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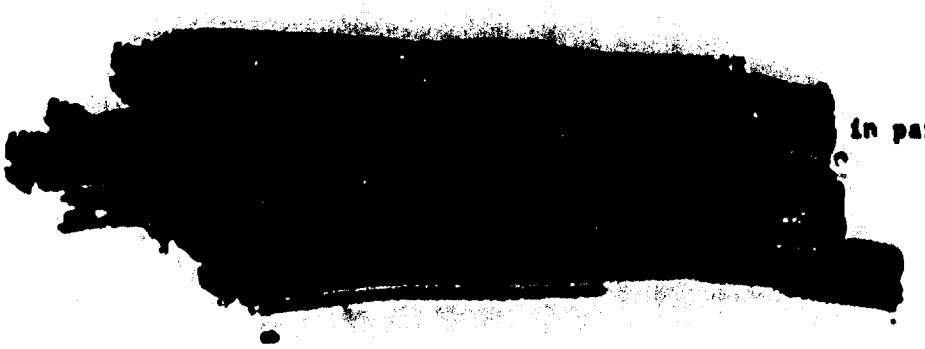

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REGARDING A TROPICAL ZOONOSIS

THE WHITMORE BACILLUS AND MELIOIDOSIS

Latest facts

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There is a renewal of current interest in the study of Whitmore bacillus and of Melioidosis. That zoonosis was confined for many years to Southeast Asia, but recently it invaded the tropical areas of Australia, of America (Dutch West Indies) and of Central Africa (Ch.); it is now considered to have ubiquitous tendencies.

In the following article we shall compound present, summing them up, current some of the recently acquired data regarding the specific germ and the disease itself.

A. - The Whitmore bacillus

1° Its place in the system of bacteria.

- Its taxonomic position has not yet been exactly set. Together with most French and American authors, we shall adopt the generic and specific names of Malleomyces pseudomallei; we shall place it in the Malleomyces genus, which belongs to the family of Parvobacteriaceae, of the order of Eubacteriales.

- Definition: a straight stick of 4 to 6 μ of length by 1 μ of width, isolated in heaps of 3 to 6 elements, negative Gram, mobile and pathogenic for man and animals. M. pseudomallei is neither capsule-shaped nor sporulated, it is endowed with one or two lashes at each pole. Certain forms of young colonies on gelose (white, opaque colonies) show individuals surrounded by a colorless pseudo-capsule of variable thickness.

- It is rational to compare this germ with the glanders bacillus (Malleomyces mallei), to which it is connected by numerous common characteristics, both cultural and immunological. The only important difference between the two species is the absence of lashes and of mobility in M. mallei.

2° Cultures

- ~~A facultative aerobic and anaerobic~~ An optional aerobious or anaerobious (=air-breathing or non-air-breathing) organism, M. pseudo-mallei develops much faster and more abundantly than M. Mallei on the usual media. Thermic optimum 37°C. pH optimum 7.5.

- the cultures are characteristi :

a) in ordinary broth : plicated veil, very special (checkered), in 48 hours. The following days, browning, smell of 'truffle'.

b) in ordinary gelose : spontaneous and immediate dissociation after 48 hours : S colonies, which are small, 1mm of diameter, swollen, fat mucuous, white, bluish reflection; the R colonies, rather voluminous (2 to 3 mm in diameter), flat, dry, gray, pleated in type of "Chinese conical hat" (Bouchard).

c) potatoes : chocolate-brown coating (like M. mallei) : coagulates the milk ; liquefies gelatine and coagulated serum. Attacks : glucose, saccharose, lactose, maltose, mannite,

dulcite, dextrine, without gas. VP : negatibve; MR:negative; Indole:negative; SH2: variable; coagulosa, ureaseis: negative; catalasis: positive.

Differential diagnosis with Malleomyces mallei

- Veil, spontaneous SR dissociation, coagulates milk (proteolytic) Braun test: positive after 4 days; negative with M mallei; positive in 24 hours with Ps aeruginosa.

Isolation : The isolation of Whitmore bacillus from samplings where it is in pure culture offers no difficulty and can be carried out on broth or nutitive gelose. But when an abundant microbian flora is detected in diract exmination in spittle or in any other product, there is advantage to use selective media. The Levine medium, or ammonium locate added with 0.0025% of 2-3-5 triphenyltetrozolium yields excellent results: the Whitmore bacillus colonies are white in it, opaque with a red center, easily recognized.

-?. Eres insists on the necessity to repeat the culture tests, applying them to various types of body humidity and excretions, for elimination is often capricious and poor in bacilli.

- After three fruitless tries with classical culture and inoculation methods, Nguyen Van Aiv and Coll obtained a positive result, by inclusion, under the skin of guinea pig, of an important amount of spittle, after incision followed with stitching.

- In purulent pathological products Whitmore bacillus is commonly often associated with numerous pyogens (staphylococci which are pathogenic and pyocyanic, Friedlander pneumobacillus, colibacilli, enterobacteria, etc.)

