OBSERVATIONS ON SOIL PROTOZOA.

By D. WARD CUTLER.

(Rothamsted Experimental Station, Harpenden.)

Introduction.

The conclusion drawn by Russell and Hutchinson that the protozoa resident in the soil are possibly detrimental to bacterial activity, and that the beneficial results which are brought about by partial sterilisation may in part be due to the killing of these organisms, has caused a great interest to be taken in the soil protozoa, and as a consequence, a good deal of literature has been produced by various observers. Much criticism, however, has been directed against this hypothesis, some workers denying that the protozoa have any reducing effect on bacterial numbers, others asserting that these organisms are present in the soil normally as cysts, and not in the active condition.

The method of investigation, in the majority of cases, involved inoculating some medium with soil or suspension of soil, and incubating for various periods of time. By this means it can be demonstrated that numerous protozoa exist in the soil, but little or no idea is given as to whether they are present as cysts or active forms—obviously a point of great importance in its bearing upon partial sterilisation.

Martin and Lewin(1), however, showed that there was undoubtedly a trophic fauna in the soil, but they were unable to arrive at any definite conclusion as to the numbers per gram of these forms. Goodey(2), on the other hand, concludes in the case of ciliates that cysts only are present.

A systematic account of the work on soil protozoa is given by Kopeloff and Coleman(3).

There is great need therefore of a method for isolating the protozoa directly from the soil within a short period of taking the sample: but it should not involve the use of the incubator or any apparatus likely to induce excystation of those forms which were present in the cystic state.

The present investigation deals with two problems requiring solution before a suitable method can be devised for directly counting the protozoa. Firstly, an efficient and direct method of counting the number

of organisms in a unit volume of a solution is needed. Secondly, the factors governing the relation of the protozoa to the soil particles require elucidation in order to explain why it is almost impossible to find the organisms in any quantity by direct examination under the microscope, although the same soil sample can be shown to contain tens of thousands, if a dilution method is employed.

The protozoa chosen for the experiments were obtained from Broadbalk field soil, and were as follows:

Amoebae	Flagellates
A. lawesiana, Goodey	*Monas termo*
A. glebae, Dobell	*Bodo sp.*
A. sp.	*Cercomonas sp.*
	Oicomonas sp.

No attempts were made to separate these one from another and grow them in "pure" culture. Although this course presents disadvantages, the treatment of the forms "en masse" more faithfully reproduces field conditions, as these organisms are representative of the soil protozoan fauna at Rothamsted.

The average sizes of the active and cystic states are:

Active amoebae, 12·5 μ; cystic stage 10·7 μ.
Active flagellates 8·5 μ; cystic stage 4·7 μ.

The investigation on the ciliates detailed in Part II of this paper was carried out upon *Colpoda cucullus*, which measured in the active condition about 45 μ and in the cystic one from 40–45 μ.

PART I.

METHOD FOR COUNTING PROTOZOA.

Kopeloff, Lint and Coleman(4) have described a direct method for estimating the numbers of protozoa in a suspension which does not involve plating on culture media and subsequent incubation. As this seemed satisfactory it was compared with the dilution method in use at Rothamsted.

The apparatus consists of a thick glass slide in the centre of which is a hollow of depth 0·1 mm. Round this hollow is a deep groove to receive any excess fluid that may be released when a cover-glass is placed upon the slide. The hollow in the centre of the slide is divided into 625 squares, each of which is 1/25 sq. mm.

A volume of the fluid to be examined, and sufficient in amount to ensure perfect contact between the cover-glass and slide, is placed in

the hollow, and covered by the cover-glass. The preparation is then examined under the microscope, the magnification generally being approximately 600 diameters.

The protozoa in each square are then counted. Estimates are made from five samples of each solution and the results averaged. The motility of the organism is usually insufficient to cause trouble; but if it does, the fluid is first exposed to osmic acid vapour, which kills the protozoa very rapidly. Kopeloff, Lint and Coleman also suggest a method by which the organisms may be stained and killed in one process, but this I find unnecessary.

TABLE I.

Method used for counting in a suspension Protozoa whose number per c.c. is greater than 100,000.

