NUMBERS OF PROTOZOA IN CERTAIN ROTHAMSTED SOILS[1].

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(With Twenty text-figures.)

INTRODUCTION.

Although during the last few years a good deal of work has been done on the soil protozoa, very little is known as yet as to their mode of life, behaviour and relations with the other soil organisms; so much is this the case that even the fact that they lead a trophic life in soil is often disputed. Since Russell and Hutchinson (16, 17) in their work on the partial sterilisation of soil first discussed the possible relations existing between the soil protozoa and bacteria, the question of the activity of protozoa in soil has become important. They found that partial sterilisation brought about an improvement in the soil as a medium for the growth of bacteria by the removal of a limiting factor, and further they showed that this limiting factor possessed many of the characteristics of a living organism. Provisionally they regarded the protozoa as constituting one of the factors limiting bacterial development in soil; the hypothesis therefore is based on the assumption that the protozoa are trophic. It has been the object of this work to ascertain whether these organisms can live and multiply in the soil under wholly normal physical and cultural conditions, and also to throw some light upon the interrelations between this group of animals and the soil bacteria. Among recent investigators Martin and Lewin (14) and Goodey (7) are practically the only ones who state that protozoa other than flagellates are trophic in soil even when the moisture content is not above the average. Martin and Lewin claim this for amœbæ and flagellates, Goodey for amœbæ only. Martin and Lewin succeeded in making preparations of trophic amœbæ, thecamœbæ and flagellates either by adding suitable fixatives, such as picric alcohol, to the soil and floating cover slips on the surface of the liquid, or by passing a stream of air bubbles through a vertical tube containing a suspension of soil in water, and letting the bubbles break on a cover slip at the top for a short time. When either of these methods results in the appearance of trophic forms on the cover slips

[1] This work was carried out before Mr Cutler's method for distinguishing between the total and the active numbers of protozoa was devised. The figures quoted in this paper always refer to total (active + cystic) numbers.

it is a satisfactory proof that trophic forms were present in the original soil, but there are certain drawbacks attached to both methods, as there appear to be physical conditions[1] under which the protozoa cannot be washed out of the soil with such ease and the cover slips remain blank, and further there is the faint possibility that sudden excystment may be induced by the treatment that the soil undergoes. According to Martin and Lewin, amœbæ, thecamœbæ and flagellates are ordinarily trophic in the soils that they used, the amœbæ and thecamœbæ being more numerous than the flagellates. Their soils included a cucumber sick soil, a soil from a seedling bed, containing sand and leaf mould, but no manure, a woodland soil very rich in leaf mould, and three of the Rothamsted field soils taken from Broadbalk, dunged plot and unmanured plot, and from a fallow plot on Agdell. Goodey, working at Rothamsted (5), thinks that ciliates are probably not trophic. Waksman working in U.S.A. (20) states that "flagellates are the most common soil protozoa found active in the soil with moisture content too low for the development of the other groups," but he makes no mention of the amœbæ, and Sherman, also working in U.S.A. (18), agrees that flagellates are in most soils the only active forms. With the heavy Rothamsted soils Martin and Lewin's methods often give negative results, but when they succeed the fauna comprises many more amœbæ than flagellates, moreover the amœbæ both from their size and from their known habit of feeding on bacteria are more likely to make an impression upon the bacterial numbers than are the very much smaller flagellates which are in some cases not even holozoic. For these reasons in the present instance the amœbæ alone are considered, except in a few cases where it has seemed useful to quote the numbers for ciliates or flagellates. It is interesting to notice in this connexion that Cauda and Sangiorgi, working at Turin (1), invariably obtain from their soils comparatively high numbers of amœbæ, and in two cases amœbæ only, the flagellates and ciliates both being absent. The thecamœbæ, which are often present in numbers up to 1000 or even more per gramme, are also neglected as they arise very late in the cultures, generally not until at least three weeks have elapsed, and to deal with them adequately requires an increase of apparatus which has hitherto been impracticable. Records of their appearance have been kept in some cases. For instance on May 3rd, 1916, 10 petri dishes containing nutrient agar were inoculated with 0·5 gramme of soil from Broadbalk, Plot 2, and enough sterile water was added to each plate

[1] Doubtless connected with surface energy (Cutler, *Journ. of Agric. Sci.* IX. part IV. 1919).

to moisten the surface. Thecamœbæ appeared on the plates marked with a × as follows:

Table I.

Thecamœbæ in soil of Broadbalk, Plot 2, *May* 1916.

	Plates									
	1	2	3	4	5	6	7	8	9	10
May 31	...	×	...	...	...	...	...	...	×	×
June 5	×	×	...	...	...	...	...	...	×	...
June 10	...	...	...	×	...	...	...	...	×	...
June 15	×	×	×	×	...	...	×	...	×	×
June 19	×	×	...	...	...	...	...	...	×	...
June 22	×	×	×	×	×	...	×	×	×	...
June 29	...	...	×	×	×	...	...	×	×	×
July 3	×	×	×	×	×	×	×	×	×	×

On September 7th, 1916, petri dishes were inoculated with 1 c.c. from some of the dilution bottles used for counting the protozoa in Broadbalk, Plot 2.

