XXXII. ON THE DIFFERENTIAL DIAGNOSIS OF THE PLAGUE BACILLUS FROM CERTAIN ALLIED ORGANISMS.

It was felt desirable that an investigation should be made of the characters of *B. pestis*, and especially of the best means to be adopted in distinguishing it from certain allied organisms liable to be mistaken for it. The tests we have selected as aids in arriving at a diagnosis will be found tabulated below, but a few supplementary remarks may be added on several points which do not lend themselves to a summary description.

It will readily appear from a consideration of the Table that the organisms we have examined may be classified into 4 groups:—1st—the plague bacillus both virulent and avirulent; 2nd—certain strains of the *B. pseudotuberculosis rodentium*; 3rd—5 organisms belonging to the "haemorrhagic septicaemia" group; and 4th—organisms conforming to the type of *B. enteritidis* (Gaertner).

I. Bacillus Pestis.

Appearances of growth on neutral agar. For diagnostic purposes cultures on dry neutral agar are fairly characteristic. Early cultures consist of delicate dew-drop-like colonies, so that the growth when looked at by reflected light somewhat resembles a ground-glass surface,—indeed, this appearance is retained even in old cultures. After several weeks' growth, isolated colonies by transmitted light are seen to be composed of a central, brownish, opaque nucleus surrounded by a thinner, translucent, granular zone; the nucleus may be eccentric. The central part has a well-defined outline, but that of the entire colony is crenulated. If a culture one month old be examined with a hand-lens, on each colony there may be seen scattered over the peripheral zone fine dew-drop-like projections giving the colony a distinctly granular look to the naked eye. Secondary colonies are frequently seen in

cultures two or three weeks old; these have a well-defined margin and contain a central thicker nucleus.

The stalactite test. The first essential towards obtaining good stalactites in a plague culture is absolute lack of vibration of the shelf on which the flask stands. To ensure this it is a good plan to have the shelves fixed to beams in the wall, instead of being supported on the floor. It is convenient also that each shelf should be separated from the wall by a space about a foot wide so that a candle can be placed behind the flask while it is being examined. The contrivance of floating pieces of cork in the broth cannot be recommended, simply because it is quite unnecessary. The addition of oil to assist stalactite formation is an advantage, but even without oil good stalactites can be obtained. When a culture is made fresh from the body stalactites may not readily form; the flask should then be shaken and its contents allowed to settle.

A highly characteristic appearance is obtained when a small quantity, *e.g.* 1 c.c. of blood containing say 10 to 100 bacilli per c.c., is inoculated into a 100 c.c. flask of neutral broth. The plasma forms a soft clot dispersed throughout the broth and if the flask be kept undisturbed each bacillus ultimately gives rise to a tack-like growth enclosed in a similarly shaped cavity. The head of the tack corresponds to the original point of growth, the vertical portion being formed by a down growth of the bacilli from this point; the cavity is presumably due to a solution of the fibrin by the bacilli.

In a typical stalactite growth the broth is clear. A difference in this respect was noted in the avirulent culture in which the broth was somewhat turbid. Again, the stalactites of the avirulent strain were not so characteristic as those of the virulent culture, *i.e.* they were not so uniformly thin and long.

Fermentation tests. MacConkey (1905) has recently pointed out that *B. pestis* and *B. pseudotuberculosis rodentium* produce parallel reactions in media containing fermentable substances, viz. the production of acid but no gas in glucose, laevulose, galactose, maltose, mannite and dextrin—lactose, cane sugar, and dulcite remaining unchanged. As will be seen from the table we have confirmed some of these reactions and find that they hold good in the case of an avirulent strain of plague isolated probably 7 years ago.

Animal tests. We do not propose to enter here into the special diagnostic points in the pathology of plague, but naturally the presence or absence of such a characteristic phenomenon as the involution forms in animal tissues is of great value in deciding as to whether or not a doubtful organism is the plague bacillus.

TABLE.