	Squares I	II	III	IV	V	VI	VII	VIII	IX	X	Total	Average	Total No. of Protozoa per c.c. of Suspension
Sample 1	8	6	6	8	9	8	8	7	5	4	69	6·9	1,725,000
,, 2	6	5	6	7	8	9	7	6	7	8	69	6·9	1,725,000
,, 3	8	7	6	5	7	9	8	8	6	4	68	6·8	1,700,000
,, 4	9	7	6	7	4	3	5	9	9	8	67	6·7	1,675,000
,, 5	5	8	5	7	9	8	6	8	5	8	69	6·9	1,725,000
													8,550,000

Average number per c.c. of suspension, 1,710,000.

TABLE II.

Method used for counting in a suspension Protozoa whose number per c.c. is less than 100,000.

	Total number of Protozoa for 500 sq.	Total number of protozoa per c.c. of suspension
Sample 1	9	4500
,, 2	8	4000
,, 3	9	4500
,, 4	9	4500
,, 5	9	4500
		22,000

Average number per c.c. of suspension, 4400.

Two methods were employed for calculating the results.

1. The number of protozoa in ten squares is counted and the average for one square found. As one square is 0·04 sq. mm. and the depth 0·1 mm. the cubical volume is 0·004 cu. mm. The number of protozoa per cubic centimetre of the suspension is found by multiplying the average count per square by $\frac{1000}{0{\cdot}004} = 250{,}000$.

2. The total number of protozoa in 500 squares is counted. This represents an area of 500 × 0·04, that is, 20 sq. mm., or 2 cu. mm. The factor, therefore, for estimating the number per c.c. of the suspension is 2500. The two methods give concordant results: the first should be used for suspensions containing over 100,000 per c.c.; the second when fewer are present. Two typical counts are shown in Tables I and II.

The accuracy of the results was shown by checking them by a dilution method. If these two very different methods of estimation gave comparable results it seemed justifiable to assume that they were fairly accurate.

TABLE III.

Showing the results obtained by counting Protozoa in a suspension by the direct and indirect method.

Sample	Number obtained by direct method	Number obtained by dilution method: Highest dilution in which growth occurred at end of 21 days' incubation	Number obtained by dilution method: Lowest dilution in which no growth occurred at end of 21 days' incubation
1	1,500	1,500	1,750
2	2,500	2,250	2,500
3	4,000	4,000	4,250
4	6,500	6,250	6,500
5	10,000	10,000	12,000
6	25,400	25,000	28,000
7	35,000	33,000	36,000
8	90,500	89,000	92,000
9	143,750	145,000	150,000
10	250,000	250,000	260,000
11	537,000	535,000	540,000
12	645,000	650,000	660,000
13	885,000	880,000	890,000
14	1,059,000	1,000,000	1,100,000
15	1,258,000	1,300,000	1,400,000
16	1,500,000	1,500,000	1,600,000
17	2,300,000	2,200,000	2,300,000

10 c.c. of a 1/100 dilution was made and further diluted to the necessary degrees. 1 c.c. of each dilution under investigation was then inoculated on to each of three nutrient agar plates, which were then incubated at 20° C. for 21 days, and examined at intervals. If growth of protozoa occurred on a 1/10,000 dilution plate, there must have been at least one organism to cause this growth, and hence it was assumed that there were at least 10,000 protozoa per cubic centimetre of the suspension. This method clearly gives only a minimum value, but if a series of dilu-

tions is employed varying only by small stages from one another an estimate of the numbers of protozoa can be made within narrow limits.

In Table III there are given the results obtained by the investigation of 17 suspensions, differing from one another by the degree of concentration.

Results 1–8 inclusive, by the direct method, were all obtained by counting 500 squares as described above, while the remaining results were obtained by counting ten squares and taking the average for one square.

The close similarity of the results demonstrated that the direct method was sufficiently accurate, and it was therefore employed for the work described in the second part of this paper. In order to obtain success with either method it is essential to secure uniform distribution of the organisms in the fluid. Now any large particle of a solid medium added to the suspension will render uniform distribution impossible by providing a substratum on which many of the protozoa will aggregate.

Therefore the best method of preparing the suspension is to add to the fluid successive loopfuls of the culture, each loopful being thoroughly emulsified against the side of the tube before entering the fluid. Even distribution is secured by shaking or by the successive use of a pipette.

