fever. The doctor who performed the autopsy, two assistants and an orderly were sick.

The third epidemic described by Back and Coll amounted to two orderlies in a surgical room through an analogous condition.

The fact that in these cases not only the doctor performing the autopsy and his assistants who had touched the corpse and especially the hands directly became sick, but also others (students) who had only looked on without direct contact, thus we must necessarily conclude that they certainly contacted the disease through inhalation of the virus, which leads one to believe;

(a) The virus concentration in the autopsical bodies and especially in the lungs, must really have been important (in the London case one
was concerned with acute edematous bronchial pulmonitis of Q fever origin).

(b) The quantities of inhaled virus sufficient to cause an infection must really be very small.

In what concerns inoculated and assigned personnel, one must exclude the possibility that the virus came into direct contact either with the mucous or with small skin abrasions.

A group which should be finally added to laboratory infections, consists of types of epidemics in slaughter houses, among the butchers and the clean-up personnel, after butchering animals (bovine and ovine) which, despite the fact that they had been passed as healthy by the veterinary examinations, resulted contaminated with Q fever, completely inapparent and despite this showed great infective power during a post-mortem examination of the blood and organs.

The first cases of this type have been described by Derrick, who refers to it as a butcher's occupational disease, very common in Australia and known as "slaughterer's fever".

In America, Topping and Coll speak of 40 cases noted among a slaughter house at Amarillo, Texas. Two of these cases died; however many cases classified as inapparent illness were only discovered by means of systematic serologic tests. Shepard describes 30 cases of Q fever all of which arose among the workers in the slaughtering section of a large Chicago meat packing plant; later during a systematic examination of the entire personnel in that section, 33 out of 61 men had a positive deviant on of the complement, proving
to be infected in an inapparent way. Geissl and Engel speak of a similar illness (20 cases with 1 fatal) in the Strasbourg slaughterhouse.

In connection with these epidemics, one should note that among the best studied American cases, the mén concerned with evisceration, those who are in more direct contact with the abdominal organs and with the lungs and the butchers who are more apt to be covered with blood, proved to be more easily infected with Q fever (Hooper, Topping and Coll).

Even though in this last group of epidemics, the relationship, that is, virus and infective relations, the simple fact that the mechanism is less simple; in addition to the inhalation way, the possibility of direct contact is excluded either through mucous membranes or a slightly abraded or open skin, contact with blood or infected material, thus these possibilities of inapparent infection, without eventual pulmonary infiltrates.

2. Another group of epidemics with sudden massive beginnings of a large number of contemporaneous patients which can be traced to direct relationship with the virus, conserved actively in the dry state. It must be said, that is, through a mechanism, a little bit more complex, that the preceding one and which is conditioned by three necessary factors:

a. The existence of the virus for a long time in the dry state, conserved actively in straw, sawdust or shavings.

b. The transport of this virus to a place distant.

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8. The course of this virus with receptive persons.

A characteristic episode of this kind is that described by Vogt at Coire in Switzerland in a mechanical office which had received machinery from Berlin. 19 cases of operation of that shop became sick after having imported the machinery which was packed in wood shavings. The virus which had been conserved in the shavings and in those packings had survived the long trip.

Such a pathogenesis can also be related in epidemic in Kaffi (Kong and Cox), 200 cases and among the military of Mt. Ceneri, Switzerland which were a motorized unit of artillery on maneuver (Geoll) and one of the other cases, the virus was probably conserved and transported along the straw, in the first case contained in a steamboat, in the second case used in military bivouac, in one of the other cases (both cases coming from places of endemic and of common infection). As we have seen, straw, shavings, packings, etc. can easily contain focuses, eggs and remnants of infective ticks which can hide during the hibernation and which, to become infected feed on the blood of infected animals.

In other cases the epidemic assumes the character even more clearly when related to specific professions dealing with wood or raw products. In both cases, whether through the cutting of the trees or along the openings or under-taken holes of these, it is easy for epidemiological arthropods to find refuge there, which when pulverized can take their place among the infected dust.
which, disturbed in the environment during drying, can communicate the infection by inhalation.

A typical example of an epidemic through workers working with raw materials is described by Forconi and Salvato among the carpenters and other workers constructing the electrical center in Alto Adige. Another epidemic ascribed to woodwork (Gsell) took place in Aarau, 45 workers in a wood furniture factory were in contact with dust and sawdust of infected wood.

A chronic epidemc focus was interesting which occurred in a hospital (Asylum). Among those working in the carpentry work, cases of Q fever were observed at diverse intervals over a period of three years and probably came from working through the demolition of war barracks, made of old wood, and probably were thoroughly infected with arthropods, which were probably infected.

3. Another group of epidemic episodes poses as a factor of the disease of man; the disease is unchanged when there happens a direct and immediate contact between a receptive man and an animal bearing the infection or the virus can serve actively in the dry state through the hair of the fleece, and when it is in condition to easily be fused into the environment through varying eventualities, and to give rise thus to infection of inhalation to many individuals at the same time and contemporaneously to create a true and proper epidemic explosion. A prototype of such is the epidemic of
Chiaravalle (Patrijmani and Bacchlocco) where a hundred or more persons were stricken at the time in that city and they all lived in a single street of the city, through which passed in the mornings to go to the pasture, a herd of sheep and goats which had recently arrived from Puglia for wintering and were shown later to be infected. Due to the preceding illustration one must think of the mechanism of inhalation of infective powder carried by the animals in the fleece in the dry state and thus easily volatile.

Thus many epidemics can be explained as observed by various authors in these latter times in the agricultural zones of central Italy, especially in the Marche and in Tuscany. The uniqueness of this mechanism is probably the thing to which can be attributed a true collective explosion, whether from a small single focus or eventually perhaps individual infection of a person, who was accidentally contacted, either casual or perhaps even momentaneous, with an animal infected and bearing the virus, perhaps by contact through the hair or coat, poorly cleaned, dusty and infected with ectoparasitic arthropods or some of their remains. This mechanism of infection can be created in addition to the animals of which typical examples are the sheep, with abundant coats, but also the equines and the dogs, that which, without having necessity to touch or reach them, a current of air can bring about gusts of infecting powder.

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4. A large group of epidemics with a mechanism even much more complex is related by the epidemics which are obtained in the city and the agglomerations which are found in regions like Sicily where there exists an endemic and zoonotic Q fever in the countryside.

These particular epidemic episodes present a particularly exceptional character that we have not been able entirely to hit upon in Sicily in our systematic study of the disease. These epidemics of the city or human agglomerations have always gone along in a markedly stage march, that is, in various stages; they are more frequent in the period which exists between the end of autumn and the beginning of spring, although its appearance at other times is exceptional but cannot be completely excluded. In general, the epidemic does not come by itself but usually is accompanied and sometimes mixed with influenza or pseudo-influenza waves, with which the cases of Q fever are mixed so that it is not possible to calculate the exact proportions in view of the patients stricken with influenza. It is not possible at the present state of conditions to determine, except only purely approximately, the numerical amounts of the three epidemics of Q fever which were observed under our direct control in the city of Palermo in 1942. In general, the patients did not show up as groups in a determinate region or groups of houses or even in a single family, it was, instead, an epidemic of various cases which were distributed throughout the city in different parts of the city but which occurred at the same time.
We cannot say that there are certain wards or sections of the city (for example, in the vicinity of the harbor, railroad yards or in the periphery next to the countryside) which in those years had larger frequency of fever cases, or that even show the particular tendency to those. Only rarely did there occur as much as two simultaneous cases in a single family except perhaps sometimes in the cases of pulmonary form, the sputum of which we know is highly infectious. Sometimes with a certain amount of frequency also in the city, there can occur sudden cases in a house which occur at different stages during and in the course of different epidemics so that the distance of time between the appearance of these single cases makes it possible to exclude decisively any possibility of any direct contact between patients or of a unique inflicted moment which could have resulted in these cases, if not at a contemporaneous time, at least, a relatively neighboring period of time.

Many of these characteristics of insurgents of these epidemic cases in the city did not seem to be analogous to those which characterized rural endemics.

From this data it would seem possible to put this group of epidemics, characteristic of the city in the agglomerations of Sicily, in single intermediate position between the epidemic form and the endemic form.

In effect, following our study, it was possible to demonstrate
that in Sicily the epidemic episode happening in the city and in the large rural agglomerations were demonstrated constantly to be in pathogenic report or accord with the rural endemic which was always produced through diverse mechanisms which are:

1. Through the transport, by means of hairy animals, different dusty objects and filth (straw, etc.), work instruments, vehicles or wood with animal traction, etc. - of the virus in the dry state, conserving its power easily to lift up into the air and to be disbursed, determining the possibility of an infection through the inhalatory means; or

2. Through the milk of infected animals, or aliments dirtied with excrements or whatever other infected materials which can create inhaled infections which can represent a possible mechanism that could be determined.

In this respect it is of interest to note in particular of no small epidemiological importance of the cities of Sicily, Palermo included, and for much of the south; it is that in the morning many groups of milking animals (milk cows and goats) come in groups from the country to be fed directly in front of the houses and that a systematic examination conducted by Mirri in Palermo showed that almost 50% of them were infected with an inapparent form of Q fever. It should be observed that the housewives having personally observed that the milk was taken directly from animals apparently in good health, commenced to put it to boiling and thus
eliminated the possibility of contagion, either by infection by those who drink it or by inhalation by those who handle it. These intermediate situations appear even more exceptional when it is considered that the determination in these groups of an epidemic factors of climate, location, etc. opposite to the other types of epidemics has such an importance that they dominate through unrecogn- nized mechanisms the whole epidemic march.

These facts are even more unusual or strange in respect to:

1. The transport of the virus from the infected countryside to the city can occur at any moment through any filth or dust carried by the workers who come into the city, or hairy animals that for different reasons can come in, and finally through the milk or other infected foods; in the infected animals which are in the continual conditions of eliminating rickettsia through the milk(jellison) and the urine (isch Wolfe, Weismann), etc. and thus to create infections from which derives the fact that cases of endemic Q fever in the country can be observed in every season.

