THE DETECTION OF ANTHRAX SPORES IN INDUSTRIAL MATERIAL.

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Anthrax is still a fairly common disease in Great Britain, both amongst men and animals, in spite of efforts to eradicate it. Some idea of its frequency may be gathered from the following tables.

TABLE I.

Number of cases of anthrax in man in Great Britain and Ireland, 1906—11.

	Great Britain and Ireland				Liverpool	
	1	2	3	4		
Year	No. in factories	No. in agriculture etc.	Total	No. of deaths	No. of cases	No. of deaths
1906	67	9	76	26	7	6
1907	58	13	71	15	15	3
1908	47	22	69	13	3	0
1909	56	15	71	15	9	2
1910	51	28	79	12	11	1
1911	64	?	?	?	5	1

Note. These figures are obtained from the reports of the Chief Inspector of Factories, of the Board of Agriculture, and the Medical Officer of Health, Liverpool, and from the Registrar General.

The figures in column 2 for 1906, 1907 and 1909 are supplemented by 9, 5, and 7 cases respectively described in the annual report of the Factories Inspector for 1907, 1908, and 1910 as *not* included in the statistics of the previous year.

The number of deaths for 1910 column 4 is obtained in the report of the Factory Inspector for 1911.

Compulsory notification of anthrax has been in force in Liverpool since June 1907.

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TABLE II.

Number of cases of anthrax in domestic animals (cattle, sheep, swine, horses) in Great Britain, 1906—10.

Year	1 No. of outbreaks	2 No. of deaths	3 No. of animals in Great Britain	4 No. of deaths per 100,000	5 No. of cases of anthrax among agriculturists	6 Total
1906	939	1330	36,323,358	306	8 farmers	8
1907	1084	1456	37,220,657	309	1 housewife 10 farmers and butchers 1 domestic	12
1908	1105	1519	38,294,017	309	12 farmers and butchers 4 housewives 3 slaughterers	19
1909	1317	1678	38,573,281	403	13 farmers and butchers 1 knacker 1 Cats' Meat Man	15
1910	1496	1776	38,035,597	406	19 farmers and butchers 5 knackers	24
% increase from 1906—10	59·3	33·5		32·6		

Note. Those figures in columns 1, 2, 3, 5 are obtained from the reports of the Chief Inspector of Factories, and the Board of Agriculture. We have calculated those in columns 4 and 6. A slaughterer kills animals for food, a knacker does not; the majority of animals killed by the latter are horses.

The causes of the dissemination of anthrax among domestic animals.

There is no doubt that industrial anthrax is contracted by handling infected hides, wool, etc., but the reason for the dissemination among domestic animals is much more obscure. Many believe such animals contract the disease by feeding upon infected pastures, but recently a considerable amount of evidence has been collected which indicates that artificial foodstuffs or manures may carry infection.

With regard to the former possibility Stockman (1911) has noted first that 83% of the outbreaks of anthrax, in the six worst infected counties in Great Britain during five years 1905–1909, occurred upon *new* farms, that is to say farms in which there had never been a previous case, consequently it was unlikely that the animals contracted it from infected *pastures*, and second that in 68% of the outbreaks the evidence pointed, after careful elimination of other causes, to infection

with "artificial feeding stuffs or manures." Again anthrax is least common from July to October when the stock are on grass, but there is a decided rise in the following months when they may be "assumed to be running in and receiving artificial food." The meal or grain from which artificial food is prepared may become infected during transit from abroad by contact with contaminated material such as dry hides, etc. or by bagging made of infected shoddy wool or horsehair, in which the food cakes are pressed during the process of manufacture. *B. anthracis* has very rarely been found in these artificial foods, though M'Fadyean (1895) once detected it in a linseed cake, which caused the death of six shorthorns, and among some oats responsible for an outbreak in London horses; similar cases have occurred in Germany (Legge, 1905).

We have recently detected anthrax bacilli in a sample of pea meal, used for feeding cattle one of which died of anthrax; the investigation will be alluded to subsequently.

The importance of ascertaining the origin of agricultural anthrax is seen from Table II, which indicates that in spite of Government inspection the disease appears to be spreading. In the last five years the number of deaths amongst domestic animals have increased from 306 to 406 per 100,000 *i.e.* by 33·5 %, the number of outbreaks have increased even more, *i.e.* 59·3 %, and lastly the number of cases amongst agricultural labourers and others have also increased from 8 to 24 per annum. Of course some of this increase may be due to more systematic notification of the disease.