If the organisms are found clumped together in a suspension it should be discarded.

PART II.

Factors concerned in the Relationship between the Protozoa and the Soil.

As is well known it is practically impossible to find any evidence of the presence of protozoa by direct examination of soil under the microscope, even after the necessary addition of water is made. The dilution method, nevertheless, demonstrates that these organisms are present in the soil in at least tens of thousands per gram. In a few cases protozoa have been observed by direct methods, but in numbers insignificant compared with those which must have been actually present.

Definite amounts of a suspension of amoebae and flagellate cysts were added to equal weights of different substances, the surface areas of whose particles varied one from the other, in order to test the action of these substances on the organisms.

The substances chosen were:

(*a*) Coarse sand: ignited and treated with hydrochloric acid.

(*b*) Fine sand, treated as above.

(*c*) Soil from Broadbalk wheat field.

(*d*) *Partially sterile soil* from the Broadbalk field treated for one hour with steam.

(*e*) Ignited soil.

(*f*) Rothamsted clay.

To 1 gram of each of these substances was added 2 c.c. of a suspension containing 1,645,000 amoebae and flagellate cysts per cubic centimetre. The mixtures were then gently agitated for 10 minutes, after which the solid particles were allowed to settle at the bottom of the tube, and the number of protozoa per c.c. of the supernatant fluid estimated by one of the direct methods described in Part I of this paper.

In all cases a control tube, containing the suspension but no solid matter, was tested at the end of the experiment to see whether many protozoa had sunk to the bottom of the tube: in no case was the rate of sinking sufficient to affect the experiment. As a further test, after each class of material had been investigated, the tube was vigorously shaken and another count made. In no case was there any evidence of sedimentation of the cysts apart from absorption by the solid matter.

Coarse Sand. The total number of cysts per c.c. in the supernatant fluid over the sand particles was 1,500,000: the suspension added contained 1,645,000 cysts per c.c.: the number taken up by the sand was therefore 145,000 cysts per c.c. of fluid.

Fine Sand. Under the same conditions the supernatant fluid contained 550,000 organisms per c.c.: the fine sand was therefore capable of withdrawing from the suspension 999,000 cysts per c.c.

Soil and partially sterilised soil. These two substances gave identical results; in each case 1,643,250 cysts per c.c. were taken out from the suspension.

Ignited soil. This was tried to ascertain whether the colloids of the soil were concerned in the withdrawal of protozoa from the suspension. If they are, ignition which destroys some of the colloid properties might be expected considerably to reduce the number of cysts taken up from the suspension. This actually happened, but the reduction in effectiveness was much smaller than was anticipated, for the ignited soil took up 1,501,250 organisms per c.c., or 142,000 per c.c. less than the partially sterilised or untreated soils.

Clay. In this case microscopic examination was rendered difficult by the non-settlement of the clay particles, but the estimation could still be made: 1 gram of clay withdrew from the suspension all the protozoa. A later experiment, however (Table IV), demonstrated that 1 gm.

of clay was capable of taking out of a suspension about 2,500,000 organisms per c.c.

It was often possible by careful focussing to see the cysts closely applied to the surface of the solid particles of matter. This was especially true of sand, but a similar result is obtained, though less frequently, with the varieties of soil employed.

It may be objected that during the course of these experiments many of the protozoa excysted and so caused an inaccuracy in the results. This is of course possible, and in order to test it counts were again made in the original suspension at the end of the experiment. In every case the second count was comparable with the first, the difference between the two being too small to affect the results. The following are typical of the difference in numbers obtained at these two counts: the second set of numbers is not always lower than the first, as would be the case had excystation occurred to any marked extent: the variations are within the experimental error.

At the beginning of the experiment	At the end of the experiment
550,000	560,000
885,000	880,000
1,645,000	1,650,000
1,980,000	1,990,000
2,800,000	2,775,000

In the next series of experiments the strength of the suspension was varied through wide limits. The results given in Table IV and fig. 1 show that however many flagellate and amoebic cysts are present in the suspension the number taken up by each substance is a constant, variations in different experiments being so small that they may be legitimately attributed to experimental error, and not to any variation in the power of the substances themselves. Sharp lines of demarcation exist between the various substances as regards their capacity for withdrawing protozoa from a suspension.

