

THE RELATIVE SUSCEPTIBILITY OF THE FIELD-VOLE TO THE BOVINE, HUMAN AND AVIAN TYPES OF TUBERCLE BACILLI AND TO THE VOLE STRAIN OF ACID-FAST BACILLUS (WELLS, 1937)

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INTRODUCTION

THE occurrence in this country of an epizootic disease among field-voles (*Microtus agrestis*), resembling tuberculosis in its anatomical features but due to an acid-fast bacillus different from the tubercle bacilli found in other species of warm-blooded animals, has raised several interesting questions. One of these is the relative susceptibility of the field-vole, and other species of wild rodents belonging to the family Muridae, to the bovine, human and avian types of tubercle bacilli and the possibility of using any of these species in place of the rabbit for differentiating between the three types. Some work on the subject was done by me in the year 1923, the rodents tested being field-voles and wood-mice (*Mus sylvaticus*). These experiments, reported in 1937, showed that the field-vole is highly susceptible to bovine bacilli, and can also be infected with human bacilli, though with less certainty and less severity than with bovine bacilli. The wood-mouse is susceptible to bovine and human bacilli, perhaps more so to the former than to the latter, and stands in an intermediate position between the vole and the white mouse in its susceptibility to mammalian tubercle bacilli.

Further experiments were necessary to determine more exactly the relative susceptibility of the field-vole to bovine and human tubercle bacilli and also to ascertain its susceptibility to the avian type of bacillus. Results obtained in

1937 established that field-voles are much more susceptible to bovine than to human or avian bacilli and in a progress report to the Medical Research Council (1938) I suggested that the vole might be used for the purpose of differentiating the bovine from the other two types.

Wells (1938) tested four strains of bovine and four strains of human bacilli on voles by the intraperitoneal method, and he also showed that bovine were much more virulent for the vole than human bacilli. In one series twenty voles were injected intraperitoneally each with 0·001 mg. of culture, ten receiving bovine and ten human tubercle bacilli, and were killed 1 month later. All those which received bovine bacilli had extensive tuberculosis, whereas none of those injected with human bacilli showed any macroscopic signs of disease. Wells suggested "that a dose of 0·001 mg. moist weight of culture of tubercle bacilli injected intraperitoneally should distinguish unfailingly between human and bovine bacilli in 1 month".

The following is an account of the additional experiments on the field-vole done by me since July 1937. Altogether fifty-five voles have been used, twenty-five for bovine (several of these died prematurely), fourteen for human and five for avian bacilli and eleven for the vole strain. Most of the voles were bred at the breeding station of the Bureau of Animal Population, Oxford, and the rest were caught in the wild state in a paddock near my laboratory at Cambridge.* The methods of infection were by subcutaneous inoculation in an inguinal region and feeding. Bovine and human bacilli were injected in the form of cultures and tuberculous fluids (cows' milk, pus or human sputum). Avian bacilli were used in the form of cultures only.

DETAILS AND RESULTS OF THE EXPERIMENTS

Inoculation and feeding experiments with bovine bacilli

Subcutaneous. Eight voles were injected subcutaneously with cultures of bovine bacilli, six of them with strains (nos. 28, 33 and 34) of direct bovine origin and two with strains derived from sputum of cases of human pulmonary tuberculosis. The results with two voles which died prematurely are excluded from consideration here.

One vole, dose 0·00001 mg. of sputum-strain C.S. 397, died in 32 days. The autopsy showed a small purulent local lesion beginning to ulcerate, and enlargement and caseation of the inguinal and iliac glands; there were no macroscopic lesions elsewhere. Tubercle bacilli were numerous in the glands and sparse in the spleen.

The remaining five voles, all injected with bovine culture of direct bovine origin, died of general tuberculosis, three (dose 1·0 mg.) in 31, 38 and 63 days and two (dose 0·1 mg.) in 93 and 107 days respectively. In each instance the local lesion was caseous and ulcerated and the inguinal, iliac and antero-ventral mediastinal glands were enlarged and caseous throughout. Other

* Since 1923 about a dozen voles found dead—most of them killed by cats—on the land around the laboratory have been examined. None showed disease.

lymphatic glands were affected, showing either simple enlargement or some degree of caseation. The lungs also showed lesions ranging from a minute tubercle or two to caseating masses replacing nearly half the parenchyma. The spleens were enlarged and in three instances contained caseous tubercles which in two were numerous. The livers were free from macroscopic lesions except one which contained a caseous tubercle. The kidneys of two voles were affected, one kidney of one animal showing a single tubercle and both kidneys of the other scattered tubercles in the cortices. In one vole tuberculosis of the right ilium was found.

These results with cultures agree with those obtained by me in 1923. Three voles were then injected subcutaneously with cultures of bovine bacilli, one with 0.5 mg. and two each with 1.0 mg. They died in 47, 47 and 48 days respectively of generalized tuberculosis, the post-mortem appearances being very similar to those just described. In one of the three voles the somatic muscles showed tubercles and three joints (two knee, and an elbow-joint) were enlarged and caseo-necrotic.