Table II.

Thecamœbæ in soil of Broadbalk, Plot 2, *Sept.* 1916.

	Dilutions			
	1/10	1/100	1/1000	1/2500
September 13	0	0	0	0
September 20	0	0	0	0
October 3	×	0	0	0
October 5	×	×	×	0
October 10	×	×	×	0

Strong evidence for the existence of flagellates and amœbæ in the trophic state is derived from observations on the fluctuations in their numbers, these fluctuations being of a very definite character and in no way due to chance.

EXPERIMENTAL.

The method used for counting the protozoa is an adaptation of the dilution method often employed in estimating bacterial numbers, and although such a means of counting can never give the absolute number of organisms present, yet it furnishes results which have a definite relative value. Each sample of soil used is composed of several six inch borings taken with a soil auger and passed through a 3 mm. sieve; 10 grammes are weighed out and shaken for four minutes in sterile tap water and this forms the initial dilution of 1/10 from which all the others are prepared. Four 1 c.c. samples are taken from each dilution bottle to give four parallel series of cultures.

The apparatus must be sterile and the suspensions thoroughly shaken so that the liquid is as homogeneous as possible when the samples for incubation are taken with the pipette. The medium used is a nutrient agar, containing 0·3 per cent. lemco, sloped in small test-tubes[1] so that there is a certain amount of agar slope above the 1 c.c. of liquid from the dilution bottle. The cultures are incubated for a minimum period of five days before examination, at a temperature of about 18° C. Samples have been taken at fairly regular intervals of seven days from Broadbalk, Plot 2, which carries wheat every year and receives 14 tons of farmyard manure per acre every autumn, and from Great Harpenden Field, which in 1915, 1916, 1917 was cropped with cereals and in 1918 with clover and had received no dung since 1914; samples have also been taken on a few occasions from Broadbalk, Plot 3, which has received no manure since 1839, and from Barnfield, Plot 10, cropped with mangolds and receiving 14 tons farmyard manure per acre every year. The numbers of protozoa fluctuate even from day to day in the top six inches of the field soils under observation; flagellates are nearly always present in numbers varying from 1000 to 100,000 per gramme, amœbæ too are generally to be found, though their numbers are lower, ranging between 100 and 50,000 per gramme and very occasionally rising to 100,000; the ciliates only appear from time to time and seldom exceed 1000 per gramme. This method of counting of course allows of no differentiation between the organisms that are present in the soil in the trophic state and those that are there as cysts; it is possible however to make such a distinction by using a device of Cunningham's (2) which depends upon heating the dilutions to 58° C. to kill off all trophic forms before inoculating the culture tubes.

Miss L. M. Underwood, B.Sc. gave valuable assistance in the routine work during the period December 1916—April 1917.

Curves 1 to 13[2] show the numbers of amœbæ in Broadbalk, Plot 2, and in Great Harpenden Field, the samples being taken at intervals of about seven days over a period extending from May 1916 to August 1919. These numbers change considerably and at first sight it may appear that the fluctuations are entirely due to chance, that at one point the soil may be rich in amœbæ while in another it is barren, implying that the amœbæ are not native to the soil but are deposited there quite fortuitously, either from the air, just as protozoa appear in a hay infusion which is left uncovered, or when manure is applied. That chance is not

[1] Mr Cutler in these laboratories uses petri dishes (see p. 133).

[2] I am indebted to Mr D. W. Cutler for Fig. 13.

alone responsible for the varying numbers and that the amœbæ are really indigenous to the soil is quite clear after a consideration of the following facts:

1. Samples of soil taken at the same time from different borings in the same field when dealt with separately yield almost identical results (Table III). In actual practice however each sample that is used consists of 6–10 such borings intimately mixed together, so that even the small differences which exist between the separate parts sink into insignificance.

Table III.

Numbers of protozoa per gramme in samples of soil taken from different parts of the same plot.

Date	Sample No.	Amœbæ	Flagellates	Ciliates
Great Harpenden Field.				
Dec. 3, 1915	1	below 100	10,000	below 100
	2	„ 100	10,000	„ 100
	3	„ 100	10,000	„ 100
	4	„ 100	10,000	„ 100
Dec. 10, 1915	1	„ 100	10,000	„ 100
	2	„ 100	7,500	„ 100
	3	„ 100	10,000	„ 100
	4	„ 100	10,000	„ 100
Broadbalk, Plot 2.				
Feb. 16, 1916	1	2500	50,000	100
	2	2500	50,000	100
	3	2500	75,000	100
	4	2500	50,000	below 100
	5	2500	75,000	100

2. The curves for the two fields in general show close similarity; this is well shown in Figs. 5 and 6, and 9 and 10, in fact out of the 27 points in Figs. 5 and 6, 24 correspond, while in Figs. 9 and 10 where 12 counts are shown, eight correspond. If chance is to explain such parallelism it is necessary to imagine that the samples taken from the two different fields on the same day both happen to be rich in cysts or both happen to be poor in them.