Name	Origin and where obtained	Neutral agar	Glucose	Lactose	Dulcite	Laevulose	Mannite	Galactose	Stalactite test	Animal test
B. pestis (virulent)	From guinea-pig Bombay		Acid; no gas	–	–	Acid; no gas	Acid; no gas	Acid; no gas	Typical	Pathogenic to rat guinea-pigs.
,, (avirulent)	Probably from Bombay—7 years old (Dean)		Acid; no gas	–	–	Acid; no gas	Acid; no gas	Acid; no gas	Typical, less so than virulent	Non-pathogenic to and guinea-pigs.
Schweineseuche bacillus I.	(Wassermann) Pasteur Institute	Like *B. pestis*	No growth	No growth	No growth	No growth	No growth	No growth	Very fair stalactites	
,, ,, II.	do. do.	,, ,,	,,	,,	,,	,,	,,	,,	Good stalactites	
,, ,, III.	do. do.	,, ,,	,,	,,	,,	,,	,,	,,	Suggestion of stalactites	
Pasteurellose ovine	(Lignières) Pasteur Institute	,, ,,	,,	,,	,,	,,	,,	,,	Negative	
,, *equine*	do. do.	,, ,,	,,	,,	,,	,,	,,	,,	,,	
B. suisepticus	(Preisz: Origin A) Pasteur Institute	,, ,,	,,	,,	,,	,,	,,	,,	Very short thick stalactites	
,, ,,	(Preisz: Origin P) Pasteur Institute	,, ,,	,,	,,	,,	,,	,,	,,	Tendency to stalactites	
B. of fowl cholera	Pasteur Institute	,, ,,	,,	,,	,,	,,	,,	,,	Good stalactites	
,, ,,	Bombay	,, ,,	,,	,,	,,	,,	,,	,,	Negative	Killed g.-pigs ac by cutaneous me
B. pseudotuberculosis (Pfeiffer A.)	More than 10 years old Pasteur Institute	Growth too luxuriant	Acid; no gas	–	–	Acid; no gas	Acid; no gas	Acid; no gas	Very fair stalactites	
B. pseudotuberculosis (Pfeiffer K.)	Pasteur Institute	,, ,,	,,	–	–	,,	,,	,,	Short thick stalactites	
B. pseudotuberculosis (Pfeiffer)	Lister Institute	Like *B. pestis*	,,	–	–	,,	,,	,,	Very good stalactites	
B. pseudotuberculosis (Singe B i)	(Binot) Pasteur Institute	Growth too luxuriant	,,	–	–	,,	,,		Short thick stalactites	
B. tuberculose zoogléique	(Nicolle) Pasteur Institute	,, ,,	,,	–	–	,,	,,		Negative	
B. pseudotuberculose coccobacillaire	(Borrel) Pasteur Institute	,, ,,	,,	–	–	,,	,,		,,	
Organism causing plague-like appearances in guinea-pigs	Bombay	,, ,,	Acid + gas	–	Acid + gas	Acid + gas	Acid + gas		,,	Produced an infe in guinea-pigs by neous method.
Same as above	Bombay	,, ,,	Acid + gas	–	Acid + gas	Acid + gas	Acid + gas		,,	
B. suipestifer (Kruse)	Pasteur Institute	,, ,,	Acid; no gas	–	Acid; no gas	Acid; no gas	Acid; no gas		,,	

Note:—The media in the above table containing sugars &c. were prepared by the methods recommended by MacConkey.
The sign "–" indicates that neither acidity nor gas production was noted.

II. Bacillus pseudotuberculosis rodentium.

The growth on agar of most of the races we have examined was more vigorous than that of *B. pestis* though a culture obtained from the Lister Institute resembled plague very closely.

The tests for stalactites gave interesting results. The culture just mentioned gave undoubted long stalactites, reaching almost to the bottom of the flask, indistinguishable indeed from those of the plague bacillus—the broth being fairly clear though later it became decidedly cloudy. *B. pseudotuberculosis* (Pfeiffer) A. grew very fair stalactites which however took a long time to develop (3 weeks). *B. pseudotuberculosis* (Binot) showed in about a fortnight very thick short atypical stalactites; while *B. pseudotuberculosis* (Pfeiffer) K. developed short thick stalactites in about 48 hours.

The various sugar reactions coincided with those of *B. pestis* as mentioned already. Fortunately we have never encountered this organism in our stock of experimental guinea-pigs. In such an event, we should rely chiefly on the effects of the injection of the culture into several *white* rats.

III. Bacilli of the "haemorrhagic septicaemia" group.

The cultures of all the organisms in this group tested by us gave a distinctly more delicate growth on neutral agar than the plague bacillus though otherwise resembling it.

