THE CULTURAL CHARACTERS AND PATHOGENICITY FOR SOME LABORATORY ANIMALS OF THE VOLE STRAIN OF ACID-FAST BACILLUS

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Introduction

Wells (1937) discovered that field voles in this country are subject to an epizootic disease due to an acid-fast bacillus. The natural disease in the vole is characterized by the deposition of a cheesy substance in the areolar tissue spaces and the formation in the organs, the lungs particularly, of lesions which have a close resemblance to those of tuberculosis.

In October 1937, Dr A. Q. Wells sent me a culture and asked me to examine it to determine whether or not it was related to any of the three well-known types of tubercle bacilli.

Cultural characters

In order to determine whether the micro-organism belonged or not to one of the three types it was necessary to study the cultural characters *ab initio*. The culture sent to me was therefore inoculated into guinea-pigs, and material from naturally infected field voles was kindly supplied to me by Dr A. Q. Wells. Cultures were made from the inguinal glands of the guinea-pigs infected with the culture and directly from the original vole material.

After 12–14 days, in which time colonies of the three well-known types of tubercle bacilli become visible to the naked eye, tubes of the egg media sown with vole material show no growth. Usually no colonies of the vole strain make their appearance until the 24th–26th day. When numerous in the material they may be seen a few days earlier or when scanty some weeks later. About the 30th day they are plainly visible to the naked eye as points which under magnification are pearly white, hemispherical colonies, in some tubes rising abruptly from the surface, on others showing either narrow, transparent frilled margins, or in a few instances widespread margins which exhibit fern-like markings on their surfaces. When the colonies are few they vary more in size and shape; some are conical and granular; some are of a blackberry outline or hollow-centred and composed of fine granules in close contact; or are umbilicated.

When fragments of tissue containing vole bacilli are implanted on the medium, numerous small colonies erupt from them as is the case when tuberculous avian tissues are implanted.

On 5% glycerin egg either no colonies appear or only one or two. The colonies are a little larger than on egg, ivory white, conical with rough or smooth surfaces and rounded, with frilled margins.

On 5% glycerin agar in subculture an extremely thin translucent grey layer is formed which at the top of the slope shows under magnification discrete, flat colonies, glistening by transmitted light. Also raised specks are seen which under 8× are found to be minute glistening colonies.

Good growth was obtained on potato, slight filmy colonies on trypinized broth, but none on the surface of plain broth. In the depth of broth a fine deposit is formed. The organism does not grow at 22° C.

The bacilli stain by the Gram and Ziehl-Nielson methods.

Morphology. The organisms are slender and vary in length, some being much longer than ordinary human tubercle bacilli. They are curved in all sorts of ways and in several directions; the most characteristic end in a crook. No branched forms were seen. Stained by the Ziehl-Nielson method some are pale pink and contain darkly stained granules which may occur in any part of the bacillus. Comma forms are frequent. Some bacilli show fine granulation and vacuolation along their whole length.

Summary. The primary colonies on plain egg have some resemblance to colonies of tubercle bacilli, but they differ from all three types in their much slower growth, taking nearly 4 weeks or sometimes longer to become visible to the naked eye instead of less than 14 days by tubercle bacilli. The organism shows no growth or only very scanty growth in primary culture on glycerin media, thus differing from the human and avian types and some bovine strains, and grows in subculture with difficulty on these media. The organism has a characteristic morphology, curved forms abounding, the most striking being in the shape of a shepherd's crook, a sickle, a spiral or the letter S. Branched forms have not been observed even in the umbilicated primary colonies on egg.

Pathogenicity for some laboratory animals

(*a*) *Rabbits*

Seven rabbits were inoculated intravenously. Two received each 1·0 mg. of culture. One died in 16 days and showed patchy hepatization of the lungs with moderate submiliary tuberculosis, an opaque area in a tracheal gland and slight enlargement of the spleen. The other died in 43 days of general miliary tuberculosis and showed very severe lesions in the liver.

Two received each 0·1 mg. One died prematurely in 16 days and showed a moderate number of minute foci in the lungs and slight enlargement of tracheal glands and spleen. The other was killed 115 days after inoculation and showed scattered tubercles in the lungs and a small abscess at the top of each caudal lobe. The kidneys showed pits and scars on their surfaces, and there was a grey prominence on papilla of one kidney. Abundant colonies of the vole strain were obtained from a lung abscess.