3. The addition of dung to the field is not followed by an immediate and sustained rise in the numbers of protozoa as it would be were the dung the chief source of the soil fauna; for instance in Fig. 9 where farmyard manure was added on October 24th and 25th, at the rate of 14 tons per acre, there is a rise in the amœba curve on the 31st which is followed by a substantial fall, and further, this rise and the subsequent one at the

end of November are paralleled in Harpenden Field (see Fig. 10) which received no manure at this date. The bacteria do apparently increase in numbers and remain fairly high from October 24th to November 10th when they too fall.

These three considerations lead to the conclusion that not only is there a protozoan fauna in certain field soils, but that this fauna is in the trophic condition and able to multiply with rapidity under certain conditions; thus in five days the number of amœbæ may rise from 5000 to 50,000 per gramme, as in Fig. 9 between October 29th and November 3rd. It may be suggested that in spite of this the amœbæ may still only be present in cysts but that their cysts are reproductive, and that where 5000 cysts each containing one amœba occurred in 1 gramme of soil in October 29th by November 3rd there were still 5000 cysts but each contained 10 amœbæ. Unfortunately our knowledge of the life-histories of these animals is insufficient to rule this suggestion out of court at once, but the investigations which have been carried out by numerous observers on free-living "limax" amœbæ hitherto have certainly not led to the discovery of such a stage of reproduction in the cyst. Further, where no amœbæ are recorded it means that although a few may have been present their numbers certainly fell well below 100 per gramme, and it is very difficult to imagine that so few cysts could give rise to 5000 amœbæ in four days, as occurs in Fig. 5 between March 8th and 12th, without excystment and a trophic period however short.

Certain experiments carried out in the winter of 1915–16 give the following results concerning the vertical distribution of protozoa in the soil:

Table IV.

Numbers of protozoa per gramme at different depths in the soil.

Date	Depth in inches 6	12	18	
Feb. 2, 1916	2500	0	0	Amœbæ
	10,000	100	100	Flagellates
	100	0	0	Ciliates
Feb. 3, 1916	1000	0	0	Amœbæ
	7500	1000	100	Flagellates
	100	0	0	Ciliates

This shows that the protozoa are practically confined to the top six inches in these field soils, and later work indicates that probably they occur very sparsely below the top four inches. Waksman (20) also finds that below

12 inches the soil is practically free from protozoa while far the greatest number is found just below the surface.

It is very difficult to give any information as to the genera and species of protozoa that can be found in soil; for practical purposes it has been convenient to divide them up into four great groups: amœbæ, thecamœbæ, flagellates, and ciliates, but this is obviously a most unsatisfactory classification. The investigation of the soil protozoa is still in its early stages and undoubtedly many new forms will be described. Unfortunately the identification of the amœbæ and flagellates presents a good deal of difficulty since it is necessary to follow out the life-history before a species can be named with any degree of certainty, and there is a striking lack of satisfactory description in the literature dealing with them. In several cases new specific names have been assigned but the data given are quite insufficient to ensure the recognition of the species by other observers.

The position as regards the occurrence of active protozoa in the soils under consideration may be summed up as follows: in arable soil, whether entirely unmanured or rich in farmyard manure, there is an extensive protozoan fauna, at least in the top six inches, which flourishes and multiplies, obtaining a great part of its food from the bacteria that are invariably present. This fauna is in great part indigenous to the soil though some of its members probably arrive there by chance. For instance it seems likely that *Chlamydophrys* sp. is introduced in dung since it is found in the dunged, but not as yet in the undunged, plot on Broadbalk; it also occurs in cultures of farmyard manure and is known to be an inhabitant of the intestine in some animals. In the types of soil examined the richer the soil in organic matter the richer it is found to be in protozoa, especially in amœbæ and thecamœbæ. Thus Broadbalk, dunged plot, gives consistently higher numbers than Great Harpenden Field (Table V) and on one or two occasions when counts have been made on glasshouse soils the numbers have been high compared with those of the two field soils.

A cursory glance at curves 1–13 shows that there is certainly some kind of interaction between amœbæ and bacteria, for where the bacteria are relatively high the amœbæ are as a rule relatively low, and *vice versa*. Given that the numbers quoted have a real meaning and that, although they do not represent the numbers of micro-organisms actually in the soil, they show the rise and fall in the numbers of those organisms, the cause of these fluctuations must next be considered. The most obvious suggestion to put forward is that the changes in both curves

are due to the changes in the physical factors that make up the environment. In the present instance only three of these factors have been dealt with, namely the soil moisture, the temperature and the rainfall. As a working hypothesis it may be presumed that a high percentage of moisture in the soil, short of water-logging, may be beneficial to micro-organic life, and that a high temperature may also promote growth and reproduction.

Table V.

Comparison of numbers per gramme of protozoa in Broadbalk, dunged plot, and Great Harpenden Field. The figures in each case are the average of ten counts.

