DISCUSSION AND CORRESPONDENCE

THE COMPLEXITY OF THE MICROORGANIC POPULATION OF THE SOIL

DURING the last few years a series of experiments have been carried out in this laboratory by Dr. Hutchinson and myself[1] which we can only interpret as showing that bacteria are not the only active inhabitants of the soil. The results in our view point conclusively to the presence of another group of organisms, detrimental to bacteria and differing from them by their larger size, slower rate of multiplication under soil conditions. and lower power of resistance to heat and antiseptics. They are, therefore, more readily killed than bacteria, and we regard their suppression as an important factor in bringing about the increased bacterial activity known to set in after soil has been partially sterilized or treated in any other way detrimental to active life. Such properties as we could ascertain agree with those of protozoa; we were thus led to look for these organisms in the soil and found numbers of them. We adduced reasons for provisionally identifying the detrimental organisms with the soil protozoa.

Recently several papers have been published in the United States controverting these conclusions. We are not satisfied, however, that the criticisms affect the validity of our arguments, and therefore desire to set out briefly the experimental facts and the conclusions we draw from them. The actual data are to be found in our papers in the *Journal of Agricultural Science;* many of the figures were also presented to the Graduate School of Agriculture at East Lansing last July.

1. We begin with the fact that partial sterilization of soil, *i. e.,* heating it to a temperature of 60° C. or more, or treatment for a short time with vapors of antiseptics such as toluene, causes first a fall and then a great rise in bacterial numbers. The rise sets in soon after the antiseptic has been removed and the soil conditions once more made favorable for bacterial development; it goes on till the numbers far exceed those present in the original soil.

2. Simultaneously there is a considerable increase in the accumulation of ammonia. This sets in as soon as the bacterial numbers begin to rise, and the connection between the two quantities is normally so close as to indicate a causal relationship; the increased ammonia production is, therefore, attributed to the increased numbers of bacteria. There is no disappearance of nitrate; the ammonia is formed from organic nitrogen compounds.

3. The increase in bacterial numbers is the result of improvement in the soil as a medium for bacterial growth and not an improvement in the bacterial flora. Indeed the new flora *per se* is less able to attain high numbers than the old. This is shown by the fact that the old flora when reintroduced into partially sterilized soil attains higher numbers and effects more decomposition than the new flora. Partially sterilized soil *plus* 0.5 per cent. of untreated soil soon contains higher bacterial numbers per gram and accumulates ammonia at a faster rate than partially sterilized soil alone.

4. The improvement in the soil brought about by partial sterilization is permanent; the high bacterial numbers being kept up even for 200 days or more. The improvement, therefore, did not consist in the removal of the products of bacterial activity, because there is much more activity in partially sterilized soil than in untreated soil. Further evidence is afforded by the fact that a second treatment of the soil some months after the first produces little or no effect.

It is evident from (3) and (4) that the factor limiting bacterial numbers in ordinary soils is not bacterial, nor is it any product of bacterial activity, nor does it arise spontaneously in soils.

5. But if some of the untreated soil is introduced into partially sterilized soil the bacterial numbers, after the initial rise (see (3)), begin to fall. The effect is rather variable, but is usually most marked in moist soils that have been well supplied with organic ma-

[1] *Journal of Agricultural Science,* 1909, 3: 111–144; 1912, 5: 27–47, 86–111; 1913, 5: 152–221.

nures; *e. g.*, in dunged soils, greenhouse soils, sewage farm soils, etc. Thus the limiting factor can be reintroduced from untreated soils.

6. Evidence of the action of the limiting factor in untreated soils is obtained by studying the effect of temperature on bacterial numbers. Untreated soils were maintained at 10°, 20°, 30° C., etc., in a well moistened aerated condition, and periodical counts were made of the numbers of bacteria per gram. Rise in temperature rarely caused any increase in bacterial numbers, sometimes it had no action, often it caused a fall. But after the soil was partially sterilized the bacterial numbers showed the normal increase with increasing temperatures. Similar results were obtained by varying the amount of moisture but keeping the temperature constant (20° C.). The bacterial numbers in untreated soil behaved erratically and tended rather to fall than to rise when the conditions were made more favorable to trophic life; on the other hand, in partially sterilized soil, the bacterial numbers steadily increased with increasing moisture content. Again, when untreated soils are stored in the laboratory or glasshouse under varying conditions of temperature and of moisture content the bacterial numbers fluctuate erratically; when partially sterilized soils are thus stored the fluctuations are regular.

