THE DISTRIBUTION OF *BACILLUS BOTULINUS* IN SCOTTISH SOILS.

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In January 1923, after the publication of *The Epidemiology of Botulism* by Dr K. F. Meyer and his colleagues in America, and after the publication of the official report to the Scottish Board of Health on the Loch Maree tragedy, it seemed obvious that an accurate knowledge of the distribution of the *Bacillus botulinus* in Scotland was very desirable. Dr Meyer had shown that this was an organism which lived in the soil, and he had already published the results of the bacteriological examination of a great number of soils in America and other countries. None of them, however, came from Scotland. I therefore suggested to the Scottish Board of Health that they should ask the Medical Research Council to undertake such an examination, and offered my own services for the purpose of collecting soils from all the Scottish counties. The Board at once agreed, and the Medical Research Council on having the request put to them also at once agreed and appointed Professor Basil Buxton, F.R.C.V.S., to undertake the bacteriological investigation. Prof. Buxton and myself arranged exactly the kind of samples which should be taken, and the method of taking them, and he supplied me from time to time with a number of sterilised containers into which samples of soil were put and forwarded to him for examination.

The following notes show in detail the exact geographical source of these samples, and the kind of soils from which they were taken.

Collecting the Soils.

In order to avoid incurring any special expense in the collection of the samples of soils, I obtained these in the course of my ordinary work, not making any special journeys for this purpose. Naturally, therefore, it took a considerable time to cover the whole of Scotland (with Orkney, Shetland and the Hebrides, which I desired to include).

My procedure was to take with me when travelling on other duty for the Board a few sterilised glass containers, and take one or more samples of soil from each county visited. These were forwarded as collected to Professor Buxton at Cambridge who carried out the bacteriological work as opportunity offered. In this way we had covered by the end of 1926 all the counties except ten, and in order to obtain samples from these and quickly complete

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the collection desired, I asked the aid of various officials of Local Authorities which was readily given.

Distribution of Soil Samples among Counties.

Aberdeen	9	Lanark	3
Argyll	1	Midlothian	4
Ayr	4	Moray	4
Banff	5	Nairn	1
Berwick	1	Orkney	3
Bute	2	Peebles	2
Caithness	1	Perth	3
Clackmannan	1	Renfrew	1
Dumbarton	2	Ross and Cromarty	13
Dumfries	3	Roxburgh	2
East Lothian	2	Selkirk	1
Fife	6	Stirling	2
Forfar	2	Sutherland	1
Inverness	4	West Lothian	1
Kincardine	5	Wigtown	4
Kinross	1	Zetland	4
Kirkcudbright	2		
	51		49

Total 100

Classification of Soils examined.

If we divide the 100 soils into four groups of cultivated gardens, cultivated ploughed fields, pasture land, and uncultivated land, they are numerically distributed thus:

Cultivated gardens	16
Cultivated ploughed fields	16
Pasture land	38
Uncultivated, waste, moorland	30
Total	100

The positive soils are:

Pasture land	3
Ploughed field	1
Total	4

Investigations already carried out by Meyer and Dubovsky[1] concerning the occurrence of spores of *B. botulinus* in the soils of Belgium, Denmark, England, the Netherlands and Switzerland[1], and almost all parts of the United States[2] as well as within limited areas by the same investigators[3] and by Burke[4], Hall and Peterson[5], Tanner and Dack[6], and Damon and Payabal[7], have shown that the spores are, in general, apparently rather widespread in nature.

The method of examination was as follows: 20 grammes of soil was placed in sterile petri dishes and dried at 37° C. After drying the sample was mixed with 30 c.c. of sterile physiological salt solution and heated in a water bath at 80° C. for 30 minutes. The mixture was then filtered through several layers of muslin in order to remove the coarse particles. The whole process was carried out in such a manner as to avoid contamination. The filtrate was

[1] Information supplied to Dr Leighton by Dr Meyer.
[2] *J. Infect. Dis.* 1922, **31**, 559 and 614.
[3] *Ibid.* 514.
[4] *J. Bacteriol.* 1919, **4**, 541.
[5] *Ibid.* 1924, **9**, 201.
[6] *J. Infect. Dis.* 1922, **31**, 92.
[7] *Ibid.* 1926, **39**, 491.

transferred to wide-mouthed bottles containing about 150 c.c. of Meyer's medium. These were incubated for ten days at 37° C. at the end of which time the contents were filtered first through paper pulp, and then through a Berkefeld filter. The filtrate was tested for sterility by sowing each of a number of broth tubes containing minced meat with 1 c.c. of the filtrate. The bulk of the filtrate was stored in the cold.