Ten voles were injected subcutaneously with sputum, milk or pus containing bovine bacilli. Three injected with sputum—from a different person in each instance—died in 15, 21 and 26 days respectively. At autopsy each showed a small local lesion—caseo-necrotic in one and purulent in two—containing great numbers of tubercle bacilli. In the first vole the inguinal glands were enlarged but not opaque (t.b. + + +) and there was nothing abnormal elsewhere; tubercle bacilli were, however, easily found in the spleen. In the second vole the inguinal, iliac and ventral mediastinal glands were enlarged and caseating and a few grey foci were seen in the liver under magnification; tubercle bacilli were found microscopically in the spleen and were obtained in culture (numerous typical “bovine” colonies) from the liver. In the third vole, which died from non-tuberculous pneumonia, the macroscopic lesions were confined to the seat of injection and adjacent inguinal and iliac glands; tubercle bacilli were, however, widely disseminated and were demonstrated microscopically in the lungs and a submaxillary gland and by cultures (moderately numerous colonies) in the liver.

Four voles were injected subcutaneously with milk from the tuberculous udder of a cow, two receiving 0.1 c.c. and two 0.01 c.c. A smear preparation of the milk showed two tubercle bacilli after some search. One vole from each pair died in 19 and 23 days respectively. In each there was a small local collection of pus and slight enlargement of the inguinal glands, those of the latter containing one or two softened foci; tubercle bacilli were present in great abundance locally and in the inguinal glands. The remaining two voles were killed 28 and 42 days after inoculation respectively. Each showed a large caseous and softened local lesion, 1 cm. in greatest diameter, enlargement and caseation of the adjacent glands, an enlarged spleen with conspicuous Malpighian bodies, some of which were opaque, and scattered greyish foci in the liver. In the vole which lived 42 days the disease was more widespread and

advanced than in the other, the lungs and a kidney each showing a tubercle and all the glands some degree of caseation.

Three voles were injected each with 0.1 c.c. of diluted pus from an inguinal gland of a guinea-pig infected with bovine bacilli (three different strains). This amount in two instances was estimated by viable counts to contain 30 and 600 tubercle bacilli respectively. One vole, dose 30 bacilli, was killed 6 weeks after inoculation. The autopsy showed a caseo-purulent local lesion, enlargement and complete caseation of an inguinal gland, partial caseation of a ventral mediastinal gland and early tuberculosis of an iliac gland, one minute tubercle in the lungs and a slightly enlarged spleen.

One vole died in 51 days and the other (dose 600 bacilli) was killed 100 days after inoculation. Both had general tuberculosis, and in each nearly all the lymphatic glands were caseous to some extent; the spleens were enlarged and closely beset with caseous softened tubercles; the liver of the former contained scattered tubercles, one of which was caseous, while that of the latter was apparently free; one kidney of each animal showed a tubercle; the lungs of the former contained six tubercles, one caseous and softened, and those of the latter numerous translucent grey masses of varying size and irregular outline and diffusely caseous.

Owing to the premature deaths of twelve voles it has been possible to follow the course of the infection in these animals after subcutaneous inoculation of bovine bacilli.

Nine of the voles had been injected with very small amounts of tuberculous pus, sputum or milk containing bovine bacilli. Three died in 3, 4 and 11 days and were found to be free from macroscopic lesions. Three died in 12, 15 and 19 days, and each of these showed at autopsy a small focus of pus at the seat of injection, slight enlargement without caseation of the adjoining inguinal gland and no macroscopic lesions in the organs. Tubercle bacilli were very numerous in the local pus and adjacent gland and were also found microscopically or by culture in the spleen, liver or a distal gland. Three voles died in 21, 23 and 26 days and each showed a small purulent local lesion and caseation of the inguinal glands with very numerous tubercle bacilli; in two of the voles the iliac and in one the ventral mediastinal glands were also caseous and the liver of one showed a few grey foci (t.b. + + +). Otherwise there were no macroscopic lesions in the organs although tubercle bacilli were abundant in them as shown by the microscope and cultures.

The remaining three of the twelve voles dying prematurely received relatively large doses of bacilli (0.1 and 1.0 mg. of culture) and died 5, 8½ and 20 days after injection. In the first vole the only macroscopic evidence of infection was a small focus of pus at the seat of injection, but tubercle bacilli were disseminated over the body and were cultivated from the spleen. The vole dying 8½ days after injection showed a small purulent local lesion and slight enlargement without caseation of the adjacent gland; in this animal tubercle bacilli were easily found by microscopical examination in the liver and marrow. The vole dying 20 days after injection showed an ulcerated local lesion, caseation of an inguinal gland and generalized lesions, tubercle bacilli being numerous in all the organs and glands.

These experiments of short duration have shown that after subcutaneous injection of relatively large doses of culture tubercle bacilli are rapidly disseminated over the body and are recoverable in culture from the spleen within

5 days of their injection. The bacilli multiply abundantly at the seat of injection and also in the neighbouring gland which shows obvious enlargement within 8 days and caseation within 20 days, when lesions may also be just visible in the organs. After very small doses nothing may be visible to the naked eye until towards the end of the second week when besides a local lesion there may be some enlargement of the inguinal gland. By the end of the third week the adjacent gland has undergone some degree of caseation, and by the end of the fourth week lesions may be visible in the organs.

Feeding. Two voles were fed each with a small mass (5–10 mg.) of culture placed upon the tongue.

One of the voles died in 28 days. At autopsy a mid-cervical gland was found to contain an irregular yellowish white area in which tubercle bacilli were very numerous. There were no macroscopic lesions elsewhere but an emulsion of the mesenteric glands yielded moderately numerous colonies of bovine bacilli. There was already some dissemination of bacilli as shown by positive cultures from the liver (three colonies grew).