3° Vitality - Resistance

- Being a mesotrophous germ, Whitmore bacillus, like most pathogenic bacteria, does not require the presence of animoated acids, It can not only maintain itself, but even multiply, in sterile tap water kept at 30°C. Its resistance against physical sterilysing agents and chemical sterilising agents is considerable

- The inhibiting action of the bile and of its components is variable. Contrary to the pyocyanic bacillus, Whitmore bacillus does not produce colonies on Difco SS medium. Contrary to the glanders bacillus, it cultivates well on bilious medium made with bright green medium (Difco B 7) and on bilious medium made with MacConkey crystal violet.

- Through repeated passage on bilious media, Chambon has obtained a stock characterised by an absence of pseudo-capsules, by weaker proteolytic properties, glucidolytic and reducing properties, and by the absence of pathogenic power. Those are not stable characteristics. A few passages on non-bilious media bring back the original morphological characteristics and the virulence.

4° Experimental pathogenic power.

- Whitmore bacillus is a pyogene and highly virulent germ.

Guinea-pig : The most sensitive animal is the guinea-pig; the infection is achieved by oral means and by a simple deposition of virulent matter in the natural cavities, on the mucuous membranes and on shaved skin; the animal loses weight and dies in 8 to 15 days.

- Subcutaneous injection provokes a local abcess with adenopathic satellite and causes death in 4 to 5 days. Autopsy reveals numerous tubercules in the spleen, in the liver, in the lungs; the diagnosis is difficult with the tubercles of M. mallei and Cillopasteurella.

- Intraperitoneal injection provokes a rapid and overwhelming infection characterized [illegible] by a generalized peritonitis, mortal in 24 to 36 hours. The adhesive suppurated [illegible] the testicles, of the type observed in the glanders (sign of Strauss), is very [illegible]. The males show an important orchitis.

Rabbit: A culture which is very violent for a guinea-pig, when injected under the skin into a rabbit, provokes a local abcess which leads to the animal's death within 10 to 15 days. The autopsy reveals tubercles in the liver and in the lungs, but the spleen is normal.

[illegible] than the guinea-pig to experimental inoculations. Infected through the digestive tract, it presents a chronical melioidosis, a progressive thinning and dies in one month. The autopsy reveals numerous tubercles in the lungs, seldom in the liver [illegible] in the spleen.

Mouse: Nigg (1958) succeeded in increasing the virulence of 8 stocks of M. pseudo-mallei through passages in series, by the cerebral way, in the mouse and in the hamster. He obtained stocks which kill with 10 to 100 germs the mouse and the hamster through the cerebral and the intraperitoneal ways.

5° Toxinogenesis.

- In old cultures, the microbes let out, through autolysis, a thermostable endotoxine.

- C. Nigg and colleagues (1958) were able to produce in vitro a lethal endotoxine, which was thermolabile, with several selected stocks of Pseudomonas pseudomallei (yielding R colonies). The production is maximum in a heart infusion added with glycerine and swine pig's gastric mucine, when cultures are made at 32°, without agitation, and during 7 to 10 days. The active factor of mucine is neither dialysable, nor precipitable by alcohol. The toxinogenesis does not seem connected either with the virulence, or with morphology.

- The same authors were able to obtain the doubling of the colonies of this raw toxine, by selective adsorption and elution on Duolite S-30 resins, in the raw filtrate of the cultures:

a) a lethal and necrosing exotoxine;

b) a lethal and non-necrosing exotoxine.

- The raw toxine is antigenic, stable between pH6 and 9. Both toxines are detoxified by formol and by phenol; but none of them is inactivated by acohol or acetone. They both are precipitated by SO4 (NH4)2 at 35% - those probably are proteins with low PM, with few ionisable radicals.

Mallemine and melioidine.

- Mallemine, when prepared in the same fashion as malleine, from the glycerinated extract of a culture, is too strongly necrosing, in order to produce clear answers.

- The melioidines which are obtained on synthetic media such as S.M.M. (Synthetic me-

lioidine medium) of Lewis and Olds (1954) are sufficiently poorly necrosing to be widely applicable to veterinarian studies.

- In Viet-Nam (1956-57), Chambon has detected the allergy with the help of purified O and K extracts: while constituting excellent antigens, they are deprived of necrosing power; they are preferable to extracts from cultures. The reactions' intensity is variable, but always easy to read.

- The intradermoreaction is the more strongly positive as it is practiced at a more recent date from the beginning of the infection. The poliomorphism of the melioidosis probably influences its sensitivity towards the antigens which are used.

6° Virulence.

- Virulence seems to be an attribute of forms rich in antigen of coating K, whether those forms be smooth or rough. Since it is rare to find a stock entirely and permanently devoid of K antigen, virulent stocks are rare. One finds only forms where it is poorly represented and which Chambon calls:

- OK minus (smooth forms provided with K antigen) or

- RK minus (rough forms provided with K antigen).

The germs in the R state seem to keep K antigen better than do the germs in S state. RK form is remarkably stable, while OK form is very unstable. This fact explains then already old notion that R stocks of Whitmore bacillus possess a special virulence; it also explains the paradoxal phenomenon according to which the R colonies generally yield homogeneous suspensions in physiological water.

- The virulence of most stocks preserved in the laboratory diminishes after some replantings. Some remain naturally virulent, if one sows them on medium Dorset (on non-glycerinated egg), or if one distributes a culture on broth in sealed bulbs, preserved at laboratory temperature.

7° Antibiosensitivity.