Three rabbits each received 0·01 mg. and were killed 105, 107 and 845 days respectively after inoculation. The first showed a small marginal patch and a few transparent foci in the lungs and three medullary tubercles in a kidney. The second had two tubercles and

a few transparent foci in the lungs and two tubercles in the kidneys and the third three minute pearly tubercles in the lungs. Cultures from the lung tubercles of the second rabbit yielded growth in the condensation water of one tube. Cultures from the lung tubercles of the third rabbit remained sterile.

One rabbit was inoculated subcutaneously with 10–15 mg. of culture. The animal died 84 days later and showed a local abscess and a few caseous foci, from which five colonies of acid-fast bacilli were obtained, in a prescapular gland.

Summary. A relatively large dose intravenously may cause death from acute miliary lesions closely resembling those of tuberculosis. Smaller doses intravenously or large doses subcutaneously produce trivial lesions and the bacilli gradually die out. An abscess in the lungs may, however, provide a nidus in which the bacilli may live for a long time. In my experiments the duration of life of the bacillus proved longer in the rabbit than in the guinea-pig.

(*b*) *Guinea-pigs*

Intraperitoneal inoculations. Relatively large doses of culture (1·0 and 0·1 mg.) intraperitoneally may cause generalized disease which resembles acute or chronic atypical general tuberculosis. For example, in one guinea-pig, which died 17 days after receiving 1·0 mg., the appearances of the lesions were as in acute tuberculosis. Another, which died 14 days after receiving 1 mg., showed a much thickened omentum containing purulent areas, grey deposit on the mesenteries, tubercles in the right manubrial glands, some hyperplasia of most other glands and numerous grey foci in the liver. An emulsion of the omentum of this guinea-pig was inoculated intraperitoneally into another guinea-pig which was killed 362 days later and showed no evidence of disease and no living acid-fast bacilli were found.

Three guinea-pigs, which received intraperitoneally 1·0, 0·1 and 0·1 mg. respectively, died in 61–90 days and showed a mild form of atypical generalized disease, the lesions in the organs being mainly grey and, except in the liver, few in number. Some of the abdominal and thoracic glands were slightly enlarged; the ventral mediastinal showed caseous foci. The spleen was enlarged and red. The omentum was not thickened in any animal, but one contained one nodule, another several nodules and the third none.

One guinea-pig which received 0·1 mg. died in 61 days with red hepatization of the lungs, and two opaque tubercles in the omentum.

One guinea-pig which received 0·01 mg. died in 84 days and showed the pyloric gland only slightly enlarged and opaque centrally. Two acid-fast bacilli were found after long search.

Intraperitoneal inoculation therefore shows that relatively large doses may cause either death from generalized disease resembling acute tuberculosis or a subacute atypical generalized tuberculosis. With smaller doses the guinea-pigs do not die and on examination some months later show traces only or no signs of infection.

The vole bacillus was recovered from three guinea-pigs inoculated intraperitoneally with cultures 61, 75 and 90 days previously and not from two guinea-pigs inoculated 84 and 156 days previously.

Subcutaneous inoculations. Subcutaneous inoculation of doses up to 5 mg. of culture have had local effects only. At the seat of inoculation a local abscess was produced which ulcerated after 10 or more days according to dose and then healed completely. The inguinal glands after a period of enlargement became normal in size.

Of seven guinea-pigs inoculated subcutaneously, two with tissue emulsion and five with culture, one, killed 46 days later, showed a small ulcer and hyperplasia of a right inguinal gland with an irregular subcapsular area of necrosis; another, killed 102 days later, after a dose of 2·0 mg., showed a linear scar locally and some hard subcutaneous nodules; a third, after a dose of 5·0 mg., died after 125 days, and showed two foci in an inguinal gland from which cultures were not obtained. Three guinea-pigs, killed 128 (inoculated with tissue), 345 (5·0 mg.) and 373 (1·0 mg.) days after inoculation, showed no sign of disease and no living bacilli were found in their glands.

The bacilli were recovered in culture from the inguinal glands of three guinea-pigs killed respectively 46, 57 and 102 days after inoculation, but not from two purulent foci (acid-fast bacilli were present microscopically) in the inguinal gland of a guinea-pig which died of pneumonia on the 126th day. Cultures were not obtained from guinea-pigs which had been inoculated subcutaneously 345 (5·0 mg.) and 373 (1·0 mg.) days previously.