It is remarkable how rarely until quite recently anthrax bacilli have been isolated from industrial material, which is known to have actually infected those who have handled it.

Methods of detecting anthrax bacilli in industrial material, etc.

The success of the efforts to stamp out anthrax in men and animals is largely dependent upon the value which can be attached to a negative bacteriological examination of suspicious material. Obviously, therefore, it is of considerable importance to discover the best method of detecting anthrax bacilli.

There are two. Cultivation, usually on agar plates, or animal inoculation.

One of the objects of this paper is to demonstrate the superiority of the latter method.

Observations by various workers.

We have summarized below the technique employed and results obtained by all the various workers who have examined industrial material for anthrax bacilli.

Rembold (1888). Material: dust from corn shed. Watery extract heated and filtered; filtrate injected into one guinea-pig. Five samples tested. Result: two positive. Several gelatine plates made from heated and unheated extract. Result: all negative.

Gruber (1895), quoted by Heim and Russ. Material: horse and buffalo hair from six different localities. Sediment of extract injected into mice. Result: nearly all died of "malignant oedema." Sediment incubated anaerobically in broth for three days, and heated for one hour at 60 to 70° C. after each day's incubation. Mice injected. Result: positive in two out of six consignments. Large agar plates inoculated with heated extract. Result: negative.

Andrews (1899). Material: China hair. No details. Result: positive.

Balfour Stewart (1901). Material: Karachi hide. No details. Result: positive.

Heim (1901). Material: goat hair. Two flasks containing a litre of broth each received two "knots" of hair respectively. One flask was heated 25 minutes at 80° C., the other was not heated. Some centrifuged deposit from 30 c.c. of the broth mixture from each flask was inoculated into one mouse and one guinea-pig. Result: negative. One agar plate made from sediment in heated flask. Result: three anthrax colonies, pathogenicity established. The broth remaining in both flasks was "enriched" by incubating for 24 hours at 37° C. and then plated on agar. Result: negative.

Heim (1906), quoted by Russ. Material: hair and bristles. Gelatine and agar plates made of heated and unheated extract; followed by sub-cultures in broth with subsequent animal inoculation. Result: positive in two out of 25 samples.

Houston (1902) isolated *Bacillus anthracis* from septic tank liquid and mud from the river Yeo, etc. by injecting $\frac{1}{2}$ or more c.c. into guinea-pigs. Cultures were not made.

M'Fadyean (1895). Material: oats, one sheep inoculated with 6 c.c. of watery extract and eight guinea-pigs with 2 c.c. each. Result: positive in the sheep and in three guinea-pigs.

Silberschmidt (1894). Material: dust and horsehair. Several samples were examined. Some watery extracts heated at 60° C. for one hour. Mice and guinea-pigs inoculated. Result: all negative. Gelatine plates made. Result: all negative.

Silberschmidt (1895). Material: horsehair and dust. February 7th, $\frac{1}{2}$ a c.c. of unheated watery extract inoculated into one mouse. Result: positive. Another mouse inoculated with 1 c.c. Result: negative. February 21st, watery extract of dust inoculated into two mice, which received 1 and $\frac{1}{2}$ a c.c. respectively. Result: the former died in seven hours. *Bacillus anthracis* was present in the latter.

Webb and Duncan (1904). Material: eight "cases" and four "bales" of China mane hair and dust. Guinea-pigs inoculated with watery extract. If death was apparently due to malignant oedema, agar plates were made from the centre of the

spleen, and anthrax bacilli were sometimes detected when apparently absent in the dead animal. Result: positive in four cases and four bales, also two samples of dust. (See under Page.)

Berka (1904). Material: horsehair. Two flasks each containing 200 c.c. of sterile water and a handful of hair were prepared. Flask *A* was digested for three hours at 37° C. and two guinea-pigs inoculated with 2 c.c. each of the extract. Result: both positive. Agar plates also made. Result: both negative. Flask *B* was left at room temperature for 24 hours, the contents centrifuged and some of the sediment injected into two guinea-pigs. Result: both positive. Agar plates also made from the sediment. Result: negative. The rest of the sediment was heated for 20 minutes from 70 to 80° C. and two guinea-pigs inoculated. Result: both positive. Agar plates also made. Result: negative.

