THE SELECTION OF BACTERIAL FOOD BY SOIL AMOEBAE, AND THE TOXIC EFFECTS OF BACTERIAL PIGMENTS AND OTHER PRODUCTS ON SOIL PROTOZOA.

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It has been shown that soil protozoa, especially amoebae, do not feed indiscriminately on any bacteria (Singh, 1941*a*, *b* ; 1942*a*, *b*). In a preliminary survey (Singh, 1942*b*) of the edibility of miscellaneous bacteria (including plant pathogens) mostly from soil a negative correlation was observed between edibility and pigment formation. A suggestion† was made (Singh, 1942*b*) that pigment formation by bacteria exerts a protective action against protozoal attacks.

An attempt has been made in the present work outlined to find out what determines whether a bacterial strain is eaten by amoebae, and to what extent amoebae are sensitive to certain metabolic products of other organisms.

A few scattered accounts of the toxic action on protozoa of the metabolic products of bacteria, and of pigments among them, are found in the literature. Thus Birch-Hirschfeld (1934), Chatton, E., and Chatton, M. (1927*a*, *b*), Cleveland (1928), Kidder and Stuart (1939) and others have carried out some inconclusive experiments to show that bacterial pigments and metabolic products of bacteria are toxic to protozoa. Chatton and Chatton (1927*a*, *b*) claim that the chromogenic bacteria *Ps. fluorescens* and *Chr. prodigiosum* exert a cytolytic effect on a number of ciliates. Although they noted that this effect was most marked in highly pigmented strains, they drew the conclusion that pigment itself was not toxic ; it was a combination of glucoside and lipoid that exerted the toxic effect upon the ciliates. They also found that saline extracts from *Ps. pyocyanea* (agar cultures) exerted cytolytic action on different ciliates. Birch-Hirschfeld (1934) obtained similar results with alcoholic extracts. They did not try to separate the various metabolic products from *Ps. pyocyanea*, and study their toxic action on protozoa.

Kidder and Stuart (1939) on the other hand, working with a ciliate (*Colpoda* sp.), claim that it is the pigment of *Chr. prodigiosum*, *Chromobacterium violaceum* and *Ps. pyocyanea* that are toxic; their conclusions are in agreement with the work carried out by the writer, but their experiments are inconclusive. They did not extract the pigments from the bacteria ; they claim that when healthy ciliates and their cysts are put into heavy suspensions of *Chr. prodigiosum* and *Chr. violaceum*, the protozoa take up the colour diffusing from the bacteria.

Recently Schoental (1941) has isolated three antibacterial substances from *Ps. pyocyanea* cultures : (1) Pyocyanin, the blue pigment which appears in the young cultures during the maximal growth of bacteria. (2) A yellow pigment appearing in

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old cultures which was found to be a derivative of pyocyanin and identified with α-hydroxyphenazine. (3) An almost colourless, chloroform-soluble bacteriolytic substance. She has compared the antibacterial activities of these substances with other disinfectants, and their action on the growth of fibroblasts in tissue culture.

1. REACTIONS BETWEEN AMOEBAE AND BACTERIA IN PURE MIXED CULTURE.

Methods.

The protozoa used in this work were two unidentified species of soil amoebae (Singh, 1941*a*). They were originally derived from single individuals, and were grown in pure culture with a species of Aerobacter as food. They are small amoebae 10–20μ long having no flagellate stage, and they are not identifiable by the characters of their cysts. In testing the availability of bacterial food the method of radiating streaks was used (Singh, 1941*a*). Sixty-three strains of bacteria* isolated from soil were used. In addition the following pigmented species, obtained from the National Collection of Type Cultures, Lister Institute, were tested : *Chromobacterium violaceum* (2537), *Micrococcus sulphurens* (1631), *Sarcina lutea* (611), *Pseudomonas pyocyanea* (1999), *Micrococcus roseus* (2683), *Torula rubra* (2627), *Chromobacterium prodigiosum* (2302), *Chromobacterium viscosum* (2416) and *Sarcina aurantiaca* (952). Other forms included two other strains of *Chr. prodigiosum* (2881, 2881C), another strain of *Ps. pyocyanea* (2650), a red bacterium (5654). The characters of bacteria 2881 and 5654 are given by Singh (1941*a*).