Date	Broadbalk dunged plot (containing 10·0 per cent. organic matter)			Great Harpenden Field (containing 5·7 per cent. organic matter)		
	F	C	A	F	C	A
Feb. 1—March 5, 1917	32,000	20	1500	13,500	10	500
March 8—April 15, 1917	29,800	40	1400	9300	0	500
April 17—June 6, 1917	23,300	130	1600	7100	20	500
June 13—Sept. 20, 1917	31,200	120	18,600	25,700	10	17,000
Oct. 10—Dec. 15, 1917	42,300	20	23,200	23,300	10	10,000
Dec. 21—March 6, 1918	20,500	40	2200	12,000	0	1500
March 13—June 5, 1918	19,700	20	5100	13,500	0	450

Rainfall. There is no definite correlation between the curves representing the rainfall and those for the bacteria and protozoa. Occasionally a heavy fall of rain is followed by high numbers but by no means often enough to justify the conclusion from these data that the numbers of either protozoa or bacteria depend upon the rainfall. On January 16th, 1918, there is a rise in the bacteria in both fields (Figs. 11 and 12), and there is also a very heavy rainfall on the 15th (Fig. 19), as the percentage of moisture for the count is low in Broadbalk and in no way exceptional in Harpenden Field, and as the temperature is only 35·5° F. it is possible that in this case the rainfall must be called in to explain the bacterial numbers. At the same time this is the only case where any change in the biological curves might be explained by reference to the rainfall, so that the chances are strongly in favour of its being a coincidence.

Moisture Content. There is a very fair degree of correspondence between the curves for moisture content and the bacteria, and as might be expected the agreement is more marked when the temperature is fairly high, from May to October, than it is during the winter months. As a general rule the amœbæ are low when the moisture content is high, while the percentage of moisture is never low enough in these soils

to act as a limiting factor to the protozoa. This conclusion is contrary to that arrived at by certain of the American soil protozoologists, e.g. Koch (9) and Sherman (18), who have found that their protozoa are often trophic only in soils with an exceptionally high moisture content.

Temperature. Temperature seems to bear no special relation to the biological curves, a fact which is not surprising on considering how very little protozoa in cultures appear to be influenced by changes in temperature.

It will be noticed that the amœbæ are apparently unaffected by any of the three physical factors under consideration. In an environment as complex as is the soil it is impossible to analyse thoroughly the action and reaction between any two of the factors that build it up. Moreover there are many other factors, both physical and biological, which have not been touched upon yet and which may well have a profound effect upon the micro-organisms. One may conclude however that a warm, moist condition of the soil is favourable on the whole to the growth of bacteria, but does not encourage the amœbæ.

In this work only two soils have been dealt with in any detail, Broadbalk, Plot 2, which affords an example of a well-manured arable soil, and Great Harpenden Field which receives a comparatively small quantity of manure; the fauna of pasture land has not been considered at all nor has that from other soils.

CONCLUSIONS.

1. Flagellates, amœbæ and thecamœbæ are usually present in these soils in the trophic condition and in comparatively large numbers, so that there is an extensive population actively in search of food.

2. The protozoan fauna is practically confined to the top six inches of the soil.

3. There is a definite inverse relation between the numbers of bacteria and amœbæ.

4. The amœbæ are uninfluenced by variations in the water content and temperature of the soil and by the rainfall.

5. The richer the soil is in organic matter the richer it is in protozoa, especially amœbæ and thecamœbæ.

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Curves 1—13 *show the moisture content and numbers of bacteria and amœbæ* (*active* + *cystic*) *in the top six inches of soil from Broadbalk, Plot* 2, *and Great Harpenden Field at various dates from May* 10, 1916 *to July* 9, 1919.

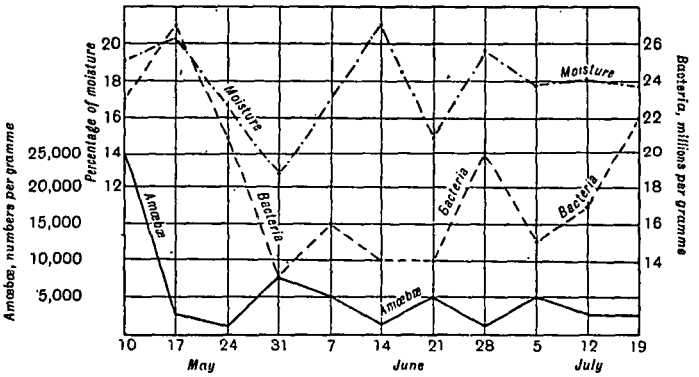

Fig. 1. Broadbalk, Plot 2, May 10—July 19, 1916.

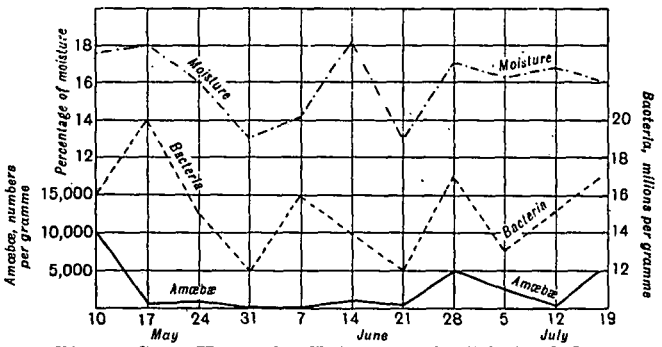

Fig. 2. Great Harpenden Field, May 10—July 19, 1916.