The fact that epidemic factors become effective only in particular conditions of season and climate makes us necessarily think too of the intervention of factors related to the life and the development of ectoparasitic arthropods since, as it is known, their importance as direct vectors of the disease, at least in America and in Europe, must not be too much of value. In the cases of the Sicilian endemic and epidemics since there was a positive
report of a massive infection of animals at the same time as
the epidemics, those occurring prevalently in the cold season
while the infection of animals through means of octoparasitic
arthropods and especially of ticks is always major in the cold
season. On the other hand, our knowledge of the virus in ticks
assures that an animal surely infected although this information
comes from non-systematic investigations of small number, are
sufficient to exclude the fact that the tick in general can
globally be infected in such a percentage that it would institute
a permanent reservoir of the virus from which the epidemic could come.

But we have not demonstrated that for an infection for Q fever
in which the inhalatory means represents the most common mechanism
for infection for man that the tick possesses importance as vector
of the virus, in maintaining and stabilizing the infection of the
wood dust or sawdust of the shavings, etc., and intervenes in the
mechanism of individual infections of epidemics, etc.

From all this it can be derived that the seasonal cycle of
development and life of the tick, also through the emission of the
eggs infected through heredity can come in some of the means from
which is due the seasonal variability of the urban epidemics. In
fact, the degree of infection of the dust capable of creating of
inhalation infections, so important in the epidemics, depends in a
major or minor characteristic of the infected remains of dead ticks
or their excrements and these are major or minor following the
seasons and with relation to the season and the climate, exercised
unto their development and the fecundity of the ticks. Since the ticks in general at the approach of winter hide in the straw, the dry wood or the barn dust and go into dormancy, it is easy to explain why the dust and powders are more infected in the cold season and that infection through inhalation of carried virus is easier during the cold season and this had been verified in the Sicilian cities.

It is also possible that, as it has been seen, the coefficients with respect to the seasonal evolution can come in, multiplying the conservation ability of the virus in the powders or dust and consequently their infecting capacity; these physical coefficients could be, for example, dry climate joined with more active conservation of the virus in specific seasons and decreased the activity with humidity.

Clinical.

The clinical aspects of Q fever are much more varied. These after all have passed the initial concepts of an acute form and of pseudo influenza frame, with a limited possibility of symptomological variations and being, in a quantitative sense, through the various gravity and intensity of the attacks; and with very little amount of variability of the symptoms in the qualitative sense, determined only by the supposition of the complications which are very rare. Actually, especially through the contributions brought by the European observers, among which the observations of my school are of no little
important, the clinical frame of this disease appears to be very varied, frequently in other than the acute form, sometimes in the subacute form, the one and the other with the possibility of focusing into clinical syndromes of diverse aspect and nature, which reach to their origins in anatomical lesions and functions of different organs or apparatuses and extending their symptomology to those of pathology from which they are not too far.

I have the clear conviction that in spite of the new and important knowledge derived from clinical and experimental contributions of these latter days, the edifice of our knowledge on Q fever cannot be considered at the actual moment still complete; and that any clinical contributions must be considered still truly important for it is exactly guaranteed that they can still inform us in regard to the pathogenesis, not only of the entire disease of the subject under discussion, but also of the particular disturbances and particular lesions that the patient possesses. In fact, it must always be maintained in mind in this particular kind of research that once it has been established with strong certainty that the patients under examination infected with a form of Burnet's rickettsia (active) it is not questioned for this reason to automatically authorize, to attribute to this infection any existing lesions in the disease since, in the majority of the cases of acute infection, it has always presented a possibility that other lesions can occur in an affected subject, lesions which may be chronic or subacute, pro-
existing of inactive or latent state; lesions which, when discrimination is applied may be erroneously attributed to new alterations of this infection, proceeding or concurrent with it, which are completely independent from it.

The diverse clinical forms of Q fever, with its many forms of symptomatology, constantly presents some common and fundamental symptoms which it should always be the practice to look for at the moment of the suspecting and the establishing of the diagnosis, which are;

1. Splenomegaly - At the beginning and under the acute form, typically soft; with a prolongation of the form, if less evident, while there is a tendency to an increase with respect to consistency; in the recurring forms (pulmonary, pseudo-Brucella) there is a reoccurrence of the fever and of the fatty bodies and a reaccentuation of the splenomegaly. The splenomegaly is a fundamental symptom and constant in all the forms of Burnet rickettsia, whether spontaneous or experimental, as well in man as in the animal.

2. Fever - It has a somewhat more varied aspect until the beginnings of the disease; a high fever to a moderate fever or the small fever; it can be continuous, intermittent continuous, relative continuous, irregular; often it has a clear undulating characteristic and is accompanied by profuse sweating and general disturbances (malaise, headaches, anorexia, nausea) which are reaccentuated each time.

The duration of the febrile period can be quite varied, from a few weeks to several months.
3. Pulmonary infiltrates - with the exception of the cases of smaller or low fever, of which we shall speak later, it is always first with major or minor evidences from the beginnings of the disease; but it can vary quite a lot as to its duration, its intensity and its localization from case to case (Longhope). In the more common cases it has typical character of a transient infiltrate. Pathologically, anatomically, speaking it is made up of concord fold of broncho-pulmonitis with a kind of isopenia extending to the bronchia and with intravascular exudations. In the majority of the cases it has a tendency to regress, sometimes up to a complete reabsorption. In the most acute forms with a bronchial pneumonia character, the infiltrate, once localized in a determined portion of the lung, as observed diffused with small sparse nodules and also numbering in the thousands. In these cases it is shown to be surrounded by large and characteristic halo of inflammatory edema. In the subacute forms it can be accompanied by peribronchial, monocytarian infiltrations or perivascular infiltrations of connective reaction, where a major persistence of the infiltrate exists, which comes to lose its characteristic of transient infiltration.

In the acute forms the transient infiltrate cannot give a symptomology to common or simple examination of the chest, since the infiltrates exceptionally are superficial and can be diagnosed in a direct clinical examination. It is easy, however, to demonstrate it through a radiological examination where this kind of transient
infiltrate appears as a tenacious shadow, undefined, with a traumatic structure, especially located in the pulmonary spaces but sometimes visible in the higher ones, non-excluding the subcarinalae. This shadow of infiltration persists differently from case to case, in the more common cases, after one to two weeks it can be considered as a relic.

It is important to point out that there does not exist any parallelism either in intensity or duration between the infiltrate and the fever.

Many infiltrates can be accompanied by an average or moderate fever, while in patients with a high fever, it is possible to have a tenacious infiltrate, small extension of very, very little symptomatology, even though radiologically significant.

Yet it can also be that even though there exists no traces of the infiltrate, a small fever can persist for months.

In the recurring pulmonary forms, the infiltrate remains unchanged for some intervals of time when there is a general disturbance and finally coughing and the expectorate disappear; the total reabsorption occurs only and solely when the recovery is definitive.

And in general the fever disappears rapidly with aureomycin (Lennette, Lennette and Coll, Roosevelt and Coll, Long and Cox); sometimes the infiltrate or complete stoppage of the inscriptive process can disappear after a few weeks or in some instances in two months.
4. Reactivity of the normal laboratory examinations - normal urine. Blood without any particular morphological characteristics, quantitative or qualitative, especially of the leukocytes. From case to case independently can be observed that a moderate leukopenia or a moderate leukocytosis; moderate neutrophilia or a relatively evident lymphocytosis. We cannot confirm the affirmation of Dohi that the active form has as a characteristic an eosinophilia; in many cases however, we have observed especially at the beginning a tendency for small eosinophilia (Ingrassia).

5. Sedimentation velocity of the "crisis"; it is constantly increased, in the repeating cases when there is a new attack it is preceded and accompanied with an increase in the sedimentation rate.

6. Hemagglutination in the cold - in Q fever on the contrary to that it is in virus pneumonia, it is constantly negative; this symptom has a differential diagnosis value. The observations of Heilmeyer of cases of positive cold hemagglutination have not been confirmed.

7. Arterial hypertension, venous hypertension, marked "astenia" persists even in convalescence.

8. Behavior of the electrocardiogram; Brawley and Kedem speak in a case of six examinations of electrocardiographic alteration (evolution of the electrical axis to the lymph). DeBrochi has followed systematically 22 cases of electrocardiographic behavior during the disease and in convalescence, in 13 cases, the graph showed no path-
ological alterations; these were light forms of moderate cases with
a transient infiltrate, rapidly recovered, either spontaneously or
with aureomycin. In 9 cases, however, there were evident alterations
present of a myocardial-coronary character. Of these cases, 4 showed
serious alterations of the T-wave in the third Bor, and in the
precordium with marked levelling of the S-T graph. In a young woman
with a pulmonary form of moderate proportion, the electrocardiograph
alterations were evident for several months after the clinical recovery
of the patient. In a case of special gravity with an edematous
bronchial pulmonitis and with circulatory collapse after the fever,
the electrocardiograph examination showed persistent alterations of the
myocardial type, these alterations remained evident for several months.
In general the alterations of the electrocardiogram observed during the
fever disease have been demonstrated to be modified by aureomycin therapy.

9. Completely negative results of the mono culture on common
medium and complete negative results of the common serological investiga-
tion, reaction of the wild phalax pad. The negative results of the
wild phalax reaction can have a discriminatory value when confronted
to other rickettsia for an eventual differential diagnosis.

With respect to the behavior of the reaction for plague and
especially of the Wasserman reaction, only Mastro has not observed
a positive result for Q fever. We have in 12 cases had positive
results at low level, limited however, during the duration of the
feverish period when the fever attains the positive result is scarce.

