STUDIES ON SOIL PROTOZOA AND THEIR RELATION TO THE BACTERIAL FLORA. II[1]

JAMES M. SHERMAN

From the Bacteriological Laboratories of the Wisconsin Agricultural Experiment Station, University of Wisconsin

VI. THE EFFECT OF VOLATILE ANTISEPTICS UPON SOIL PROTOZOA

Introduction

It is claimed by Russell and Hutchinson and their co-workers that soils partially sterilized with volatile antiseptics are entirely freed from protozoa. Hutchinson (1913) further claims that the larger types of the soil protozoa are killed by the treatment of soil with caustic lime. On the other hand, the results reported by Gainey (1912) and by Grieg-Smith (1911) indicate that the application of such amounts of volatile antiseptics as are used in practice does not exterminate the protozoa. Even if it be acknowledged that some types of the soil protozoa are able to resist the process of partial sterilization by antiseptics, we must still consider the contention of Russell and Hutchinson that the harmful factor is inactivated for a considerable period, when not exterminated. Further, the possibility exists that the kinds of protozoa most detrimental to the bacterial flora are peculiarly susceptible to the antiseptics. Since the greater part of the protozoan fauna of the soil is inactive, the mere survival of certain types is not necessarily important, but the effect of volatile antiseptics upon the *active soil protozoa*, on the other hand, would appear significant.

Experiments

Tests were made of partially sterilized soils to determine the number of protozoa and also the types. These tests were made

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with pots each containing one kilogram of soil. The number of protozoa was determined by the dilution method, while the types were determined by the inoculation of 25 grams of soil into sterile hay infusion.

The effect of volatile antiseptics upon the active protozoa was determined by the treatment of soils with carbon bisulphide and toluene and by determining the number of protozoa one day after treatment and again after two months. The results (Table XXVI) show that the active protozoa are not exterminated and again multiply to numbers equivalent to those found in normal soils. *Monas sp.*, *Dimorpha radiata* and *Flagellate A* were all observed on the 1/10,000 dilutions after two months.

TABLE XXVI

Effect of toluene and CS_2 on the soil protozoa

POT NO.	TREATMENT	NUMBER OF PROTOZOA PER GRAM	
		1 day	60 days
1	2 per cent toluene.....	Less than 10	10,000
2	2 per cent toluene.....	Less than 10	10,000
3	2 per cent CS_2.....	Less than 10	10,000
4	2 per cent CS_2.....	Less than 10	10,000

In another experiment toluene, carbon bisulphide and chloroform were used, and samples were taken at the end of one month to determine the number and types of protozoa present. In this test it was found that the protozoan fauna had not been simplified, as far as could be noted by microscopic examination, there being present a very complex mixture of ciliates, flagellates and amoebae. At the end of a month the active protozoa were again present in just as large numbers as are found in untreated soils.

In the foregoing tests the antiseptics used were left in the soil. It was also thought desirable to treat some soils by the method followed by Russell and Hutchinson. These workers usually employed 1 per cent toluene and then after one day spread the soil out to allow the antiseptic to evaporate. Four pots of soil were treated after this manner and four other pots

TABLE XXVII

(Effect of toluene, CS_2 and $CHCl_3$ upon the soil protozoa (one month after treatment)

POT NO.	TREATMENT	PROTOZOA PER GRAM	TYPES OF PROTOZOA
1	2 per cent toluene	10,000	C. F. A.
2	2 per cent toluene	10,000	C. F. A.
3	2 per cent toluene	10,000	C. F. A.
4	2 per cent CS_2.	10,000	C. F. A.
5	2 per cent CS_2	10,000	C. F. A.
6	2 per cent CS_2	10,000	C. F. A.
7	2 per cent $CHCl_3$	10,000	C. F. A.
8	2 per cent $CHCl_3$	10,000	C. F. A.
9	2 per cent $CHCl_3$	10,000	C. F. A.

C = Ciliates; F = Flagellates; A = Amoebae.

were treated with 1 per cent toluene but not evaporated. As is shown in Table XXVIII the results after one month were similar to those obtained in the other experiments.