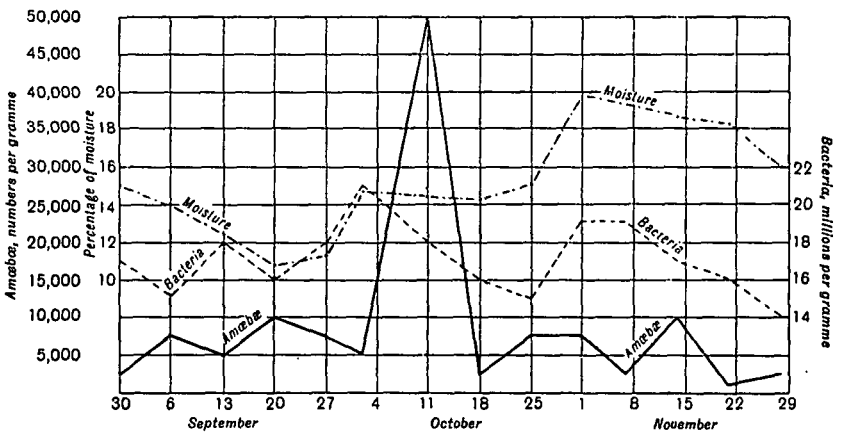

Fig. 3. Broadbalk, Plot 2, August 30—November 29, 1916.

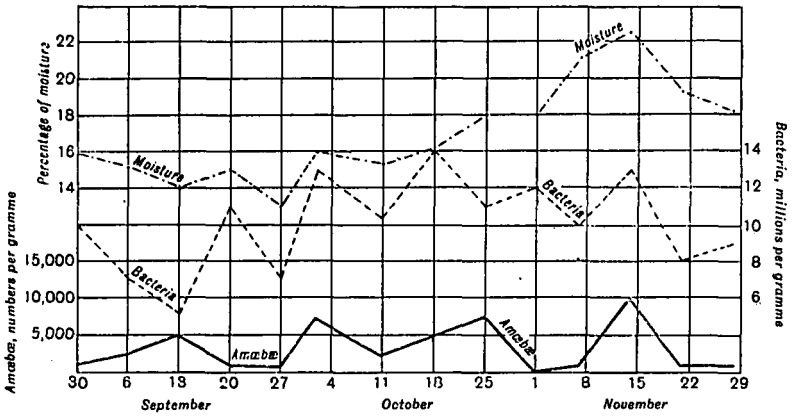

Fig. 4. Great Harpenden Field, August 30—November 29, 1916.

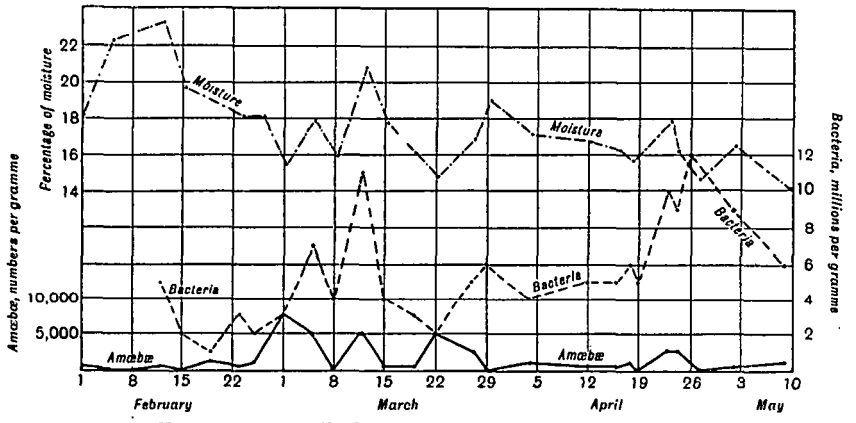

Fig. 5. Broadbalk, Plot 2, February 1—May 10, 1917.

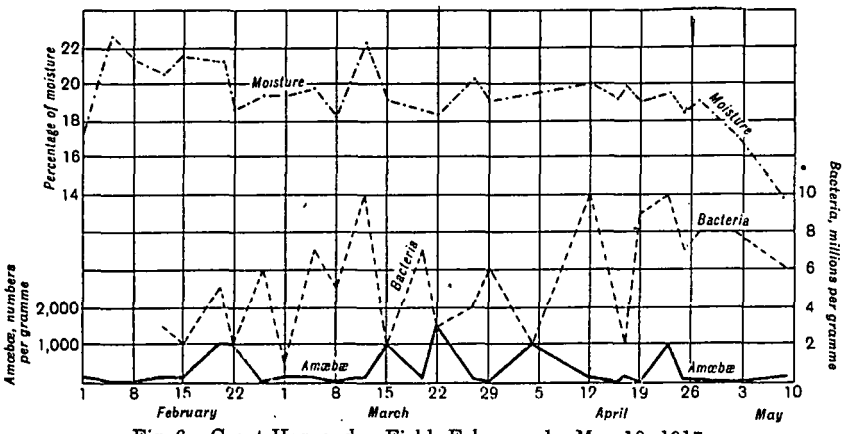

Fig. 6. Great Harpenden Field, February 1—May 10, 1917.