It seems to us that this problem is worth studying systematically in other cases of Q fever: in other cases of a typical pulmonitis with positive Wassermann reaction, the eventual positivity of the serological reaction for Q fever must be studied systematically for typing it in with the biological proof in guinea pigs in cases where this would be positive. In such a way it would be possible to correlate the reports for Q fever and virus pulmonitis described by Hoglund, Grumba and Biacconi.

In the clinical study of the cases of Q fever which have been reported in the world literature and including the contributions of our personal files, it is possible to distinguish among the many forms which can be related to the constant presence of some common symptoms that we have considered as fundamental and as disease.

These different clinical forms are:

1. Pseudo-influenza form.
2. A settic form.
3. A broncho-pulmonitic form.
4. A nervous form.
5. A pseudo-brucillus form.
6. A form of small fever (Burnet's little fever)
7. A sub-acute pulmonary infiltrate.
8. A form of latent disease with apparent symptomology.
1. The pseudo-influenza form was first described by Derrick. It occurs easily in the cases reported by American and Australian authors, frequently it is presented in epidemic episodes and especially in those where there is particular gravity of the situation. The general aspect of this form falls very well within the framework of a common attack of influenza and is differentiated from it only through the duration of it, which is always major or larger.

This form has always an acute beginning, at the time of good feeling and without any previous period of attack; it is accompanied by shivering, of a larger or smaller intensity, which can be more or less generalized, there develops malaise, debility and of general depression. There is also myalgia and diffused arthritis of the rheumatic type, sometimes there are chest pains (Robbins and Regan) which provoke exacerbating respiration pains of the pleuritic type (Finestine and Coll). In addition to the fever in some epidemics there are some kinds of catarrhal, more or less intensive in the pharynx (Robbins and Regan, Finestine and Coll, Kaldolcsi) and of the higher respiratory tracts. Some of these lack in other epidemic episodes; not infrequently there is a persistent cough, dry or with little expectorate, sometimes mixed with blood. Not too rarely the beginning is underlined with evident hemorrhagic manifestations; these can be more or less intense or of small importance (Irons and Hopper, Kaldolcsi).
The general appearance of the patient is very similar to that of one suffering from influenza, congested face, red conjunctivitis. In the first two to three days there can be, not infrequently, a cutaneous rash on the face, breast, shoulder, back (Raleigh and Modern, Derrick); almost always there is a feeling of mental and sequelae confusion, especially on the bronchial locations, also in the retroorbital location; in any case there is observed a pain upon movement of the ocular bulb, typical of influenza. There can also be a state of disquietude and insomnia, but it can be observed not infrequently a true and proper stupor state or a state of mental confusion and also of intense agitation (Derrick); in some cases it can also be added a generic sign of meningeal suffering; photophobia (Derrick) and nucorrigidity (Honebruck and Nelson, Robbins and Ding).

The tongue is always diffusely patent, there exists sometimes digestive disturbances more or less marked; anoxoria, nausea even sometimes vomit; also sometimes diffuse pains in the abdomen. Many times there is evidence of diarrhea (Robbins-Regan, Bramley and Modern) but rarely is there a marked constipation. Especially there exists a burning urine, a symptom that the Anglo-Saxon authors have underlined with insistence. In any case, Raleigh and Modern have encountered a micropoliadenia which is very painful and diffused to the major part of the lymphatic glands. The objective examination is completed with this in addition to the splenomegaly and the pulmonary
infiltrate which is more or less marked and more or less persistent from case to case.

The fever is always rather high and continuous; it is remittently accompanied by profuse sweating. The duration of the period of fever varies from case to case; from 10-15 days, from rapid exhaustion to complete recovery, without any type of relapse, excepting a profound asthenic which lasts for a few weeks.

2. The settic form is characterized by generic impression of great seriousness which manifests in type from all the symptomatology, completely pervaded by the profound and serious general sotthical character; definitely settic tongue; evident and remarkable depression of the general condition, of the sensoria, of the circulatory functions; high continuous or intermittent fever; constant myocardial coronary changes which can be shown electrocardiographically (Johrend), marked arterial hypotension, venous hypertension. The duration will be at least from 4-5 weeks. Complications can set in very easily, the most frequent and the most characteristic among these is broncho-pneumonia of a migratory nature, with reactive pleuritis (Moldolesi, Negrassi and Coll, Coppo and Coll, Irons and Hooper, Koechlin and Kossowski), and above all phlebitis, (Hubens and Stickmen, Negrassi and Coll, Coppo and Coll, Moldolesi) periarticular (Moldolesi), orchitis (Irons and Hooper) and also Moldolesi, Csoll; Koechlin and Kossowski; angiitis, (Moldolesi)
pancreatitis (Morsbach and Nossewski). The convalescence under these forms is very slow; functional reconstruction is slow. A certain percentage of deaths exists.

3. The broncho-pulmonary (broncho-pulmonary edematous) form represents a characteristic of Baranet's disease, either the clinical study or anatomopathological discovery.

It has always a rapid beginning, resembling true pneumonia; in full health with no premonitory disturbances, with sudden shivering and immediate state of exceptional gravity and of profound depression of the general state and of the circulatory function; high fever, continuous and noticeable arterial hypotension. Low, frequent pulse, which is thick, filiform, rapid; dyspnea and violent cyanosis; persistent and tormenting coughs, with serious expectoration of pink coloring, in every way like the characteristic type of acute pulmonary edema.

During an examination of the thorax, a reduced tympanic sound came to light, spread through the entire pulmonary region. With great attention during the examination, at a certain point here and there one is able to detect the vaguely traceable sounds of bronchial pulmonary confluence. However, the vascular tautness completely vanished and was replaced by fine regular rales; breathing similar in all ways to that of acute pulmonary edema. This simiological report true to characteristic, finds a perfect interpretation in anatomopathological report (Jinlir and Coll; Hamana; Kaldolasi) which brings into evidence spread here
and there, multiple nod of broncho-pulmonitis, of confluent character, enclosed by a large halo of highly contagious odors.

Six such cases have been brought to our attention, which can be compared to the case of contagion from a laboratory described by Lillie and Coll and to Karman's cases. Cases of extreme seriousness are always discussed, which can easily result in death, as in the case of Lillie, in that of Karman and two of ours.

Such a case in our experience demonstrated an even greater and more precipitous clinical syndrome without referring to particulars of the individual. It concerned a young artillery officer, 26 years old, no particularly weak background traits; he was not plagued and not a smoker; strong with no original deficiencies, with no previous weaknesses of any kind. Attacked by the illness in full health, his friends and doctor were impressed from the very start by the sudden state of depression and of seriousness. By the third day of sickness, conditions were suddenly precipitated to the point of a state of collapse, protracted 48 hours, with a hippocratic face, very dangerous arterial hypotension; the pulse hardly noticeable, rapid, irregular and arrhythmic, but above all with complete suspension of the diuresis and a very dangerous state of secondary hyperazotemia. He slowly recovered with symptomatic treatment. The electrocardiographic examination showed grave myocardio-coronary changes of the myocardial types (DaGrcbi).
In certain cases, especially if one uses aureomycin, the fever begins to drop rather rapidly, after 2 to 3 weeks, leaving the patient in such worn out condition, from a general point of view and because of circumstances as to necessitate a few months of convalescence and a series of symptomatic treatments before there can be a sign of recovery.

However, in other not rare cases, a typical symptomatology of successive relapses is observed, each lasting from 3 to 4 weeks, recurring at intervals of varied length of one or more weeks during which the patient, while enjoying a relative lull in the symptoms of the illness, mostly the burden of the respiratory system (coughs, dyspnea, expectorations) does not indicate any lasting improvement.

Then, at every relapse, the high fever, the sudden gravity, the heart and breathing difficulties and the burden of the respiratory system reappear suddenly.

To the characteristic clinical and anatomopathological discovery concerning the lung of this form correspond, a radiological discovery even more characteristic, i.e., a shadow, not uniform, in the two pulmonary cavities, more marked at the cases, so as to nearly always leave the top sections unaffected. This irregular diminishing of transparency of the pulmonary areas is due to two different conditions:
a) to a circulatory effect, of infectious pulmonary oedema, which is the more striking and more evident, depending on the acuteness of the type, whether at the start of the disease or of every eventual successive relapse.

b) to the combinations of very numerous dying particles, roundish, of the sublobular or lobular type and sometimes also sub-lobular which are especially more noticeable in the high regions of the pulmonary areas where they always join smaller and therefore, more isolated; while in the lower regions where the tendency is to combine, they do not appear unless from the extended shadows, irregular in distance and transparency. The more the illness is separated from its beginning, the less diffused appears the denitrous area, while on the contrary, the shadows of infiltrative effects, sub-lobular, become more and more dense and with more and more blurred contours. In a successive period the character of the micronodular diffusion continues gradually to disappear because of the strengthening of the phenomenon of confluence.

The type, which is typified by relapses, presents noteworthy modifications from one period to the next. During the intervals, the oedema fades, however despite the stopping of the dyspnea, from the cough, expectoration, the nodular shadows not only do not vanish, but they have a tendency to become more intense and larger, with ever more precise contours, while at the same time a conjunctive reaction of the peribronchial and perivascular character appears in the background.
which progressively grow in intensity and magnitude. During each
new relapse, the clinical features return, with the reappearance of
acute clinical symptoms on the basis of this radiological
background. However, the clinical phenomenon shows a constantly
lesioned intensity after each new relapse, while, even their
sub-nodular shadows, recent and with blurred intervals joins the
existing ones. Only at the final healing of the disease do those
infiltrates resolve, more or less fast, while the peribronchial
reaction remains repeated at length.