TABLE XXVIII

Effect of toluene left in and evaporated upon the soil protozoa (one month after treatment)

POT NO.	TREATMENT	PROTOZOA PER GRAM	TYPES OF PROTOZOA
1	Left in	10,000	C. F. A.
2	Left in	10,000	C. F. A.
3	Left in	10,000	C. F. A.
4	Left in	10,000	C. F. A.
5	Evaporated	10,000	C. F. A.
6	Evaporated	1,000	C. F. A.
7	Evaporated	10,000	C. F. A.
8	Evaporated	10,000	C. F. A.

Another test was made in which large amounts of volatile antiseptics were used in order to see if the protozoa could be entirely eliminated from soil. Soils were treated with 5 and 10 per cent of toluene and carbon bisulphide and the antiseptics left in the soil. Even with such large amounts of antiseptics the protozoa were not entirely eliminated, although the fauna was considerably simplified, especially with carbon bisulphide. As is shown in Table XXIX, ciliates, flagellates and amoebae

were found in every case except in the one treated with 10 per cent carbon bisulphide in which no amoebae were observed. In all of these soils *Monas sp.*, *Dimorpha radiata* and *Flagellates A* and *B* were present.

TABLE XXIX

Effect of large amounts of toluene and carbon bisulphide upon the soil protozoa

TREATMENT	5 PER CENT TOLUENE	10 PER CENT TOLUENE	5 PER CENT CS_2	10 PER CENT CS_2
Types of protozoa.....	C. F. A.	C. F. A.	C. F. A.	C. F.

Several attempts were made without success to demonstrate a stimulation of the protozoa, similar to that of the bacteria, subsequent to the application of volatile antiseptics to the soil. Moore (1912) in an address on the "Micro-organisms of the Soil" stated that results obtained in his laboratory indicated that the protozoa in soil not only withstood the action of antiseptics but that they might be increased by such treatment. Woodruff (1908) has shown that the multiplication of infusoria may be stimulated by small doses of alcohol. The dilution method for the determination of the number of protozoa is far too crude to measure small differences so the fact that it failed to demonstrate any increase in the number of protozoa following the application of volatile antiseptics to soil cannot be considered of much importance.

Discussion

From the results herein reported it may be concluded that volatile antiseptics in the amounts used in practice do not free the soil from protozoa. The active soil protozoa not only survive, but multiply rapidly and again attain their normal numbers, usually within a month after treatment. It is difficult to explain the failure of Russell and Hutchinson to find protozoa in the soils which they treated. They noted the survival of certain flagellates which they do not, however, associate with the "detrimental factor." The failure of these workers to find ciliates and amoebae may be due to insufficient samples. The ciliates and amoebae are greatly reduced by the treatment of

soil with volatile antiseptics; these organisms, being inactive in most soils, do not increase subsequently and so it is obviously necessary to use a larger sample in order to demonstrate their presence.

That the presence of protozoa in the partially sterilized soils used in this work was not due to contamination was shown by holding ten pots of sterilized soil under identical conditions for one month and then taking samples for protozoa. Nine of these pots were found to be free from protozoa, while the tenth contained one small flagellate.

These results argue strongly against the protozoan theory as an explanation of the phenomena of partial sterilization, but it cannot be said they positively disprove it, since, as was pointed out before, the particular kinds that are detrimental, if such exist, may be very sensitive to volatile antiseptics.

VII. EXPERIMENTS RELATING TO THE POSSIBLE EXISTENCE IN SOIL OF A HARMFUL BIOLOGICAL FACTOR WHICH IS DESTROYED BY THE ACTION OF VOLATILE ANTISEPTICS

Introduction

The experiments made in his part of the work were planned in an effort to determine whether the beneficial action of volatile antiseptics upon the soil bacteria is due to the destruction of a detrimental factor which is antagonistic to them. This problem was attacked in much the same way as was the study of the soils containing protozoa and free of protozoa (Part IV). If normal soils contain a bacterial-limiting factor while partially sterilized soils do not, it would seem that that fact could be quite definitely established by a comparison of the numbers of bacteria found in these soils when subjected to various conditions. It should also be easy to demonstrate the presence of this harmful factor by the reinfection of the partially sterilized soils with a small amount of untreated soil.