Experiments with Active Flagellates and Amoebae.

These experiments were carried out in nearly the same manner as the preceding, except that for greater accuracy the animals in the supernatant fluid were first killed by osmic vapour.

The results are given in Table IV: the number of active forms withdrawn from 1 c.c. of the fluid by the solid particles is similar to the number of cysts taken by the same substance.

It may be concluded therefore that the capacity of sand, soil and clay for retaining flagellates and amoebae is independent of the condition of the organisms, whether they are in the cystic or active form, but varies with the size, as experiments with ciliates demonstrate.

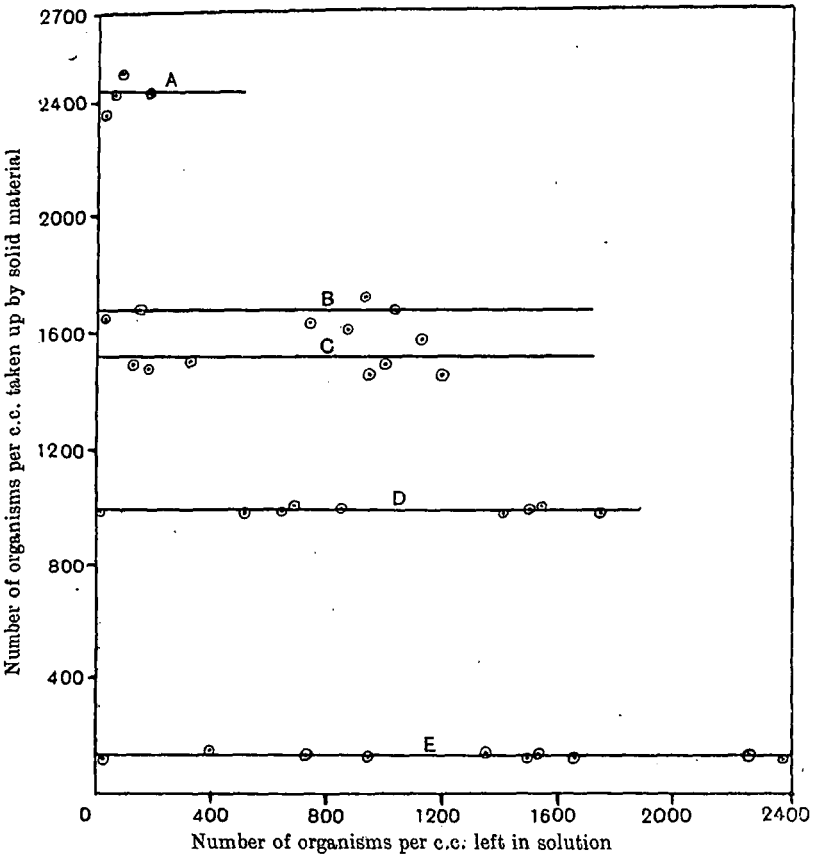

Fig. 1. Showing the number of amoebae and flagellates withdrawn from suspensions of varying strengths by the different types of solid matter. A = clay, B = untreated and partially sterilised soil, C = ignited soil, D = fine sand, E = coarse sand. Since complete retention occurs when the number of organisms added is less than the capacity of the solid matter, the first part of each of the above curves is coincident with the ordinate. The numbers of organisms are given in thousands.

Experiments with varying amounts of solid matter.

In these experiments 2 c.c. of suspension was added to weights of solid matter varying from 1–0·1 gram. Again the results demonstrate that solid matter has a specific capacity for withdrawing a definite

TABLE IV.

Cysts of Flagellates and Amoebae.

