THE LONGEVITY OF DRY SPORES OF *B. ANTHRACIS*.

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The spores of *B. anthracis* are well known to be very resistant, and able to survive for long periods, especially when dry. Some of the older workers have quoted instances of their long survival, but none seem to have ascertained the limit of their survival under definite conditions.

Pasteur (1881) found them to be alive after 12 years, Sirena and Scagliosi (1894) showed that they could survive in moist or dry earth for 33 months, in distilled or sea water for 20 months, and in sewage for 16 months, and Wencke (1900) and his collaborators give an instance of their apparent persistence in soil for 15 years over a place where animals dead of anthrax had been buried, and another of their presence in gravel in which such carcases had been buried 20 years previously.

Swann (1924) seems to have been the first to study either the length of the germination period or the death-rate in spores. "Direct microscopic observation at 37° C. was used throughout. The spores were sown on agar, employing a method described by Graham-Smith (1910), which allows of continuous examination with a 2 mm. oil immersion objective." Swann defined the germination period as "the time elapsing between the commencement of incubation and the first division of the newly germinated bacillus."

He found that the germination period was related not so much to the age of the spores as to the conditions under which they were kept. Spores from moist cultures 1 to 117 days old showed a very uniform germination period of from 60 to 90 minutes. Spores from cultures 6, 8 and 2 months old, dried slowly at room temperature, dried and kept over calcium chloride and dried rapidly at 37° C. respectively, did not show this constancy in the germination period. In all cases some spores germinated early (110–160 minutes), others germinated late (6 hours), and between these were others showing "a complete range of germination periods." "The method of drying had no apparent effect on the length of the germination period." He came to the conclusion "that a spore, if not showing signs of life after $6\frac{1}{2}$ to 7 hours' incubation, is incapable of germination and must be regarded as dead."

Swann further made the unexpected observation that an appreciable mortality occurs even amongst young, two to three day old, spores, for he found that about 5 per cent. are incapable of germination in 7 hours. Also 970 "spores one year old on agar, dried over calcium chloride, were examined and of these 538 or 55·5 per cent. failed to germinate in 7 hours."

Swann called attention to the fact that spores found in older and drier cultures have a different appearance to spores found in young, moist cultures.

"The old spore is small, oval, highly refractile, and shows no trace of the existence of a spore-envelope. The young recently formed spore is large, more oblong than oval, slightly refractile and the spore-envelope can be easily made out. The difference in appearance is suggestive of a difference in the moisture content of the young and old spores. To use a homely adjective, the young spores look 'juicy'."

In the observations here recorded material from a young potato culture, containing very numerous spores, was inoculated on to dry, sterile pieces of thin canvas, measuring about 0·5 × 0·5 inch, on July 7th, 1907. These pieces were placed in a sterile Petri dish, which was kept throughout in a cupboard with a glass door in the laboratory. The spores were therefore exposed to diffuse daylight and remained at all times dry and at room temperature. At intervals pieces of cloth were removed with sterile forceps and either placed in sterile Petri dishes and covered with nutrient agar, melted and cooled to 50° C., or placed in broth tubes.

For about 10 years confluent growths were obtained in the former, showing that many of the spores had germinated, and heavy growths of the usual type in the latter. During the next 7 years no cultures were made.

In 1924 the observations were continued. At this time it was noticed on examining, under a low power of the microscope, the Petri dishes inoculated with pieces of cloth, that about 10 to 30 colonies developed on each piece, showing that only a few of the spores, probably less than 0·001 per cent., were then capable of germinating.

In 1926 and 1927 the mean number of colonies developing on each piece of cloth was six.

In 1929, 22 years after inoculation, eleven cultures in agar remained sterile, but three pieces of cloth produced one, one and three colonies respectively. Similarly eight broth cultures remained sterile, but in two growth occurred. Subcultures showed morphologically typical bacilli, which formed spores in the normal manner, and exhibited the usual cultural characters, except that the lateral projections in gelatin stabs were short and scanty and liquefaction was slow. The virulence of these cultures was not tested.

In January 1930, 22½ years after inoculation, broth cultures inoculated with ten pieces of cloth and agar cultures inoculated with eight pieces all remained sterile, though all were incubated at 37° C. for 3 weeks.

After a few days' incubation the surfaces of some of the pieces in the broth cultures were scraped with a strong sterile platinum needle in order to free the spores or the bacilli, if any had germinated, from the meshes of the cloth, and perhaps assist them in growing. No growth, however, occurred.

At the end of 3 weeks' incubation pieces in other broth tubes were scraped. The tubes were then violently shaken and the pieces of cloth removed. The fluid remaining was then centrifugalised and smears made from the sediment. On examining stained specimens bacilli, often in pairs, were found to be not uncommon. Some were characteristic in appearance and retained the stain

by Gram's method, others were distorted and retained the stain only in patches, whilst others were completely Gram negative. In fact the bacilli presented the appearances often seen in bacilli from potato cultures a few days old. The bacilli therefore seem to have remained for this long period in the condition in which they were when applied to the cloth, which is not surprising when it is remembered that dried films remain unchanged for very long periods. Some small, refractile, ovoid bodies, less than half the length of young spores, were also present, and probably represented dead spores, though they failed to stain in the usual manner by Muller's or Anjeszky's methods.

No contaminating organisms appeared in any of the cultures. It would be of interest to ascertain if the longevity of the spores would be greater in a damper atmosphere.

CONCLUSION.

If spores of *B. anthracis* are kept dry at room temperature and exposed to diffuse daylight about 50 per cent. seem to be incapable of germinating within a few months. Of the remainder a considerable proportion is capable of germinating for 10 years. Subsequently the proportion of living spores decreases, until all seem to be dead in about 23 years.

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