Andrews (1906). Material: muddy water from Suffolk stream contaminated with waste from horsehair factories, inoculated into one mouse and one guinea-pig. Result: both positive.

Page (1909). Material: 54 samples of bristles and dust and 29 of horsehair and dust: many samples from China, Russia, and Siberia. Technique: the material was extracted with salt solution from $\frac{1}{2}$ to 24 hours, and frequently agitated. Agar plates were made; some were examined after 18 hours, others apparently much later. Page attempted to kill the anthrax-like bacilli, whose colonies appeared on the plates so frequently, by (1) inoculating bristles into carbolic broth (1 in 1000), (2) heating of watery extracts at 50° C., (3) soaking samples from $\frac{1}{2}$ to 24 hours at a temperature varying from 45 to 60° C. In many instances the material was centrifuged and the deposit inoculated upon agar plates. Result: no anthrax bacilli detected in any specimen; none of them were blood stained.

Russ (1907). Material: horsehair. Sample mixed in 100 c.c. of salt solution, heated to 80° C., then allowed to sediment for 24 hours. 50 c.c. of the supernatant fluid were removed and the remainder shaken up with the sediment; 40 c.c. of this were distributed upon 10 agar plates. Result: two colonies of anthrax; pathogenicity established. The remaining 10 c.c. were inoculated into three guinea-pigs and seven mice. Result: all negative.

Reichel (1911) examined a large number of hides and wools, chiefly from Servia and Syria, a few from Macedonia, Bulgaria, and Italy. Technique: the sample was washed in from 10 to 50 c.c. of sterile water, heated at 65° C. for three-quarters of an hour and centrifuged for 10 minutes. The resulting sediment was smeared on the surface of dry agar plates, 8 inches in diameter. The plates were examined from 14 to 16 hours later. The anthrax-like colonies were sub-cultured, and their pathogenicity tested; according to the author this latter proceeding is absolutely essential. Result: these are rather difficult to classify owing to the confusion between the terms "consignments," "lots," and "samples," but a careful analysis gives the following:

27 lots "Partien" examined. Anthrax found in ... 13 lots = 48 %
15 of these lots examined more carefully, *i.e. at least* 10 samples taken. Anthrax found in ... 10 lots = 66·6 %
270 samples "Proben" examined altogether. Anthrax found in ... ... 64 samples = 24 %

The high percentage of positive results is mainly due to the fact that one

consignment "Sendung" of Servian hides contained anthrax in 23 out of 60 samples, "Proben," and one consignment of Syrian wool in 15 out of 23.

Laubenheimer (1912). Material: hair and wool which had been "disinfected," in most cases by steam at 105° C. Pieces of hair 1 cm. long, unheated, were mixed with agar at 60° C., distributed in several Petrie dishes and incubated at 37° C. from 12 to 20 hours. Suspicious colonies were examined by the hanging drop method, because pseudo-anthrax bacilli are usually motile. The cultures were further tested by inoculation into mice, giving positive results in 24 to 48 hours. Result: spores were demonstrated in many samples of disinfected hair by this method. "Direct" inoculation into animals failed frequently, when spores proved to be present by the culture method. The author concludes, "cultures are a more delicate test than animal inoculation." Unfortunately he gives no figures; the animals used were almost certainly mice, not guinea-pigs, and he omits to describe the technique of "direct" inoculation.

Dr Eurich (1906–11), Bacteriologist to the Anthrax Investigation Board, Bradford and District, has conducted a systematic examination of wool and hair for many years. He has kindly given us the following particulars regarding his technique.

The raw material is soaked for an hour in a dilute watery solution of caustic potash "to dissolve blood clots and prevent coagulation of albumins." The mixture is then heated at 80° C. for several minutes and the wool well teased. The watery extract was not centrifuged and about ½ c.c. was distributed upon each of five or more agar plates and examined after 18 hours incubation.

The number of plates varies from five to 50 according to the amount of material teased as a few strands of hair or the whole fleece. Dr Eurich states that if anthrax is present, colonies generally appear on 50% of the plates, usually four or five on each. The material examined is classified by Dr Eurich into blood stained and non-blood stained, and I am informed that the term "blood stained" is applied when the condition can be recognised by the naked eye, though a certain amount of practice is necessary, for the appearance of the blood varies with its "age, exposure, and admixture of dirt."