The other vole died in 88 days of general tuberculosis. The small intestines showed five widely distributed lesions; two were disk-shaped, about 1.5 mm. in diameter, and contained opaque foci; the others were minute opaque foci in the submucosa. All the glands of the body were caseous to some extent, mostly throughout. The spleen contained a moderate number of small caseous tubercles; the liver, two tubercles; a kidney, one tubercle in the cortex. The caudal lobes of the lungs were about half replaced by six caseating masses, one adherent to ribs. Tubercle bacilli were very numerous in the lesions, many in dense clumps and many intracellular. Confluent colonies were obtained in culture from a cervical gland.

The results of the experiments with bovine bacilli confirm those obtained in 1923 and show clearly that the field-vole is highly susceptible to infection with tubercle bacilli of the bovine type. Large doses—1.0 or 0.5 mg.—of culture determine a progressive infection with death within 9 weeks. With smaller doses ranging from 0.1 mg. of culture to 30 bacilli in a tissue emulsion the result is the same—generalized progressive tuberculosis—but death may be delayed for more than 100 days. Rapidly fatal generalized progressive tuberculosis has also been produced by feeding with a single dose of culture. Characteristic features of bovine infection are extensive caseation of lymphatic glands associated with enormous multiplication of bacilli and, in advanced cases, large caseating masses in the lungs also crowded with bacilli. Caseous tubercles also occur in other organs, most regularly in the spleen.

Inoculation and feeding experiments with human bacilli

Subcutaneous. Six voles were injected with cultures of three eugonic human strains from sputum, two with each strain, the dose in each case being 1.0 mg. They died in from 27 to 151 days after inoculation, but death in none of them could be attributed to tuberculosis. No local lesions were found in two voles

and only small local lesions, two slightly ulcerated, were present in four voles. The inguinal glands were little if at all enlarged, and there were no lesions in other glands or in the organs, except the lungs of one which showed two small tubercles and red hepatization of one lobe. Tubercle bacilli were, however, demonstrated microscopically and/or by culture in the organs, in some in considerable numbers, and it is possible that this multiplication of bacilli may in some of the voles have been a contributory cause of death.

Two voles were injected subcutaneously each with 1.0 mg. of a dysgonic human strain from sputum. One died in 7 days as a result of a fibre-ball in the stomach, and except for a small collection of thick pus at the seat of injection there was no sign of disease; tubercle bacilli were, however, easily found microscopically in an inguinal gland and the spleen, and moderately numerous colonies were obtained on egg tubes sown with inguinal gland and liver.

The second vole died in 116 days and showed at autopsy a linear ulcerated caseous local lesion, a minute opaque focus in an inguinal and a similar one in a ventral mediastinal gland and caseation of two lymphoid follicles in the wall of the caecum; there were no lesions elsewhere. Microscopically tubercle bacilli were numerous in the caseous lesions and sparse in the spleen. Cultures from the liver yielded numerous colonies.

One vole was inoculated subcutaneously with 1.0 mg. of a slightly attenuated eugonic human strain from a case of lupus. Killed 180 days later, the animal showed no lesions at autopsy, although tubercle bacilli were present in moderate number in the glands and organs.

Three voles were inoculated subcutaneously with human sputum from three different persons, the doses being 0.05, 0.1 and 0.1 c.c. The cultures obtained directly from these sputa were typical "eugonic human". Two of the voles were killed 51 and 113 days after inoculation and one died in 177 days. In each vole there was a small local lesion (in two this was a cyst with purulent contents) and no sign of disease elsewhere. Tubercle bacilli were, however, present in varying number in the inguinal glands and the spleen and (in two instances) the liver, from which regions they were cultivated in each case.

Feeding. Two voles were fed each with a small mass of culture placed upon the tongue. The two voles died in 104 and 185 days respectively. The former showed no sign of disease but tubercle bacilli were numerous in the organs and glands; one tubercle bacillus was seen in a film from the marrow and one in a film from the blood. Numerous colonies were obtained from the liver. The other vole also showed no macroscopic lesions except in a mesenteric gland which contained a pin-point white focus. The submaxillary and mesenteric glands and the spleen were slightly enlarged, the liver was congested and the lungs contained some red airless patches. Tubercle bacilli were moderately numerous in the organs and glands and were obtained in culture from the spleen (moderately numerous colonies) and from a submaxillary gland (abundant colonies).

Summary. None of the twelve voles inoculated or fed with eugonic human

bacilli (nine as culture and three in sputum) showed caseation of the lymphatic glands, either locally or elsewhere, and no macroscopic lesions in the organs, except one vole whose lungs contained two grey tubercles. The only signs of infection in the remaining eleven voles were a trivial local lesion and slight enlargement of the adjacent inguinal glands and of the spleen, and these were not present together in all the voles. These results are in striking contrast to those obtained with bovine bacilli. In all the voles inoculated with human bacilli, however, these bacilli could be demonstrated microscopically and were recovered in culture from organs and glands, sometimes even from glands so remote from the seat of injection as the submaxillary. The numbers of bacilli in the organs and distal glands varied from relatively sparse to numerous and were in no instance found in such great abundance as in voles fatally infected with bovine bacilli.