- Chloramphenicol, tetracyclines and novobiocine appear to be the most active antibiotics in vitro and in vivo. According to Chambon, kanamycine exercizes in vitro a highly bactericide action even on resistant stocks chloro-ure which have little sensitivity to chloramphenicol; but in vivo it does not give good results. Neomycine, D. cycloserine, framycetine, oleandomycine only act at high concentrations. The other antibiotics have no action on it.

- Framycetine may have great usefulness in localized suppurations (various abcesses, pleurisies, meningitis, arthritis...) and especially in the lung abcesses, where Whitmore bacillus is often associated with with germs that are highly sensitive to framycetine, such as staphylococci and pyocyanic.

- Some combinations of anti-biotics yield interesting results: chloramphenicol+tetracycline seem to be compounding their effects. Chloramphenicol + aygnamycine in equal parts are an especially active synergy. Base tetracycline and aigmamycine in equal parts a 2/1 proportion have a clear bactericide power.

- The adjunction of sulfadiazine, of septoplix or of rufol to the treatment by chloramphenicol may have beneficial effects.

8° Structure and antigenic properties.

- This study is the object of many very important research work by J. Fournier and L. Chambon, at the Institut Pasteur of Saigon: antigenic constitution, identification of pseudo-capsules and of K antigen, morphological and biochemical variations, in function of antigenic structure; serological heterogenity, enzymatic system.

- The morphological and cultural characters of M. pseudo-mallei suggest the existence of 3 antigens; they are, starting from the periphery and moving towards the center:

a) a mucous antigen M, very superficial or even exterior to to the bacteria, considered as product of autolysis. Its properties are comparable to those of antigen M of the enterobacteries. It give an agglitunation shaped like a crepe;

b) an antigen of coating K, a prop for toxicity and virulence, behaving like a capsular antigen. It masks the somatic antigen O and makes it inagglutinable.

When the emulsion is boiled, antigen K is destroyed and antigen O is agglutinated;

c) a thermolabile flagellary antigen H. As antigenic flagellary function was noted, which was common to both Whitmore bacillus and pyocyanic bacillus. A serum H antipseudo-mallei can agglutinate certain stocks of Ps. pyocyanea; but the serums O antipseudo-mallei and anti-Ps pyocyanea are strictly specific;

d) and e) the somatic antigens O and R which manifest themselves during the classical dissociation into colonies which are "smooth" and into "rough" colonies. The common thermostable of all M. pseudo-mallei, similar to that of negative Gram bacteria, the O antig. gives a granular, no-dissociable agglutination. Being an antigenic complex of polyosidic nature, devoid of toxicity, it yields agglutinative anti-bodies, precipitating but not protecting.

9° Antigenic communities between M. pseudo-mallei and M. Mallei and other germs.

Verge and Pairemaure (1928 to 1930) have made known to the world the existence, among horses, of immunological reactions crossed between M. mallei and M. pseudo-mallei. More recently close antigenic common properties between the two germs have been clearly established and shown by means of the crossed agglutination, of the saturation of the agglutinins and of the fixation of the complement by Cravitz and Miller (1950), as well as by Alexander and his colleagues (1955).

-- Let us note, also, that there an antigenic fraction common to M. pseudo-mallei and to certain stocks of Achromobacter, Escherichia, Aerobacter, Klebsiella, of Salmonelles (Fournier, Brygoo, 1957), and that this community interests complex O.

10° ~~Immunologique~~ Serological heterogenity.

- Contrary to what had been believed at the beginning of the studies on M. pseudo-mallei, this species does not present a serological homogeneity. Alexander and colleagues (1955) have noticed that two stocks of Whitmore bacilli are serologically different from the others, and have concluded from that that the stocks of M. pseudo-mallei form a het

rogenous group, from the point of view of antigens.

- Fournier and Chambon (1957-1958) have undertaken a seriological classification of M. pseudo-mallei, taking into account separately the antigens α O, K, M and H. They have experimented with 96 stocks issued from autochtonous cases in Viet Nam and in ww other South East Asia countries, in Australia and in tropical America. The results which were obtaine show that an isolated stock in a goat in Australia (Cottew) (or: a stock isolated in a goat...) beaves differently from the other nine which are antigenically identical to th control stock. The others authors have dedicted from that fact that somatic antigen O of M. pseudo-mallei is divisible at least into two parts which are serologically different and which the authors call a and b.

Heterogeneity also exists as regards to lysotypy.. At the Institut Pasteur of Hanoi, Leclerc and Sureau (1956) have isolated bacteriophagi which seemed specific for Whitmore bacillus. They give the lyse that is confluent with all the stocks of Whitmore bacillus isolated in North Viet Nam, but which is confluent only with one part of the stocks isolated in South Viet Nam.

B. - THE DISEASE : MELIOIDOSIS

- An infectuous, virulent, inoculable disease, due to Whitmore bacillus, melioidosis is common to man and to various animal species, in particular to rodents, equides, ovines, caprines and porcines.

- It is characterised, clinically, by a septicaemy with cutaneous and visceral localizations, especially pulmonary, hepatic and splenic, and anatomically by milliary pseudo-tubercles of the type of glanderous lesions. That is clearly the pseudo-glanders.