Dr Eurich's results are given on page 240.

Observations in Liverpool.

A systematic examination of material supposed to have given rise to "industrial" anthrax in Liverpool has been carried on since 1904 in the University Laboratory under the direction of the late Sir Rubert Boyce until recently City Bacteriologist. During the years 1904–6 inclusive, plate cultivations were employed, but since 1907 animal inoculation has been substituted.

Plate culture method.

A watery extract was made of the suspected material, the mixture heated at 75 to 80° C. for 30 minutes and plated in at least six Petrie dishes. Next day after at least 18 hours had elapsed the plates were examined with a lens, and suspicious colonies tested microscopically, and in gelatine cultures. If any of these still resembled anthrax their pathogenicity was ascertained. Speaking generally we may say that about one anthrax-like colony appeared on every other plate, and during the three years about 12 were considered after thorough microscopical and cultural examination to merit injecting into guinea-pigs. The time and material employed in the investigation of each sample were considerable. Anthrax bacilli were never found.

Inoculation method.

Fifteen grammes of hair or wool or in the case of hide about 4 square inches were soaked in approximately 100 c.c. of tap water. The mixture was heated from 75 to 80° C. for 15 minutes on a water bath, during which time the hair or wool was well teased or the hides scraped. The mixture was then allowed to stand for an hour or two. About 8 c.c. of the supernatant fluid were injected subcutaneously in two guinea-pigs, 4 c.c. into each, occasionally the whole 8 c.c. were injected into one guinea-pig.

In the examination of the first 50 samples all the watery extract was centrifuged, and a considerable part of the sediment injected into guinea-pigs. If an animal died the following routine was adopted to distinguish an infection by *B. anthracis* from one by the spore-bearing anaerobes, so common in industrial material as *B. oedematis maligni*, *B. enteritidis sporogenes*, and *B. aerogenes capsulatus*.

1. *Macroscopical evidence of B. anthracis infection.* (*a*) The spleen is soft, of dark colour, and enlarged two or three times. The spleen is not perceptibly enlarged in infections with the anaerobic bacilli. (*b*) There is extensive inflammatory oedema at the site of inoculation. This appearance is quite different from the "haemorrhagic gelatinous mass" and "thin blood stained" odourless fluid (Klein) found in animals inoculated with *B. oedematis maligni*, or "the extensive gangrene of the subcutaneous and muscular tissues, the abundance of gas" and the "copious malodorous more or less viscid sanguineous fluid" (Klein) found with *B. enteritidis sporogenes* and *B. aerogenes capsulatus*.

It is generally assumed that the only large spore-bearing pathogenic bacillus present in hides, wool, etc., besides *B. anthracis* is *B. oedematis maligni.* But other anaerobes pathogenic to guinea-pigs are also found in dust and dirt. Thus *B. enteritidis sporogenes* has been shown by one of us, E. G. (1901), and Hewlett (1899) independently to be frequently present in dust, and in the alimentary tract. This organism is closely allied to, if not identical with, *B. aerogenes capsulatus* (Welch and Nuttall) and *B. perfringens.*

2. *Microscopical evidence of B. anthracis infection.* Stained smears from the spleen of guinea-pigs dead from anthrax are usually crowded with the characteristic bacilli. Often these bacilli cover a larger area of the microscope field than the cells of the spleen itself. The anaerobic bacilli on the other hand are very scanty, and in a case of malignant oedema long unseptate threads may appear: if the animal has been dead some time. When the bacilli in the spleen were few, and atypical and their nature doubtful, the peritoneal fluid was examined. In the case of the anaerobes many bacteria were usually present.

As a rule Gram's stain was employed; most of the anaerobic bacilli proved to be Gram positive.

3. *Cultivation.* If the microscopical examination was typical of *B. anthracis* infection, cultures were made upon agar slants, if atypical upon agar plates, and the characteristic colonies looked for and verified microscopically. The first dozen strains of *B. anthracis* isolated were further confirmed by re-inoculation into guinea-pigs, but this practice was afterwards usually omitted. The usual appearances in a guinea-pig dead of anthrax are so characteristic, viz. the local subcutaneous lesion, the enlarged spleen swarming with Gram positive bacilli, giving typical colonies on agar, that further investigation is unnecessary. Only in rare instances, where the slightest doubt obtained regarding the nature of the bacillus, was the pathogenicity of the sub-cultures tested.