The vole injected with a dysgonic human strain showed a caseous focus in each of two local glands and caseation of two caecal lymphoid follicles, in which tubercle bacilli were very numerous. There was slightly more obvious disease in this vole than in those infected with eugonic human bacilli, but nothing comparable to that found in voles infected with bovine bacilli.

These results are in agreement with those previously reported (1937) with one exception. Seven voles were injected with two strains of human tubercle bacilli and six developed no more than was found in the ten voles already mentioned; one vole in fact showed no lesions or bacilli. The remaining one died in 235 days of severe generalized tuberculosis which was indistinguishable except in its slow course from that produced by bovine bacilli. In view of the clear-cut and decided differences between human and bovine infections in all other experiments on voles one may ask whether the disease in this exceptional case was in reality caused by eugonic human bacilli. The culture used for the vole was not a mixture of human and bovine bacilli, since another vole injected at the same time with the same strain of bacilli in equivalent dose survived 284 days after injection and at autopsy showed local disease only. A bovine bacillus might, however, have gained access to the tissues of the vole in some way or another during the long course of the experiment, and by reason of the great sensitiveness of the vole to infection with this type of bacillus succeeded in starting a progressive bovine infection. On the other hand, it is possible that a human strain may occasionally and after a long interval exert in some individuals an action similar to that of bovine bacilli. Unfortunately, the result was not questioned at the time and cultures were not made from the lesions for the purpose of comparing the characters of the bacilli with those of the original strain.

Inoculation and feeding experiments with avian bacilli

Subcutaneous. Three voles were inoculated subcutaneously with avian bacilli, the doses being 2.0, 1.0 and 1.0 mg. respectively. They died in 80, 105 and 154 days. In each of two, beyond a small local ulcer, there was no sign of

disease, and microscopically tubercle bacilli were scanty in the organs. In the third vole (dose 1.0 mg.), which died in 154 days, there was a local focus of pus and each inguinal gland contained a minute purulent focus; in the substance of the liver near the attachment of the ligament there was a yellow body the size of a millet seed with tough capsule and purulent contents; the lungs showed under magnification two minute grey foci. Film preparations of the liver tubercle and inguinal gland tubercle showed numerous tubercle bacilli; in the spleen and lung they were easily found but were not numerous.

Avian bacilli were recovered in culture from the liver of each vole.

Feeding. Two voles were fed each with a small mass of culture (5–10 mg.) placed upon the tongue.

One vole died in 103 days and showed no macroscopic lesions. Scanty tubercle bacilli were found in the mesenteric glands. The other was killed 186 days after being fed. At autopsy an opaque gritty focus was present in each submaxillary gland, and two minute opaque white foci were seen in the mesenteric glands; there were also a few small grey translucent areas in the lungs. These were the only lesions visible to the naked eye. Microscopically tubercle bacilli were moderately numerous in a submaxillary gland focus and sparse in the mesenteric glands, a Peyer's patch and a translucent area in the lung; no tubercle bacilli were found in smears from liver, spleen or normal-looking submaxillary gland tissue. Avian bacilli were recovered in culture from one submaxillary gland (twenty colonies) and the mesenteric glands (moderate number of colonies).

Summary. The pathogenic effects of avian tubercle bacilli in the vole resembled, in their scanty macroscopical features, those produced by human bacilli. There were, however, in the avian infections, on the whole, greater tendency towards retrogression and less abundant multiplication of bacilli in the tissues.

Inoculation and feeding experiments with the vole strain of acid-fast bacillus

Inoculation. Three voles (29, 28 and 27) were inoculated subcutaneously with the vole strain L.V. 285, the dose being 0.5, 0.1 and 0.01 mg. respectively.

Vole 29, died 105 days. *Autopsy:* Between umbilicus and pubis large ulcer, 1.5 × 1.0 cm. floor dirty yellow, base composed of firm necrotic substance which extended under skin into both groins, involving inguinal glands and infiltrating thigh muscles and also encircling the anus; each groin region was filled with a firm mass of greyish white necrotic substance which on cross-section had a triangular outline. In both axillary regions and in the subcutaneous tissues of the back there were irregular tracts of dry friable necrotic substance. In the coeliac region there was an irregular mass of dense cheesy substance, measuring 7 × 5 mm. The iliac glands were slightly enlarged and showed opaque white areas. Mesenteric glands normal. The two antero-ventral mediastinal glands, each 1.5 mm. in diameter, were composed throughout of dry cheesy substance. Two submaxillary glands were opaque-white centrally and translucent around the margins. Liver showed under magnification a fine dust-like speckling. Spleen, medium enlarged, showed in pulp numerous minute opaque foci. Lungs

and kidneys appeared normal. Small intestines showed through serosa a greyish white mottling similar to that seen in coccidial disease of intestines in rabbits.

Vole 28, died 115 days. *Autopsy*: Small elongated caseous collection in right groin. Inguinal gland on this side not obviously affected. In areolar tissues of left groin and axillae a good many minute slightly opaque tubercles were seen. In the region of the iliac glands and probably involving them there was a small opaque white mass from which thickened white lymphatic vessels proceeded anteriorly; between it and the kidneys were also isolated small masses, opaque centrally, translucent round the margins, and some spherical tubercles, less than a pinhead in size. There were two small masses in the portal fissure. The abdominal masses were rigid and composed of dry 'caseous' gritty substance. The ventral mediastinal glands, the size of pins'-heads, were opaque-white throughout. Spleen, liver and kidneys normal to the naked eye. Lungs, crepitant, showed two minute translucent foci. Nothing abnormal seen in the neck. On the serous surface of the small intestines and mesentery there were about two dozen small tubercles with opaque centres and grey margins.

