

THE SUSCEPTIBILITY OF THE WATER (OR GRASS) SNAKE (*TREPIDONOTUS NATRIX*) TO THE AVIAN TUBERCLE BACILLUS AND TO REPTILIAN STRAINS OF ACID-FAST BACILLI

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THE joints of rabbits are predilection sites of disease in chronic avian infections and are nearly always found affected in rabbits which have lived longer than 3-4 months after an intravenous or subcutaneous inoculation of fully virulent avian strains. Highly attenuated avian strains, namely, those which have ceased to be infective for fowls, also give rise to joint disease in rabbits when injected intravenously; for example, twelve rabbits which lived over 5 months after intravenous inoculation of 2.5-10.0 mg. of attenuated avian strains all showed joint tuberculosis, and in eleven of these the joints were the only affected parts (Griffith, 1925).

The production of joint disease in rabbits by acid-fast bacilli from two spontaneously infected snakes therefore raised the question of the relationship of reptilian strains with the avian type of tubercle bacillus. The snakes from which the acid-fast bacilli were cultivated died at the Zoological Society's Gardens, London, and were examined by Col. A. E. Hamerton, F.Z.S., to whom I am indebted for the material.

Zoo 95. African python (*Python sebae*) died 9 January 1935. Autopsy: The liver contained fairly numerous evenly distributed opaque whitish tubercles, majority miliary, a few larger in size up to that of a split pea; some on the surface were flattened and loosely attached. On crushing, the caseous substance was dry and that of the larger nodules was obviously gritty.

Smear preparations showed moderately numerous acid-fast bacilli varying in length, mainly longish, rather thick and much vacuolated; in some the vacuolation was regular and the protoplasm was in the form of narrow bars; in others vacuolation was irregular and the protoplasm was reticulated or fragmented. The bacilli were bluntly rounded at the ends and many were curved.

The culture was slimy and yellow and grew at 25 and 37° C.

Zoo 99. African python (*Python sebae*) died 22 October 1935. Autopsy: The liver was closely beset with opaque greyish white or greyish yellow nodules, 1-3 mm. in diameter, some confluent. The lungs contained similar nodules not so numerous as in the liver.

A smear preparation of emulsion of liver nodules showed numerous acid-fast bacilli similar to those in Zoo 95 but shorter on the whole.

The culture was slimy and slightly creamy.

#### PATHOGENICITY OF THE SNAKE STRAINS

*Zoo 95.* Rabbit 4209 was inoculated intravenously with 1.0 mg. of culture and died 141 days later. The autopsy showed scattered tubercles in the lungs and disease of both knee-joints, very slight in right and more extensive in left.

The test was repeated on two rabbits which, however, died prematurely 16 and 26 days after inoculation.

*Zoo 99.* Rabbit 4351 was inoculated intravenously with 1.0 mg. of culture, and after remaining well for a long time died with swollen joints 753 days after inoculation. At the autopsy no tuberculous lesions were found in the thorax or abdomen. An elbow and a knee-joint and both ankle-joints were enlarged and nodular in outline; on section the swellings contained a rather dry, whitish, caseous substance, there being an absence of synovial fluid; the bony surfaces of the joints were roughened and appeared necrosed and the femur epiphyses showed purulent foci. In the pus of the left knee-joint acid-fast bacilli were not numerous but easily found; they were longish, rather broad, mainly a little curved—one long S-shaped organism being seen—much vacuolated, protoplasm segmented or barred, some quite regularly, others irregularly; stained parts narrow with large vacuoles or broad with small vacuoles; in others protoplasmic segments had chequered appearance.

The following experiments were carried out with strain Zoo 95.

*Rabbit 4302.* 1.0 mg. intravenously, died in 16 days. Autopsy: Lungs, pink and crepitant, showed ( $\times 8$ ) moderately numerous translucent foci. Liver speckled grey foci. Spleen enlarged. Kidneys, moderate number translucent foci. Microscopically acid-fast bacilli were scanty and vacuolated.