Throughout the experiments 1 per cent agar containing 0·5 per cent NaCl was used. This medium checks the growth of bacteria. The temperature of incubation was 20°–21° C., and bacterial cultures of the same age (2–7 days) were always used to compare their edibility by amoebae.

Results.

The bacteria tested fall into three groups ; some of them are completely eaten (readily or slowly), others are partly eaten for a few days, after which the amoebae encyst or die, while the rest are either inedible or are eaten very slightly on rare occasions. The two species of amoebae resemble one another closely in their reactions, differing only in the case of seven of the white or yellow bacteria. These are only partly eaten by the large amoebae, while they are eaten slowly but completely by the small one.

Table I shows the relation between pigment formation by bacteria and the feeding reactions of the two amoebae ; among a total of 56 colourless strain 71 per cent were eaten ; among 32 yellow, orange and brown 75 per cent proved suitable. One of the pink strains (*Micrococcus roseus*) was edible, while of the remainder red, violet, blue, green and fluorescent organisms were not eaten. Some among them also have an inhibitory effect on the amoebae, for when such species as *Chromobacterium violaceum* or *Chr. prodigiosum* are mixed with a strain that is readily eaten and inoculated with amoebae on non-nutrient agar plates, the amoebae in most cases die out within seven days without apparently destroying any of the bacteria.

Oehler (1916, 1924*a*, *b*) claimed that amoebae showed a preference for Gram-negative bacteria ; the results for 92 strains given in Table II show that no such correlation exists, as was pointed out from less extensive earlier data (Singh, 1941*a*).

* These bacteria were kindly given to me by Miss L. M. Crump. They comprise dominant and rare species of soil bacteria isolated from various soils. The details of their morphological and physiological characters will be published separately by Miss Crump.

TABLE I.—*Showing the Relation between Pigment Production by Bacteria and the Feeding Reaction of Two Amoebae.*

Bacteria.	Partly or completely eaten.	Not eaten.	Total.
Colourless	40	16	56
Yellow	16	7	23
Orange and brown	8	1	9
Red	—	6	6
Pink	1	2	3
Violet blue	—	2	2
Green (*Ps. pyocyanea*)	—	2	2
Fluorescent	—	1	1

(Among the 102 bacterial strains included in Table I, 29 were recorded in an earlier paper (Singh, 1942*b*); of these 17 were colourless, 7 yellow, and 5 red or violet.)

TABLE II.

	Partly or completely edible.	Inedible.	Total.
Gram-negative species	31	16	47
Gram-positive species	30	15	45

2. TOXIC EFFECTS OF BACTERIAL PIGMENTS AND OTHER METABOLIC PRODUCTS ON SOIL PROTOZOA.

The protozoa used were two amoebae previously described, a flagellate, *Cercomonas crassicauda*, and a ciliate, *Colpoda steinii*.

The species of inedible pigmented bacteria can be classified into two groups:

(1) Those that produce pigments readily soluble in water.

(2) Those whose pigments are only partly soluble or are insoluble in water.

The toxic effects of the metabolic products of the following inedible pigmented bacteria were studied: *Pseudomas pyocyanea* 2 strains, *Chromobacterium prodigiosum* 3 strains, *Chromobacterium violaceum* 1 strain, and a red bacterium (5654).

Ps. pyocyanea.

This produces water-soluble pigments. It was grown on nutrient agar for 3–7 days to allow the pigment to diffuse through the agar, and the bacteria were then scraped off. The agar was then melted and poured into a petri-dish. One or two loopfuls of edible bacteria growing on nutrient agar (2–7 days old) were spread on this agar in the form of a disc or " bacterial circle " and amoebae were inoculated in the centre of them. The amoebae died without destroying the edible bacteria, and no cyst formation could be observed. If the amoebae were inoculated into closely parallel streaks (Singh, 1941*a*) of *Ps. pyocyanea* and of an edible organism on nutrient agar, the amoebae placed in the latter streak died or encysted without destroying the edible organism in appreciable amount. Thus it is clear that a diffusible toxic substance is produced by *Ps. pyocyanea*.