1.- Geographical distribution

- Detected in 1912 at Rangoon (Burma), by colonel-physician Whitmore and by Krishnamani, the disease has remained, until 1949, practically localised in the whole Southeast Asia: Burma (1912), Malaya (1917), South Viet Nam (1926), Ceylon (1927), North Viet Nam (1929), Cambodia (1930), Central Viet Nam (1937), Indonesia (Celebes q 1933, Java 1935)), Thailand (1947), The Philippines (1948).

- From 1949 on, one saw a diffusion of the malady in Australia : in 6 years the disease spread through 15 Queensland 'stations' and provoked 5 ovine epizooties, 7 caprine epizooties and 1 porcine epizooty. One human case was observed at Charters Towers, an important stock-raising area.

- The cases registered in Africa deserve thought. In 1936 G. Girard isolated a stock of Whitmore bacillus on a pig in Tamatave (Madagascar). A case on a serviceman was observed in 1944 in South Africa, but was contracted in Singapore. Recently the Laboratory of Veterinary Research of Farcha, in the Chad (Central Africa) has isolated two stocks of M. pseudo-mallei:

- The first on a goat (an autochtonous case) in 1956,

- the second, in 1959, on a Negro soldier originary from the Chad, who had made a sojourn in Viet Nam, about one approximately 5 years ago.

Is the existence of melioidosis in Africa an old fact which escaped the researchers, or must one see in it a zoonosis of recent importation?

- Since the end of the War in the Pacific, that disease has invaded the American continent, 2 cases of servicemen on Guam Island in the Marianas Archipelago in 1946; isolated human cases in Saint Louis (1947), in Missouri (1948), in Colorado (1951), in Louisiana ~~(1947)~~, (1954). Certain among those observations concern patients who had never left the United States. More recently, in 1957, Sutmoller and colleagues have isolated in Aruba, in the Dutch West Indies, near Panama Canal : 17 stocks of M. pseudo-mallei on ovines, caprines and porcines carriers of ganglio-lymphatic abcesses and slaughtered in slaughter houses; on the same island, and at the same epoch, an epizooty hit a sheep herd of aboriginal sheep, killing 25 of the 90 animals of the herd. One is entitled to think that a systematic research of the germ and of the disease would detect them in the neighboring countries, in inter-tropical America.

- Europe has not been spared : melioidosis cases have been tracked : in France (4 cases in 1952, 1953, 1956), in England (one case in 1953) on servicemen who had returned after serving in the Far East . According to Grenier de Cardenal, a melioidosis (?) epidemic appeared in Berlin at the end of the Second World War, among the starving people who lived amid the ruins.

"Far from being a disease of the future, melioidosis is becoming a malady of the present", says Phung Van Dan. From its original hotbed of Monsoon Asia, it tends to reach certain tropical zones of Australia, of America and of Africa. The perfecting of the means of diagnosis shows it to be much more widespread in those regions than commonly imagined.

II. - EPIDEMIOLOGY

- Melioidosis appears as a hydro-telluric disease. The main reservoir of germs is the earth- soil and the waters (stagnant waters of the ponds, of the rice paddies, of the river banks), as it is proven by the direct method of research of the bacillus and the indirect method of research on specific phages, carried out by the Institut Pasteur of Hanoi and the Institut Pasteur of Saigon. The bacteria, although it is non-sporulated, and is only surrounded by a pseudo-capsule, nevertheless puts up considerable resistance against natural physical and chemical destruction agents. Mesotophous, it grows and multiplies on the poorest media.

- Spontaneous animal melioidoses appear principally during the rainy season, a time when contamination through polluted waters reaches its paroxysm. They notably hit the animals who are compelled by their habits or by the condition of their raising to a 'semi-aquatic' life, or who must consume vegetables, fodder or grasses which may be soiled by the dormant waters; among such animals are the rodents (rats, guinea-pigs, rabbits), the porcine, the ovine and the caprine.

Human melioidosis is recognized to have the same hydro-telluric origin. One is struck by the important number of human cases provoked by open fractures, by multiple contusions which are consecutive to a car accident in a muddy pond; the hunting wounds incurred in

swamps infested with leeches. The military operations which took place in Viet Nam until 1954 have multiplied this kind of observations : wounds by mines (land mines) and projectiles which were soiled with mud, marches through flooded terrain, sojourns in humid trenches. However, the effects of the penetration of the germ into human organism or animal organism are far from always being immediate. In certain cases, an operation shock (laparatomy, ablation of a bone splinter, regularization of a sore...) or simply a soiled operating meteriel ("epidemic of the senngue" (?)) unleash a grave melioidosis, originating from an infection which was, if not latent, at least localized and relatively tolerated.

- The terrain plays a considerable part in the etiology of the disease : it needs stimulating causes which put the receptive host in a state of inferiority towards the aggression by a strongly pathogenous germ that was, nevertheless, quite tolerated until then... Let us mention in the first place the parasitary maladies (malaria and dyssentry), or infectious diseases (syphilis, gonococci, staphylococci, pneumococci, whooping coughs, ricchettioses, typhoid fever...) then the chronical intoxications (opianomania, morphinomania, alcoholism), the diathenes (diabetis), the pregnancy, the alimentary deficiencies, the malnutrition, the fatigue, the acclimatisation crises. In 1959 a first deathly melioidosis epimoty occurred in Cambodia, in three stock-raising stations, reaching in the first place the bears and the sows imported from Australia and from Japar, while the aboriginal porcine stock possesses a certain degree of relative immunity against the disease.