Summary. The micro-organism in doses of 1·0 and 0·1 mg. intraperitoneally may cause death from generalized disease, the lesions of which resemble tuberculosis and microscopically have a tuberculous structure. The lesions, however, are retrogressive, and those produced by small doses intraperitoneally or large doses subcutaneously heal completely, the bacilli dying out.

(*c*) *Rats*

Subcutaneous inoculation. Two rats were injected subcutaneously, one with a dose of 5·0 mg. and the other with a dose of 10 mg. The first, killed after 190 days, showed no signs of disease, but very small numbers of acid-fast bacilli were found in smears from the lungs, inguinal, iliac and axillary glands. In a culture from the inguinal gland seven colonies developed and in a culture from the spleen four colonies. The second rat died on the 562nd day. There were no lesions due to the vole strain and no acid-fast bacilli were seen in smears from the organs, glands or atrophied fatty tissue.

Intraperitoneal inoculation. Four rats were given intraperitoneal inoculations, three received doses of 1 mg., and the other a dose of 5 mg. Of the rats which received 1 mg. one was killed after 190 days. The omentum and peritoneum appeared to be normal. The spleen was firm and appeared enlarged. Abscesses were present in the lungs. Acid-fast bacilli were found in smears from the portal glands. Cultures from the spleen showed a moderate number of colonies. The second rat was killed on the 468th day. There was a very small nodule with a fibrous wall, containing pus, at the site of inoculation and a very small abdominal nodule. In smears from the abdominal nodule numerous acid-fast bacilli were found, and in smears from the crushed lungs few, but none were found in smears from the liver and spleen. Cultures from this abdominal nodule showed numerous colonies, from the lung and spleen moderate numbers, and from the liver nine colonies. The third rat was killed on the 470th day. All lobes of the right lung and the cephalic lobe of the left were expanded and congested, and showed, mainly on their dorsal surfaces, grey, lobular, translucent areas up to 1 mm. in diameter, some of which showed whitish points. The left caudal lobe contained cheesy pus (not due to the vole bacillus). Smears from the right lung showed moderate numbers of acid-fast bacilli. Cultures from the right lung produced moderate numbers of colonies of the vole bacillus, and from the inguinal gland, liver and spleen small numbers of colonies. The rat which received 5 mg. died on the 493rd day. The carcass was emaciated and there was alopecia but no ulcers

or thickening of the skin. The subcutaneous fat in three regions was examined for acid-fast bacilli, but none was found. The omentum was normal. The spleen was enlarged and the liver normal, but acid-fast bacilli were found in moderate numbers in both. The lungs were expanded, slightly congested and mottled with grey, translucent foci similar to those found in the third rat. A few showed very minute whitish spots. Cultures from the lungs showed numerous and from the spleen moderate numbers of typical vole-bacillus colonies.

Summary. After intraperitoneal injection the vole bacillus may produce in the lungs of the white rat lesions resembling macroscopically those produced in this species by the mammalian tubercle bacillus.

(*d*) *Fowls*

Two fowls were inoculated intravenously each with 0·1 mg. of culture. One died from toxaemia in 27½ days and showed minute grey points in lungs and liver, acid-fast bacilli being fairly numerous in the liver and spleen. The other was killed after 141 days and showed only one minute opaque tubercle in the lungs (acid-fast bacilli). Cultures from the spleen were sterile.

Two fowls each received 0·01 mg. intravenously. One died in 96 days and showed sparse grey foci up to 1 mm. in diameter in the lungs and a disk-shaped grey opaque surface lesion of doubtful nature on one caudal lobe in which no acid-fast bacilli were found. The liver showed some opaque marginal lesions (5 mm. in diameter) and four or five translucent grey foci. Numerous colonies were obtained in cultures from the spleen, but only five colonies on two tubes from the liver.

Two fowls each received an intramuscular inoculation of 10·0 mg. of culture. They died in 54 and 115 days and each showed a small local infiltration only. Cultures from the spleen and liver of the former and from the local pus of the latter were negative though numerous acid-fast bacilli were found microscopically.

Summary. These experiments show that the vole strain is not virulent for the fowl. After a large dose the bacilli remained alive in the apparently unaltered spleen for 141 days.

(*e*) *Other animals*

The vole bacillus was also tested on the field vole (1939*a*) and golden hamster (1939*b*, 1941) and, in collaboration with Dalling, on the calf (1940).

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