*Rabbit 4303.* 1.0 mg. intravenously, died in 26 days. Autopsy: Lungs crepitant, posterior parts caudal lobes congested and a little oedematous, no definite tubercles, but a few light grey foci were seen in congested parts (two vacuolated acid-fast bacilli found in smear preparation). Spleen slightly enlarged.

*Chicken 219* was inoculated intravenously with 5.0 mg. of culture and was killed 158 days later. The autopsy showed no sign of tuberculosis.

*Guinea-pig 7026* received 10.0 mg. subcutaneously and died 77 days later of pseudo-tuberculosis. There was no local disease and cultures from the inguinal glands (treated KOH) remained sterile.

The snake strains resembled avian strains only in their slimy cultural characters and in their ability to cause joint disease in rabbits. They did not produce branched forms, could grow at low temperatures, were not virulent for the fowl, and did not absorb the agglutinin from an avian serum. If of avian pedigree the bacilli, therefore, had undergone considerable modification.

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No typical avian strain has, however, been obtained from the lesions of a naturally infected snake and, furthermore, it was not known whether or not snakes could be infected with avian tubercle bacilli. Having at this time a grass snake available I was able to put the question of its susceptibility to avian bacilli to the test of experiment. At the same time I inoculated two toads with equal doses of the same suspension of culture. (Unhappily when I returned to the laboratory after a long illness, the toads could not be traced.)

*Grass-snake 9* was inoculated subcutaneously in the back, 1 ft. from the snout, with one-third of a primary culture of avian bacilli and was found dead 105 days later. Autopsy: At the site of injection there was a haemorrhagic area with superficial necrosis of vertebral bones beneath. Lungs, congested, showed sparsely scattered minute opaque whitish foci. Liver, speckled with minute yellowish white foci, ranging from microscopic to 0.5 mm., some with grey marginal zone. Spleen (? enlarged) filled with miliary caseous tubercles. Kidneys, no lesions seen.

*Film preparations.* Spleen: smear resembled a pure culture of tubercle bacilli; the micro-organisms were mainly short; some were of good length, but with little beading. Liver: swarms of tubercle bacilli, many clumps. Kidney: a few bacilli found after long search.

*Histological investigation* (by Dr W. Pagel). *Lungs:* No tissue reaction, but tubercle bacilli were found in capillaries and in the alveolar septa.

*Spleen:* This organ was full of nodules consisting of large mononuclear cells with pycnotic nuclei (beginning necrosis); no caseation. The cells of the foci were crowded with acid-fast rods which were also found outside the foci in the reticulo-endothelial cells, where they were much less in number than in the foci.

*Liver:* Full of small nodules of similar structure to those found in the spleen. In some of the nodules there was central necrosis reminiscent of caseation. The foci were packed with acid-fast bacilli. There were also some basophil elements resembling coccal emboli.

*Kidneys and heart:* No changes.

### SUSCEPTIBILITY OF THE GRASS SNAKE TO REPTILIAN STRAINS

The two strains—Zoo 95 and Zoo 99—were not tested as to their virulence for the grass snake, but two other strains—Zoo 75 and Zoo 81—obtained from snakes were so tested. The autopsies on the snakes and details of the bacteriological investigations of the cultures are summarized.

*Zoo 75.* Chequered Keelback (*Natrix piscator*), died February 1929. Autopsy: the lungs showed a number of grey tuberculomata, 2–3 mm. in diameter, from some of which a small quantity of caseo-pus could be squeezed. The pus contained moderately numerous acid-fast bacilli, rather thicker than ordinary tubercle bacilli, ranging from coecal to moderately long forms, the latter beaded, vacuolated or bent at an angle.

Cultures from the lung of Zoo 75 became contaminated, but one glycerin egg tube showed in 18 days at 20° C. two pinhead white colonies composed of acid-fast bacilli.

*Grass snake 2* was inoculated in the subcutaneous tissues of the tail with a small dose of a partly acid-fast culture from the lung of Zoo 75 and was found dead 16 days later. Autopsy: Lungs congested and showed under magnification ( $\times 8$ ) minute grey tubercles. Liver enlarged, pale and speckled; when  $\times 8$  closely beset with spherical grey tubercles and resembled a fish roe. Spleen not found, kidneys pale. No local lesion seen.