In these cases of edematous broncho pneumonia we have made a
singular and characteristic discovery. If, at the beginning of a
relapse or during the acute phase of the illness, we injected the
sputum of the patient using the necessary technical precautions,
into the peritoneum of a guinea pig, we could establish a typical
Burnet’s disease (in the animal), which could easily be transferred
into the animals in series and even into the embryonic chicken egg
until the pure culture has been obtained. Already after the first
test, in the guinea pig’s spleen, which has reached far larger
proportions than the normal biological reactions, coloring bands of
pulp using Macchiavello’s stain or better, using that of Giemsa,
the undisputable presence of rickettsia. I repeat, that through
our personal experience, it is not an ordinary happening during
biological problems in which common experimental materials are used.
Thus it is proven that among patients of this singular
particularity, a direct elimination of rickettsia takes place
through the sputum. The amount of virus in the sputum of these
patients must rise immediately, for, very frequently, the guinea
pig will, after first injection, demonstrating the
characteristic rosette-pat:ent report of rickettsia Frome ti.
A reaction was noted by the common biological experiments
rickettsia Frei epidemic for this, in type, is quite easily
tolerated by the guinea pig and not infrequently, with the passing
of time-hardly of any clinical illness. Another effect, singularly
important, is seen observed in these patients. In one of our
patients, a clear record, muscular dangerous and gravity,
at the beginning of disease, the sputum which contemporaneously
to the biological experiment has shown that it contained a large
amount of the infective to the guinea pig, examined on an ordinary
swab colored with Macchiavello or Giemsa, showed a
great quantum of minute muscles, which could be identified as
rickettsia their short size, a unique report in science
today(Butt). 4.
4. Nervous tics are not very frequent. It was first
observed by in a particular clinical syndrome. Symptoms of
nervous centre peripheral, during Q fever have been
recorded by authors in cases of epidemic episodes of
lesser gravity; headache, vertigo, a feeling of general malaise, profound asthenia (Hornibrook and Nelson), intense and persistent headache (Derrick; Robbins and Hagan; Card; Hornibrook and Nelson; Shepard; Bradley and Modern) which frequently constitutes the most obvious symptom during the entire most acute part of the illness and can also persist for a long while during the convalescence, after complete lapse of fever (Kaldeski). In acute cases, more or less important changes affect the sensorium (existing, a stupor; Derrick speaks at length of the possibility of a true and actual typhoid state, accompanied in many cases by photophobia and by rigidity of the neck (Bradley and Modern). However, we have always discussed highly fevered patients, acute, of particular gravity, in which the nervous state can only represent the expression of the general toxic state or yet, of the acuteness of the danger of the disease.

While the nervous state described by us always has a rather less acute character and manifestations, it nearly always manifests itself with low fever, in certain cases with only a little fever; however, in every case the fever sequence appears in waves of varying duration, recurring at intervals of various length, during disorders, always of organic nature, present ulterior rekindlings. Characteristic of these nervous manifestations for the entire duration of the illness is the constant presence and persistance
of splenomegaly or of pulmonary infiltrate. The disturbances, in the
form of lesions of menopilite character, always begin in a sudden
manner, without ever being accompanied by attacks, even minute, of the
sensorium, the sleep and of the exsensiliorius synery.

A characteristic form, noted by us frequently, is represented
by the grouping of paralytic in the areas of the pharynx, the larynx
and of the velophalai with phonia, oesophagus, dyspnea; frequently
unilateral but bilateral in some cases. The appearance of an
associated syndrome of this type, completely isolated, during the
course of an illness with hardly any fever, without any disturbances
of the sensorium must make us think of Q fever. In one such case observed
by us, the motor difficulty in the area of the larynx, which started
suddenly, was so serious as to cause a paralysis in the area of the
vocal cords and to force an emergency tracheotomy, to avoid serious
asphyctic possibilities.

Usually, the disturbances of impulses, especially under
treatment with aureomycin and Vitamin E, but sometimes also spontan-
eously, can regress slowly and without after effects. Wegmann,
Koschalin and Kossova describe a typical encephalitic extrapyramidal
syndrome of Q fever, with dyopia, reversal of sleep, speech and
sensibility disturbances with slight motorial deficit of the
pyramidal type, this syndrome too can regress completely. Another
not infrequent, form manifests itself by diffused pains, of poly-
necrotic type, frequently accompanied by a certain sign of meninges.
suffering. The pains sharpen every evening, with the rise of temperature, which remains low, they also reappear with each new fever period, during the not infrequent relapses. By type, these pains remain for a very long time and decline very slowly.

Another form is characterized by headaches, which can appear:

a) either as an attribute of a common acute attack, pseudo-influenza and later even after this is exhausted, continues as a low fever, persistent for weeks or months.

b) or as a phenomenon, alone and isolated, only accompanied by enlargement of the spleen, by pneumonia infiltrates, by waves of fever, after assuming a violent hectic character, especially during the heightening of the fever, which are also noted by the transient appearance and flight type of meningeal irritations (photophobia, light rigidity of the neck, trifling hypertensive state of the spinal fluid.

In both of these forms, the headache is always persistent and slow to regress. We have noticed it sometimes continues slightly modified, a long time after the disappearance of fever and of the pneumonia infiltrate, when one could even have stated that the clinical healing had been completed. Koschlin and Koszowski describe during a case of Q fever, a typical form of acute lymphocytic meningitis, with a rise in the spinal fluid of both the cellular elements (58) and the proteins (125mg.); Roch and Coll, and Koch
have noticed so much.

In all septic forms, whether observed by us or others, the infec-
tion has always shown significant changes which could have a
differential value. We have never observed the presence of antibodies
reverable by complement fixation with following Robbins and Regan, a
positive biological reaction when injected into a guinea pig.

5. The pseudo-brucella form is not infrequent. It follows the
clinical pattern of brucellosis completely, accompanied by typical
fever waves, pro- and remission, prolonged course, through successive
waves for months and months. A differentiation between the two forms
is only possible through diligent, precise and repeated laboratory
serologic and microbiologic research.

6. The fever form is also not too frequent. Here it is isolated
from among the speckled groups of patients who present themselves to
the doctor for the solution of the complex and difficult problem of
long lasting fevers, resistant to a great variety of treatment and
not resolved through the usual and more exact clinical and laboratory
examinations.

The Burneti fever, usually lasts for months and months without
greatly upsetting either the general state or that of nutrition or
blood count; it is never accompanied by particular subjective or
objective disturbances with the exception of some shivers with the
rise of temperature and slight sweats with fever decrease and by
some general disturbances, of mostly conesthesiopathic character. The
objective report, if one excerpts the enlargement of the spleen,
which is often also negligible, is practically negative. No trace
of the pulmonary infiltrate is even found during the radiological
examination even if the latter is conducted at the beginning of the
illness.

The existence of a Burnet fever, without appreciable pulmonary
localization, even at the beginning of the illness, has found the
most luminous confirmation in an endemic focus of Q fever recently
identified by Battavina and Battista in a penal camp (Augusta);
of the constancy of 52 cases, all positively proven, at high titer,
as well by the seroagglutination as by the complement fixation.
The biologic experiment was also possible in 6 cases, positive reaction
from the patient's blood; all had a persistent fever for more than a
month, with slight splenomegaly with no subjective or objective
disturbances and mainly with no signs of pulmonary infiltrates. Even
in the 6 cases, while still at the beginning and with the biological
proof from the blood still recent, the radiological examination for
pulmonary infiltrate appears negative.

The major part of these patients presented, during the
illness, a singular exanthema, from bladder elements, on a normal
skin, spread here and there especially on the trunk, which greatly
resemble the rickettsial pox. We should remind ourselves that elements
similar to those noticed in the Augusta epidemic have been observed by us in other cases and by Patrignani and Bacchiocco in an epidemic in the Turin.

At the Augusta epidemic, fever soon gave way under aureomycin treatment.

7. The subacute pulmonary infiltrate is not even very infrequent. It has as an anatomopathological basis a mononuclear infiltration of the blood vessels and of the bronchi, with a secondary connectival reaction. Radiologically the infiltrate is more dense than in the acute form; more consistent and with well defined limits. It can be localized either parenchymal or in lymphatics; in which case it is accompanied by secondary atelectasis, caused by the compression of the bronchial tubes by the force of infiltrated glands and by remembering the plastic or tumor forms. Its persistence and its radiological characters can embarrass in the confusion of specific and neo-plastic shapes.

a) a peribronchial type, with irregular regional alterations of the pulmonary tissue; with typical images of bronchi with inspected walls, either taken in a file (annular shadows, with central transparent light) or in a minor perpendicular to the section of the rays (repetitive images). Such images, with linear diffusion are similar to those encountered in peribronchitic changes of various types, and are usually localized in the two outer thirds of the corresponding pulmonary section.
b) a circumscribed alveolar type; characterized by the presence in the pneumonic area, of a round shadow, compactly blurred, with irregular outlines, of slight and uniform density. The seat of this shadow is frequently local, but it sometimes can also present itself high and even in the area known to radiologists as "subclavicular".

c) an ill-pneumonic type, sometimes bilateral, but also frequently unilateral, characterized by massive hilar images, with direct digitation towards the various regions of the relative pneumonic area, along the vasculo-bronchial fillets. They may vary in density and consistence also appearing at more or less defined intervals, so as to simulate with a narrow radiologic point of view, the images of processes either neoplastic or specific infiltrates and with great difficulty to establish a differential diagnosis.

From a strictly clinical point of view, the sub-acute infiltrate of the lung always represents a more or less intensely feverish illness, of a progressively evolving character, even though slow; after a few months it can also be accompanied by malnutrition and anemia, at the expense of the general condition; it may appear with breathing difficulties, with insistent cough, with more or less abundant expectoration, also sometimes of a definitely hemorrhagic character. Even if we wanted to evaluate the efficacy of the sulfonycin cure, so as to obtain a criteria ex adjuvantiibus, even at the first treatment a decline in the fever can be observed, the infiltrate frequently remains completely unmodified. Sometimes it is necessary to insist
on 2 to 3 successive cycles of the antibiotic so as to establish
a safe beginning of regression and later after a few months, the
complete clearing of the latter. Reference is made to cases of hardest
and riskiest interpretation.