Vole 27, died 126 days. *Autopsy*: In left groin ulcerated area over an angular mass filling groin space and measuring 1.5 × 1 cm. composed of dry cheesy substance. In left axillary region extending from last rib to front of shoulder were tracts of dry greyish white friable substance surrounded by thin connective tissue. Lungs, crepitant, showed sparsely scattered minute translucent foci. Spleen, slightly enlarged. Nothing abnormal elsewhere. No glandular enlargements or opacities.

Two voles were cutaneously inoculated by scarifying the skin and rubbing in a small drop of a suspension of strain L.V. 285, 1 c.c. of which contained 1.0 mg. of culture.

Vole 34, scarified on forehead, died 144 days later. *Autopsy*: No cutaneous lesion. A gland at left angle of jaw, size of split wheat-grain, was composed throughout of white cheesy substance. No other glands enlarged except portal which however was not opaque. Lungs: left lung red and hepatized; right lung crepitant showed at margin of caudal lobe one minute translucent tubercle. No sign of disease elsewhere. Acid fast bacilli (A.F.B.) were very numerous in the submaxillary gland and numerous in the portal gland, lung tubercle and spleen pulp.

Vole 33, scarified over rump, died 263 days later. *Autopsy*: The body was plump with a good deal of subcutaneous fat. The right groin was filled with an irregular nodular mass, 2 cm. in length, of firm caseous substance replacing the fatty tissues. In left groin the fatty tissues were not replaced but contained numerous caseous nodules, majority 1.5–2 mm. in diameter. In each axilla, between scapula and ribs, there was a mass about 1.5 cm. in greatest diameter composed of closely aggregated creamy-white nodules, 2 mm. in diameter. Smaller similar masses on axillary vessels and in posterior triangles of neck. Between the pectoral muscles, particularly for about 1 cm. over the costal cartilages and ends of ribs, there was an infiltration of small opaque tubercles. The fatty tissues of the neck and the connective tissue under the pad of fat on sternum were speckled with fine greyish white foci. In the abdomen there were irregular caseous masses of various sizes up to 1.5 cm. in diameter in the pelvis, on the iliac vessels, along the lumbar spine, in front of left kidney and between the layers of the mesentery—and small nodules were seen in the portal fissure and around the coeliac vessels. The mass in the mesentery, 1.5 cm. in length, had evidently caused vascular obstruction, as there was blood-stained fluid between it and the serous membrane.

Lungs, crepitant but congested in places and patchily hepatized, showed under × 8 magnification numerous disseminated translucent tubercles which were not visible to the naked eye; there were besides three opaque nodules, 1.5–3 mm. in diameter. Spleen, moderately enlarged, no foci. Liver normal in appearance. Kidneys, one minute opaque focus in left kidney, none in right. Intestines normal.

Lymphatic glands could not be seen in the groin, axillary and iliac regions, and no affected glands were found except one submaxillary gland which was opaque in the cortex.

Feeding. Six voles were fed with the vole strain.

Vole 14 was fed once with 3.0 mg. of vole culture (strain G 2). Found dead 63 days later the vole showed no lesions: but A.F.B. were found microscopically in mesenteric glands (A.F.B. + + +) and were cultivated from these glands and the liver; thirty-two colonies grew from a fragment of liver.

Five voles—the mother (vole 15) and her four young (*a, b, c, d*), 4–5 weeks old—were fed with vole culture (strain G 2) suspended in saline and given on bread on eight occasions between 2 July and 11 August 1937.

Vole 15a, found dead 101 days after the experiment began. *Autopsy:* Right submaxillary gland a little enlarged but not opaque (A.F.B. + + + +). Mesenteric glands not enlarged (A.F.B. + +). Organs normal; no A.F.B. in liver or spleen.

Vole 15b, the skin only was found on the 136th day, the rest of the carcass having been eaten.

Vole 15c, found dead on the 138th day, lower part of abdomen and intestines having been eaten. The mesenteric glands were enlarged, firmer than normal and showed diffuse whitish opacities mainly cortical (A.F.B. + + +). Right submaxillary showed a minute opaque focus (A.F.B. + + + +). Spleen seemed slightly enlarged (A.F.B. +). No sign of disease elsewhere. No bacilli found in liver or lungs.

Vole 15d, found dead on the 154th day. The mother had eaten the spleen and most of the intestines. The liver (no A.F.B.) and kidneys remained and showed no macroscopic lesions. In the coeliac region were two slightly enlarged glands which showed under magnification a yellowish white mottling due to small foci and irregular patches. Right submaxillary gland showed an opaque focus (A.F.B. + + +). Lungs, right anterior lobe showed a subpleural greyish yellow focus, a little over 1 mm. in diameter; under $\times 8$ magnification this lesion showed the interlobular septa surrounding yellow lobules.