The effect of volatile antiseptics upon the subsequent development of bacteria and protozoa in soil

It was thought that some light might be thrown upon the protozoan theory by making bacterial and protozoal counts on soils subsequent to treatment with volatile antiseptics. If this theory is correct we would expect to find the greatest number of bacteria in partially sterilized soil at a time when the

TABLE XXX

Effect of volatile antiseptics upon the bacteria and protozoa in soil

POT	TREATMENT	BACTERIA PER GRAM	PROTOZOA PER GRAM
	Fifteen days after treatment		
1	Control	15,000,000	20,000
2	Control	14,500,000	20,000
3	2 per cent toluene	14,000,000	100
4	2 per cent toluene	15,000,000	1,000
5	2 per cent CS_2	13,000,000	100
	Thirty days after treatment		
1	Control	20,800,000	20,000
2	Control	20,200,000	20,000
3	2 per cent toluene	48,000,000	20,000
4	2 per cent toluene	49,300,000	20,000
5	2 per cent CS_2	44,400,000	20,000
	Forty-five days after treatment		
1	Control	17,000,000	20,000
2	Control	21,000,000	20,000
3	2 per cent toluene	45,000,000	20,000
4	2 per cent toluene	46,000,000	20,000
5	2 per cent CS_2	110,000,000	20,000

protozoa are depressed. This, however, does not appear to be the case as is shown by the following experiment. Determinations were made of the numbers of bacteria and protozoa in treated and untreated soils at intervals of fifteen days after treatment. The results of this test are given in Table XXX.

This table shows that the maximum number of bacteria is not found while the protozoa are depressed, but rather that

the development of the two classes of micro-organisms subsequent to treatment with volatile antiseptics runs parallel. This experiment was verified by another test in which normal and carbon bisulphide-treated soils were compared. In this test (Table XXXI) the number of bacteria in the treated soil rose above that of the control soil by the fifteenth day, but at this period the protozoa in the treated soil had also returned to their normal level. It is seen also that the number of bacteria continued to increase after the protozoa had again become as numerous as in untreated soil.

TABLE XXXI

Effect of CS_2 upon the bacteria and protozoa in soil

POT	TREATMENT	FIFTEEN DAYS		THIRTY DAYS	
		Bacteria	Protozoa	Bacteria	Protozoa
1	Control.....	23,000,000	10,000	60,000,000	20,000
2	2 per cent CS_2.....	94,000,000	20,000	240,000,000	20,000

The reinoculation of partially sterilized soils

In their work at the Rothamsted Station Russell and Hutchinson (1913) claim to have demonstrated that the soil contains a detrimental factor since the bacterial content of partially sterilized soil may be reduced by reinoculation with untreated soil. It is pointed out that when soil treated with a volatile antiseptic is reinoculated with 5 per cent of untreated soil the number of bacteria is reduced, while if only 0.5 per cent of normal soil is added no such reduction takes place. These observations are explained by the assumption that when only 0.5 per cent of untreated soil is added the harmful factor is not transmitted, but when 5 per cent is used for the inoculum the treated soil again becomes infected with the undesirable group of organisms. The soundness of this view may certainly be questioned, as it is difficult to understand why it should be necessary to use such a large amount of untreated soil in order to insure the presence of a factor which is supposed to exist in amount sufficient to suppress the bacteria. A review of the work of Russell

and Hutchinson reveals the fact that in some of the tests the treated soils which were reinoculated with 5 per cent of untreated soil did not show an appreciable depression in the number of bacteria, and they qualify their conclusion on this point with the statement that, "the harmful factor is not invariably transmitted to the same extent from the untreated to the partially sterilized soil and in a few cases indeed it is not transmitted at all."

In the experiments which were carried out in this laboratory the partially sterilized soils were reinoculated with 1 per cent of untreated soil; since at least 1 kgm. of soil was used in each pot the inoculum never consisted of less than 10 grams of normal soil. It could hardly be doubted that this amount of soil would be sufficient to transplant the group of organisms, if such exist, which act as a limiting factor upon the bacterial flora.