6. The form of latent illness, with unapparent symptomatology
(sub-clinical Q fever) is discussed today by all world literature
(Bock and Coll) and its existence is also confirmed by our case
history. We are concerned with individuals who, in cases of
epidemiological research, show strong enough proof of positive
serologic (agglutination, fixation of complement) reactions of
rickettsia Burneti, without ever having suffered in recent months
disturbances or symptoms pointing to any illness, either as such or as a
possibility, a typical case, in our records, is that of a stableboy
working in a stable with animals proven to be infected and whose
wife washed his work clothes, contracting a disease of a typical
form of Q fever, also proven at the biologic examination. This
stableboy, in excellent health for his years, showed a highly
positive reaction by the agglutination and fixation of complement
in the serum; the biological examination was negative. He showed
signs of having had the illness recently, while it was not possible to
demonstrate that it was still active considering the negative
biological test.

An even more experimental case stands out among our case
histories. Our bacteriologist, dedicated to research in the field
of rickettsiae, are always kept under clinical observation. They are
subject to serologic examination every month (agglutination, fixation of
complement) during the beginning in 1949 having succeeded through
some of our problems to pass the organism from the guinea pig to
cultures in eggs; after approximately a month, two of our bacteriolo-
gists, suddenly presented positive serologic reactions, of a rapidly
and progressively growing nature. Having been subjected to radio-
graphic examination of the thorax, no signs or traces of pulmonary
infiltrates existed in either of these; however, the spleen seemed
slightly enlarged in volume and of soft consistency. One of the
two bacteriologists had a light daily fever in the evening, not
accompanied by disturbances of any weight. However, the other,
despite the most accurate measuring, showed no fever changes at any
time of the day, had no disturbances or ill feeling of any type and
felt in perfect condition to continue work and research. In both
cases the positiveness of the biologic test reached by injecting
the subject’s blood into a guinea pig, gave proof of an active infec-
tion, which in the first case caused a typical febrile form, with the
characteristic absence of the pulmonary filtrate; in the second case
it produced a typical form of subclinical, asymptomatic, inapparent
Q fever.

From the epidemiologic point of view, research in sub-clinical
cases, both active and anamnestic, holds the greatest value in closing
the link of infection from animal to man.
We hold that in the pathologic determination of the symptomato-
tologic multiplicity of Q fever besides the common individual
factors and other factors; circumstantial, professional, etc.,
great importance is assumed by the variety of means of infection.

From the experimental reports, we know that, for example, the
percutaneous or transcutaneous infection, which can also be
considered that caused by haematophagous arthropods, determines as the
greatest lesion that which can remain in some cases, from a
morphologic point of view, the black spot of *Culex beutonncus*
As general reaction of this type of inoculation, the splenomegaly
is moderate. The fever is very low and in many cases can be
completely nonexistent, the pneumonic infiltrate is absolutely
missing; while on the other hand the immune reaction, shown by
the positivity of the sero reactions, is always very strong.

On the other hand, the contagion, through inhalation,
especially from infected dust, always causes an intense fever and above
all the formation of a pneumonic infiltrate.

Among our bacteriologists, who worked under sterilized glass
caps, which eliminated nearly all possibility of contagion by
inhalation and managed large quantities of virus, it is not
improbable that the infection took place transcutaneously, though
only through the intact skin of the hands and especially through
the fingertips. The milk clinical form which appeared in them fit
such a pathogenesis perfectly. Thus, in the endemic focus of the
Augusta penal camp, the most accurate epidemiologic data were

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useless in establishing the most common causes of the epidemic; nearly all possibility of infection from animals and from their products was excluded; by breathing of infected dust, etc. Given the particular atmosphere of a penal camp, the hypothesis of an infection by way of bites from ectoparasites remains most probable, also in view of the singular cutaneous vascular eruption.

The great frequency, among cases of Q fever, of types with presence of infiltrate and with the character of an acute illness with more of less high fever corresponds in this way with the great prevalence, in the pathogenic of Q fever, of contagion through breathing.

During the clinico-epidemiologic study of Q fever, the problem of eventual reports of clinical forms presented by the patient and the eventual means of contagion represent also from a practical point of view, one of the most important and the most interesting areas of study.

**Serological and Microbiological Facts**

The former are possible by soragglutination and fixation of complement; the second by means of biological experiment in the guinea pig. Hirt's ophthalmo-reaction can finally be used in animals.

Soragglutination was the first method used, in order of time, to study rickettsia Burnetii; Freeman and Burnet used this to reach the identification of rickettsia Burnetii. However, this method, as
opposed to complement fixation, has lost favor in practice, both medical and epidemiological, it causes some practical difficulties for those who try to execute it, especially in reading the results.

In 1914, however, Giroud propounded a new technique of micro-agglutination, to an utmost clarity of reading and above all requires minute quantity of antigen, which is not without its practical advantage.

We have used Giroud's micro-agglutination for experiments, paralleled with the fixation of complement, using the specific technique proposed by Battitza for the preparation of the antigen.

By which in the guinea pig, the comparison of the agglutinated antibodies makes somewhat more slowly the comparison of fixed antibodies, also, in practical results, direct agglutination has given us results in complete agreement with those obtained from fixation of complement, therefore, we are discussing a method of great practical value and one not to be forgotten, especially when one has to examine small quantities of serum at a time.

The fixation of the complement, because of the facility of execution today has become the method of choice and nearly of habit in the serologic determination of Q fever. All authors agree in their preference of the reading "cold" technique described by Jacobson and today named the Kolmer technique by Anglo-Saxon authors.

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All authors have used this method in diagnosis, both
clinical and epidemiological, not only Sandel and Coll; Bengston.
This method of research contains insurmountable qualities of preci-
sion and attention (Irons and Coll).

Like Sandel and Coll, we in our case histories, and especially
in dubious and difficult cases, have systematically controlled the
results of agglutination and of fixation of complement with those
of the biological experiment in the guinea pig. In no case were any
differences found between the sera agglutinations and fixation
of complement and the results of the biological experiment.

As to precocity, the appearance of noticeable antibodies
in the serum by fixation of complement, would start as of the
9th day of illness, according to Drumley and Modani; as of the
13th day or later according to Bengston. Our experience with
cases not only localized in Sicily, but also in other sections
of Italy, discovered an important diversity, whereas precocity of
reactions is concerned, depending on the antigen used. With the
"Monterling" we reached positive reactions in 5 to 6 days from the
start of the fever condition. As such as was found in the case
of the antigen "Palermo", prepared by us beginning with the
rickettsia virus isolated from a patient of our of endemic type
and carried into cultivation in a fertilized egg (Battitta). To
us also, the "Nine Mile" antigen, appeared less sensitive and
less specific.

As Dr. Cohn, Huping and Codd in parallel research executed with the antigens "Panama", "Nine Mile", Henneberg", some cases which with "Nine Mile" and with "Henneberg" gave a completely positive reaction, gave negative with "Panama"; thus with us, certain cases during the first 4 days of the illness, had a negative reaction with the "Nine Mile" and positive with "Henneberg", then the separation blood was more sensitive with our "Palermo".

In a veterinarian, a colleague of ours, sick with a laboratory type and with positive biological proof from the blood in a guinea pig, we obtained a positive complement fixation with the antigen "Palermo" a week ahead of the others.

Caminopetros states that he discovered two goats with mild infection by rickettsia Burneti and a negative fixation of complement in the blood. We never were able to confirm a report of this type, during a vast experience in the field. However, we frequently discovered non-infected milk in animals with a positive complement fixation in the blood, while in all the animals with milk shown to be infected by the biological test, we constantly found the fixation of complement in the blood to be positive as also observed by Kiesner and Codd.

According to Bengston, the positivity of the serum augments in magnitude progressively during the first 22 to 25 days of the fever condition; then declines. Our experience also vary in this
case, it has happened, as a matter of fact, to observe patients who did not react positively to the complement fixation during the first days of illness, to react later, in a progressively growing manner. As a matter of fact, we consider, as a general rule at the bed of the patient, a negative serological report during the first days as not eliminating the possibility of Q fever; if it becomes positive during the following days, this represents a truly convincing datum. Thus a positive complement fixation of low type at the start of the illness is considered quite sufficient to evaluate the possibility of Q fever, while on the contrary, now convincing for the diagnosis is a positive reaction of high type during the first days of illness, unless, during the remainder of the course, the type does not give signs of greater increase. As a rule, one should never decide on a diagnosis of rickettsia Burneti on the basis of case of agglutination or of fixation of complement; even though at first it might be of high type it is necessary to ascertain that it will have a tendency to rise during the following days. This is because, in the case of Q fever, as in all rickettsiae, the complement fixation may remain positive long, even months, after the apparent clinical healing (Sulkin and Strauss); and only diminishes slowly and after a long period of time. Thus the danger exists in practical to meet positive fixation of complement, referring not to the active
illness and the origin and nature of which are being investigated but to past infection, clinically healed. The positivity of the sero reaction of which only represents an anamnestic reaction.

In connection with this, one should remember that Dauplon found the fixation of complement to be highly positive, in 2 cases, respectively, 385 and 380 days after the start of the fever. We have also observed analogous cases. The greatest persistence found by us in a patient from the start of the illness amounts to 324 days; we were concerned with a nervous form, which continued for more than 3 months with light fever attacks and relapses. The fixation of complement, still appeared highly positive 8 months after all trace of fever and of appreciable nervous changes had disappeared. All our Q fever patients, after leaving the institute, were systematically examined serologically every month, until the complete disappearance of all positivity. Among more common cases, the complement fixation presents a negative reaction after the first examination, it can be stated; a month after the clinical cure, while in cases with persistent pulmonary infiltrates especially in subacute types; in cases with fever recurring in waves; or even with recurring pulmonary episodes; and mainly with changes of the nervous type; the persistence of positive serological reactions beyond the apparent clinical cure is the rule. In view of this, we must ask whether the common explanation given for these facts
can still be held true; that the persistence of antibodies in the blood circulation, detectable by deviations of the complement, represent the expression of an immunity reaction, to be considered on the same level, or on a parallel level with those phenomena which would lead to a permanent assured immunity after infection by rickettsiae in general and also by Q fever in particular.

