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THE EFFECT OF BACTERIAL PRODUCTS ON AMOEBIC GROWTH

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(With Four Text-figures.)

The interrelationships that exist between various groups of Protozoa in nature and the bacteria which occur in the same environments are undoubtedly of more than academic interest. This is especially the case perhaps in soil, where both types of organisms are present in very large numbers, and where a marked degree of activity is evidenced by the continual fluctuation to which these numbers are subject. The food relationships of one member of the soil population, the amoeba *Hartmanella hyalina*, was described in a previous paper (2), where it was shown that certain kinds of bacteria afforded a more efficient food supply than others as judged by the reproductive rate of the amoeba. *A priori* this might have been due to the fact that the bacteria giving a high reproductive rate to the amoebae were of greater food value than the others; or that the by-products formed by the less valuable bacterium in the course of growth were deleterious in their effects; but preliminary experiments indicated that for the species of bacteria concerned it was a true food value effect and not one caused by the presence of any by-products.

The type of problem is seen in the following inconclusive experiment where single amoebae (*Hartmanella hyalina*) from a pure culture with "YB"[1] bacteria were isolated into soil extract to which approximately equal numbers of bacteria of four kinds were added. The bacteria selected were "YB," "SE,"[1] *B. prodigiosus* and *B. fluorescens liquifaciens*. After 24 hours' growth the reproductive rates of the amoebae were very different in the four cases (Table I). The reproductive rate for any time is calculated from the formula $\frac{\log B - \log A}{\log 2}$: where A is the number at the beginning and B that at the end of the period.

Table I. *Average reproductive rate of* Hartmanella hyalina, *single-cell cultures, with four species of bacteria in* 24 *hours.*

	"YB"	"SE"	*B. prodigiosus*	*B. fluorescens liquifaciens*
Average reproductive rate	3·24	1·68	0·5	1·75
No. of cases	4	4	4	4

[1] For description of these bacteria see Cutler and Crump (1927, p. 157).

It is impossible to say whether the "YB" bacteria alone provided a really adequate food supply, or whether the other three types in varying degrees produced conditions inhibiting the reproduction of the amoebae. Since in all cases a certain number of "YB" bacteria are present, carried over with the single amoeba, it is probable that at least in the case of *B. prodigiosus* there was a definite check, other than lack of food, on the growth of the amoebae. On the other hand, further experiments have shown that by continuous subculturing it is possible to keep *Hartmanella* alive in pure culture with *B. prodigiosus*[1]. If amoebae that have been grown with *B. prodigiosus* and "YB" respectively are isolated into soil extract containing on the one hand *B. prodigiosus* and on the other "YB," the results suggest that continuous growth with the former has produced a tolerance to the otherwise unsuitable bacterium (Table II).

Table II. *Average reproductive rates of single-cell cultures of* Hartmanella hyalina *with* B. prodigiosus *and* "YB" *in* 24 *hours.*

	Parent grown with	
	B. prodigiosus	"YB"
Subculture fed with "YB"	3·56	3·93
,, ,, *B. prodigiosus*	2·09	1·15

In the experiments so far quoted the bacterial numbers were not counted; but in all parallel experiments the initial numbers were the same and the reproductive rates are therefore comparable.

If growth products are responsible for the lowering of the reproductive rate of the amoeba a similar effect should be obtained by using a filtrate made from a culture of the appropriate bacterium. Previous work with the bacterium "SE" and *B. mycoides* showed that this only obtained when old cultures were used; further work with *B. prodigiosus* has given the same result (Table III). In this experiment

Table III. *Average reproductive rate of* Hartmanella hyalina *in* 48 *hours in presence of filtrates from bacterial cultures.*

	Parent *B. prodigiosus* Fed *B. prodigiosus*		Parent "YB" Fed "YB"	
	Filtrate "YB"	Filtrate *B. prodigiosus*	Filtrate "YB"	Filtrate *B. prodigiosus*
Average reproductive rate in 48 hours	5·25	6·19	7·89	7·45
No. of cases	5	5	5	6

amoebae from two pedigree strains of *Hartmanella*, one with *B. prodigiosus* and one with "YB," were isolated into soil extract containing 50 per cent. filtrate of each of the two species of bacteria prepared from young cultures. Each subculture so

[1] In an earlier paper (2) this was stated to be impossible; experiments in that case were carried out on nutrient agar.

made was fed with the type of bacterium to which it was accustomed. From the results it would appear that the effect of the filtrate was negligible compared with that produced by the direct feeding.