Vole 15 (the mother) died on the 190th day. *Autopsy:* the areolar tissues of the groins, axillae, posterior triangles of the neck and around the base of the tail showed opaque whitish sheets or tracts of dry friable substance occupying exactly the distribution of the adipose tissues in these areas. The small intestines showed eight creamy-white projecting nodules up to 2 mm. in diameter. Distributed along the colon from the caecum to the pelvis were half a dozen projecting nodules up to a barley-grain in size, closely resembling the intestinal nodules of fowls. The end of the caecum and also the part close to its junction with the small intestine was thickened and opaque whitish. Spleen, much enlarged, beset with firm white nodules, up to 3 mm. Liver peppered almost as closely as possible with grey foci. Lungs, pink and crepitant, two minute glassy foci seen with a lens. Kidneys, no lesions visible. Antero-ventral mediastinal glands, size of small pins' heads, opaque white. All other glands were affected in some degree; those in groins and axillae were lost amid the necrotic substance occupying these regions. Extending anteriorly from the iliac glands, which were opaque and not much enlarged, were thickened semi-opaque lymphatic vessels. In the pelvis there was a small mass of caseous substance. The glands at angles of jaw, the mesenteric and coeliac and other glands in connexion with intestines were much enlarged and composed throughout of dry creamy-white friable substance.

Microscopical examinations

Film preparations of the subcutaneous, glandular and visceral lesions of the injected voles and of vole 15 showed extraordinarily numerous acid-fast bacilli. Acid-fast bacilli were also found, but less abundantly, in organs and glands which showed no macroscopic lesions, and in the faeces of several of the voles.

HISTOLOGICAL OBSERVATIONS

Dr W. Pagel* has written a description of the histological appearances in voles infected with human and bovine tubercle bacilli and with the vole strain of acid-fast bacilli and has kindly sent me the following summary. Only one vole injected with (dysgonic) human bacilli was examined histologically. The full details will be published later:

There are marked differences between the tissue response elicited in voles by the vole strain of acid-fast bacilli on the one hand and human and bovine tubercle bacilli on the other. Infection with the former leads to extensive necrosis with accumulation of enormous numbers of acid-fast bacilli. These are also found outside the lesions in the cells of the organs or in small groups of proliferating reticulo-endothelial cells. No epithelioid cell tubercles were observed in infections with the vole bacillus. Tubercles were, however, very marked in voles infected with human and bovine bacilli. In this group bacilli were sometimes very numerous, but never found outside the foci.

Apart from the formation of epithelioid cell nodules the extent of caseation in bovine infections is impressive, particularly in the lung and liver, although it is not as regular and marked as tissue necrosis in infection with the vole acid-fast bacilli.

Infection of voles with the human and bovine types of tubercle bacilli is therefore characterized by the formation of foci; in infection with the vole strain of acid-fast bacilli a septicæmia with subsequent irregular tissue necrosis is characteristic.

Tissue reaction in voles

	Tubercle formation	Necrosis	Caseation	Giant cells	Reticulo-endothelial proliferation	Number of acid-fast rods in lesions
Vole bacillus	—	+++	—	—	+++	Enormous in all lesions and outside lesions
Bovine and human	+++	—	Varying	—	—	Numerous only in some lesions, in others scanty. No bacilli outside lesions.

SUMMARY OF PATHOGENIC EFFECTS OF THE VARIOUS TYPES OF BACILLI IN THE VOLE

The results show that the vole strain of bacilli can produce by any of the methods of infection employed a slowly progressive generalized disease which in its later stages is characterized by enormous accumulations of acid-fast bacilli in the areolar tissues of the groins, axillae and other regions, where they form dense irregular or nodular masses or tracts of dry opaque friable substance. The slow course of an infection with the vole bacillus was clearly shown by the results in four young voles which were fed and one which was cutaneously inoculated with culture. Two of the fed voles died 63 and 101 days respectively after the experiment began and at autopsy showed no lesions anywhere though acid-fast bacilli were found microscopically in the mesenteric glands of both and by culture in the liver of one. The other two fed voles died in 138 and 154 days and in these there were lesions—diffuse necrotic areas—in the cortices of the mesenteric glands and a small opaque focus in a submaxillary gland of each.

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There were no lesions in the organs, except a small focus in the lungs of one, and no naked eye lesions in the areolar tissues. The vole infected cutaneously died in 144 days and showed caseation of a submaxillary lymphatic gland and a minute tubercle in the lungs and nothing elsewhere. Two further voles, one fed and one cutaneously inoculated, died 190 and 263 days respectively after infection and in both of these subcutaneous lesions were found.

Areolar tissue lesions appear therefore to be late manifestations of an infection by vole bacilli when these gain access through a natural channel in relatively small numbers. They appear earlier in animals which have been injected subcutaneously with relatively large doses and begin apparently in the lymphatic spaces as colonies of bacilli which increase in size, coalesce and eventually replace the adipose tissues in these regions. Lesions in the organs are also tardy in their appearance and are not of regular occurrence or always of the same character in the same organ. The lungs most frequently showed lesions, usually in the form of inconspicuous translucent foci and once only as opaque white nodules. The spleen of one vole contained numerous minute yellow foci and that of another numerous firm white nodules. The liver in two instances showed a fine grey dust-like speckling. The kidneys were free from lesions except one which showed a minute opaque focus in the cortex. Glandular changes were not a conspicuous feature except in vole 15. In the early stages the glandular lesions were diffuse opacities in the cortices and were semi-purulent. Later the glandular tissue became replaced by a dry whitish friable substance consisting largely of acid-fast bacilli.