The work which has been done on the reinoculation of partially sterilized soils (Tables XXXII to XXXIV) fails to give any indication that a harmful factor is thus introduced. It would appear, on the other hand, that if reinfection of the treated

TABLE XXXII

Effect of reinoculation of treated soil with untreated soil (treatment of 2 per cent toluene)

POT	TREATMENT	NUMBER OF BACTERIA PER GRAM		
		30 days	60 days	90 days
1	Control	56,000,000	80,000,000	69,000,000
2	Control	66,000,000	75,000,000	62,000,000
3	Reinoculated	57,000,000	82,000,000	79,000,000
4	Reinoculated	62,000,000	100,000,000	92,000,000

TABLE XXXIII

Effect of reinoculation of treated soil with untreated soil (treatment 1 per cent toluene: evaporated)

POT	TREATMENT	NUMBER OF BACTERIA PER GRAM	
		15 days	30 days
1	Control	149,000,000	95,000,000
2	Control	127,000,000	81,000,000
3	Reinoculated	152,000,000	130,000,000
4	Reinoculated	178,000,000	92,000,000

TABLE XXXIV

Effect of reinoculation of treated soil with untreated soil (treatment 2 per cent CS_2)

POT	CONTROL	AVERAGE	REINOCULATED	AVERAGE
1	273,000,000	255,300,000	247,000,000	392,000,000
2	218,000,000		317,000,000	
3	285,000,000		422,000,000	

Incubation period after reinoculation: 2 months.

soil has any effect it is to increase the number of bacteria rather than to decrease it. However, the data on this point are doubtless within the boundaries of experimental error. It is difficult to reconcile these findings with the theory of Russell and Hutchinson.

The number of bacteria in partially sterilized and normal soils at different temperatures

One of the strongest points in the evidence produced by Russell and Hutchinson to prove that the soil contains a harmful biological factor was the difference in the behavior of untreated and partially sterilized soils when incubated at different temperatures. Their results indicated that the maximum development of bacteria in the untreated soil was at low temperatures (5° to 12° C.), while in treated soil the greatest number was found at 20°C., and at 30°C. there was a marked increase over that found at 12°C.—the maximum in the case of the untreated soil. This phenomenon they claim shows that the bacteria under normal conditions are limited by the detrimental factor and that their maximum development takes place under conditions unfavorable for the harmful factor.

This point has been tested by the comparison of toluened and normal soils at 10°, 22°, and 37°C. The treated soil used had been treated with 2 per cent toluene three months previously. These soils were incubated for one month at their respective temperatures and then sampled and their bacterial counts determined. The results are given in Table XXXV.

TABLE XXXV

The number of bacteria in treated and untreated soils at different temperatures

TREATMENT	NUMBER OF BACTERIA PER GRAM		
	10° C.	22° C.	37° C.
Untreated	21,000,000	23,000,000	22,000,000
2 per cent toluene	64,000,000	49,000,000	36,000,000

These data are not sufficient to base any conclusions upon but it can not be said they indicate very much, either in favor of the protozoan theory or against it. It will be seen that the greatest difference in the numbers of bacteria in the treated and untreated soils was at 10°C., a point not in favor of the protozoan theory. On the other hand, the least difference was found at 37°C., which point may support the theory of Russell and Hutchinson.

It was decided to carry out another experiment at 37°C. in order to throw more light on this point. Instead of using soils which had been previously treated, the soils were first placed at 37°C. and allowed to incubate at that temperature for one month. Half of them were then treated with 2 per cent carbon bisulphide. If the protozoan theory is correct the antiseptic should have very little effect at this high temperature. One month after treatment bacterial counts were made. The results obtained are given in Table XXXVI.

TABLE XXXVI

Effect of CS_2 upon the number of bacteria in soil at 37°C.

