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EXPERIMENTAL AND BIOCHEMICAL STUDY OF A STRAIN OF PLAGUE BACILLUS DERIVED FROM A STRAIN OF THE MALASSEZ AND VIGNAL BACILLUS

[Following is a translation of a report made by Georges Blanc and Henri Mollaret, of the Pasteur Institutes of Morocco and Paris, at the 13 June 1962 meeting of the Societe de Pathologie Exotique (Society of Exotic Pathology), and published in the French-language periodical Bulletin de la Societe de Pathologie Exotique (Bulletin of the Society of Exotic Pathology), Vol LV, 1962, pages 323-329.]

In 1912, S. Rowland (12) [numbers in parenthesis refer to numbered items in the bibliography] reported that by cultivating the plague bacillus in a solution of its own nucleoprotein he had succeeded in transforming it into Malassez and Vignal's bacillus. Since the discrimination criteria admitted by Rowland rest only on degrees of virulence, they keep us from subscribing to his conclusions. The same is true of the variants of Malassez and Vignal's bacillus that Zlatogorov and Moghilevskaya (14) obtained in 1928 and Pokrovskaya (10) in 1934. Nevertheless, these experiments opened the disturbing chapter of the eventual passage from one to the other germ, a possibility admitted by Levine (9) in 1913 and by Boncinelli and Aradas (5) in 1933, from a theoretical point of view.

In 1936, Beszonova, Lenskaya, Molodtzova and Mossolova (1) reported five cases of spontaneous transmutation of Pasteurella pestis into Malassez and Vignal's bacillus that occurred between 1929 and 1936 in 214 strains kept in a collection.

Tumansky (13) reported, two years later, a case of transformation, under the influence of a plague bacteriophage, of P. pestis into a germ that "resembled pseudotuberculosis bacilli very closely", a result that was close to Korobkava's (8) findings, who, in 1937, produced a series of modifications among which, in addition to the loss of virulence, the acidification of media to which rhamnose had been added occurred, by also subjecting P. pestis to the action of a plague bacteriophage.

A particularly pertinent discussion of these facts and their importance will be found in Pollitzer (11) and mainly in G. Girard (7). We limit ourselves to state that the above-cited observations all report the passage of Yersin's bacillus to Malassez and Vignal's bacillus.

In 1944, one of us, in collaboration with Baltazard, pointed out that inverse phenomenon, the transformation of a Malassez and Vignal's bacillus into a plague bacillus (3). We believe it necessary to review the history of this strain. In the course of investigations made for the purpose of studying the behavior of Malassez's bacillus on the Xenopsylla cheopis flea, we performed the following experiment: a strain of B. pseudotuberculosis rodentium, whose biochemical characteristics and pathogenic power were verified very frequently [See Note 1], is transmitted by fleas. Since guinea-pig septicemia is weak, the fleas were applied to bite a rabbit that had been subjected to experimental septicemia (2). The animal received ten billion germs in the marginal vein of the left ear. A quarter of an hour later, 200 Xenopsylla cheopis fleas [See Note 2], inserted in a Borrel tube closed with a piece of sifting silk, are applied to gorge on the ventral wall of the rabbit. The operation lasts one half hour, after which it is easy to verify by binocular examination that all the fleas are quite distended. A hemoculture obtained by a heart puncture and the seeding of a drop of blood taken from the right ear of the rabbit will both be positive. Finally, ten fleas that have just bitten are crushed in a few drops of bouillon. Seeding on agar yields about one hundred colonies of a germ identified as Malassez and Vignal's bacillus.

([Note 1:] This strain had all the usual characteristics of P. pseudotuberculosis, particularly mobility at 27° C., fermentation of rhamnose and glycerin.)

([Note 2:] It is a question of young fleas, recently hatched, that have never bitten.)