III - HUMAN MELIOIDOSIS

- It is one of the most polymorphous diseases that exist. Collomb and Boube classify its multiple manifestations in three groups, as follows: septicaemic forms, septico-pyohemic forms and local forms.

Septicemic forms

- Very rapid evolution; death in 3 or 4 days in the acute forms -- from 8 to 10 days in the less acute forms. If the patient does not succomb after 10 days, he has passed the septicaemic form and has moved into the septico-pyohemic form.

- Strong hyperthermy, cephalees, dispnea, delirium, prostration, coma, death by cardiac collapsus. Cutaneous eruption is frequent : purpura, scarlatiniformous or morbilliformous rash, exanthems of the face, hemorragic bubbles, pustules, which are sometimes numerous, and which simulate small-pox.

- Among young children, frequent digestive troubles : abundant glanderous stools, gastro-intestinal paresis, toxic syndrome. In the adult one notes sometimes some diarrhea and some icterus (jaundice).

- One must make a differential diagnosis with the cholera, with the plague, with typhoid fever, with typhus, with spirochetoses and with the eruptive fevers. In certain cases, the laboratory examinations detect the coexistence of melioidosis with one of these latter diseases.

Septico-pyohemic forms

- These are by far the most numerous forms. A brutal period of invasion; a state period

marked by grave general troubles, by visceral suppurated metastases and by a rapid emaciation. Duration and evolution are very variable. Pulmonary localisation is unique or predominant, remarkable by its frequency and its gravity.

- The differential diagnosis is to be made with the staphylococci, lung tuberculosis, acute brocho-pneumonia, casseous pneumonia, the abcesses or the cysts of the lung.

Local forms

- Sometimes, a single abcess interests either the superficial or deep cell tissue, or a muscle, a ganglion or a lymphatic territory ... At other times, it is a hotbed of osteiti which generally fistulises itself. The fever is absent or moderate. The other general signs are in relation with the seat, the extent and the duration of the suppurating hotbed.

- The purulent collections may have a seat:

a)at the head ~~ow~~ and on the neck : abcesses of soft cervical parts, of neck or under the chin ; paroditis, adhenitis, osteitis of the skull with juxta-osteic abcess;

b)at the thorax : lung abcesses which often are multiple, purulent pleurisy;

c) at the abdomen : abcesses of the liver and the spleen, generalized peritonitis,

d)at genito-urinary organs : abcesses of the kidney with pyuria, abcess of the prostate with retention of urine, archiepidymite, suppurated vaginalitis , pseudo-puerperal infection;

e) to the members : lesions of the cell tissue and of the skin, abcess of soft parts, osteites, arthrites, myosites, torpid ulceration of the lower member, of the root ~~ow~~ of the buttock or of the thigh, polyfistulisates, eczematoses (?), with or without ~~lesions~~ underlying bone or articular lesions.

- It happens for the deep or extensive collections to evolve during months, even years, and to require several surgical interventions. Huard has, for a long time, drawn attention of practicing physicians on the great importance of those various cases of surgical melioidosis.

- The differential diagnostic is to be made, according to the case, with the mycoses, with syphilis, with tuberculosis, with suppurations with pyogens and notably with staphylococci. As always, the laboratory is alone capable to establish this diagnostic.

- In sum, "the only fixed characteristic of the ailment is not to be allo a standard description" (Collomb and Boube). The septicaemic form may shift to septico-pyohaemic form and for the latter to be grafted on a local form.

Treatment

*Chloramphenicol currently constitutes the most efficient therapeutic weapon against melioidosis under all its forms. The clinical results have widely corroborated the tests in vitro of the sensitivity of Whitmore bacillus towards antibiotics, and no other among these can be substituted to it" (Fournier and Chambon).

In order to avoid the relapses, the cures must be important (3 to 4 g per day) and protracted sometimes for several weeks. An intensive vitaminotherapy will then concurrently be administered, and control of the hemogram will tend to spot early a possible anemia.

The danger is to see the appearance, in the course of the research, a resistence of the Whitmore bacillus to chloramphenicol. Certain combinations of antibiotics are then synergic, in particular chloramphenicol + sigmamycine in equal parts ; or chloramphenicol (3 g) + erythromycine (1 g 50) per day; or chloramphenicol + tetracycline.

"Sulfadiazine has given some good results in certain local forms, it has seemed to be a useful additive to the treatment by chloramphenicol" (Farinaud and colleagues).

"The localised abcesses may be treated by aspiration of the pus and instillation "in situ" of formycetine.

" The greatest number of forms localized forms deserve surgical treatment which, as a rule, general rule, associated with the medical cure, brings about the recovery, but sometimes at the price of several interventions, at more or less long intervals. The patient must be warned of the necessity of a protracted medical surveillance.

IV. - ANIMAL MELIOIDOSES

1° Horses:

Several cases were observed in Malaya (1927, 1947 to 1949 on racing horses imported from Australia), in the Philippines (1938, 1947), in Java (1932) and in Cambodia (1952).