POT	NUMBER OF BACTERIA PER GRAM			
	Untreated	Average	2 per cent CS_2	Average
1	21,000,000	21,000,000	208,000,000	228,000,000
2	21,000,000		248,000,000	

The results are very striking; a difference of over ten fold in the number of bacteria in the treated and untreated soils being found. This observation indicates strongly that the beneficial action of volatile antiseptics in soil is not to be explained by

its effect upon the protozoa. Soil extract and hay extract cultures made from untreated soil and incubated at 37°C. have failed entirely to reveal the presence of any of the active types of protozoa which have been mentioned as especially abundant in soil. In such cultures only a very few types of protozoa appear at all and these only slowly and in small numbers.

The number of bacteria developing in sterilized soils reinoculated with untreated and with partially sterilized soils

The preceding experiments appear to demonstrate quite conclusively that the beneficial effect of volatile antiseptics in soil is not due to the destruction of a biological factor, unless it be assumed that the treatment of soil so changes it that the harmful organisms are no longer able to develop in it, even though it is reinoculated with them. An experiment was planned in order to see if this explanation is a true one. Two pots of sterile soil were inoculated with 1 per cent of normal soil, while two other pots were inoculated with 1 per cent of a soil which had been treated with 2 per cent toluene. In case the antiseptic really destroys a harmful factor that fact should be indicated by a much greater number of bacteria in the soils inoculated with the treated soil. This result, however, was not obtained; on the contrary, the counts made at thirty and forty-five days after inoculation showed no practical difference between the numbers of bacteria in the two soils, as is shown in Table XXXVII.

TABLE XXXVII

The number of bacteria developing in sterilized soils inoculated with normal and with toluened soils

POT	NUMBER OF BACTERIA PER GRAM			
	35 days		45 days	
	Normal	Toluened	Normal	Toluened
1	127,000,000	112,000,000	126,000,000	102,000,000
2	208,000,000	148,000,000	110,000,000	104,000,000
Average.....	190,000,000	130,000,000	118,000,000	103,000,000

The effect of carbon bisulphide on the number of bacteria in sterilized soils reinoculated with normal soil and with protozoa-free soil

Another experiment performed in order to detect the presence of the "harmful factor" was to inoculate soils sterilized by steam with normal soil and with the protozoa-free soil described in an earlier part of this paper. These soils were allowed to stand three weeks and were then treated with 1 per cent of carbon bisulphide. Bacterial counts were made before the soils were treated and then at fifteen and thirty days after treatment. According to the phagocytic theory, it would be expected that the number of bacteria in the soil inoculated with normal soil would subsequently be greatly increased while the soils inoculated with the protozoa-free soil should not be appreciably affected.

As in the previous experiments, the results of this test give no indication that there exists in soil a biological factor which is harmful to the bacterial flora. It will be seen upon examination of Table XXXVIII that the soils free of protozoa and those containing protozoa behaved in exactly the same way.

TABLE XXXVIII

Effect of CS_2 upon sterilized soils inoculated with normal soil and with protozoa-free soil

POT	NUMBER OF BACTERIA IN MILLIONS PER GRAM					
	Before treatment		15 days		30 days	
	Without protozoa	With protozoa	Without protozoa	With protozoa	Without protozoa	With protozoa
1	178	120	228	140	166	109
2	172	110	182	142	144	91
Average.....	175	115	205	141	155	100

The results of this test add further weight to the preceding experiments all of which point to the non-existence of a detrimental biological factor in soil. The fact that volatile antiseptics have no appreciable effect in soils which have been sterilized by steam and then reinoculated with normal soil would appear to indicate that the beneficial effects derived by the use of these

substances are due to some action of the antispetics on the soil itself rather than to a simplification of its micro-organic population.