Strength of suspension per c.c.	No. per c.c. taken up by coarse sand particles, 0·1–0·2 mm.	No. per c.c. taken up by fine sand particles, 0·2–0·04 mm.	No. per c.c. taken up by ordinary soil and partially sterilised soil	No. per c.c. taken up by ignited soil	No. per c.c. taken up by clay
96,800	C*	C	C	C	C
130,000	C	C	C	C	C
155,000	142,000	C	C	C	C
550,000	150,000	C	C	C	C
885,000	148,000	C	C	C	C
1,000,000	145,000	995,000	C	C	C
1,500,000	150,000	985,000	C	C	C
1,645,000	145,000	995,000	1,643,250	1,501,250	C
1,690,000	150,000	1,008,750	1,665,000	1,498,250	C
1,832,000	146,000	1,000,500	1,686,250	1,506,250	C
2,399,999	148,000	980,000	1,637,499	1,445,000	2,368,749
2,500,000		1,000,000	1,630,000	1,500,000	2,430,000
2,656,250		1,100,000	1,740,000	1,450,150	2,456,000
2,736,250		998,542	1,687,250	1,587,000	2,550,000
		Active Flagellates and Amoebae.			
20,000	C	C	C	C	C
35,000	C	C	C	C	C
50,250	C	C	C	C	C
168,500	139,500	C	C	C	C
230,000	149,000	C	C	C	C
560,150	150,150	C	C	C	C
1,005,000	142,250	999,950	C	C	C
1,640,000	148,276	1,006,425	1,639,950	1,580,625	C
1,980,250	142,150	988,000	1,690,150	1,487,342	C
2,670,000	147,365	996,560	1,600,000	1,560,000	2,560,250
2,800,000	139,295	1,005,245	1,763,150	1,499,950	2,489,350
Experimental error	7 %	11 %	9 %	9 %	9 %

* In the above table C denotes that the supernatant fluid was devoid of protozoa and that therefore the solid matter had completely withdrawn them from the suspension.

number of organisms from a suspension. Thus when ·25 gram of either sand, soil or clay was employed there was retained only 1/4th of the number of organisms retained by 1 gram of the substance. For these results see Tables V and VI.

Effect of varying the Time Factor.

The preceding investigations were all performed with the time factor constant, which had been arbitrarily fixed at 10 minutes. In these final experiments this factor was varied. The results were not

affected, showing that the action between the surface particles and protozoa is practically instantaneous.

TABLE V.

Strength of suspension	Amount of solid material	Time of action	Coarse sand	Fine sand	Soil	Ignited soil	Clay
	gram						
550,000	1	F*	140,000	C	C	C	C
885,000	1	F	147,000	C	C	C	C
1,700,000	1	F		1,005,250	1,665,000	1,560,000	C
1,236,000	1	F	145,275	1,000,000	C		
1,837,500	1	5 min.	145,000	1,062,500	1,675,150	1,506,250	
1,235,000	1	2 min.	146,150	1,016,667	C		C
550,000 (killed)	1	5 min.	142,500	C	C		C
2,200,000 (killed)	1	7 min.	147,250	1,000,150	1,600,000	1,530,250	C
855,000 (killed)	1	1 min.	143,150	C		C	
2,700,000 (killed)	1	4 min.			1,625,350		2,535,000
2,975,000 (killed)	1	5 min.		1,100,000			2,435,150
575,000	0·2	3 min.	30,000	203,000	335,150		500
60,000	0·25	2 min.	36,500	C	C	C	C
325,000	0·25	F	35,000	230,000	C	C	C
1,300,000	0·5	F	74,250	500,000	815,000	780,000	1,150,000

* In the above table F indicates that the suspension was filtered through the solid material.

Effect of filtering suspension through soil.

The soil or sand was placed in the bulb of a 20 c.c. pipette, and the suspension allowed to filter through. Examination of the filtrate showed that the number of organisms retained by the solid matter was the same as in the experiments detailed above.

Further the results obtained by using a suspension of protozoa previously killed by heating at 80° C. for 5 minutes were identical with those obtained when the organisms were alive: the action is therefore physico-chemical and is not determined by any "vital factor."

In Table V are given in tabulated form the results obtained when the various factors described above are varied.

Experiments with Ciliates.

Both active and cystic forms of *Colpoda cucullus* were investigated. The procedure was that employed for amoebae and flagellates, except that the counting was always done by the 500 square method described

in Part I of this paper. Immediately before examination the fluid was subjected to the action of osmic vapour for a few seconds to kill the ciliates.

TABLE VI.