De rod has been able to prove, during experiments in curing Q fever with aureomycin, frequently and rapidly changes the sero-agglutination and the deviation of the complement to negative, even in highly positive cases. In light and non-dangerous cases and mainly with an early start, it is sufficient to administer one dose during the 5 and 6 days of treatment, in medium weight patients, so as to obtain both the disappearance of the fever and the rapid vanishing of the pneumonary infiltrate, and the negation of the sero-agglutinations.

In relapses, of long duration, and of pneumonary changes of less acute duration, the phenomenon unfolds with less evidence, and mainly much more slowly. Even if the fever vanishes after the first administration, or at least drops enough to assume the character of a light fever, the infiltrate is hardly affected, while the nervous and respiratory disturbances are attenuated but not eliminated.

In such cases it is necessary to try various (2 or 3) cycles of aureomycin at intervals of a regular number of weeks, so as to observe, the negation of the deviation of the complement, with the disappearance of the clinical factors and mainly of the pneumonary infiltrate.
From my point of view, this observation by Deorch is of great importance, especially when tied in with a series of already positively demonstrated facts, i.e.:

1) That prolonged and mainly recuring forms of Q fever exist, forms which do not comply to the closed cycle, considered as typical of rickettsiae until today. (see for example the dermotypus, both, historical or enclosed);

2) That the presence of rickettsiae can be proven by the biological test (in the sick men or animals), even long after the vanishing of the fever and the apparent clinical cure;

3) That in many cases after the disappearance of fever and the apparent clinical healing, an illness of a type of insistant character and with definite localization, especially pneumony or nervous, may still cause subjective disturbances of a certain importance (acute asthedia, general ill feeling, important incapacity to functional resumption, etc.) and evident objective phenomena (coughs with expectoration, in the breathing types; violent headache, possibly cold spells, insufficient functional condition mainly of the larynx and pharynx muscles, in nervous forms); subjective and objective disturbances, which sometimes surpass, also in their insistance, the properties of a single convalescence, be it even prolonged.

4) That all these phenomenon - meaning the prolonged forms as much as the persistence of both the objective and subjective disturbances,
and the elimination of rickettsia through the urine after decrease
of temperature disappear rapidly and definitely if sufficient doses
of aureomycin are administered, with the characteristic test such
disappearance is definite only when the reaction to the complement
fixation is definitely negative.

All these facts lead one to believe that the persistence of a
positive reaction to the complement fixation in our patients does
not necessarily constitute a phenomenon of anamnestic nature and
origin. Referring to cured or regressed infection, but that it shows a
phenomenon analogous to that which we and others, Beck and Coll have
been able to demonstrate finally; that is, that in certain domestic
animals, (horses, bovines, dogs) the rickettsia Burnotii infection
may reside in the stage of an apparent disease. Of the remainder,
unless the knowledge in the field of Q fever broadens, unless the
number of observed patients with positive complement fixation and
with completely negative anamnesis in the sense of having suffered,
at a more or less close time of morbid form which can be approximated
to an attenuated form of Q fever. (Beck and Coll; Holdolesi;
Doddinasaya) but he admitted that Q fever in man, not only may in
certain cases unfold as a completely unapparent illness, one may say
completely asymptomatic, but it can also pass at a certain moment,
from a state apparent illness to a state of sub-clinical illness, so
as to simulate a clinical cure. These hypothesis do not contrast in
fact with our actual knowledge, which is proved in the fact that
Roper, for diphtherial, stated in 1929 and proves in 1946 concerning
a case of Brill's disease, which appeared spontaneously in Zurich
(Doosor and Looffler) and which had all humoral signs of a
rickettsia prowazekii; that there exists a possibility, that following the apparent clinical cure, the infection passes to the inapparent (subclinical) state, so as to be able to have, after 27 years of apparent complete health, the resumption of a typical Brill Norkus, without the necessity of any epidemiologic link with some recent case of diconphylid, therefore, without necessity of reinfection.

In the particular case of Q fever, the persistence of positive serologic reactions long after the apparent clinical cure and the vanishing of all changes in temperature, would indicate in certain particular cases, the passing of the infection to a sub-clinical state. The administration of aureomyein would eliminate the sub-clinical infection, and with final neutralization of the sero reactions could lead to the final destruction of the infection and therefore to a true cure.

By these observations we would like to increase the practical value of serological reactions for Q fever diagnosis, and to greatly diminish the range of error due to positivity of anamnestic origin.

The proof of rickettsia burnetii in the patient - man or animal - constitutes the more precise search for confirmation, either in the clinical field at the patient's bedside or your experimental research.

The choice animal is the guinea pig, which in cases of positive biological tests, always shows, with rare exceptions, a feverish disease, with characteristic symptoms easy to recognize as
rickettsia Burneti. The guinea pig stands the injection of infected matter in the peritonium very well, because of which—in the case in which spouts is the material for inoculation—it is conveniently protected from other eventual concomitaneous infections, by an amixture of antibiotics (penicillin 10,000; streptomyein 10 c.ℓ).

We have never noticed an evident negative reaction caused by the streptomyein on the development of rickettsia Burneti, as have been observed (Kohnen and Coll).

In the clinical and experimental field the blood, urine, sputum and also the milk of the patient may be used as inoculation material. The test has a good chance of success, with anyone of these. In the experimental field sterilised pop of organs can also be inoculated, especially pop of splenic pulp; the latter is exclusively used for infections in series from animal to animal. After 4 to 5 successive days in the guinea pigs, one may attempt a passage into the embryonic chicken œgg, following Coxe's technique; here, however, research might really meet unexpected difficulties as the adaptability of rickettsia Burneti proves to be different from forces to forces. Among those known and studied today "New Dale" proves to be the easiest to cultivate in an œgg, while Humorizing only adapts itself with great difficulty. In our experience, we were successful 5 times, after repeated passing through guinea pigs, in isolating a strain of rickettsia Burneti from patients with foma unlike C foma.
However, only twice did we succeed in transplanting from the guinea pig to the fertile egg, so as to obtain a pure culture, with which, using the technique standardized by Cox we were able to isolate an antigen, which, on our clinical material - human and veterinary - proved to correlate the human MC strain very closely, which was proved by Cox, however, with some characteristic differences, with even greater precision. In certain cases, as stated, the complement fixation executed with the antigen derived from the Pul вне strain gave a positive result with the same number or less days than it took the antigen of American origin and successively gave much higher results. Everyone of the results arrived at with the antigen of the Pul вне strain was in perfect harmony with the results of the biological test in the animal. In the other three cases, however, despite the more scrupulous technique, despite the fact that the eggs showed no sign of deformity; despite the fact that the illness in the animals for experiment had shown all the characteristics of the ricketsial Barton infecion; despite all this, we were unable to transfer the strain into the embryonic chicken's eggs. A few rare elements of ricketsial aspect were observed during the coloring of the slides with Hechtavello's method, after the first passage, however, during the successive transplantings the eggs remained sterile, even though some gave birth to apparently healthy chicks. In two cases we were concerned with strains obtained from patients originating in different sections of Italy;
in one case, with a strain isolated from a patient's blood, sent for examination from the Republic of St. Marino, by Dr. Susi-Valli.

Some authors (Caspero and Castaçart) proposed to simplify the biological test, by stopping with first inoculated a llama pig and seeding the rickettsiae on slides from splenic pulp removed from the latter, coloring them by Bacchiavalle's method. We can not accept such a proposal; in the first place, in all such, as by our personal experience, the appearance of rickettsiae in slides of splenic pulp from an inoculated animal, contrary to the affirmations of some authors, mostly inerent, has been only in exceptional cases and precisely only for inoculation of sputum from patients with edematous broncho-pneumonia, sputum which we know to contain such a highly infectious amount, to result most of the time in the death of the first inoculated llama pig after 4 to 5 days; in the second place, were not consider as sufficiently guaranteed, a diagnosis of rickettsiae Burneti, based only on a microscopic report of a slide of splenic pulp, colored by Bacchiavalle's method for, as once occurred to our collaborator, Prof. Mirri and we have also had occasion to note this among other researchers - it is never possible to exclude with complete certainty that, despite the most accurate controls, llama pigs contaminated with pseudo-tubercular bacilli might enter the stable; indicating the possibility of finding acid resistant elements in the spleen slides, which, when colored by Bacchiavalle's method, could erroneously be interpreted as being caused by rickettsiae Burneti.
In proper circumstances, this biological test in guinea pigs is accomplished with certain ease, so that this method of research can be carried into clinical routine, as a means of research and proof, indispensable in obvious cases, of control in cases of particular interest.

However, for the routine execution of these tests, a particular laboratory arrangement is necessary, lacking which completely false results can be obtained; either all positive or all negative. This is not only limited to the specific competence of the person conducting the observations, the inoculations in the animals and the passing in series from animal to animal and eventually also from the animal to the egg. But beyond this must concern the efficiency of the stable hands, who must guarantee the analyst a strict isolation and a continuous and systematic surveillance of the animals on their arrival and during their stay, so as to be able to have the assurance with every experiment that the animal:

1) is not already infected with *Rickettsia Burneti*, in a non-evident form, before the inoculation;
2) is not exposed to some type of infection, after inoculation;

Indispensable for this, is:

1) that the stable hand have, beyond the usual furnishings necessary for any work of microbiological type, a particular section where the animals after injection can be isolated in special tiles lined cement cubicles, separate for every inoculation; that these cubicles guarantee
both complete isolation and the possibility of easy sterilization
after every experiment, either by flames or by means of a germicide
lamp;
2) that every animal be tested by means of the complement fixation or
by sero-agglutination both on arrival at the stable and systematically
every week, during the entire period preceding the inoculation, so as
to make certain that it is not a carrier of a latent rickettsia Burneti
infection.