Stalactite test. The bacillus of German swine plague (I) formed good stalactites in 3 days though later the broth showed clumps. No. II also gave good stalactites indistinguishable from those of plague but in this case their formation was delayed for at least 3 weeks. *B. suisepticus* of Preisz A. and P. showed very short thick stalactites after a fortnight's growth. Stalactites formed in a culture of the fowl-cholera bacillus obtained from the Pasteur Institute, which could not be distinguished from those of *B. pestis*; on the other hand a recently isolated strain failed to produce them.

It is noteworthy that of all the cultures tested for the production of stalactites only those included in this group and certain strains of the pseudotubercle bacillus showed any tendency to their formation—a fact

which serves to confirm the close relationship of these organisms to the bacillus of plague.

Sugar media test. The differentiating test of this group was furnished by the employment of MacConkey's sodium taurocholate medium. We failed to obtain any growth of these organisms in various modifications of this medium.

Animal tests. An opportunity was afforded of testing by the cutaneous method the effect on guinea-pigs of a spleen from a goose recently dead of a haemorrhagic septicaemia. The spleen was rubbed into a shaved area of 2 guinea-pigs weighing about 200 grammes each. One died in 26 hours showing *post-mortem* a local cutaneous reaction, marked general subcutaneous oedema, a considerable amount of fairly clear peritoneal and pleural effusion, and congestion of the inguinal glands. The spleen was not enlarged. On microscopical examination no bacilli were seen in the spleen, but small organisms showing bipolar staining were noted in the heart blood. A good culture was obtained from the heart blood on agar and in broth. The second guinea-pig died in 3 days showing an intense local reaction, general subcutaneous injection, small double inguinal, pelvic and axillary buboes, and pleural and peritoneal effusion. The spleen was not much enlarged and showed no nodules but contained fairly numerous organisms. The heart blood showed numerous very small diplo-bacilli, and gave a good culture on agar. The glands showed a few bacilli. These results correspond with those obtained by Fritsche.

IV. Bacilli of the B. enteritidis (Gaertner) group.

1. *B. suipestifer* (Kruse) is unlikely to be mistaken for *B. pestis.* Its growth on agar is too vigorous and opalescent, and the colonies never assume the typical appearances of those of the plague bacillus. The test for stalactites proved negative. The sugar reactions carried out by us differed from those of *B. enteritidis* (Gaert.) in that no gas development was observed in glucose, mannite and laevulose.

2. Another organism belonging to this group is of practical importance in our work as it was the cause of an epizootic amongst the young stock guinea-pigs. The pathological features of the disease markedly resembled those of plague. Smears of the spleen after death frequently showed bipolar-stained bacilli very like *B. pestis.* It may be mentioned that the disease was given to a healthy guinea-pig by cutaneous inocu-

lation, the spleen of an animal showing numerous nodules being used for the purpose.

A similar organism has been isolated on one or two occasions from rats brought dead to the Laboratory for examination. On two occasions the spleen of a rat which did not prove to be plague infected when inoculated cutaneously into a guinea-pig gave rise to a subacute infection. In one case an inguinal gland was enlarged and the spleen contained a nodule the size of a millet seed.

From the foregoing details it may be asserted that organisms of the "Gaertner" type occasionally occur in rats, and that if the cutaneous method in guinea-pigs be employed for purposes of diagnosis, an infection by this organism may result; confusion from this cause may easily be avoided if the possibility of such an infection be remembered.

Into this group, or more broadly into the *coli* group, probably fall many of the so-called plague-like bacilli described by various authors. We can merely state our experience that we have never encountered any organism of the group which with ordinary care is in the least likely to be mistaken for *B. pestis*.

Summary.

We have no desire to claim for the tests described above that they will be found applicable to every strain of organism belonging to any particular group. Our experiments were necessarily limited to a few races of each—many of these being probably avirulent from long sub-cultivation on artificial media. We merely wish to indicate the general methods we would adopt in any case of difficult bacteriological diagnosis, although as a matter of fact the only tests we found it necessary to use in the course of our work were the method of cutaneous and subcutaneous inoculation into animals and the stalactite test.

The tests referred to in this paper may be summarised thus:

1. The plague bacillus gives a fairly characteristic type of colony on neutral agar; it forms typical stalactites in neutral broth; it gives certain definite fermentation reactions.

2. *B. pseudotuberculosis* resembles *B. pestis* more closely than any other organism. The animal test on white rats is probably the best for its differentiation.

3. Bacilli of the haemorrhagic septicaemia group appear to be inhibited in their growth in media containing sodium taurocholate.