- The disease began with fever, lack of appetite, cough. The horse presented an intermittent purulent gleet through the mouth, but nothing at the level of nasal cavities ; in the pus of the gleet, one was able to isolate the Whitmore bacillus. In one case, the start was marked by the incontinence of urine, in another case by a swelling of the belly. Death came in 6 to 18 days, except in one case which ended in recovery, after a dragging evolution lasting for 18 months. This last horse had been malleinated on several instances, without success, with malleines of various origins.

- Penicillin averred inefficacious and chloramphenicol was not tried. Sulfadiazine has appeared to prolong the life of the animals who were affected.

- The autopsies showed lung abcesses, hemorragic adenomegalies, an inflammation of the mucuous membranes : pharyngeous (?), nasal and laryngeous (?). The horse afflicted with urine incontinence presented, beside lung abcesses, a strong congestion of the vesical mucuous membrane, and the horse afflicted with the swelling of the belly presented necrotic areas on the mucuous membranes. The horse who recovered was killed and found carrier of a fibrous cicatrice in a lung and of a hypertophy of the ganglions of the hile with islets of necrosis.

- The malady imitates the glanders, and the lesions of upper respiratory tract, while sometimes they lack clearness in the live animal, are always present in the autopsies; they quite justify the name of "pseudo-glanders".

2° Bovines and bubaline animals

One case has been reported up to now. It is the case of a cow observed by Nicholls (1930) in Peyton Colombo (Ceylon), and who died suddenly. The autopsy d uncovered a big abcess of the spleen open into the peritoneum, the pus contained whitmore bacillus.

- In the Far East of Asia, the disease must pass unnoticed among the bubaline stock,

mistaken for other infections. For the buffalo loves to wallow in the muddy waters of the swamps, leeches sting him frequently and produce many infected sores. It is easy to imagine that animal being a carrier of germs, such as those for the trypanosomiases. At the Institut Pasteur of Saigon, Phung Van Dan has examined the serums of 8 buffaloes presumed healthy, killed at the slaughter house : 3 contained active agglutinies at a rate of over 1/80, the reactions of fixation of complement were negative. Those first results already show the abundance of natural agglutinies in the serum of buffaloes in Viet Nam. It would be worth while to continue this research on a larger scale.

3° Porcines.

- Epizooties of porcine melioidosis have been reported in the course of these last years in Australia (1953), in Viet Nam (1955, isolated cases in 1958 and 1959) and in Cambodia (1959). The infected pigs showed lack of appetite, hyperthermy (i.e. they ran temperatures , dyspnea, a deep prostration, a progressive weakening, the paresis of the hind quarters , sometimes diarrhea. Shrey Thonn and his colleagues have observed abortions among the sows, and suppurated orchitis among the boars. Death followed in 10 to 15 days. In Cambodia as well as in Viet Nam the disease primarily struck the porcine belonging to selected breeds, imported a few months earlier from countries immune to melioidosis (Japan, Australia).

- Autopsy showed numerous abcesses in the lungs, in the spleen, in the liver, in the intestines, in the epiploon and in various lymphatic ganglions; the culture of the abcess, as well as hemaculture led to the isolation of Whitmore bacillus in pure state or associated with staphylococci, with colibacilli.

- In Saigon the research of Fournier (1952), Luong and Dan (1955) have revealed agglutinies which were active at a rates of above 1/80 among 28 % of the pigs of an infected farm , among 9.5 to 11.35 % of the hogs earlier presumed to be healthy and who were bled at the slaughter house; the limit rate of 1/160 was observed among 1.43% of the latter.

4° Ovine and Caprine stock.

- The first ovine epizooties and caprine epizooties occurred from 1949 to 1955 in Australia, in the State of W Queensland. More recently, in 1957, an epizooty occurred in the Dutch West Indies, in a herd of indigenous sheep, killing 25 out of 90 animals. Sutmoller and his colleagues reported the frequence of abcesses ganglio-lymphatic abcesses am found among the ovine, the caprine stock and among hogs in the slaughter houses; they were able to isolate 17 stocks of M. pseudo-mallei from 15 sheep and 2 goats.

- The prevailing symptoms are dyspnea, arthrites, prostration, emaciation. The infected animals have a difficult respiration, a rebel cough which is exhausting their forces, rattling in the throat during auscultation, difficulty to walk, paresis of the back quarters or nervous troubles.

- Autopsy reveals chronical abcesses, that are voluminous, of the vital organs (lungs, spleen, liver, kidneys, mammal glands), adenomegalies (of the mediastine, of the shoulder) , swellings of the articulations, suppurated lesions of the nasal cavities, of the maxil-

lungs. Cottew notes that melioidosis lesions among the goats were found to be very similar to the chronical glanders.

- In many infected herds, except for the clinically sick animals, there is a great number of subjects in a state of inapparent infection, reactining positively to melioidine, presenting high agglutination figures, varying between 1/80 and 1/60. The systematic slaughter of such animals reveals frequently important melioidosic lesions.

5° Cats and dogs.

- Stanton and Fletcher have detected some cases of melioidosis during a minutious examination of the cadavers of a great number of those animals. The main ~~suspected~~ symptoms indicated by the owners of those pets were diarrhea, icterus, pustules on the abdomen.

- Autopsies ~~showed~~ bacilliferous granulations in the lungs, the hypertrophy of the spleen and of the liver, an inflammation of the intestinal tract.

- Differential diagnostic must be made with the infancy disease in the dog and the infectious gastro-enterite in the cat.