The fleas, left fasting for 24 hours, are applied to bite, on four occasions during four days, on a young guinea-pig that does not become infected, whereas the inoculation of a few crushed fleas yields an abundant culture of Malassez's bacillus. The same biting operation is performed on a young guinea-pig that does not become infected, but the Xenopsylla culture turns out positive. The biting experiment is performed two more times without infecting the guinea-pig, although the fleas are always carrying Malassez and Vignal's bacillus. A final experiment produces new data. The guinea-pig that is bitten on three occasions in three days becomes infected. After three days of incubation without fever, its temperature goes up on the following days to 40.4° C., 40.7° C., 41.3° C, drops to 40° C., then to 39.6° C. on the tenth day, the day on which it is killed. Autopsy reveals the presence of a very large inguinal ganglion of the size of a small nut located to the left, that is to

say, on the side where the tube containing the fleas were applied on the ventral surface. When it is sectioned, the ganglion shows an aveolar structure. There is a little pus in the crypts. The spleen is very large, weighing 3.50 g., and is filled with abscess. The liver is interspersed with very many small abscesses. Spleen smears show numerous short bacilli with bipolar staining; smears of the ganglion display countless bacilli of the same type. Cultures of the heart blood, of the spleen and of the inguinal ganglion are positive. The isolated germ has all the characteristics of the plague bacillus. It is pathogenic for the guinea-pig and the rat.

As soon as fever appears in the guinea-pig that has been bitten by the fleas, the remaining fleas (about 100) are crushed and after being cultivated, with positive results, they are inoculated intraperitoneally in a young guinea-pig.

This guinea-pig becomes infected; its temperature is 40.4° C. two days after inoculation, 41° C. on the third and fourth day, 40° C. on the fifth day. A very large inguinal ganglion is found on the left side. The animal is sacrificed on this same day. Autopsy reveals the presence of a subcutaneous absecess at the level of the point of inoculation and a large left absecessed inguinal ganglion, the size of a walnut. Very numerous short bacilli with bipolar staining are seen on spleen and inguinal ganglion smears. The blood and spleen cultures yield, in a pure state, very numerous colonies of plague bacilli.

Therefore, it is indeed a question of a plague strain that appeared in the course of experiments conducted with a Malassez and Vignal's bacillus, while at that moment no experiment had been performed with the plague bacillus at the Pasteur Institute in Morocco.

Let us add that the strain of Malassez and Vignal's bacillus used in these experiments always retained its specific characteristics.

After isolating a plague bacillus derived from Xenopsylla cheopis fleas, that have been infected by a Malassez and Vignal's bacillus, the strain is preserved in the laboratory. Very many Xenopsylla cheopis fleas, young recently grown fleas, are put in a vat that has been described elsewhere (4). A first guinea-pig inoculated with plague is put in, then a second one (P.P. strain), and even a third one. When an examination of the fleas shows that they are infected, only young guinea-pigs are put in, in accordance with the following rhythm: on Saturday a guinea-pig is put in the vat, on Tuesday it is found dead, exceptionally on Wednesday. The autopsy, hemoculture and splenoculture, organ smears confirm that it really is plague.

This strain, originally a Malassez and Vignal's bacillus, retains a very great virulence, and is transferred only by a rodent and fleas.

It has been used to prepare a very active antiplague serum. Three cases of laboratory plague, arrested in a few days by treatment with serum in heavy dosage and with streptomycin, are imputable to it. We believed it to be interesting to study its biochemical characteristics.

This strain, whose appearance, both of the germ and of its colonies and of its cultures in a liquid medium, does not offer any peculiarity, is strictly immobile and not ciliated at any temperature, by way of contrast with the obvious mobility of the original strain at 18° C. and 27° C.

Nitrates are reduced, sodium citrate is not used, there is no decomposition of urea. Litmus milk and buttermilk are not modified. The reduction of methylene blue is more rapid and more intense than the reduction usually produced by the majority of Pasteurella pestis strains.

The action on carbohydrate substances conforms with the usual behavior of Pasteurella pestis; the following are fermented: arabinose, esculin, galactose, glucose, levulose, maltose, mannitol and xylose. The following are not decomposed: adonitol, dextrin, dulcitol, glycerin, inositol, lactose, rhamnose (in contrast with its fermantation by the original strain), salicin and sucrose.