Criticisms of our technique.

The technique we have employed for isolating anthrax bacilli, though capable of improvement in detail, is simple, and as the figures demonstrate very efficient.

Several points, however, require special consideration.

Value of centrifuging.

As previously stated the extract from the first 50 samples was centrifuged, and 8 c.c. of the sediment injected, 4 c.c. into each animal, but afterwards the use of the centrifuge was abandoned for various reasons. The effect of this change of technique is seen below.

Comparison of the percentage of deaths from anthrax and spore-bearing anaerobes inoculated with centrifuged and uncentrifuged extract.

Centrifuge used

48 samples inoculated into 96 guinea-pigs.		Deaths	63	=	64·3 %
Anthrax present 15 samples	= 31·2 %	,,	21	=	21·8 %
Pathogenic anaerobes present 23 samples	= 47·9 %	,,	42	=	43·7 %

Centrifuge not used

73 samples inoculated into 146 guinea-pigs.		Deaths	57	=	39 %
Anthrax present 13 samples	= 17 %	,,	19	=	13·7 %
Anaerobes ,, 29 ,,	= 39 %	,,	38	=	26·1 %

The higher percentage of positive results in centrifugalised series may be due to centrifuging or infection of a larger number of samples. The latter explanation is very improbable because all the samples tested during the last six years were of approximately the same class of raw material, and imported into Liverpool from approximately the same foreign countries, and there is no apparent reason why such material should be more extensively infected with anthrax during 1907.

Secondly there is no definite relationship between the number of cases of anthrax in Liverpool and the number of samples of raw material found to be infected each year.

Lastly the remarkable increase in the mortality of guinea-pigs from pathogenic anaerobes can only justly be ascribed to the centrifuge, and it is only reasonable to attribute the increase mortality from anthrax to the same cause.

Duration of life after inoculation with the spores of anthrax or of anaerobes.

Several observers have noted that guinea-pigs or mice inoculated with extracts of wool, etc., die sooner from the spore-bearing anaerobes than from anthrax. An analysis of our figures demonstrates that the average duration of life in 80 guinea-pigs dying from anaerobes was

2·4 days, 3 surviving 7, 5, and 5 days respectively. On the other hand the average duration of life in 40 animals dying from anthrax was 4·2 days and 3 survived 8, 7, and 7 days respectively. There is no reason to doubt that the percentage of deaths from anthrax would be higher but for the presence of the pathogenic anaerobes. Recently a guinea-pig inoculated with extract from a decomposed ear of a horse died from anaerobic infection, six agar plates were made from its peritoneal fluid. Only one suspicious colony appeared, this was proved by cultivation and animal inoculation to be anthrax. The importance of examining the peritoneal fluid is further emphasized by the following:

A guinea-pig inoculated with the centrifuged extract of some raw hair died within 20 hours after inoculation from an extensive oedema; there was no macroscopic or microscopic evidence of anthrax bacilli. A series of agar plates were however made from the peritoneal fluid, and one of the resulting colonies was observed to be somewhat irregularly thicker and greyer than the others, suggesting it was impure. *B. anthracis* was finally obtained from this impure colony by sub-cultures, and confirmed by animal inoculation. Webb and Duncan had a similar experience.

The value of duplicate inoculations.

Owing to the tendency of guinea-pigs to die from anaerobes before they die from anthrax, it was thought advisable whenever possible to inoculate two animals from each sample. The value of the duplicate inoculations is clearly demonstrated by the following figures:

141	samples	inoculated	into	1 or 2 guinea-pigs,	positive	in 30	= 21·3 %
19	,,	,,	,,	1 guinea-pig,	,,	,, 2	= 10 %
122	,,	,,	,,	2 guinea-pigs,	,,	,, 28	= 23·3 %

The advantage of duplicate inoculation is still better shown by further analysis of the 28 positive cases in which *two* guinea-pigs were inoculated. The figures may be grouped as follows:

(Series A)	Both animals died of anthrax in 16 cases	=	57 %
(Series B)	One animal ,, ,, ,, ,, 12 ,,	=	43 %

In other words if in Series B only *one* animal had been inoculated, *B. anthracis* might not have been detected in several, probably half the samples.