Extraction of crude pyocyanin.

Ps. pyocyanea (strain 2650) was generally used because it produces more pyocyanin than strain 1999. It was grown on glycerol-peptone-agar medium in 30 to 40 boiling tube slope cultures. The medium had the following composition : Glycerol 2·5 per cent, peptone 1·0 per cent, agar 1·5 per cent. It gave a profuse formation of pyocyanin and practically no fluorescent pigment. Crude pyocyanin was extracted by chloroform from cultures about 14 days old. Chloroform was left for several hours in the cultures with an occasional shaking till nearly all the blue colour was extracted. The solution was poured, the chloroform removed by evaporation and the pigment redissolved in distilled water. This was cleared by centrifugation and finally evaporated slowly to obtain crude pyocyanin.

From this dry substance solutions of known strength were made to test the toxic effect of the crude extract on protozoa. It is not pure pyocyanin because it contains a light tea-coloured substance (to be described later), and probably α-hydroxyphenazine and other substances. The solution was blue and had a pH of about 7·6.

Toxic effect of the crude extract of pyocyanin on soil protozoa.

The toxic effects of the extract from cultures of various ages were not studied separately.

TABLE III.—*Showing the Toxic Effect of the Crude Extract of Pyocyanin on Soil Protozoa.*

Pyocyanic extract.	Big amoeba.	Small amoeba.	*Colpoda steinii.*	*Cercomonas crassicauda.*
Dilution :				
1/1000	Dead 1–2 hrs.	Dead 1–3 hrs.	Dead 1 hr.	Dead 1–3 hrs.
1/2000	,, 3–6 ,,	,, 3–8 ,,	,, 2–4 hrs.	,, 2–5 ,,
1/4000	,, 12–18 ,,	Mostly dead in 48 hrs.	,, 24 ,,	,, 24 ,,
1/8000	,, 48 ,,	Mostly alive after 48 hrs.	No effect	,, 24 ,,
1/16,000	No effect	No effect	,,	Mostly dead 48 hrs.

The effect of the crude pyocyanin extract is given in Table III. The experiments were carried out in hollow-ground slides in moist chambers, and normal NaCl was used to make the necessary dilutions. The experiments were performed in duplicate, and controls were always kept in which protozoa were suspended in normal NaCl. The medium containing protozoa also contained a little of the edible bacterium, and so it is possible that the bacteria inactivated the pyocyanin to some extent, but from the point of view of comparing the toxic effects of the various substances this is immaterial because the conditions were the same in all the experiments. The toxic effects of this substance on different numbers of protozoa were not tried ; in all the experiments large numbers of protozoa were always used. To make sure that pyocyanin extract contained nothing toxic coming from the medium used to grow the cultures of *Ps. pyocyanea*, glycerol-peptone-agar was extracted with chloroform for the same period as was used in the extraction of pyocyanin. After evaporating the chloroform a little brown oily or fatty substance was left behind. This was insoluble in water and had no toxic effect on the protozoa.

The cultures were microscopically examined at various intervals of time.

From Table III it is clear that the small amoeba is more resistant to the action of the crude extract of pyocyanin than the large one; the flagellates are more easily killed than either the ciliates or the amoebae. When the crude extract of pyocyanin is autoclaved at 15 lb. pressure for 15 minutes its toxicity is to a great extent destroyed (Table V).