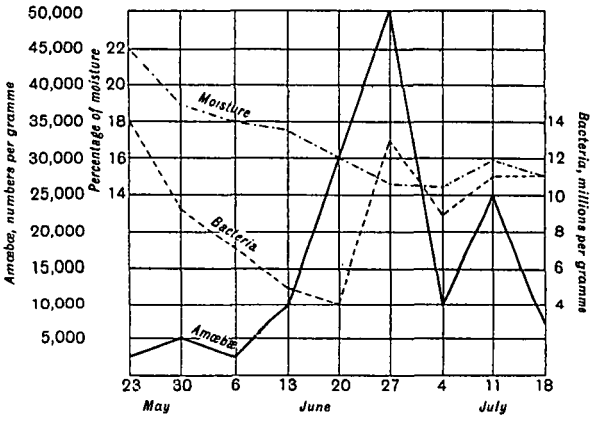

Fig. 7. Broadbalk. Plot 2, May 23—July 18, 1917.

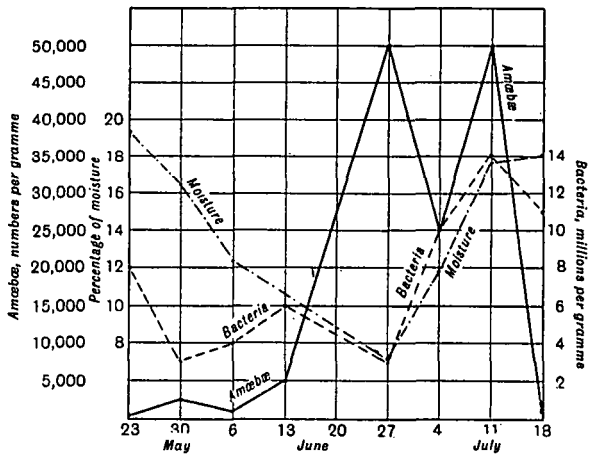

Fig. 8. Great Harpenden Field, May 23—July 18, 1917.

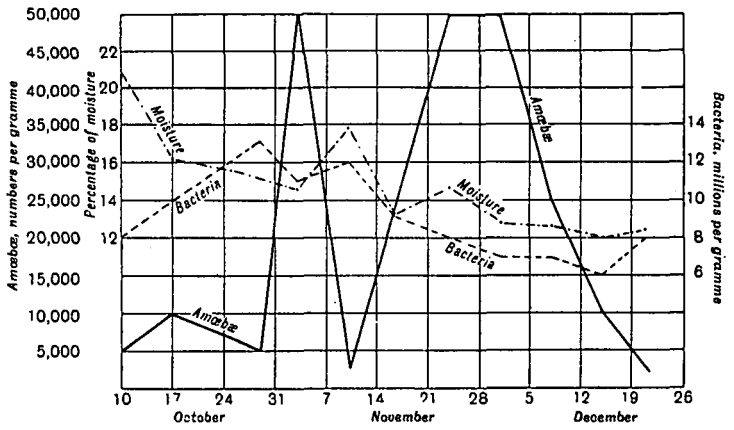

Fig. 9. Broadbalk, Plot 2, October 10—December 21, 1917.

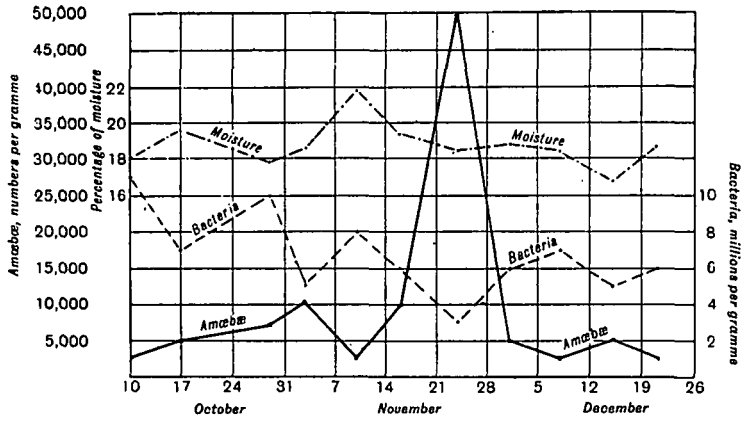

Fig. 10. Great Harpenden Field, October 10—December 21, 1917.

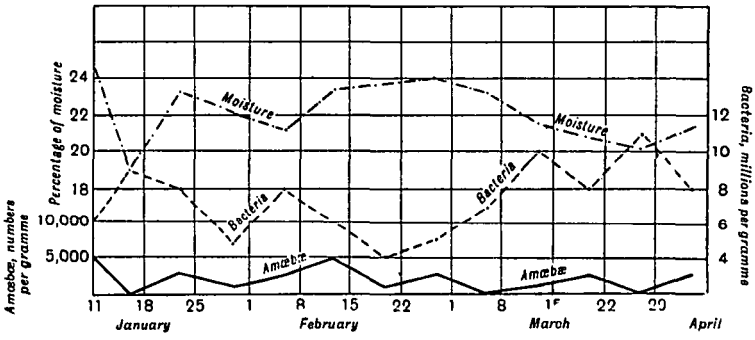

Fig. 11. Broadbalk, Plot 2, January 11—April 4, 1918.