In the earlier experiments duplicate bottles of Meyer's medium were inoculated with soil and incubated for 14 days at 20–22° C. It was subsequently found that this was unnecessary.

In the event of the filtrate proving to be sterile it was injected into guinea-pigs.

In the earlier experiments eight guinea-pigs were used for each sample. Six were passively immunised against *Bacillus tetani*, vibrion septique, *B. welchii*, and *B. oedematiens* by means of one dose of a mixed antiserum (T.V.W.E.). In addition two were immunised against the toxin of *B. botulinus* type *A*, and two against the toxin of *B. botulinus* type *B*. Two were used as controls. One of each pair was injected subcutaneously with 5 c.c. of filtrate, and the other with 2 c.c. This procedure was subsequently modified owing to the infrequency with which the control guinea-pigs showed symptoms of intoxication. Latterly, two normal guinea-pigs only were employed for the preliminary injection of filtrate. One received 5 c.c. subcutaneously and the other 2 c.c. In the event of one or both showing symptoms of tetanus, the filtrate was subsequently injected into guinea-pigs which had previously received an injection of tetanus antitoxin. In all one hundred and six samples of soil were examined for the presence of spores of *B. botulinus*. One hundred of these were from various parts of Scotland, the Orkneys, Shetlands and Hebrides, and six were from the Isle of Man.

Two samples (5 and 12) were found to produce the toxin of *B. botulinus*, type *A*. One was obtained from ploughed land in Peeblesshire, and the other from old pasture on the east coast of Aberdeenshire.

One sample (4) yielded toxic cultures type *B*. This was obtained from hill soil near St Mary's Loch, Selkirkshire. One sample (13) obtained from old pasture at Stranraer yielded a toxic filtrate which was neutralised by both type *A* and type *B. botulinus* antitoxin.

Although not part of the investigation it is interesting to note that only eight samples of soil gave filtrates containing tetanus toxin. Four of these were from manured cultivated land—one from old pasture, and one from moorland.

In no instance was toxin produced which could not be identified by antitoxin protection tests.

Perhaps the most interesting point brought out is that in the present investigation two soils have yielded type *A* of the bacillus, the first time this has been found in a European soil. All the other positive soils showed type *B*.

Comments.

Although this Scottish series yields only 4 per cent. positive results as compared with 5·5 per cent. in Denmark, 7·8 per cent. in England, 20 per cent. in Holland, and 23·5 per cent. in Switzerland, it would not be safe to conclude that the organism is thus relatively and proportionately rare in Scotland. What is definitely shown for the first time is that *B. botulinus*, types *A* and *B* occur in Scottish soil.

It must be remembered that each sample of soil is a very small amount (3 to 4 ounces), an almost negligible portion, one might say, of a field, garden or moor. Of this a still much smaller portion (a few c.c.) is used in the bacteriological examination. Therefore it is obvious that the first collection of a positive sample and the following examination of it is largely a matter of chance as to its content. Thus, if one had stopped at the first five samples collected, the result (2 out of 5) would give 40 per cent. positive. If one had stopped at the first ten, 20 per cent. would be the answer. If one stopped at twenty, the result would be also 20 per cent. But if one had examined only the last eighty samples, the result would be 0 per cent.

Analyses of European Soils.

(K. F. Meyer and J. Dubovsky)

Country	Locality	Specimens	Total number of specimens examined	Number of toxic cultures	Total number of typed cultures	Type *B*	Untyped	Percentage total toxic cultures	Percentage of typed cultures
Belgium ...	Unknown	Soils	3	2	2	2	—	—	—
Denmark ...	Copenhagen	Garden soil 8		2	1	1	1	6·8	3·4
	Amager	Field soil 12 Sewage 4 Compost 5	29	—	—	—	—	—	5·4
	Different parts of country	Field soils	25	4	2	2	2	16	8
England ...	Different parts	Garden, orchard 47 Field soil 7 Virgin soil 7 Sewage farms 3	64	9	5	5	4	13·1	7·8
Netherlands	Different parts	Meadow soil	10	6	2	2	4	(60·0)	(20·0)
Switzerland...	Vicinity of Berne and one Canton (Tessin)	Soils 16 Vegetables 18	34	11	8	8	3	35·2	23·5
		Total	165	34	20	20	14	20·6	12·7

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