Our tables show that materials from Singapore were most frequently contaminated; in many instances, however, it was impossible to identify

the country of origin. Hides owing to the presence of blood give, as one would expect, the largest number of positive results.

Frequency of anthrax in industrial material in Liverpool.

Table of results of examination of industrial material in Liverpool for anthrax by plate cultivation and animal inoculation methods respectively.

	Plate cultures 1904—6		Animal inoculation 1907—12		
Material	No. of samples examined	Result	No. of samples examined	Result	Percentage
Hides and skins	32	0	21	6	28·6
Wool	14	0	72	16	22·2
Hair	8	0	34	7	20·6
Bones	—	—	14	1	7·1
	54	0	141	30	21·3

Note. Wool in the technical sense includes hair of sheep, goat, mohair (Angora goat), camel etc.

Table of nature and source of industrial material.

Hides and Skins			Wool			Hair		
Source	No. of samples	No. positive	Source	No. of samples	No. positive	Source	No. of samples	No. positive
Singapore	5	2	E. India	11	2	German disinfected	6	2
E. India	3	1	Karachi	3	1	Russia	1	1
Karachi	1	1	Bombay	7	1	Singapore	1	1
Unknown	12	2	Persian	8	1	Karachi	5	0
			Unknown	36	8	Unknown	21	3
			Goat Karachi	4	1			
			Goat unknown	3	2			
	21	6		72	16		34	7

GENERAL SUMMARY.

6 samples of industrial material from Singapore positive in 3.
13 ,, ,, ,, ,, ,, Karachi ,, ,, 3.
14 ,, ,, ,, ,, ,, East India ,, ,, 3.

It is important to describe the method by which our samples were collected, in order to obtain some idea of the frequency of anthrax spores among hides, etc., imported into Liverpool. Almost all the cases of anthrax occur among dock labourers or warehousemen. Immediately after notification, the inspectors attempt to discover the material which the infected labourer was handling. If they are successful, samples are taken from the different bales, lots, etc.; several days must, however, elapse between the date of infection and the notification of the disease;

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consequently it is often impossible to find the suspicious material. Although, therefore, only wools, hides, etc., apparently responsible for a case of anthrax were examined, the method of collecting samples is necessarily a haphazard one. Thus it is clear that anthrax spores are present in a fairly large proportion of industrial material imported into Liverpool.

Approximate number of anthrax spores.

It is advisable to obtain some idea of the number of anthrax spores in imported infected material. The fact that colonies of anthrax were never discovered by the plate method demonstrates that they cannot be very numerous, as a rule.

A very rough idea of the minimum number of spores may be gathered as follows: 15 grammes of each sample were soaked in 100 c.c. of water, and 4 c.c. of this uncentrifuged extract, that is to say $\frac{1}{25}$ part, injected into guinea-pigs. If the animal died of anthrax there must have been *at least* one anthrax spore in $\frac{1}{25}$ of a gramme, or five spores in 3 grammes. This rough estimate is probably much too low, and is based on the unlikely assumption that all the spores are washed off the infected sample, and only one spore is necessary to kill a guinea-pig.

Our figures indicate anthrax spores are present, though probably in small numbers, in a fairly large proportion of industrial material imported into Liverpool. It is at first sight rather remarkable that the disease is not, therefore, more common among labourers and others. Yet the chances of a labourer contracting anthrax from handling a particular hide are really small. First the bacilli must enter his system by actual contact with the contaminated material, and second, as in every bacterial infection, other determining factors are the dose of the bacilli, their virulence, and the individual's own resistance. Fortunately man is more resistant than most domestic animals.

The relative value of plate cultures and inoculation methods.

Among observers who have tried both methods Berka, Rembold, Silberschmidt, Gruber, and ourselves have found inoculation of animals more efficient. On the other hand Heim discovered anthrax in one sample by plate culture when inoculation into a mouse and a guinea-pig was negative. Russ obtained two anthrax colonies by plating 40 c.c. of watery extract but failed to demonstrate the bacilli by inoculating the

remaining 10 c.c. amongst three guinea-pigs and seven mice. Lastly, Laubenheimer concludes that cultures are a more delicate test than animal inoculation, but he gives no figures, and almost certainly employed mice not guinea-pigs, a serious defect in technique.