4. Bacilli of the *B. enteritidis* (Gaertner) group are the most readily distinguished of all; the appearance of agar cultures, the negative stalactite test, and their fairly definite fermentation sugar reactions are sufficient to mark them off from the plague bacillus.

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CHART I

BOMBAY,

1897—1906

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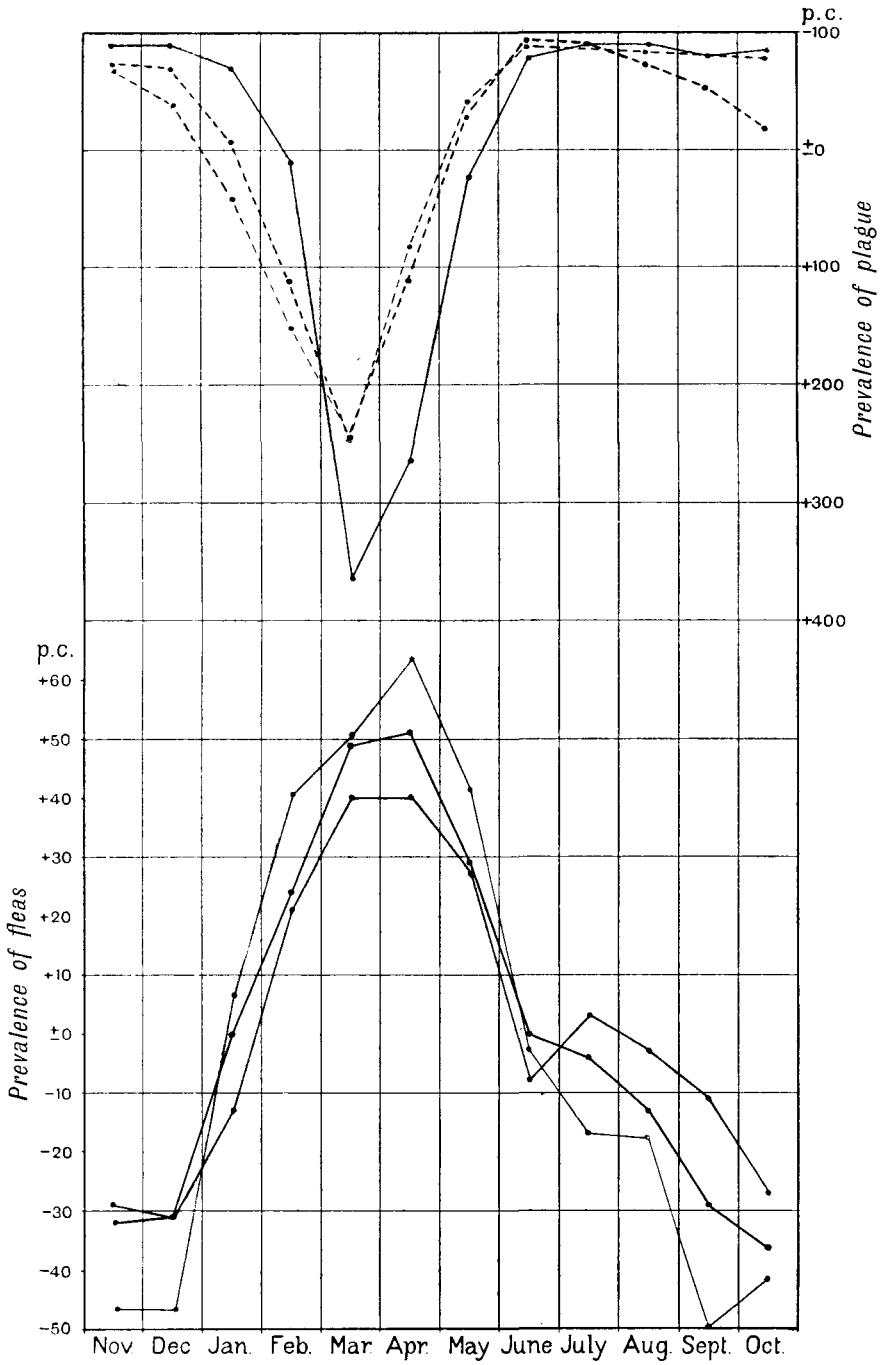
CHART VII



PREVALENCE OF FLEAS IN BOMBAY

Human plague

Plague in M. decumanus

Plague in M. rattus

Fleas on all rats

Fleas on M. decumanus

Fleas on M. rattus

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