VIII. RÉSUMÉ

Discussion

The results of the foregoing experiments appear to establish quite definitely that protozoa in the soils which have been studied do not have a detrimental effect upon the bacterial flora. It is difficult to see how the action of an important phagocytic agent could have escaped detection by the methods employed unless the factor is unable to increase in soils which have been previously sterilized with steam or partially sterilized with volatile antiseptics when again introduced into these soils with an inoculum of normal soil. This restricted power of growth would be very different from the properties of micro-organisms in general, either of animal or plant nature, and there is no evidence, as far as we are aware, that a group of organisms with such peculiar characteristics exists in the soil. As has been pointed out, the soil protozoa, at least those types which appear in liquid cultures, grow better in soil which has been previously subjected to steam sterilization just as do the bacteria. Aside from the evidence that soil does not contain a biological factor which is inimical to the bacterial flora, the facts that volatile antiseptics do not exterminate the soil protozoa, and that partial sterilization of soil under conditions unfavorable for the action of protozoa (e.g., at 37°C.) is followed by the characteristic rise in the number of bacteria, would appear to cast serious doubt upon the theory of Russell and Hutchinson as an explanation for the effect of volatile antiseptics upon the soil bacteria.

Cunningham (1914) has recently published the results of some work which he thinks proves that protozoa act as a limiting factor upon the bacterial flora in soil. The fact that his data on this point are derived from only two experiments, one of which gave negative results, would preclude his conclusions

from very serious consideration. A study of the methods he used indicates, however, that the difference found in the soils with and without protozoa might have been due to a difference in the complexity of the two flora, as was the case in the experiments reported in Part IV (see Table XI) of this paper. In fact, his manner of attack was very similar; sterilized soils were employed as a substratum, and inoculations were then made into these soils of cultures containing protozoa and free of protozoa. "One flask was inoculated with bacteria plus protozoa from a culture of protozoa from soil, the other received as nearly as possible an equal inoculation from the same culture of bacteria alone." It is not clear from this statement how he obtained the bacterial culture free from protozoa, but it is very certain that a protozoa-free culture could not be obtained which would contain as complex a bacterial flora as did the original culture from which it was derived. As was previously pointed out, a difference in the complexity of the bacterial flora in different soils may cause a great disparity in the counts obtained by the plate culture method. This fact was apparently not recognized by Cunningham as he concluded that "the reduction in bacterial numbers in the soils inoculated with protozoa is very marked and lies well outside the limits of experimental error." A review of the data in Part IV of this paper will show, on the contrary, that his results may fall well within the limits of experimental error.

It is believed that the conclusions drawn from the work herein reported will hold in general for the cultivated soils in this country, but it is not desired to make too broad an application of them. Many of the "sick" soils which have been studied at the Rothamsted Experimental Station are very different from the ordinary American soil. Martin and Lewin (1914) describe a sick cucumber bed which was made up of one part of light pasture soil, one part of heavy pasture soil and two parts of horse manure, and had an optimum moisture content of 62 per cent. The assumption that the biological conditions in such a soil are the same as in the average soils of the United States (which contain about 2 per cent organic matter and the optimum

moisture content of which is from 16 to 18 per cent) would be obviously unwarranted. That a difference in the micro-fauna does exist under various soil conditions is indicated by the fact that Martin and Lewin have found amoebae to be the predominating types of protozoa in the soils they have studied, which are very rich in organic matter, while the results reported here, as well as the data obtained by Cunningham on German soils, indicate that the flagellates occur in greater numbers than do the amoebae. It appears possible that in the rich soils and green-house beds, which have been studied extensively at the Rothamsted Station in connection with soil sickness, there might be a phagocytic agent which is not active in ordinary soils. This possibility, however, should not make us unmindful of the fact that no direct evidence has as yet been produced which indicates that such a factor exists in any cultivated soil. It should also be remembered that the beneficial effects of partial sterilization of soil—for the explanation of which the protozoan theory was advanced—have been observed in all localities in which the problem has been studied and in nearly all types of soil.

The question of the activities of the protozoa which lead an active existence in soil is a problem upon which much work could profitably be done. The active protozoa which occur in soils in large numbers certainly have functions there, some of which in fact may be very important. It is not desired to give the impression that because the protozoa which have been studied do not exert a limiting action on the bacteria in soil that it is thought that they do not ingest bacteria at all. Some in all probability do not, while others (e.g., *Monas*) it would appear undoubtedly do. Why active protozoa which feed upon bacteria should not cause a measurable decrease in the number of bacteria in soil is difficult to explain. It would seem that the excretory products of the protozoa which feed upon the soil bacteria would increase the amount of available energy for the rest of the bacteria so that a condition of metabiosis would be established which might offset the antagonistic action of the protozoa. This hypothesis does not appear unreasonable when it is remembered that the chief limiting factor upon the bacteria