6° Small rodents.

- Fletcher and Stanton, in Malaya, have observed, in several instances (1913, 1917 to 1919, 1921, 1932), epizootics occurring among the small laboratory animals (rabbits, guinea pigs, rats), They then produced the hypothesis that melioidosis was a disease natural to these rodents.

- In Viet Nam, in 1954 and 1955, several grouped cases have been reported, during the dry season, among the rabbits and the guinea-pigs of the Institut Pasteur of Saigon. The epidemiological inquiry has revealed the presence of contagion in the potato leaves and of water bind-weeds soiled by the muddy water of the swamps.

- English authors have well described the symptomatology : oculo-nasal flow of milky aspect, muco-purulent, bacilliferous, dyspnea, death in a few days. In Saigon, Destombes did not note the nasal and ocular outflow; on the contrary, he observed cervical and axillary adenopathies and infected sores on members.

- Autopsy showed abscesses on various vital organs : lungs, spleen, liver (mammary gland in one case), purulent overflows in the pleura, in the pericardium, suppurated localizations on the members, on mesenteric ganglions, on mediastine (mediastinal) and cervical ganglions.

- Among rats, the evolution is slower : 3 or 4 weeks, lack of appetite, immobility, emaciation coming rapidly. The necropsic constatations are the same. However the affection is not known among the muridæ (?).

* * *

- It can be seen that, in natural conditions, cases of melioidosis are rather frequently observed among domestic animals (horses, swine, goats and sheep) and among rodents of laboratory herds (guinea-pigs, rabbits). The small laboratory epizootics are ephemeral and their inroads remain very limited. The extensive and deathly epizootics hit the porcine, the ovine and the caprine stocks, and in the first place the animals of selected

race, recently imported into endemic-enzootic or epi...otic regions.

V. - Bacterio-Immunological diagnosis

- The research carried out at the Institut Pasteur of Saigon from 1953 to 1955 have notably improved the laboratory techniques used in the detection of melioidosis both in man and in animals. These techniques include :

1° The search for the germ in the blood or in the pathological products (cultures, inoculations) ;

2° The bringing into evidence of specific antibodies in the serum of the infected subject (sero-diagnosis);

3° Or the revelation of a state of allergy towards the reactives issued from Whitmore bacillus.

1° Search for the germ

- In all local forms, both e septicemic and septico-pyohaemic, the hemoculture must be practiced; it is most often positive at the first onset. But sometimes, it is found to be negative several times, before the germ is isolated. "There is always advantage in setting into action simultaneously the hemoculture and the peritoneal inoculation of the guinea-pig with the blood of suspect subjects". (Fournier and Chambon).

- The spittle in man, the pus of the abcesses, the ocular secretions, the nasal and pharyngical secretions among the animals, are pathological products which are the most interesting for the search for the germ. The urine yields positive results only in case of localization of the infection on the urinary duct. The fecal matters always remain negative, even in case of gastro-intestinal troubles.

2° Search of the anti-bodies.

There are three different serological tests :

a) Sero-agglutination of bacterian suspensions which are stabilized and periodically controled with the help of experimental serum of known grade.. It is admitted that a grade of 1/80 has an indicative value, that one of 1/160 has an almost absolute value. The sero-agglutination is lacking in sensitivity : "it is possible to register sometimes negativ e results in septicemic forms in short evolution and in chronic forms without important impact on the general state of the subject." (Fournier and Chambon).

b) The hemagglutination practiced with human globules (cells) OBh (or with horse cells) seri-stabilized by a trichloracetic extract of M. pseudo-mallei. This is the most sensitive reaction and the most specific one. It is considered as positive if one notes a total agglutination of the hematies (+++) for the weakest dilution of the serums (1/4).

c) The fixation of the complement utilises the same antigen (?) as the hemagglutination. It is sensitive, but poorly specific. .. "The percentage of falsely positive results is of 5.2 (4 among among the non-febricitants and 11 (?) among the febricitants (=those running a fever))" (Fournier and Chambon). In the presence of a febrile (=running a fever) patient who is suspected of having melioidosis, one should proceed with tghree reactions on at least two samples of blood, taken at about 10 days of interval, and to prac-

tise concurrently a Widal-Felix sero-diagnosis, in order to eliminate serums containing antisalmonella O agglutinines; and thus lowering the proportion of falsely positive reactions.

- In man these three serological tests make possible the diagnosis, before the search for the germ itself is positive. In animals, on the contrary, they appear very dissociated, and inapplicable, because of the frequency of non-specific natural agglutinines, and of the belated appearance of specific antibodies; furthermore, they do not allow the differential diagnosis between melioidosis and glanders.

3° Search for the Allergy.

- The intradermic reaction is still in a stage of experimental research. Whitmorine, extracted from cultures in glycerinated broth requires, because of its considerable necrosing power, the use of high dilutions, and the results have poor clafrity.

- The extracts from bacterian bodies are to be preferred to the extracts from cultures. At the Institut Pasteur in Saigon, Chambon has sought the allergy separately with purific O antigen (trichloracetic extract, neutralized and isotonic), and with antigen K which had been purified (extract from capsulated forms, in a 1/2,000 dilution). The answers were of variable intensity, but always easy to read, because of the absence of the necrosing power. The positive reaction is marked, at the spot of injection, by a purple-red pimple, which is painful, and is surrounded with an erythematous areola, which is more or less wide, and which appears on the 24-th hour, and which may persist unti the 4-th day.