TABLE IV.—*Showing the Toxic Effect of the Chemically Pure Pyocyanin on Soil Protozoa.*

Chamically pure pyocyanin.	Big amoeba.	Small amoeba.	*Colpoda steinii.*
1/1500	Dead 3–4 hrs.	Dead 3–6 hrs.	Dead 2–4 hrs.
1/2000	,, 6–10 ,,	,, 8–24 ,,	,, 6–8 ,,
1/4000	Some alive 24 hrs.	No effect 24 hrs.	,, 24 ,,
1/8000	No effect	No effect	No effect

TABLE V.—*Showing the Toxic Effect of Autoclaved Crude Pyocyanin (15 lb. pressure for 15 minutes) on Soil Protozoa.*

Autoclaved pyocyanin extract.	Big amoeba.	Small amoeba.	*Colpoda steinii.*	*Cercomonas crassicauda.*
1/750	Mostly dead 48 hrs.	Some dead 48 hrs.	Dead 6–24 hrs.	All dead 48 hrs.
1/1000	Ditto	Ditto	Dead in 48 hrs.	Mostly dead 48 hrs.
1/2000	Mostly alive 48 hrs.	No effect	Movement slowed down 48 hrs.	No effect
1/4000	No effect	,,	No effect	,,
1/8000	,,	,,	,,	,,

Toxic effect of chemically pure pyocyanin.

Pyocyanin chloride was dissolved in distilled water, and was roughly adjusted to the same pH as the crude extract (pH 7·6). The solution of pyocyanin is red when it is acid and turns blue when alkaline. Table IV shows the toxic effect of pure pyocyanin on two species of amoebae, and on *Colpoda steinii*. A comparison of Tables III and IV makes it clear that pure pyocyanin is less toxic to various protozoa than is the crude extract; it is less toxic to the small amoeba than to the large one, as was the case with the crude extract.

Toxic effect of the light tea-coloured liquid present in the watery solution of the crude pyocyanin extract.

When a watery solution of the crude extract is passed through an L5 Chamberland bacterial candle, the blue pigment is adsorbed by the candle and a light tea-coloured liquid with a pH of about 4·6 is obtained. The blue pyocyanin passes through the candle if a solution of 5 per cent. KH_2PO_4 is added. The toxic effect of the tea-coloured liquid on protozoa was tested after adjusting its pH to roughly 7·6 (the same pH as the crude extract). It is toxic to amoebae, flagellates and ciliates. The toxicity of this liquid is increased nearly twice by autoclaving it at 15 lb. pressure for 15 minutes; no visible change takes place in the colour after autoclaving. As the amount of

liquid obtained was so small it was not possible to find its nature, and its effect at various dilutions could not be studied. When diluted two or three times with water it still retains its toxic action. If differs in its action from pyocyanin in the following respects :

(1) Its effect on *Colpoda steinii* is somewhat different, these ciliates remaining oval after death and did not become rounded as they do in the presence of pyocyanin.

(2) Its toxicity is increased by autoclaving and not reduced.

Toxic effect of α-hydroxyphenazine on soil protozoa.

As α-hydroxyphenazine is only slightly soluble in water a saturated solution was made, and the strength of the solution was found to be nearly 1/2000. The pH was adjusted to 7·6 (the same as the crude extract), and the colour of the solution was

TABLE VI.—*Showing the Toxic Effect of α-Hydroxyphenazine on Soil Protozoa. As α-Hydroxyphenazine is Partly Soluble in Water, its Effect at Lower Dilutions could not be Tested.*

α-Hydroxy-phenazine.	Big amoeba.	Small amoeba.	*Colpoda steinii.*	*Cercomonas crassicauda.*
1/2500	Mostly alive 24 hrs.	Mostly dead 10–24 hrs.	Dead 9–12 hrs. ; burst 24 hrs.	Some dead 24 hrs.
1/3000	As above	As above	Dead 9–18 hrs.	Few dead 48 hrs.
1/4000	Some dead 48 hrs.	Dead 48 hrs.	Dead 12–24 hrs.	No effect 48 hrs.
1/8000	No effect 48 hrs.	No effect 48 hrs.	No effect 48 hrs.	Ditto

yellow. Table VI shows the toxic effect of α-hydroxyphenazine on the protozoa. By comparing Tables IV and VI it appears that the chemically pure pyocyanin is slightly more toxic than α-hydroxyphenazine. A strict comparison between the toxicity of the light tea-coloured substance and the other substances obtained from the cultures of *Ps. pyocyanea* could not be made owing to the difficulty of getting them in sufficient amount.