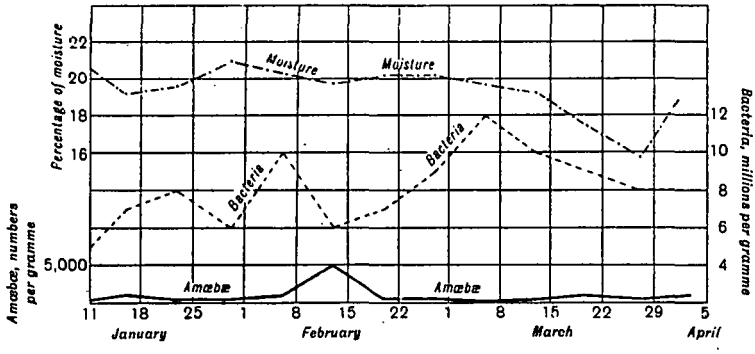

Fig. 12. Great Harpenden Field, January 11—April 4, 1918.

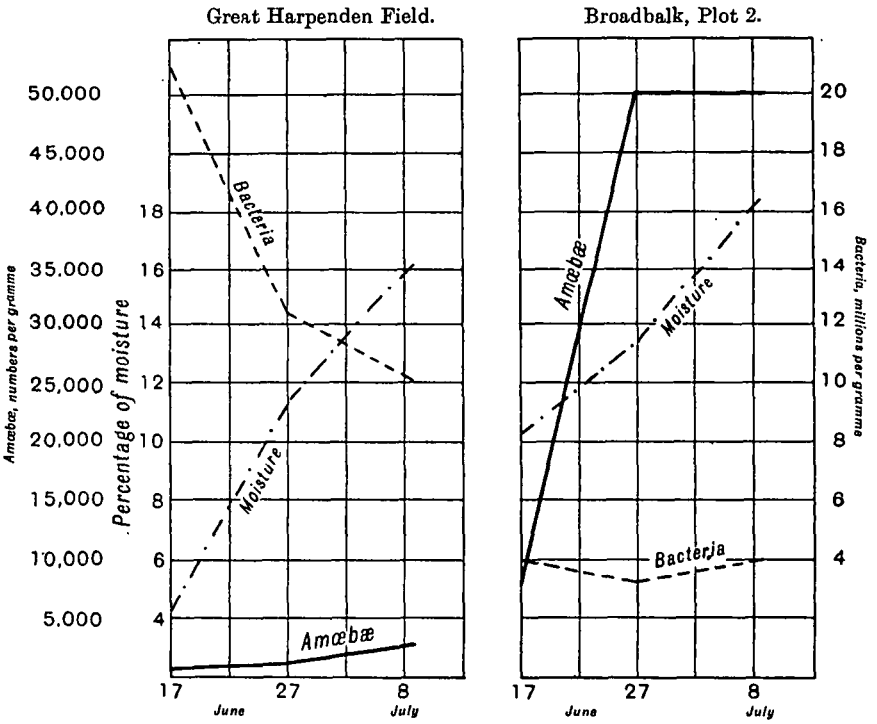

Fig. 13. Great Harpenden Field and Broadbalk, Plot 2, June 17—July 8, 1919.

Curves 14—20 *show the temperature and rainfall at various dates from May* 10, 1916 *to July* 9, 1919.

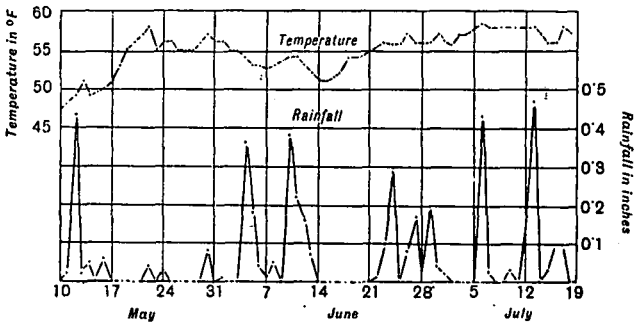

Fig. 14. May 10—July 19, 1916.

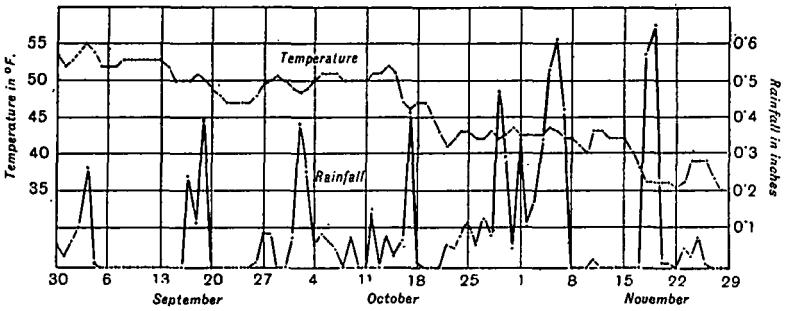

Fig. 15. August 30—November 29, 1916.