- In veterinary medecine, Olds and Lewis, declare they have obtained good results in the tracking of the winfection among the goats with 2 types of melioidine.

1° Melioidine in diluted bureth (dilute broth melioidin D.B.M.),

2° Melioidin in synthetic medium (synthetic medium melioidin S.M.M.),

-Each animal was submitted to three successive intredermic injections : intrapalpebral, intranasal and at the edge of the ear. The reactions were marked on the 1st, 2nd, 4th an 8-th days, sometimes until the 16-th day after the injection : oedema, erythem local erythema, sometimes a purulent outflow, hypertrophy of lymphatic ganglions and regional ganglions, and of the paretide. In the infected herds those reactions have uncovered with good precision the animals in a state of asymptomatic disease. The slaughter and the autopsy of these animals have revealed suppurated lesions, from which Whitmore bacillus was obtained in culture.

- It is certain that the polymorphism of melioidosis on the one hand, and the state and characters of the stocks put into culture, on the other hand, have their repercussion on the sensitivity towards the antigens which reveal the allergy.

VI. - PROPHYLAXY

1° Medical prophylaxy.

-In the face of the recrudescence of epizootics of melioidosis in the Australian stock farms, Cottew (1953b) has deplored the imperfection of our knowledge in the matter of vac

cination against Whitmore bacillus, and has urged studies of this immunization.

- Nigg and his colleagues (1955-1958) have underlined the interest of filtrates detoxified with formol and phenol : mice having received injections of de-toxified filtrates of pseudo-mallei were tested 2 to 4 weeks later with inoculations of the virulent germ; they survived in the proportion of 12 out of 18, while all the control animals succumbed.

Levine and his colleagues (1958), through U.V. (?) irradiation of a virulent stock of Whitmore bacillus have isolated a mutant requiring for its culture adenine or hypoxanthine. The virulence of this variant for mice is clearly diminished. Nevertheless, it recovers when the stock recovers in vitro its independance from purines. It does not seem that the same thing occurs in vivo : the mutants persist in the mouse about 20 days after the inoculation of 10^7 germs. After intraperitoneal injections of this same dose, an effective immunization appears, verified by the parenteral injection of various virulent stocks. Nevertheless the mice thus immunized succumbed to the infection by respiratory organs.

- At the Institut Pasteur of Morocco, Blanc and Baltazard (1941) have immunized a sheep with 4 injections in growing x doses of germs killed by the heat, followed by 3 injections of live cultures. The sheep yielded an agglutinating serum, but we do not know what his survival fate was.

- The antigenic heterogeneity of the germ requires that in each country and even in each region of a country, the selection of the vaccinal stocks be based on the typing of locally isolated stocks.

In the absence of specific vaccination, only the sanitary measures must retain the attention.

2° Health Prophyllaxy.

Taking into account the hydrotelluric origin of the infection and of the part played by the reserves of viruses of the muridae and of certain hematophagist insects, prophylaxy must benefit from the sanitation and drainage works : drainage of stagnant waters, of swamps, systematic destruction of rats, elimination of brush, insecticide campaign.

- In human medecine there is a great interest to track as soon as possible the patient or patients by means of serological and intradermic tests, and take steps to isolate the patient or patients, and to disinfect the locals in consequence. Laboratory infections being frequent, serious precautions must be taken in the course of the manipulations and of the autopsies of experiment animals.

- The detection of the meats and of the milks which are bacilliferous is mandatory for veterinarians, for slaughter house inspectors and for dairy farm inspectors. In the zones of endemicity, one must avoid going on marches on through stagnant waters and the soiling of sores or wounds with these waters ; one must also prevent, through antibiotic treatment, the melioidoses which are consecutive of surgical operations, especially in cases suppurated collections.

- In veterinary practice, the systematic tracking of infected animals of all species (solipedes, bubaline, bovine, porcine, ovine and caprine) is primarily required, in ende-

mic and 4 enzootic regions, with the aid of conveniently prepared melioidine, or with other active antigens extracted from the Whitmore bacillus. Merciless elimination of the reacting animals, even in the absence of clinical signs, is the only efficacious measure to arrest the enzooty. It must be completed with severe desinfection measures : deep burial, between two layers of quicklime, of the cadavers and of the dejections of the infected animals ; disinfection of the stables and of stalls, and, if possible, of the pasturages, of the tending and feeding material ; sterilization of the products of the farm products, in particular of the milk. The re-stocking must be done with young animals issued from the healthy part of the herd, or coming from unscathed herds.

The inter-human contagion being unknown, it is the prophylaxy of the animal disease which is the foundation of the prophylaxy of the human disease. In endemic or epizootic tropical regions one should plan registering melioidosis on the list of legally contagious diseases of the stock.

CONCLUSIONS

Melioidosis, a hydrotellurie tropical zoonosis, tends to gain terrain on various continents.

The dreadful extension of the diseases presents certain particularly acute problems : the perfection of the tellurie test ; research concerning preventive and curative vaccination ; the carrying out of vague measures of prophylaxy general prophylaxy and the promulgation of a severe legislation destined to protect efficiently farm stock.

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