All Page's observations with plate cultures upon bristles and horse-hair were negative, and likewise the great majority of Eurich's when non-blood stained was examined. On the other hand we demonstrated by animal inoculation anthrax in 22·2 % of 72 samples of wool, none of which were obviously blood stained, though they were not examined chemically. We have recently found anthrax in two specimens of horse and cow hair which showed no macroscopic blood stains, and were negative with the Guiacum test.

Though no workers have yet undertaken a large series of examinations of equal portions of the *same* material by both methods simultaneously, yet the available evidence indicates that animal inoculation is decidedly better, provided that guinea-pigs, not mice, are employed. If, on the other hand, plate cultures are made, the experience of Reichel and Laubenheimer indicate how essential it is to examine them after a short incubation, probably 12 to 16 hours is most suitable.

The skill of the observers in recognising the anthrax colonies must also be a factor of importance.

In our opinion the inoculation of guinea-pigs is more successful than the use of plate cultures, mainly because a larger quantity of watery extract is usually examined. This more than counterbalances the disadvantage that some of the guinea-pigs died from anaerobes before they develop anthrax.

It is doubtful, however, if it is always possible to identify with certainty *B. anthracis* without demonstrating its pathogenicity to animals. According to Reichel, Muir and Ritchie, this is essential to distinguish anthrax from pseudo-anthrax bacilli.

Dr Eurich's figures prove beyond question that anthrax is more common on blood-stained wools, a discovery of great practical importance to the trade. In Bradford there has recently been a very marked diminution in the percentage of blood-stained wool contaminated with anthrax, partly owing to stricter supervision and to the smaller quantity of blood upon the specimens. It is clear, however, from the tables that we have demonstrated anthrax bacilli in a larger proportion of samples than any previous worker, where a large series of samples have been examined, except Reichel's, but in his case the high figures were mainly due to some badly contaminated Servian hides and Syrian wools.

In all probability our percentage of positive results would have been higher had two guinea-pigs always been inoculated, and the watery extract always centrifuged. The results obtained by the principal workers with the plate and inoculation methods have been summarised below.

Table of the percentage of cases in which anthrax was demonstrated in five or more samples of industrial material, by the plate cultivation and animal inoculation method.

	Plate cultures				Animal inoculation			
Author	Year	No. of samples	% of positive	Remarks	Year	No. of samples	% of positive	Remarks
Rembold	1888	5	0	Gelatine plates	1888	5	40	1 guinea-pig each sample
Gruber	?	6	0	—	?	6 consignments	33	Mice inoculated with anaerobic heated broth cultures
Heim	1901 1906	2–25	3·7	—	1901	2	0	1 guinea-pig, 1 mouse each sample
Webb and Duncan	1904	—	—	—		8 cases 4 bales	66	Guinea-pigs
Eurich	1906–8	600	0	—		—		—
		139	14·1	Blood stained		—		—
	1909	68	0	- -		—		—
		719	3·2	Blood stained		—		—
	1910	111	1·8	—		—		—
		318	8·5	Blood stained		—		—
	1911	90	1·1	—		—		—
		786	3·8	Blood stained		—		—
Page	1909	83	0	Samples not blood stained; plates incubated at least 18 hours		—		—
Reichel	1911	270	24	8 inch agar plates incubated; 14–16 hours samples from Servia and Syria		—		—
Laubenheimer	1911	?	Several	Agar plates incubated 12–20 hours. "Disinfected" material	1912	A few	?	States that inoculation less delicate than cultures, but mice used
Liverpool	1904–6	54	0	No obvious blood stains, 6 agar plates, each sample incubated about 18 hours	1907–12	141	21·3	No obvious blood stains noticed. Usually 2 guinea-pigs inoculated and much more satisfactory than one

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The disinfection of industrial material.

The results of various workers differ greatly as to the suitability of the ordinary modes of disinfecting hair, etc. Laubenheimer, the most recent writer on the subject, after a series of exhaustive investigations, concludes that the complete sterilization of horsehair is not practicable, but he recognises the utility of imperfect sterilization in diminishing infectivity. We have isolated anthrax in two of six samples of horsehair, "disinfected" in Germany by steam probably at 105° C., and in one sample of hair treated in a 5% solution of a high coefficient disinfecting fluid for 36 hours at 212–214° F.