7. When the curves obtained in (6) are examined it becomes evident that the limiting factor in the untreated soils is not the *lack* of anything[2] but the *presence of something active.*

8. This factor, as already shown, is put out of action by antiseptics and by heating the soil to 60° C., and once out of action it does not reappear. Less drastic methods of treating the soil put it out for a time, but not permanently: *e. g.*, heating to 50°, rapid drying at 35°, treatment with organic vapors less toxic than toluene (*e. g.*, hexane), incomplete treatment with toluene. In all these cases the rise induced in the bacterial numbers per gram is less in amount than after toluene treatment and is not permanent; the factor sets up again. As a general rule, if the nitrifying organisms are killed, the limiting factor is also extinguished; if they are only temporarily suppressed the factor also is only put out for a time.

9. The properties of the limiting factor are: (*a*) It is active and not a lack of something (see (7)); (*b*) it is not bacterial (see (3) and (4)); (*c*) it is extinguished by heat or poisons and does not reappear if the treatment has sufficed to kill sensitive and non-spore-forming organisms; it may reappear, however, if the treatment has not been sufficient to do this; (*d*) it can be reintroduced into soils from which it has been permanently extinguished by the addition of a little untreated soil; (*e*) it develops more slowly than bacteria and for some time may show little or no effect, then it causes a marked reduction in the numbers of bacteria, and its final effect is out of all proportion to the amount introduced; (*f*) it is favored by conditions favorable to trophic life in the soil.[3]

10. We see no escape from the conclusion that the limiting factor is a living organism. We were, therefore, led to search for organisms not bacteria, slower growing, less resistant and larger. Protozoa naturally suggested themselves. We soon found numbers of ciliates, amœbæ and flagellates and induced Mr. Goodey to study them in detail. This work is still continuing, and promises highly interesting results: some remarkable forms have been picked out, and it is already evident that the zoological survey of the soil will be a prolonged business but will be eminently worth while. The ciliates and amœbæ are killed by partial sterilization. *Whenever they are killed the detrimental factor is found to be put out of action,* the bacterial numbers rise and maintain a high level. Whenever the detrimental factor is not put out of action the protozoa are not killed. To these rules we have found no exception. Some exceptions have been found to the converse proposition,

[2] The soils we used included fertile loams well supplied with organic matter, calcium carbonate, phosphates, etc.

[3] This is dealt with fully in the *Journal of Agricultural Science,* 1912, 5: 27, 86.

i. e., we have sometimes found ciliates and amœbæ in soils in which the detrimental factor had been put out of action, but our present methods do not enable us directly to discriminate between protozoan cysts and active forms, nor to estimate the numbers present, nor, on the other hand, to determine how completely the detrimental factor is put out of action. But in general the parallelism between the detrimental factor and the soil protozoa is so complete that we are justified in provisionally regarding protozoa as the detrimental organisms we have been seeking.

Such is a short statement of the main lines of the work. I have omitted the subsidiary issues: the vain search for bacteriotoxins,[4] for evidence of bacterial stimulus, of improvements in the bacterial flora, etc. The identification of the detrimental organisms with the soil protozoa is provisional only; in the nature of the case a rigid proof would be very difficult even if it were possible.

I now turn to some of the criticisms that have been passed on this work by my American colleagues. Dr. Jacob G. Lipman at the New Jersey Station, in conjunction with Messrs. Blair, Owen and McLean, carried out some experiments,[5] the results of which they consider to be in direct opposition to ours. They added pasteurized and untreated soil infusions respectively to mixtures of dried blood and sterilized soil (heated under a pressure of 1.5 atmospheres of steam). After seven days the pasteurized infusions had induced the formation of no more ammonia than the untreated infusion. These results, they say, "do not bear out Russell and Hutchinson's contention as to the part played by protozoa in depressing the activities of soil bacteria."

[4] This result is not necessarily in contradiction with those obtained by the Bureau of Soils. I understand that Dr. Schreiner's toxin is obtained from badly drained, badly aerated soils deficient in calcium carbonate: our soils, on the other hand, were well drained, well aerated and well supplied with calcium carbonate.

[5] Bull. 248. 1912.