*Microscopical examination.* Liver: Acid-fast bacilli extremely numerous, singly and in large masses; varied much in length, some resembling short threads, protoplasm much beaded and vacuolated.

Cultures from the blood (treated with KOH) of grass snake 2 yielded on glycerin egg one colony of acid-fast bacilli (mainly short and a little thicker than bovine bacilli) and numerous colonies of bacilli which were not acid-fast. Cultures from the liver yielded numerous colonies, none of which was composed of acid-fast bacilli. Owing to other work these cultures were neglected and a pure culture of the acid-fast bacilli from the blood could not afterwards be obtained. I cannot say, therefore, what was the type of acid-fast bacillus which caused the disease in grass snake 2. It may be mentioned, however, that a pure culture of a yellow strain (*M. marinum* Aronson) was eventually obtained from the original snake material.

Zoo 81. Say's King Snake (*Lampropeltis getulus holbrooki*), died November 1930. Autopsy: The lungs were moderately closely beset with submiliary yellow tubercles. The liver contained minute grey tubercles with opaque centres; they were fewer in number and smaller in size than those in the lungs.

In a film preparation of the lungs acid-fast bacilli were very numerous; in one of the liver they were scanty.

The bacilli were pleo-morphic and rather thick; the protoplasm was fragmented or finely vacuolated and divided into segments by large clear spaces; some contained deeply stained granules with a wider diameter than the thickness of the bacilli; palisade arrangement of the bacilli was common.

Zoo 81 produced a slimy cream-coloured paint-like growth which did not absorb the agglutinin from a serum made with an avian tubercle bacillus or from a serum made with *M. marinum* (Aronson). The strain has not been identified with any other reptilian strain of acid-fast bacilli.

Grass snake 7 was inoculated subcutaneously with 10.0 mg. of culture from Zoo 81 and was found dead 276 days later. Autopsy: At the seat of inoculation there was a small quantity of caseo-pus spread out over an area 1 in. in length by  $\frac{1}{4}$  in. in breadth, the tissues around showing no sign of reaction. The pus was composed in the main of short plump acid-fast bacilli. The liver was pale and under magnification ( $\times 8$ ) showed innumerable minute grey foci. The lungs were congested, but no tubercles were seen. The parietal pleura presented three translucent tubercles. Kidneys normal. Owing to unfamiliarity with

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the anatomy of the snake I did not find the spleen. A smear preparation of liver showed fairly numerous acid-fast bacilli which varied in length and were vacuolated and beaded and some were curved.

Cultures of the acid-fast bacilli were recovered from the blood (numerous colonies) and the liver (very numerous colonies).

*Histological investigation* (by Dr W. Pagel). There were two organs looking like liver. One of them showed no tubercular changes. The other contained well-encapsulated foci, consisting of spindle cells with hyperchromatic nuclei. Some of these foci showed a large central area of necrosis with many acid-fast bacilli. There were smaller numbers of acid-fast bacilli in the cells of the foci.

*Lung*: No changes.

*Kidneys*: No changes in the organ proper, but there was a roundish hyaline-necrotic nodular mass adjacent to the pelvis of the kidney which contained many acid-fast bacilli.

There was, finally, one large organ with glandular structure, but without tuberculous changes. I am afraid I did not know what it was.

The results of the experiments on grass snakes with these two reptilian strains (Zoo 75 and Zoo 81) are given to show that the grass snake is susceptible to infection with some strains of acid-fast bacilli which occur in the lesions of spontaneously infected captive snakes. Not all acid-fast strains from spontaneously infected captive snakes are, however, virulent for the grass snake, which might therefore prove of value for differentiating between pathogenic and saprophytic acid-fast strains. Unfortunately, no grass snakes could be obtained to test this hypothesis.

### CONCLUSION

The grass snake (*Trepidonotus natrix*) is highly susceptible to experimental infection with fully virulent avian tubercle bacilli and with reptilian strains.

### REFERENCE

- GRIFFITH, A. S. (1925). Serological classification of mammalian and avian tubercle bacilli. *Tubercle, Lond.*, 6, 417-36.

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