Comparing experimental infections in the vole with the vole bacillus and bovine bacilli, one finds that both infections give rise to generalized disease with macroscopic lesions. The two infections, however, differ one from the other in important respects. The bovine infection runs the more rapid course and is characterized by caseation of the lesions, particularly the glandular. Infection with the vole strain, apart from its slower course, is distinguished from bovine (and human and avian) infections by the formation of subcutaneous lesions composed largely of acid-fast bacilli. Human and avian infections, though followed by generalization and multiplication of bacilli, are rarely associated with macroscopic lesions, and these when present are generally of a trivial nature.

COMPARATIVE SUSCEPTIBILITY OF VOLES AND GUINEA-PIGS TO HUMAN AND BOVINE TUBERCLE BACILLI

In a certain number of experiments a guinea-pig was inoculated at the same time as a vole (or voles) with an equal dose of infective material. The results of these experiments, the details of which are set out in Table I, enable one to compare the susceptibility of the two species of animals to the human and bovine types of tubercle bacilli respectively.

In three out of the five experiments with human tubercle bacilli the dose for each animal (six voles and three guinea-pigs) was 1.0 mg. of culture and in

two (two voles and two guinea-pigs) it was a small quantity of sputum. Of the five guinea-pigs one, injected with sputum, died prematurely in 26 days and four died of general tuberculosis in from 58 to 110 days. None of the voles, with the exception of vole 36, in which there were a few small foci in the lungs, showed disseminated lesions visible to the naked eye, although one at least in each experiment lived longer than the corresponding guinea-pig and all had tubercle bacilli in their organs, demonstrated by microscopical as well as by cultural examination.

It is evident from these experiments that the vole is much less susceptible than the guinea-pig and reacts in a manner different from that animal to infection with tubercle bacilli of the human type.

In eight experiments with bovine tubercle bacilli ten voles and eight guinea-pigs were used and in all but one of the experiments, where culture was injected, the infective material was sputum, pus or cows' milk.

In two experiments the animals were allowed to live until one of each pair died, when the remaining animal was killed. Both animals of each pair showed general tuberculosis.

In the rest of the experiments most of the voles died prematurely. Two died in 11 and 15 days, too soon for the development of disseminated lesions in the guinea-pigs which were kept until they died of general tuberculosis. In each of three experiments a vole died or was killed 23, 28 or 32 days after inoculation and the corresponding guinea-pigs were killed after the same intervals of time. Disseminated lesions were visible to the naked eye in all three guinea-pigs, but in only one of the voles, the macroscopic disease in two voles being confined to the seat of infection and adjacent glands.

In the remaining experiment (included in Table II also) one vole and one guinea-pig were each injected subcutaneously with an amount of tuberculous pus estimated to contain about thirty living tubercle bacilli and were killed 42 days later. The guinea-pig showed slight generalized tuberculosis while in the vole the macroscopic lesions were confined to the seat of injection and the lymphatic glands draining that region.

It would seem from these results with bovine bacilli that disseminated lesions are later in appearing in the vole than in the guinea-pig, but that ultimately the disease progresses to a fatal termination in the vole after about the same interval of time as in the guinea-pig.

THE USE OF THE FIELD-VOLE IN DIAGNOSIS AND IN TYPING

The results of the experiments with bovine bacilli have shown that an extremely small number of bacilli of this type suffice to set up generalized progressive tuberculosis in the vole. This fact suggested that the vole might be used in place of the guinea-pig as a test for the presence of bovine bacilli in specimens, for example, of cows' milk, where these organisms cannot be found by microscopical examination.

TABLE I. *Comparison of susceptibility of voles and guinea-pigs*

Strain and dose	Voles			Guinea-pigs		
	No. of Vole	Duration of life	Result	No. of guinea-pig	Duration of life	Result
			<i>Eugonic human bacilli</i>			
M. 937 1.0 mg.	{20 21	D. 74 D. 151	Local lesion only No macroscopic lesions	7275	D. 87	Severe G.T.
Scot. 483 1.0 mg.	{35 36	D. 27 D. 138	Local lesion only Local lesion and a few foci in lungs	7308	D. 65	G.T. moderately severe
C.S. 351 1.0 mg.	{37 38	D. 69 D. 76	Local lesion only	7310	D. 58	G.T. moderate
A. 317 sputum	48	K. 51	Local lesion and slightly enlarged inguinal glands	7336	D. 26	Local tuberculosis only
C.S. 388 sputum	51	K. 113	Local lesion only	7340	D. 110	Severe G.T.
			<i>Bovine bacilli</i>			
M 951 G.P. pus	45	D. 51	G.T.	7334	K. 51	G.T. moderate
M. 953 G.P. pus	60	K. 100	Severe G.T.	7353	D. 99	G.T. severe lungs and spleen
Sputum	61	D. 11	—	7354	D. 72	Severe caseo-necrotic G.T.
Sputum	49	D. 15	Local lesion and enlarged inguinal gland	7339	D. 57	G.T. severe
Milk 0.01 c.c.	{56 57	D. 23 K. 42	Local lesion and gland G.T. not severe	7351	K. 23	Severe local and early G.T.
0.1 c.c.	{58 59	D. 19 K. 28	Local lesion only. T.B. disseminated Local tuberculosis and early G.T.	7352	K. 28	Local T. and early G.T.
Milk G.P. pus 30 T.B.	77	K. 42	Local lesion and caseous adjacent glands	7469	K. 42	Early G.T.
C.S. 397 1/100000 mg.	62	D. 32	Local lesion and caseous inguinal and iliac glands. T.B. in spleen	7355	K. 32	Early G.T.