The argument is ingenious, but it does not appear to us to bear on the question. In the first place, soil sterilized by heating under a pressure of 1.5 atmospheres has undergone very considerable decomposition. We have obtained evidence that such highly heated soil is altogether different from normal soil as a medium for the growth of microorganisms. Failure of protozoa to develop in the highly heated soil would be no evidence at all of their inability to develop in ordinary soil. As a matter of fact the nitrifying organisms do not seem to have developed; would Dr. Lipman argue that the results "do not bear out the usual contention as to the part played by the nitrifying organisms in the soil"? Secondly, even if the detrimental organisms could develop in highly heated soil they were not given the chance: we have never observed any development in anything like so short a period as seven days, our experiments have always been continued much longer. Lastly, the action of the detrimental organisms is to keep down the *numbers* of bacteria. Now the rate of ammonia production is not necessarily a measure of bacterial numbers and therefore affords no rigid test of the activity of the detrimental organisms.

Dr. G. E. Stone, of the Massachusetts Experiment Station,[6] who has had great experience of soil sterilization and informs us that he has "experimented with practically everything there is in this line," is convinced that protozoa "have little or no part in accounting for the increased number of bacteria in our soils." The evidence is based on some experiments by Messrs. Lodge and Smith. Decoctions were made of untreated soil and of soil heated for 45 minutes to 250° F.; into each of these decoctions soil bacteria were introduced. Greater bacterial development occurred in the decoction of the sterilized soil than in the decoction of the untreated soil. (A subsoil behaved differently.) The authors state that protozoa were absent and that the results must be due to other causes. With this I entirely agree; a decoction of a highly

[6] Twenty-fourth Annual Report, 1912.

heated soil is manifestly very different from a decoction of untreated soil; it contains much larger quantities of dissolved substances and may be expected to behave differently as a medium for bacterial development. The experiment proves conclusively that heating a soil to 250° F. causes decomposition, but I can not see that it helps us to find out what is going on in an unheated soil. The authors go on to say that protozoa are "uncommon in their soils" and "very few forms were found." It would be interesting to find what is the difference between their soil conditions and those at Michigan where Dr. Rahn[7] found protozoa of the same types occurring in numbers of the same order per gram as we find at Rothamsted.

Professor G. T. Moore, writing in SCIENCE,[8] disagrees wholly and absolutely with our work; indeed he thinks that in the tangled maze of microbiological problems "the one fact which does seem to be fairly well established is that the temporary removal from the soil of the protozoa has but little bearing on the problem." We should not feel that we had lived in vain if we had merely been the humble instruments by which such a proposition was established, but again we are not satisfied as to the evidence. Professor Moore asserts that soil protozoa are not killed by toluene, carbon disulphide, etc., but are only temporarily depressed, and after three days their numbers may equal or even exceed those originally present. Never on any occasion have we observed anything of this kind.

In an admirable paper[9] on the effects of heat on the soil Drs. Seaver and Clark attribute to us the claim that the increased productiveness of heated soils is due to the destruction of protozoa. We wish to point out that we have always regarded the destruction of detrimental organisms as only one factor in the case, and have fully recognized the effects of the decomposition brought about by the heat. In order to minimize these decomposition effects we generally treat our soils with vapors of antiseptics rather than by heat, but here also we do not lose sight of the possibility of other changes being induced besides the destruction of life.

Finally, we may be allowed to remind the reader that the adverse effect of our detrimental organisms is on the numbers of bacteria, but that the relationship of bacterial numbers to soil fertility is by no means simple. Fertility is determined by any of the factors capable of limiting plant growth. In some soils it may be the supply of phosphates, of potash, of water that is inadequate; if so, soil bacteria may show little or no connection with fertility. Only when the supply of nitrogen compounds becomes a limiting factor do the soil bacteria come in, and even then the relationship between their numbers and their activity is not quite straightforward. We have traced out this problem in detail in our paper in the *Journal of Agricultural Science*, 1913, p. 152.

We do not underrate the complexity of soil fertility problems and, above all, we do not assert that our destructive organisms are the only things involved in the matter, but we do claim that they are an important factor. Our only hope of getting any further with the complex problems of the soil is to study the factors one at a time. We must not be confused by the circumstances that other factors remain to be studied, nor, on the other hand, must we lose sight of the possibility that these other factors may vitiate some of our experiments.

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[7] *Centr. Bakt. Par.*, 1913, 36: 419–421.
[8] November 8, 1912.
[9] *Biochemical Bulletin*, 1912, 1: 413.

TWO ADDITIONS TO THE MAMMALIAN FAUNA OF MICHIGAN

THE northern pine vole, *Microtus pinetorum scalopsoides* (Audubon and Bachman) has apparently not been recorded from Michigan, and up to last year no Michigan specimen had been secured by the museum. In April, 1912, a specimen (No. 42,558, Museum of Natural History, University of Michigan) was taken by W. A. Brotherton, near Rochester, Oak-