It is interesting to note that α-hydroxyphenazine is more toxic to the small amoeba than to the large amoeba, and the flagellates seem to be more resistant than amoebae to the action of this substance. This is contrary to what was found in the case of the crude extract and the chemically pure pyocyanin. (Compare Tables III, IV and VI.)

In addition to pyocyanin and α-hydroxyphenazine Schoental (1941) isolated a third product, an almost colourless chloroform-soluble, bacteriolytic substance. This has not yet been tested on protozoa.

Toxic effect of the fluorescent pigment on soil protozoa.

For the production of fluorescent pigment *Ps. pyocyanea* (Strain 2650) was grown in flasks containing the liquid media of Nicolle and Zia Bey (1896) and Turfitt (1936, 1937). In all these media there was a good yield of the fluorescent pigment, but occasionally traces of pyocyanin were also formed. After two weeks' growth the

medium was evaporated and the pyocyanin was removed by chloroform, in which the fluorescent pigment is insoluble. This fluorescent pigment was toxic to amoebae and flagellates. The small amoeba is more resistant to the action of this pigment than the large one, and the flagellates are more easily killed than the amoebae or the ciliates. This compares with the toxic effect observed in the case of pyocyanin.

Chr. prodigiosum (*Strains* 2881, 2881*C* and 2302).

This bacterium and the others to be described produce pigment only slightly soluble in water or insoluble.

When amoebae are inoculated in the centre of circles consisting of highly pigmented strains of *Chr. prodigiosum* on non-nutrient agar plates they are unable to move far from the place of inoculation, and within a short time begin to look unhealthy and rounded. Finally they die or encyst without apparently destroying this bacterium. To demonstrate the toxic effect of the pigment the following experiments were made : *Chr. prodigiosum* was grown on the surface of nutrient agar blocks in boiling tubes for 2 weeks at 25° C. Very little diffusion of the pigment through the agar was seen. Thin slices were cut from the top of the solid agar and melted and poured in petri dishes. Bacterial circles of an edible bacterium were made on this agar and amoebae were inoculated in the centre. The amoebae died in a few days. This effect is only seen in the agar block within ½ in. or less from the top. When slices from ½ in. and downwards were used, the amoebae could feed and multiply on an edible species of bacterium. Thus it seems that the slight diffusion of the pigment makes the agar toxic for protozoal growth within ½ in. It may be that the production of exotoxin and not the pigment is responsible for the death of the amoebae, or both the exo-toxin and pigment may be responsible for the toxicity of the agar.

To rest this point *Chr. prodigiosum* (Strains 2881, 2881C and 2302) was grown on the following medium : Agar 15 g., mannitol 10 g., K_2HPO_4 0·5 g., $MgSO_4$ 0·2 g., NaCl 0·2 g., $CaCO_3$ 3 g., $CaCl_2$ 0·2 g., yeast water 100 c.c. and tap water 900 c.c. Strains 2302 and 2881 completely lost their pigment in several sub-cultures and 2881C became pale pink. These strains were spread on 1 per cent. non-nutrient agar in the form of radiating streaks (Singh, 1941*a*), and amoebae were inoculated in the centre. Strain 2881C (pale pink) is partly eaten by both the amoebae ; 2881 is also partly eaten (the small amoeba does better on this strain than the large one), and 2302 is slowly and completely eaten by the small amoeba and partly by the large one. When the non-pigmented strains were sub-cultured on nutrient agar and they remained non-pigmented, the amoebae could still eat them partly or completely as described before.