Concerning the consideration of the spread of rickettsia Burneti
by zoonosis throughout the animal population, indifferent of the various
types; considering the great ease with which the infection spreads from
animal to animal; given the enormous frequency, among animals more than
among men, of latent infections, completely inapparent, however always
able to spread the disease; the possibility of an entire stable becoming
infected with Q fever is great. It is sufficient for one animal with
an uncontrolled, inapparent infection to enter. Considering the
enormous resistance which rickettsia Burneti may demonstrate against
common physical agents and common disinfectant, once a stable is infected,
it may remain that way for years, and even if detected as such, it is not
easily sterilized. An infected stable may lead to colossal errors,
well-known cases of which exist in literature, both in the clinical and
experimental fields. Furthermore mention has been made of the practice
of inoculation of animals and of successive transplantings, for which a
particular laboratory set up is also necessary, allowing such maneuvers

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to be made sterile under glass hoods, kept sterile by means of ultraviolet germicide lamps.

In particular, foresight exists in collecting samples for proof of rickettsia Rettii, except for the common norms of sterility; so far as blood is concerned, we had the best results in using complete pip, instead of blood smears. In general, it is preferable to use recent and fresh blood; it is most important that it be obtained sterile and if possible kept in a refrigerator. However, we also had positive results in the biological test in guinea pigs with blood sent by mail in a sterile container. However, according to Ciamblesco and Bidentesco, the infectious capacity of sterility obtained blood kept at room temperature, in a sterile test tube covered with vaseline oil, remained unchanged for 6 months and 8 days. After this date and up to 9 months and 16 days the infectious quality was not destroyed, but only weakened and that by doubling the amount of blood (by 50%) the guinea pig became infected, even though only in a non-apparent way, but showing the presence of specific antibodies in the serum, revealed by complement fixed on.

Diagnosis.

Diagnosis of Q fever is frequently difficult; often frankly obscure.

It is always based on epidemiological, clinical and laboratory data; among the latter, the radiological and seriological and the biological tests in the animal (guinea pig) are of great importance.

The epidemiological data is always sought out. Before rendering a clinical verdict it is necessary to determine whether a rickettsia
Borrelia infection is possible, in general. For this it is necessary, as for any contagious disease, to know the epidemiology of the type perfectly, so as to be able to apply to the study of the single patient, the epidemiological knowledge acquired by the case.

Having put the first difficulty of generic proof of Q fever, the difficulty of ascertaining the particular clinical appertains in the case under study, amounting to the difficulty made greater by the multiplicity of clinical fields to which rickettsia Burneti may tend, none of which fields, with the possible exception of adenomous pneumonia, have such a precise and definite aspect, so as to be directly isolated, for immediate identification. However one may suspect Q fever, at the patient's bedside, especially if the epidemiology agrees; however, certainty is never possible without the backing of laboratory tests.

Among the laboratory tests, serological reactions (agglutination, fixation of complement) are most important, in the practical field. As we have seen, despite their persisting to positivity long after the apparent cure, the dangerous possibility of error in referring to anamnestic and not active disease conditions because of their positivity in considering them non too important; however, one can not exclude them a priori.

In doubtful cases, one should try the biological test in the guinea pig, which, when definitely positive, gives certain proof of an active infection and can be considered a direct diagnosis.

However, we should clearly define what is to be understood.
by direct diagnosis. We may state that if the biologic test gave positive results, the direct diagnosis of the active infection can be considered reached; however, we are not authorized to hold that every lesion, every disturbance shown by the patient under observation, is directly connected, in a casual sense, with the rickettsia Burnetii infection, as it could be a case of lesion or of disturbances caused by an illness active before the infection, and which continues its evolution despite the contracted infection.

Also, because of this, in the case of Q fever, the microbiological or serological diagnosis alone, cannot and must not be considered sufficient for a final diagnosis, without the complete explanation and interpretation of the pathologic state of the patient. Also, in the case of Q fever, the actual complete clinical diagnosis must be exclusively guided by the clinical criterion, namely by the weighed consideration of the entire mass of data at our disposal, obtainable both during the direct clinical examination of the patient and from laboratory findings, without excluding the epidemiological contribution; even in lighter and apparently simpler cases, this must be the conclusion of an attentive and observant diagnostically differential deliberation.

Differential Diagnosis.

Usually the diagnosis is much easier in the epidemic forms, in which obvious and acute, even if dangerous, forms are more frequent; while, in the endemic forms or typically long drawn-out, with
attenuated and merged symptoms, light fevers of worn out or unapparent forms.

An acute case of Q fever at the start reminds one, in a general way, of an acute infectious disease and which gives rise to most diverse etiological hypotheses, which then usually collapse, as the common serological and cultural tests prove to be negative.

From a common influenza attack, the emphasis might be on the rash, the typical movement of the leukocytes, the, alike in the notion of the ocular globes; only rapid recovery is lacking, for Q fever always lasts at least 1 to 2 weeks, barring complications. Then one can prove the presence of the characteristic pneumonia infiltrate, one must immediately concentrate on the serological microbiological tests and await their positive result, and the successive rise in intensity or in other words, the diagnostic laboratory proof. The more serious forms and more accentuated breathing difficulties, would more likely advance the hypothesis of an atypical pneumonia and above all of a virus pneumonia, which differs from Q fever, given the enormous resemblance down to the radiological character and the localization of the pneumonia infiltrate, is mainly based on the reaction of the agglutination under cold, definitely different in the two types (negative in Q fever), and on the microbiological and serological data, negative in virus pneumonia.

A direct pneumonia could never be confused with Q fever, not even in the central form, given the great diversity of anatomic-pathologic
lesions, of clinical symptomatology, of laboratory reports.

The hypothesis of pneumonia or of secondary broncho-pneumonia will find the best clarification in other concomitant facts. The septic types of Q fever are almost always difficult to diagnose, because their symptomatology is completely pervaded by the profound and serious septic chemical character. One will think of Q fever in those cases where the etiology does not find an explanation in the common laboratory tests, especially if, from the start, they resemble phebitis or acute orchitis (otherwise unexplained). The pneumonia type of Rickettsia burnetii (closed, broncho-pneumonia) shows characteristic clinical (a state of great seriousness, intense cyanosis, dyspnea of sometimes asthmaform aspect; acute toxic reaction of pneumonia edema) radiological (micronodular infiltrate, with diffused pneumonia edema) and laboratory reactions, which can lead to a direct diagnosis, especially if confirmed by the cultural reaction of the sputum.

The problems may become difficult when it appears in relapses, especially if so is frequent, each relapse is accompanied by serious breathing difficulties, high fever and hemoptysis, thus reaching the possibility of orchitis (which may be excluded from the relative specific reactions), or even, if during the continuation of the illness, the infiltrate, composed of micronodular fog, should create an evident polybromentric situation, often taken for a tubercular etiology. In such cases, the laboratory data should be of great value and
precisely the negative search of Koch's bacillus in the sputum and the positive reaction of the rickettsia Burneti both to serological (agglutination, fixation of complement) and even more to the biological reaction in the guinea pig. The differentiation of the pseudo-brucellar type, frequently presents difficulty, because it causes the same symptomatology, the analogous epidemiology and mainly the non-infrequent possibility of interference between the two types, both in animals and man, so the control test by Mirri. Only the direct certitude of either brucellae or rickettsiae in the blood or the urine would clear up all difficulty.

The problem of light fever caused by rickettsia Burneti is really difficult; given the negative pneumonia reaction in these patients (lack of infiltrate); considering the lightness of the subjective disturbances, most of the time exclusively followed from purely osteopathic character; given the absence of objective symptoms, exceptive the light swelling of the spleen, which for example, can also be found in healthy subjects, malaria or endemic brucellosis countries. In these cases, one may suspect Q fever only after completely eliminating other diagnostic possibilities, not only by positive serological tests, repeated and agreeing among themselves, but also by means of the biological test in the guinea pig. It reactions of the skin ensue, you will have to extend the research to the possibility of rickettsia Conori or of rickettsial pox.
One may only think of a nervous type after having ascertained the presence of the pulmonary infiltrate and the positivity of repeated serological reactions (agglutination, fixation of complement). However also in these cases, it would be good to move as cautiously as ever and always to think of the possibility of neuritis and of encephalitis of a different type, infective and toxic. The exclusively parallel form of the lesions, of a definitely unclear character, limited especially to the bulbar nerves; the characteristic association of simultaneous paralysis of the larynx, pharynx and velopharynx which sets in suddenly, without other phenomena of foci or without even the slightest effect on the sensorium and with hardly any fever changes, are all facts which might indicate Q fever. The negative laboratory results, on the basis of the spinal fluid including specific agglutinations and above all the normalcy of the glycorrhachia, are data of a certain value. I advice in such cases, before affirming or negating, to always attempt to isolate the rickettsia Burnetii from the spinal fluid despite the fact that we have never been successful in this. The rather slow decrease if the type; the fact that the evidence slowly and completely regresses without leaving a trace; its influenzability under therapy, first with aureomytin and later administering vitamin B; can be useful criterions in a final confirmation of the diagnosis.

In the case of both presitve headaches and those following an active pseudo influential form, just as in the presence of nervous and radiological disturbances, the suspicion of Q fever can only be
justified if all the other symptoms, both clinical and laboratory, characteristic of the illness, are present, and especially if the serological reactions and biological tests are positive. The latter taken from the blood or better yet from the spinal fluid.