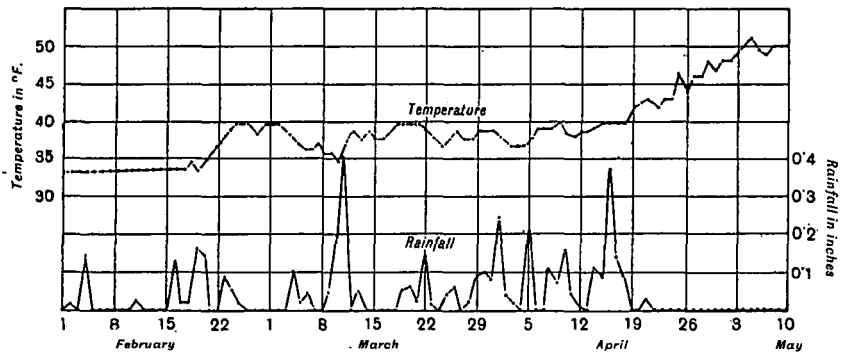

Fig. 16. February 1—May 10, 1917.

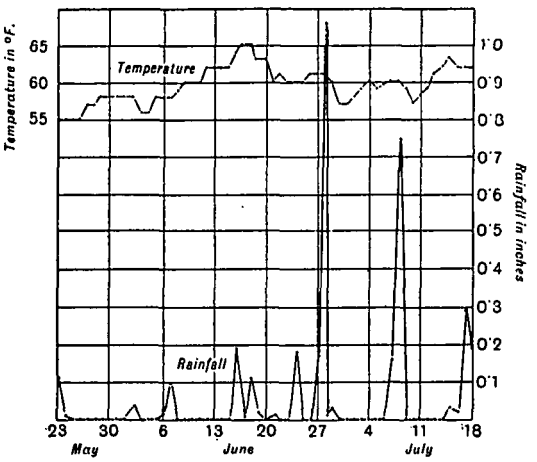

Fig. 17. May 23—July 18, 1917.

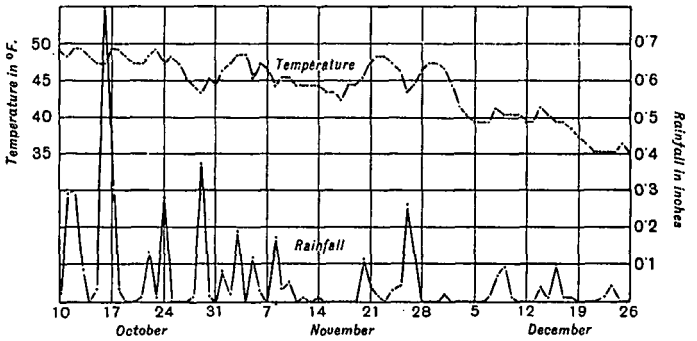

Fig. 18. October 10—December 26, 1917.

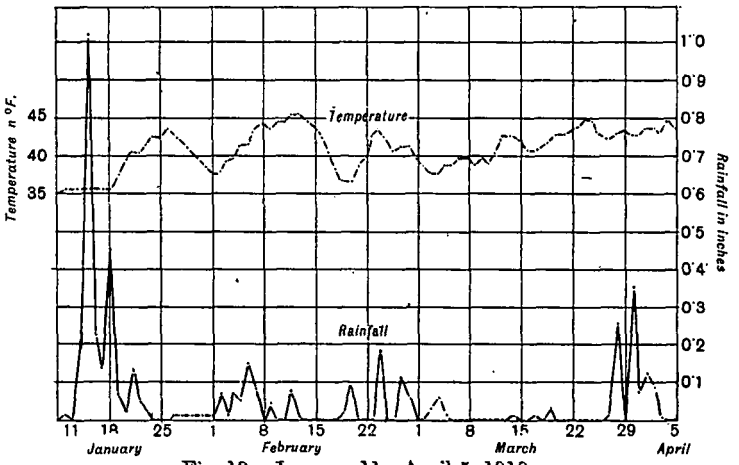

Fig. 19. January 11—April 5, 1918.

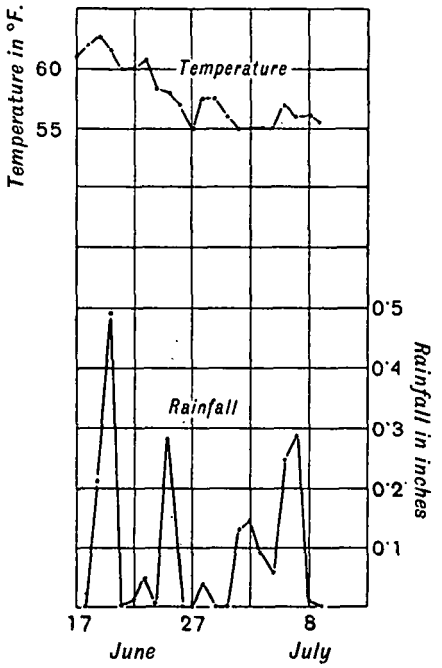

Fig. 20. June 17—July 8, 1919.

(*Received* 8*th December* 1919.)