Finally the pigment was extracted with absolute alcohol from a large number of cultures on nutrient agar. The pigment, which is very insoluble in water, was dried and crushed in a glass bacterial mill with a few drops of water and several loopfuls of an edible bacterium (N16 (i)). The thick paste thus obtained was spread in the form of circles on non-nutrient agar and amoebae were inoculated in the centre. The small amoeba was able to eat the edible bacterium partly within a period of 7 days and finally encysted or died. All of the large amoebae died without destroying the bacteria in appreciable numbers.

When amoebae, flagellates and ciliates were placed in heavy suspensions of *Chr. prodigiosum* (2881) in normal NaCl, no toxic effect was observed within 48 hours. No healthy individuals or healthy cysts could be seen taking a pink colour as claimed by Kidder and Stuart (1939). *Colpoda steinii* could eat the bacteria slightly, and a few reproductive cysts were formed. The slight pink colour in the cytoplasm of the ciliates was due to the accumulation of bacteria and not to the diffusion of the pigment.

When protozoa were put in NaCl solution containing finely divided suspension of the pigment, no toxic effect was observed within a period of 48 hours. This is probably due to the fact that the pigment is very insoluble in water.

Bacterium 5654.

No diffusion of exo-toxin through nutrient agar could be demonstrated in the case of this bacterium, as the pigment is insoluble in water. When amoebae were placed in the centre of bacterial circles on non-nutrient agar, they died or encysted within a day without moving far from their place of inoculation. The behaviour of the extracted pigment with absolute alcohol was the same as in the case of *Chr. prodigiosum.*

Chromobacterium violaceum (2537).

When amoebae were inoculated in the centre of bacterial circles consisting of *Chr. violaceum* on non-nutrient agar, they moved in all directions and appeared healthy for several hours, but within a day or two they died. No exo-toxin production on nutrient agar could be demonstrated by this bacterium. The pigment extracted with absolute alcohol is very slightly soluble in water. The toxic effect of the extracted pigment was studied in the same way as in the case of *Chr. prodigiosum.* In the presence of crushed pigment the amoebae ate the edible bacteria very slightly within a period of 3–4 days and then died or encysted. The cultures were examined up to 8 days, and in no case were the edible bacteria consumed. The toxic effect was more marked in the case of the big amoeba than in the small one.

When protozoa were suspended in normal NaCl containing the crushed pigment of *Chr. violaceum* in hollow-ground slides, the protozoa were killed within a day or longer, depending on the concentration of the pigment. In very few amoebae could the pigment be observed in the cytoplasm.

When amoebae, flagellates and ciliates were placed in a thick suspension of *Chr. violaceum* in normal NaCl, no apparent effect could be seen in the small amoebae up to 24 hours, but the others were killed (the flagellates within 5–12 hours, the ciliates within 1–2 hours and the big amoeba within 4–10 hours). The ciliates could be seen actively eating this bacterium, but soon became rounded and motionless and finally burst. No staining of the cytoplasm of healthy protozoa or healthy cysts could be seen as claimed by Kidder and Stuart (1939).

3. EFFECT OF CERTAIN SUBSTANCES PRODUCED BY FUNGI (PENICILLIC ACID, CITRININ AND Ca SALT OF PENICILLIN) ON SOIL PROTOZOA.

The effect of these substances on protozoa has not been studied in detail, but it may be of interest to record a few observations. Penicillic acid and citrinin at about neutral pH have a toxic effect on amoebae, flagellates and ciliates up to a dilution of 1/1500, while the Ca salt of penicillin seems to have no toxic effect on protozoa, even in strong concentrations. A ciliate (*Colpoda steinii*) could feed on edible bacteria and reproduce in the presence of strong concentrations of the Ca salt of penicillin.

Effects produced on protozoa by toxic secretions.

In the presence of toxic substances, amoebae and flagellates became rounded and finally burst. The contractile vacuoles in the amoebae were very much enlarged, after a time depending on the concentration of the toxic substances, and cease to function.

In many amoebae no contractile vacuole can be seen in the rounded individuals; probably they burst before the cell wall bursts. The amoebae and flagellates stop their movement some time before the contractile vacuole begins to swell. The ciliates gradually become rounded and motionless in the case of most of the toxic substances tried, and no cilia can be seen in the rounded individuals; they too finally burst.