The subacute nonspecific infiltrate, especially if atypical by structure and position, always represents a great difficulty to diagnosis, always having to be differentiated from processes of different types, radiologically the same. Each of the three radiological types (peribronchial, circumscribed alveolar, lobar-pneumonic), typical of this form, always give birth to different diagnostic problems, and should be separately considered.

The peribronchial type of the subacute infiltrate has many points of contact, in its radiological aspect, with specifically tubercular or lusitcal fibrosis; of carcinomatous lymphangitis; of the initial forms of pneumococcosis and finally, of some chronic circulatory changes.

Early localisation can be of value against tubercular fibrosis, very frequent in Q fever, and the reason for other pulmonary manifestations which can eventually result in a specific pulmonary process.

The lack of simultaneous vascular or mediastinal changes contrasts with this hypothesis of a leucic form.

Carcinomatous lymphangitis always has a diffuse character and at the root is accompanied by lesions of the pulmonary flanks.

The changes, both chronic circulatory and from the initial pneumonococcosis, never show a definitely interstitial type, as is
characteristic, in this type of rickettsia burnetii infiltrate. The problem of the alveolar circumscribed forms is even more difficult; which from a strictly radiological point of view, greatly compare with any pulmonary infiltrate, of any type. The infiltrates connected to circulatory disturbances (multiple or repeated pulmonary infarctions; Morgagni's infiltrate; Best's infiltrate) are all characterized by the simultaneous presence of both diffused pulmonary oedema and of cardiac defect, or of serious alterations of the circulatory functions, noticeable at the general clinical examination.

Among the specific infiltrates, the lytic (pulmonary gamma) as distinguished by the characteristic aspect of both the edges and the contuise; it is characterized by changes of the same type, affecting other organs and apparatus and especially the large vessels and the mediastinum.

The tubercular infiltrates, both of the Ascani type and the type surrounding the pulmonary flanks (peri-ilar), constitute a far more complex problem, in desiring to differentiate them from a Burnetii infiltrate.

Our experience offers similar cases of infiltrates of uniform opacity, round isolated, with now well defined edges, found in perfectly healthy pulmonary areas at the base, but frequently also subclavicular, with all the characteristics attributed to the Ascani infiltrate. One must add that frequently in such cases a hemoptysis
occur at the beginning of the illness, quite similar to a true
and proper "alarm hemoptysis", occurring namely, in a serene atmosphere.
The temperature may vary from high to low; the velocity of sedimentation
is always noticeably high; it may also be a definite lymphocytosis.

In certain cases of this type, the infiltrate acts like a
transient infiltrate. It appears with high fever, slightly depressed
general state, dry cough and negative complement fixation. However,
in little more than a week the complement fixation becomes definitely
positive. The fever falls rapidly, when aureomyein is administered,
while the infiltrate shows a rapid tendency to vanish completely and
without relapses.

Finally, these transient infiltrates, greatly clarify their
pathogenesis by rickettsia burnetii.

In other cases, however, the infiltrate is perhaps accompanied
by lighter fever, sometimes also by evening fevers, by very rare dry
coughs, by headache and by some diffuse muscular pain. The radiological
examination reveals a more dense infiltrate, more evident,
of less uniform structure and less fused edges, which shows to further
modifications in the following weeks, that is no tendency either to
spontaneous evolution, or to forming a central negative picture of the
exanthem type. Considering such behavior, not infrequent for our
accented infiltrate, one may really advance a suspicion of rickettsia
burnetii. Thus one has a clear contrast between certain clinical and

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radiological data (especially with the morphological characters of the infiltrate) and the seriological and microbiological data; because of this the case is really doubtful. Clarification will result only after the administration of aureomycin. The fever is out of the first series and the reabsorption of the infiltrate begins, a process which however seems decisive only after the second or third series of the antibiotic, that is when the fixation of complement is negative. Thus we have the corroboration of the rickettsial pathogenesis of the infiltrate.

A last group of infiltrates of subacute fever, and sometimes with morphological characteristics not far removed from those of the proceeding groups, however, shows a persistence for the same number of weeks, without the least tendency to either spontaneous involution or to excavation; it is in no way affected by aureomycin therapy, even though repeated during three successive series. In all these cases, even despite the definite positivity of the serological test and of the biological test in the guinea pig, the infiltrate must be considered as being of tuberculous etiology, and interpret the case as a rickettsial Eburnei infection, taking place in a patient suffering from active pulmonary lesions.

The ile-pulmonary forms are also full of diagnostic difficulties. Their radiological picture, varying in different cases, always shows certain morphological analogies to the view of primitive or secondary neoplastic morbid conditions; with infiltrating tubercular forms; with pulmonary localization of the lue or of certain fungidito
have to consider as very slim the morphological differences offered
by radiology, on which to base a differential diagnosis. Here also
we are concerned with a general clinical problem and not with a purely
radiological argument.

In a case of our experience, the differentiations from a primitive
bronchial tumor as really difficult and laborious and was only reached
at the price of diligent and protracted observation, with the help of all
possible means of research, not excluding bronchoscopy and pneumography.

The case was of a middle-aged individual, affected for five
months by an irregular high (38-39) fever, with quite intense breathing
difficulties (cyanosis cough with abundant expectorate, sometimes mixed
with blood), also observed by us to be slightly emaciated, and anemic
and in a general state of depression. Dense irregular opacity of the
flank, the size of a large egg and completely unilateral was discovered
radiologically; from which non-vascular liver, irregular in form and
size, radiated into the pulmonary region, without being able to call
then actual "spider leg" digitations. At the beginning an evident
insobriety of the hemidiaphragm was noted on the affected side, and
later it resembled a strong pleuritic reaction of exclusively pleuritic
caracter. Lesions of the bronchial walls were evident neither during
the bronchoscopy nor during the pneumography. The serological
reactions and the biological blood test gave definitely positive results
for rickettsia burnetii, at the administration of aureomycin, in three
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three different doses, the fever vanished rapidly, while the general state improved considerably, so that the patient was soon able to leave his bed for a long convalescence. At the same time, the shadow showed an immediate tendency to decrease, which, however, only happened slowly, so as to be considered definite only after four months. The patient took up his usual occupation completely cured before the year's end. He showed no sign of relapse, while kept under strict ambulatory control. We, therefore, consider justified the affirmation of a rickettsia Burnetii pathogenesis, for this neoplasticiform ile-pulmonary infiltration.

As to the possibility of distinguishing subacute infiltrates of Q fever from ile-pulmonary localization of secondary neoplasias, we quote the case of a 57 year old woman, sick for six months with a definitely undulating high fever with profuse sweating and noteworthy dyspeptic disturbances, but without the least respiratory disturbance. Suspecting a brucellosis, despite negative serological reactions, various cures were attempted, vaccine included, with no result. The patient came to us in a generally poor state of health and anaemia; an enlarged liver, but no change in the consistence, which was smooth and painful to palpation. The spleen was slightly enlarged in volume and consistence. The basic laboratory tests were indifferent. The radiological examination negative for the other organs and systems, showed at the thorax a unilateral mass, thick, dense, non-uniform, with irregular edges, clear; simultaneously a thin shadow, irregular, less dense, with fuzzy edges are found in each of the pulmonary fields.
The presence of *Rickettsia Burneti* in the blood and a high positive complement fixation were proven. In view of the obscure nature of the case, an aureomycin cure was started and repeated three times. After the first series, the temperature vanished, while the patient entered into a stage of real recovery. The liver soon resumed its normal size, losing the painfulness. The pulmonary infiltrates had completely vanished after three months; the shadows had also lost all pathological character, in volume and intensity. The patient, continuously kept under observation, is well and has had no relapses of any type.

We have observed cases of differentiation from infiltrant ileo-tubercular form in three girls with fever for a few months, undernourished and a bad state of anemia and generally, with a high ilar infiltrate, neoplasticiform, dense, irregular, lymphocytes separated from the blood, definitely positive. It should be noted that radiographically visible were both the residue of a primary illness, apparently cured, and numerous calcifications, in the ilar area, pointing to cured tubercular activity. Serological examinations and biological tests for *Rickettsia Burneti* were definitely positive. Aureomycin was administered and checked the fever quickly and definitely after the first cycle, while the patients improved in the general state and in strength. The radiographic report showed certain regressive changes in few days after the first series for the use of aureomycin; the complete disappearance is only noticed after a few months and after two complete cycles of aureomycin. By the end of six months all the
patients were cured to confirm the diagnosis, the complement fixation for ricketsia Burnetii became negative, simultaneously to the vanishing of the infiltrate.

The distinction from a free of disturbance never offers real difficulty even in the case of serologically positive reactions for lab, which can be observed in the febrile phase of ricketsia Burnetii. The alterations of the infiltrating part of the lungs vessels were never infected as would be the normal case of tertiary lues.

The last possibility of differential diagnostic concerns pulmonary mycosis and mainly moniliasis, which frequently appears with large amounts of infiltrate, pseudo-tumor, mono- or bilateral; with pleuritic reactions of a plastic nature, with irregular, constant and often high fever; in a generally upset state; with dyspnea, continuous coughs, abundant expectorate, frequently bloody sputum, the negative results of which, either microscopically or by culture, in favor of a Burnetii infiltrate speak; the intraderm reactions of monilia ex recto; the examination of the nervous system, which is always characteristically upset during chronic moniliasis.

The serological research, the biological test, the influence of aureomycin are of value as positive data.

In conclusion of this diagnostic study of our, on subacute infiltrates of ricketsia Burnetii, we insist that, in this field, a final decision can only be reached through steady and prolonged clinical examination, not only based on radiological, serological and microbiological data, but also on a complete examination of the patient.

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In this connection, one must not forget the most important fact, that only radiological or just one serological report are never sufficient; that one should always exclude the less infrequent possibilities; that it is always useful to note the therapeutic effect of aureomyein, without being limited to just one administration and mainly giving great weight to the eventual joint report of the regression of the infiltrate and of the negativization of the complement fixation.