The results of the experiments with milk, quoted in Table I, give support to this suggestion, but in these particular experiments the number of bacilli was not inconsiderable. Before one could recommend the vole as a substitute for the guinea-pig in diagnosis, it would be necessary to show that the vole can be infected with as small a number of bovine bacilli as the guinea-pig.

In order to gain some information on this point a preliminary experiment on voles and guinea-pigs (three pairs) has been done with diminishing doses of bovine bacilli. The following is a description of the experiment. A very small quantity of pus from the tuberculous inguinal gland of a guinea-pig, which 6 weeks previously had received a subcutaneous injection of cows' milk, was diluted with salt solution. A rough estimate of the numbers of bacilli was first made by microscopical examination and the primary suspension of pus was then diluted by tenths with the aim that the second pair of animals should

each receive less than 10 bacilli. It was estimated by viable counts that the first pair of animals each received about 30, the second 3 and the third no bacilli. The results are presented in Table II.

Of the pair which received the crucial dose, namely, that estimated to contain not more than three bacilli, only the guinea-pig became infected. The failure to infect the vole may have been due either to the capacity of the animal to destroy one or two bacilli introduced into its tissues or to some technical reason whereby no bacilli were actually injected. The dose of dilution was 0.1 c.c. and a glass syringe was used the piston of which was flat at the end. It is quite possible that any bacilli which were drawn into the syringe may have been retained by adhesion to a surface or in a crevice. The latter is, I think, the more likely explanation since the great abundance of tubercle bacilli in the lesions of vole 77, which received about 30 organisms, does not suggest that the vole has any power to restrain the multiplication of bovine bacilli introduced into its tissues. Further experiments are therefore necessary to ascertain whether or not the vole is as reliable as the guinea-pig for demonstrating the presence of small numbers of bovine bacilli in a material. We also do not yet know what is the minimum infecting dose of the human type of bacillus. Experiments have shown that bacilli of this type can multiply in the tissues of the vole and can be demonstrated microscopically though no macroscopic lesions may be present. Should the vole prove as dependable as the guinea-pig for testing the presence of either type of bacillus, the vole would have the advantage over the guinea-pig—where human material is concerned—of indicating at the same time the type of bacillus causing the infection.

The vole may also prove of value in yet another way, namely, in confirming the classification as "bovine" of strains which, though culturally of the bovine

TABLE II. *Diminishing doses experiment.*

Dose of tubercle bacilli estimated by viable count	Voles			Guinea-pigs		
	No.	Duration of life in days	Result	No.	Duration of life in days	Result
30 T.B.	77	K. 42	Caseous local lesion and inguinal gland; partial caseation left iliac and ventral mediastinal gland; T.B. very numerous in lesions	7469	K. 42	Small ulcerated caseous local lesion. Inguinal, one xiphisternal and one manubrial glands extensively caseous. Early caseation of portal and bronchial glands. Spleen, 5 caseating nodules. Liver, 3 foci
3 T.B.	76	K. 30	No sign of disease. Culture from spleen negative	7468	D. 30	Small purulent local lesion. One inguinal gland, small caseous area. T.B. easily found in both
No T.B.	75	D. 38	No sign of disease	7467	K. 85	No tuberculosis

type, fall considerably below the standard of bovine strains in virulence for the rabbit. Four such strains—three from human sputum and one from tuberculous meningitis—have already been tested and have each produced in a vole the general caseation of lymph glands which is characteristic of infection in this species with bovine bacilli.

CONCLUSIONS

1. The vole is susceptible to infection with all the four types of acid-fast bacilli used.

2. The bovine tubercle bacillus is the most virulent, and when injected parenterally or introduced by feeding gives rise to generalized progressive tuberculosis which runs a rapid course and is characterized by caseation of the lesions, especially the glandular, and great multiplication of the bacilli.

3. The human tubercle bacillus can multiply in the tissues of the vole and cause death but does not, except locally and occasionally elsewhere, provoke tuberculous tissue changes leading to the formation of visible lesions.

4. The avian bacillus acts in much the same way as the human bacillus, having little tendency to produce macroscopic lesions.

5. The vole strain of bacillus can infect the vole through abrasions in the skin, or when taken with the food, or injected parenterally. The generalized disease produced experimentally by the vole bacillus is distinguished from that set up by bovine bacilli by its more chronic course and by the occurrence in the areolar tissues of masses of necrotic or caseous substance largely composed of acid-fast bacilli.

6. The vole appears to react in a characteristic manner (general lymph-gland caseation) to all strains of bovine tubercle bacilli, whether these are fully virulent for the rabbit or so reduced in virulence for that animal as not to be clearly distinguishable by the rabbit test from tubercle bacilli of the human type.

7. These results suggest that the vole may prove of great value in ordinary typing of tubercle bacilli and for testing strains which, though culturally bovine, have given equivocal results in rabbits.

REFERENCES

- GRIFFITH, A. S. (1937). Experimental tuberculosis in field-voles and mice. *Vet. Rec.*, **49**, 982-4.
— (1938). *Rep. Med. Res. Coun.* (1936-7), p. 85.
WELLS, A. Q. (1937). Tuberculosis in wild voles. *Lancet*, **1**, 1221.
— (1938). The susceptibility of voles to human and bovine strains of tubercle bacilli. *Brit J. exp. Path.* **19**, 324-8.

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