Strength of suspension	Amount of solid matter	Time of action	Coarse sand	Fine sand	Soil	Ignited soil	Clay
	gram						
Active ciliates.							
10,000	1	10 min.	C	C	C	C	C
15,000	1	10 min.	C	C	C	C	C
25,000	1	5 min.	C	C	C	C	C
35,000	1	F*	27,500	C	C	C	C
45,000	1	F	25,150	C	C	C	C
200,000	1	7 min.	27,000	185,000	C	C	C
400,000	1	1 min.	26,150	184,500	280,000	270,000	C
500,000	1	10 min.	28,000	190,000	280,500	270,250	450,000
45,000	0·2	5 min.	5,000	37,000	C	C	C
45,000	0·1	F		18,500	28,322	26,900	45,000
Ciliate cysts.							
5,000	1	10 min.	C	C	C	C	C
20,000	1	10 min.	C	C	C	C	C
32,500	1	5 min.	28,000	C	C	C	C
400,000	1	F	27,500	184,000	280,150	270,000	C
500,000	1	3 min.		190,000	280,000	275,250	450,000
600,000	1	F	28,000	185,150	282,000	214,250	440,150
5,000	0·1	F	2,500	C	C	C	C
20,000	0·5	5 min.	13,500	C	C	C	C
300,000	0·25	7 min.	7,000	46,500	70,000	67,250	100,000

* F indicates that the suspension was filtered through the solid material.

Experiments in which the strength of suspension, time of action, and amount of solid matter used were varied demonstrated that the different kinds of materials per gram were capable of retaining specific numbers of organisms per c.c. (see Table VI). For the sake of convenience the numbers below are given to the nearest thousand.

Coarse Sand	Fine Sand	Soil	Ignited Soil	Clay
27,000	185,000	280,000	270,000	450,000

These figures are much lower than those obtained with experiments on amoebae and flagellates, as was expected on account of the enormously greater size of the ciliate.

The ratio which one substance bears to another as regards capacity

for retaining amoebae and flagellates is practically the same as the ratio of their capacities to retain ciliates:

	Ratio of			
	Coarse Sand to Soil	Fine Sand to Soil	Soil to Ignited Soil	Soil to Clay
Amoebae and Flagellates	1: 6·7	1: 1·6	1: 1·06	1: 1·5
Ciliates	1: 6·8	1: 1·5	1: 1·04	1: 1·6

The ratio of the mean diameter of the amoebae or flagellates to that of ciliates is as 1 : 5, while the ratio of the mean volume of the amoebae or flagellates to that of the ciliates is as $1 : 5^3$. On the other hand the ratio of the holding power of the various substances used is for ciliates and amoebae or flagellates as 5 : 1 approximately. Thus the ratio of the retaining powers of the various substances is inversely proportional to the ratio of the diameter of the protozoa and to the cube root of their volumes. Some relationship between these variants seems probable, but at present it has not been discovered.

Discussion.

The foregoing results demonstrate that the factors governing the relation between soil protozoa and soil particles are largely physico-chemical and primarily of the nature of surface action. As the size of the particles diminishes so the number of protozoa retained increases, till finally 1 gram of clay withdraws 2,500,000 flagellates and amoebae from 1 c.c. of the suspension. Different types of soil probably differ in their capacities according as their content of sand or clay was high or low, for it has been shown that the results are the same if the suspension is allowed to filter through the soil as would occur in a field.

The surface action, however, between the protozoa and the soil particles appears to differ from ordinary adsorption. The action is linear up to the point when a suspension is used of a strength less than the retention capacity of the substance, then complete withdrawal of the organisms from the suspension takes place. This is in sharp contrast with adsorption, which is never complete. Also there is no similarity between a typical adsorption curve and those given in fig. 1. Nor could any be expected. Rothamsted soil is estimated to contain some 12,000 million particles per gram, possessing an area of the order of 2,500 sq. centimetres: 18 per cent. is clay with particles of 2 μ downwards; 53 per cent. is silt with particles of diameter 25–6 μ. The average diameter of the protozoa is much greater than that of the clay particles and equal or only slightly less than that of the silt particles. Thus any

attempt to regard the action between protozoa and soil particles as one of adsorption is rather hopeless, and the fact that experiment negatives such a view is not a matter for surprise.