There is, however, the further possibility that though a filtrate from a growing culture gave no effect, yet one obtained from disintegrated bacterial cells would give a positive result. *B. mycoides* was chosen to test this point, the cells being thoroughly ground in a glass bacterial mill with hay infusion and the resulting suspension filtered. At this time a culture of *Hartmanella* was not available, but another common soil amoeba (*Naegleria gruberi*) was used. The active forms of these two species are almost indistinguishable, although the cysts are different, and the latter reproduces more slowly than the former. For example, with similar bacterial feeding the average reproductive rate in twenty-four hours for eight single-cell

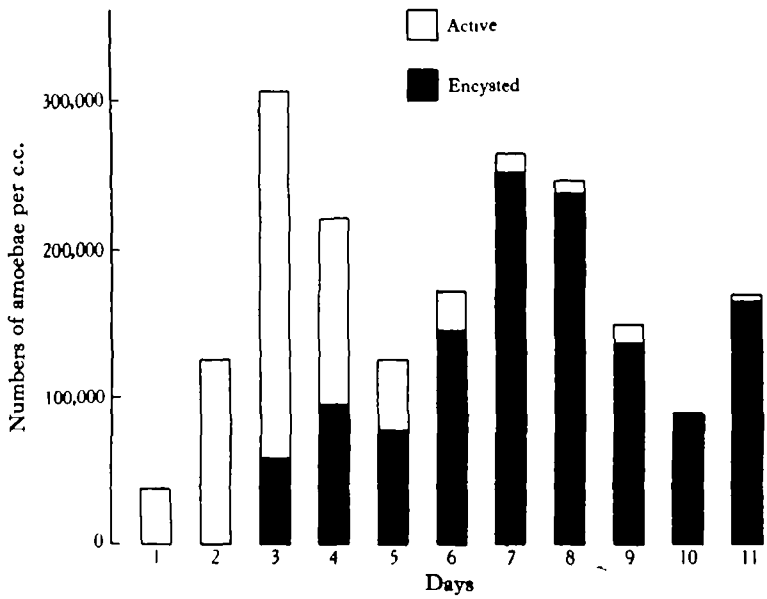

Fig. 1. Control culture in hay infusion.

cultures of *Naegleria* was 2·93, while that for *Hartmanella* was 6·63. On this occasion mass cultures instead of single cells were used, as the cultures were to be kept for some time so that daily observations could be made to determine the changes in the numbers of the population and the time of the onset of cyst formation.

In the control (Fig. 1) the end of the initial growth period is reached on the third day, and of the total population of 307,500 amoebae, 57,500 were encysted. The toxin culture (Fig. 2), however, was in comparison much less active and the initial peak was not only delayed by twenty-four hours but was much lower, the total number present being 140,000 of which all were encysted. After these periods the numbers in both cultures agree in falling, and in the toxin culture remain more or less constant to the end of the experiment, while with one exception 100 per cent. are in the form of cysts; whereas in the control, although the percentage of cysts still remains high, there is definite evidence that reproduction is still taking place since the numbers are rising and falling.

As it was evident that the toxin was exercising an inhibiting influence this culture was centrifuged to collect the amoebae and the supernatant fluid was boiled for half an hour; to this material the amoebae were returned and the experiment continued (Fig. 3). Boiling had no appreciable effect.

The control culture was similarly treated and behaved in the same manner. The apparent discrepancy between the numbers at the end of Fig. 1 and at the beginning of Fig. 3 in this culture is due to the fact that the volume of the fluid was reduced and therefore the concentration of organisms per cubic centimetre was increased. After seven days the cultures were again centrifuged and boiled, and on this occasion the amoebae from each culture were divided and while half were returned to their own fluid (Fig. 3) the other half were put into sterilised hay infusion (Fig. 4). Again in the case of the original toxin solution boiling had no