Experiments have recently been made by one of us (F. C. L.) with a new form of sterilizing apparatus. The results are so far encouraging, numbers of anthrax spores were destroyed in two minutes, and no damage was done to the horsehair so treated. The observations are being continued.

The detection of anthrax bacilli in a sample of cattle food.

We have already alluded to this case, the details are as follows: 14 cows were kept in a Liverpool shippon, and one of these, which had been confined for five months, died of anthrax. We, therefore, examined samples of all the foodstuffs which constituted its diet, viz. pea meal, Makhar meal, Bibby cake, nutrimol meal, and clover hay. The technique was the same in the examination of each kind of foodstuff. Fifteen grammes were mixed with 150 c.c. of water and heated at 75 to 80° C. for one hour; 100 c.c. of this mixture was centrifuged, and 90 c.c. of the supernatant fluid poured off. The remaining 10 c.c. and the deposit were shaken, and two guinea-pigs inoculated with 3 and 1 c.c. of the mixture respectively. All the 10 animals survived except the one receiving 3 c.c. of the pea meal extract, it died of anthrax.

The evidence strongly points to the pea meal being the infecting agent, for other sources can be excluded. All the shippons in Liverpool are under the supervision of the Health Department; no case of anthrax has ever occurred in the one in question. The cows have been strictly confined in it for periods varing from 2 to 18 months. None of them came from infected farms.

The pea meal is the mixed product of materials imported from various countries, and is sold in sacks. A fresh sack of the meal was

bought by the cowkeeper every week, kept in a covered bin, and about a pound given to each cow in the shippon twice a day, mixed with its other food.

If, as appears most probable, the pea meal was contaminated with anthrax bacilli from the sack, the number so introduced would necessarily be small. Obviously the number was small because the guinea-pig inoculated with 1 c.c. of the extract survived, and the other cattle in the shippon, although fed upon the same small quantities of meal, did not contract the disease. Of course the resistance of these animals may have been greater, or the dose of bacilli administered less.

We have examined foodstuffs used for cattle which had contracted anthrax on other occasions but with negative results.

Summary and conclusions.

(1) There are two methods of examining industrial material for anthrax spores, viz. agar plate cultures and the inoculation of guinea-pigs.

(2) Though no worker has yet made a large series of examinations of equal portions of the same material by both methods simultaneously, yet the available evidence indicates that animal inoculation is superior, provided that guinea-pigs, not mice, are employed.

(3) It is better to inoculate two guinea-pigs than one, and to use the centrifuged deposit of the watery extract, though this practice may also increase the mortality from pathogenic anaerobes.

(4) The inoculation of guinea-pigs is more successful than plate cultures mainly on account of the larger quantity of watery extract usually tested, which more than counterbalances the disadvantage that some of the animals died from anaerobes before they develop anthrax.

(5) If plate cultures are employed it is essential to examine the superficial colonies after a short incubation, probably 12 to 16 hours is the most suitable.

(6) Anthrax spores have been demonstrated by the inoculation method in 21·3 % of 141 samples of industrial material, supposed to have produced anthrax in Liverpool amongst those who handled them. Of these samples 28·6 % were from hides, 22·2 % from wool, 20·6 % from hair and 7·1 % from bones. The largest proportion of infected samples came from Singapore.

(7) Anthrax spores have been demonstrated in one sample of hair "disinfected" with 5 % fluid of high coefficient at 100° C. and in two out

of six samples of hair disinfected in Germany probably by steam at 105° C. It is practically impossible to completely sterilise certain industrial materials without seriously damaging them; yet partial disinfection should on no account be abandoned, because the perfect method has not yet been discovered.

(8) Anthrax appears to be steadily increasing among domestic animals, and consequently a larger number of agriculturalists are becoming infected. The reason for this dissemination amongst animals is still obscure.

(9) We have found *B. anthracis* in a sample of pea meal used for feeding cattle confined to a shippon, one of which died of anthrax. The meal was probably infected from the sack.

(10) Anthrax is preventible among men and domestic animals, and its ultimate suppression depends largely upon the certainty with which bacteriologists can demonstrate the presence or absence of bacilli in suspected industrial food, or other materials.

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