A further point of interest at present inexplicable is that so few organisms are retained by the soil. Assuming the approximate area of 1 gram of Rothamsted soil to be 2500 sq. cm. this figure is much larger than the total area of the amoebae and flagellates retained by the soil, which is only 4·2 sq. cm. approximately. In the case of fine and coarse sand the area covered by the retained organisms is much larger, though still below what might have been expected.

Examination of the five columns in Table IV shows that as the number of particles in the material increases so also does the number of organisms taken up. It is remarkable, however, that fine sand proves so effective as compared with coarse sand, and that ignited soil has a capacity so nearly equal to that of the untreated and partially sterilised soil. The particles of fine sand appear to be of sizes most suitable for retaining the organisms. With ignited soil the power of retention is almost as great as is that of untreated soil, thus indicating, under the conditions of these experiments, that the effective agent is the surface area of the particles irrespective of their colloidal properties.

Part I of this paper showed that the dilution and direct methods are comparable. It is safe then to assume that the number of protozoa found per gram of soil by the dilution method probably represents fairly accurately the actual numbers in the soil sample. Since various observers have shown that the number of amoebae and flagellates usually present in the soil is between 10,000 and 100,000 per gram, it is evident that the number of protozoa in an average sample of soil is far less in number than the soil is capable of retaining. Russell and Golding(5) found numerous protozoa in sewage sick soils and by the use of the centrifuge they were able to obtain some of the active forms free from the soil particles. Probably the conditions were such that excessive reproduction of the protozoa occurred until the numbers were greater than the retaining power of the soil. Protozoa would then be found lying free from soil particles and would be acted upon by the centrifuge. Further investigations on these lines are in progress.

Part II of this paper demonstrates that the protozoa are normally resident on soil particles, therefore their environment may be of a different nature from that sometimes assumed. Russell and Appleyard(6) showed that the "free" air of the soil was approximately that of the atmosphere, but that there was also a second atmosphere dissolved in the colloidal

substance surrounding each particle, which was characterised by an increased percentage of CO_2 and nitrogen and the absence of oxygen. If therefore there are anaerobic protozoa in the soil, and experiments in this laboratory indicate that such is the case, this second dissolved atmosphere provides a suitable environment. Also the physical conditions of the water around soil particles may differ from those in the free spaces of wet soil: how far, however, these factors will influence the life of the protozoa requires investigation.

Finally, these experiments have a distinct bearing on the physiological condition vaguely termed "positive *thigmotaxis*," or the tendency for small living organisms to adhere to hard surfaces. This is a widespread phenomenon occurring both in plants and animals. A case is recorded by Verworn of a small ciliate—Oxytricha—which coming into contact with the egg of a rival mussel (Anodonta) remained on the surface for four hours, unable to leave it until a piece of mud drifted sufficiently near to the egg to allow escape. Jennings has also described how Paramoecia will adhere in countless numbers to a piece of filter paper introduced into the fluid in which they are living.

Also there is the well known phenomenon of the spermatozoa clustering and adhering to the egg during the process of fertilisation. This is no place to enter into the discussion of this physiological question, but it may be pointed out that the observations can be explained on surface action factors probably of a kind similar to those governing the relation between protozoa and the soil particles.

SUMMARY.

1. It has been shown that the direct counting method for soil protozoa devised by Kopeloff and Coleman for use in liquid media gives results entirely comparable with those obtained by a dilution method.

2. The factors governing the relation between the protozoa and the soil particles are those of surface action, and the capacity of various substances, sand, soil and clay, for retaining these organisms is specific and constant.

3. Coarse sand is capable of withdrawing per gram approximately 145,000 amoebae and flagellates per c.c. from a suspension of any strength. Fine sand withdraws approximately 980,000 per c.c.: soil and partially sterilised soil 1,650,000, ignited soil 1,500,000 and clay 2,450,000.

4. These figures are constant for given material and organisms and

are independent of the concentration of the suspension, the time of action, or whether the suspension contains cysts or active forms of the amoebae and flagellates investigated. Also the action is the same when the experiment is performed with a suspension of living or dead organisms.

5. Experiments with the ciliate—*Colpoda cucullus*—show that coarse sand per gram retains 27,000 per c.c.; fine sand per gram 185,000 per c.c.; soil and partially sterilised soil 280,000 per c.c.; ignited soil 270,000 per c.c. and clay 450,000 per c.c.

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