in the soil is the food supply. In liquid cultures, on the other hand, the limiting factor is not the food supply but the accumulation of detrimental by-products; the number of bacteria soon reaches its maximum and then begins to decline gradually. It can readily be seen that if predatory protozoa are added to liquid cultures, in which the bacterial flora is in a comparatively inactive condition due to the presence of harmful by-products, a very striking reduction in bacterial numbers will be noted. Whatever the effect of protozoa on bacteria in solutions may be the results herein reported appear to indicate that under ordinary conditions they are not able to limit the bacterial flora when acting in soil.

Summary

1. Determinations made by means of the dilution method indicate that the normal fertile soil has a protozoan content approximating 10,000 per gram.

2. In the soils studied the flagellates were the predominating type of protozoa and not the ciliates nor amoebae.

3. *Colpoda cucullus* appears to be the most widely distributed ciliate in soil and is occasionally found in numbers approximating 1,000 per gram.

4. Certain of the soil flagellates are active in soils of normal, and even subnormal, moisture contents.

5. Tests made with the ciliates *Colpoda cucullus, Balantiophorus elongatus* and *Oxytricha sp.* show that these organisms are not active under ordinary soil conditions.

6. *Colpoda cucullus* is probably active whenever the moisture content is much above normal, but not under ordinary conditions of moisture.

7. Active soil protozoa attain greater numbers when inoculated into previously sterilized soil than in normal soil.

8. Sterile soils when inoculated with normal soil and with an artificial soil culture which is free of protozoa show a difference in the total number of bacteria as determined by the plate culture method, due to a difference in the complexity of the two flora.

9. A great difference may exist in the number of bacteria as determined by the plate culture method, due to a difference in the complexity of the flora, between soils which are free of protozoa.

10. Experiments with soils containing protozoa and free of protozoa showed that the bacterial flora in the two soils behaved in exactly the same way when exposed to different conditions of temperature and moisture content.

11. The data obtained indicate that soil does not contain a biological factor which is harmful to bacteria.

12. Pure culture tests with the ciliates, *Colpoda cucullus* and *Balantiophorus elongatus*, showed that these organisms are very detrimental to bacteria in solutions. In soil, since the ciliates are inactive, they are unable to affect the bacterial flora.

13. Pure culture tests with four types of active soil flagellates showed that these organisms were not capable of limiting the number of bacteria when acting in soil. One of the cultures, however, had a very marked limiting action upon the bacteria when tested in soil extract.

14. Treatment of soil with the ordinary amounts of volatile antiseptics (1 to 2 per cent) does not appear to simplify the protozoan fauna. A complex mixture of ciliates, flagellates and amoebae is to be found in cultures made from soils partially sterilized with volatile antiseptics.

15. As much as 10 per cent of carbon bisulphide and toluene when added to soil fails to exterminate the protozoa entirely.

16. The active soil protozoa which are at first suppressed by treatment with volatile antiseptics soon begin to multiply so that they are again found in numbers equal to those of untreated soil within one month after treatment.

17. The maximum number of bacteria in partially sterilized soil is not found while the protozoa are suppressed but after they have again returned to their normal level. It appears that the development of these two classes of micro-organisms subsequent to treatment with volatile antiseptics runs parallel.

18. The reinoculation of partially sterilized soils with 1 per cent of normal soil fails to decrease the number of bacteria.

19. The treatment of soil with carbon bisulphide at 37°C. gives a very marked increase in the number of bacteria in the soils treated.

20. Sterilized soils which are reinoculated with normal soil and with partially sterilized soil show no essential difference in the numbers of bacteria which develop.

21. When volatile antiseptics are applied to sterilized soils reinoculated with and without protozoa no difference is to be noted between the behavior of the bacteria in the different soils.

22. No evidence has been obtained which indicates that the beneficial effect of partial sterilization is due to the elimination of a biological factor which is harmful to the bacteria.

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