Thus, according to these experiments and to the biochemical characteristics of the two strains, everything happened as if there had been a transformation of Malassez and Vignal's bacillus into plague bacillus. We emphasize the fact that when this "transformation" occurred there were no strains under experimentation at the Pasteur Institute in Casablanca and that, moreover, Yersin's bacillus and Malassez and Vignal's bacillus have never been isolated out of several thousand guinea-pigs used annually in that same institute.

SUMMARY

Experimental and Biochemical Study of a Strain of Plague Bacillus Derived from a Strain of Malassez and Vignal's Bacillus

According to the authors' experiments and to the biochemical properties of the two strains, everything happens as if Malassez and Vignal's bacillus had been transformed into plague bacillus. The authors point out that as this "transformation" was taking place, no strain of plague bacillus was under experimentation at the Pasteur Institute, Casablanca, and that, on the other hand, among several thousand guinea-pigs used every year in this Institute, neither Yersin's nor Malassez and Vignal's bacillus has ever been isolated.

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DISCUSSION

M. G. GIRARD. -- It is regretable that a discovery that I do not hesitate to call sensational due to the views that it summons up was not the subject of a separate report at the time that it was made. Since it was inserted without more ado within an article dealing with "the behavior of pathogenic microbes in hematophagous insects", I overlooked it, all the more so because no one had drawn my attention to it. Otherwise, I would not have failed to take it into account in my teaching at the Pasteur Institute as well as in my publications on the relationships between P. pestis and P. pseudotuberculosis, that were filled with critical analyses on the supposed "mutations" of the former toward the latter, occasioned by research undertaken in this field in my laboratory.

R. Pollitzer does not mention it either in his monograph that is, nevertheless, so rich in references on this problem. After an interval of eighteen years, while the original strain of Malassez and Vignal's bacillus has not been preserved, it would be useless to pass judgment on the circumstances that surrounded the observation by Messrs. Blanc and Baltazard, that was at least unforeseen. Nevertheless, I am struck by a curious coincidence: the P. pseudotuberculosis strain, isolated on the spot in a rabbit, was the first and only one that has never been found in Morocco and it is precisely that one that behaved, after transfer by X. cheopis, like an authentic P. pestis strain of the oceanic variety, the only type found to date in the modern plague manifestations on the African coast.

Parallelly, from 1940 to 1945, the plague prevailed in North Africa, especially in Morocco where from the interior it reached the coast, struck Casablanca in 1942 and did not disappear until after a fresh epidemic start in 1945 (R. Pollitzer).

This situation implied, fundamentally, the existence of an active reservoir of viruses and, although it is known that X. cheopis, so subtle in its behavior, is the common flea on rats in Morocco, the infection of other species of animals cannot be excluded a priori. Although the domestic rabbit is very little susceptible to experimental or natural plague, save for exceptions of which we have had some examples in Madagascar, it is capable of showing, like the guinea-pig, anatomic lesions analogous to the ones found in pseudotuberculosis with a plague bacillus of weak pathogenic power, but the characteristics of the etiologic agent continue being the characteristics of P. pestis. In addition to biochemical reactions, antigenic analysis probably would allow a better discrimination now than formerly between the two Pasteurellas in doubtful cases. An increase in virulence of a weakened strain, that is recuperated after massive inoculation in the blood of a rabbit, perhaps more so than by means of its passage by the flea, would probably provide a solution to the enigma that is created for the lemologist by the report of our two colleagues, if I can judge by what experiments of this type with EV virus-vaccine have taught us. But this would be doubting the authenticity of the germ isolated originally in a rabbit, which I refrain from admitting, in the absence of proof.

In conclusion, I believe, along with G. Blanc and H. Mollaret, that new studies are necessary and must be accomplished in accordance with the procedures that have been revealed to us and under conditions that do not leave room for any doubt as to the interpretation of results that would tend to confirm the ones recorded in 1944 in Morocco by Messrs. Blanc and Baltazard.

The exceptional interest in the problem as it is stated will give rise indubitably to these studies. With commendable prudence, G. Blanc puts forward that the events occurred as if ... A confirmation of his discovery would permit what is probably only a presumption for most informed readers to be transformed into certainty, with the import that this phenomenon of mutation would probably have in epidemiology.

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