DISCUSSION.

The effect that micro-organisms and their by-products have on one another are varied and complex; it is, moreover, becoming increasingly evident that they are of great practical interest to mankind; this is true in many industrial processes, in the soil, in animal tissues, and hence in the treatment of disease. The interactions may be due to straightforward competition between organisms with the same needs occupying the same habitats, or to the fact that one group of organisms prey upon the other, or to the effect that the by-product of one may have upon the existence of the other. It is the last type of interaction, demanding close collaboration between biochemists and biologists for its understanding, which is of the greatest interest from all points of view. The reactions of free-living amoebae to various types of bacteria serve to emphasize the complicated nature of these problems, for although at first sight it may seem here to be a clear case of the relation between predator and its prey, it is well known that amoebae are not nourished indiscriminately by all bacteria, though the reasons underlying their apparent choice of food are as yet by no means clear. It has been shown (Singh, 1941*a*, *b*; 1942*a*, *b*) that certain types of pigments and exo-toxin production give a satisfactory explanation of the causes of inedibility in some of the bacterial species. The writer could not find in a large number of bacteria any particular character, such as Gram staining, motility, presence of proteolytic ferment, etc., correlated with inedibility to amoebae. It may be possible that some of the inedible species in whose association the amoebae remain alive for 7–10 days (like nodule organisms (Singh, 1942*b*)) without apparently destroying the bacteria are not digested by the amoebae. Thus protozoa may, in such cases, die or encyst owing to starvation.

During the last several years the therapeutic value of the substances produced by micro-organisms has attracted much attention of the various workers. It has been shown that there is a great possibility that some of the substances produced by micro-organisms may have a chemotherapeutic value. Unfortunately the effects of these substances have not been investigated on protozoa in detail. The study of the chemistry of certain types of pigments which have been found to be toxic to soil protozoa may lead to the discovery of substances of therapeutic importance in the cure of certain diseases caused by pathogenic protozoa.

As protozoa and other micro-organisms live in close association in intestines of human beings and animals, a detailed study of their inter-relationships, along similar lines as has been carried out by the writer, may lead to the discovery of organisms whose metabolic products may have a curative value in protozoal diseases. Moreover, the encouragement of such organisms in their natural habitat may have a protective value against the disease caused by intestinal protozoa.

Some earlier work at Rothamsted showed that the fluctuations in bacterial numbers have no correlation with moisture content, temperature or other physical conditions of soil up to a considerable limit. Thus the dominance of certain bacterial species or groups must be due to their successful competition with other micro-organisms. A detailed knowledge of the causes of dominance of bacterial species in various soils would be of considerable importance to agriculture. Amoebae may be an important

factor in bringing about qualitative changes in the bacterial flora of soil, which is more important than the quantitative changes in the bacterial population. This problem is being studied by the writer.

SUMMARY.

(1) Amoebae are extremely selective in the type of bacteria that they will eat. Bacteria fall into three groups in this respect: (*a*) those that are edible (partly or completely), (*b*) those that are inedible but whose presence in the environment is not otherwise harmful to protozoa, (*c*) those whose presence alone or in a mixture with edible bacteria is definitely toxic to protozoa.

(2) In a survey of 103 miscellaneous bacteria mostly from soil, those producing red, violet, blue, green or fluorescent pigment were inedible to the protozoa.

(3) Various methods have been devised to show that bacterial pigments of *Chr. prodigiosum*, *Chr. violaceum* and a red bacterium (5654) are toxic to protozoa.

(4) Metabolic products (pyocyanin, crude extract, α-hydroxyphenazine, tea-coloured liquid, fluorescent pigment) of *Ps. pyocyanea* are toxic to soil amoebae, flagellates and ciliates.

(5) The calcium salt of penicillin is not toxic to protozoa even in strong concentrations, while penicillic acid and citrinin seem to be toxic up to a cencentration of 1/1500.

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