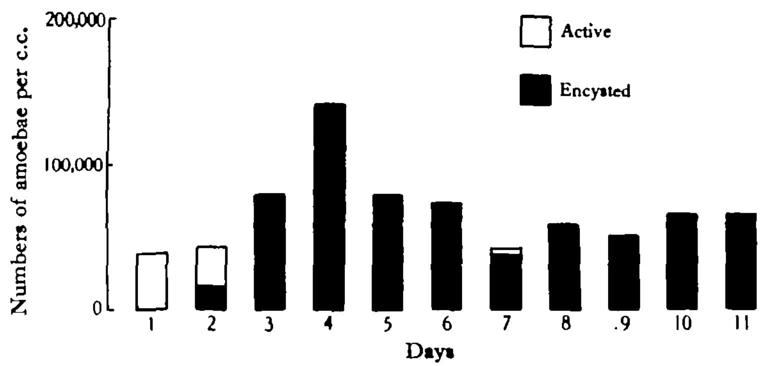

Fig. 2. Toxin culture in 50 per cent. hay infusion and 50 per cent. *B. mycoides* toxin.

effect, although on the twenty-second day there is a drop in the numbers, implying that a small amount of excystation had occurred followed by death since on the previous day there were 100 per cent. cysts.

The original control, which had also been boiled, gave much the same result, though there was an initial excystment, accompanied by slight reproduction, after which it behaved like the toxin culture, though to a greater extent, there being excystation followed by death.

The cysts from both cultures which had been put into new medium showed an entirely different picture as regards activity, for not only did considerable excystation occur, but this was accompanied by a reproductive rate of 0·94 in one case (control) and 2·59 in the other (toxin).

Judging from the results obtained from the treated culture the effect of the *B. mycoides* toxin was twofold, firstly it undoubtedly depressed the whole level of activity, and, secondly, it appears to have encouraged cyst formation. The question of the causation of cyst formation is very much debated; an idea that adverse external conditions such as lack of food, drought and toxic conditions are the sole

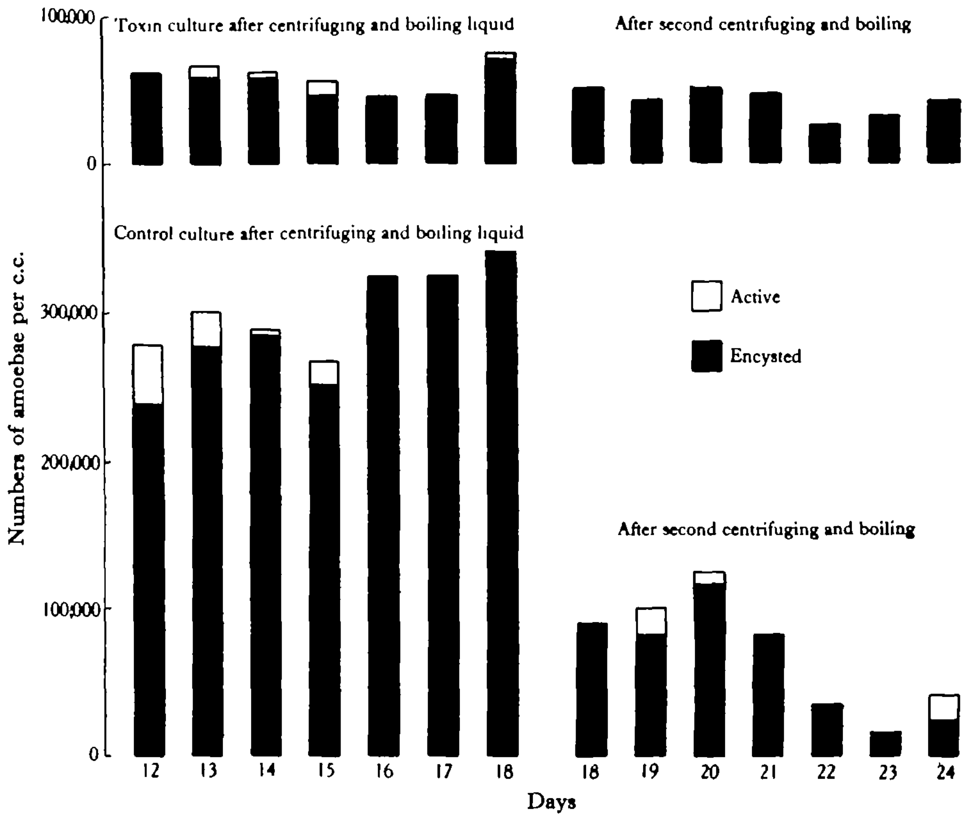

Fig. 3. Growth of amoebae in control and toxin cultures after centrifuging and boiling supernatant fluid and returning the organisms to the boiled liquid.

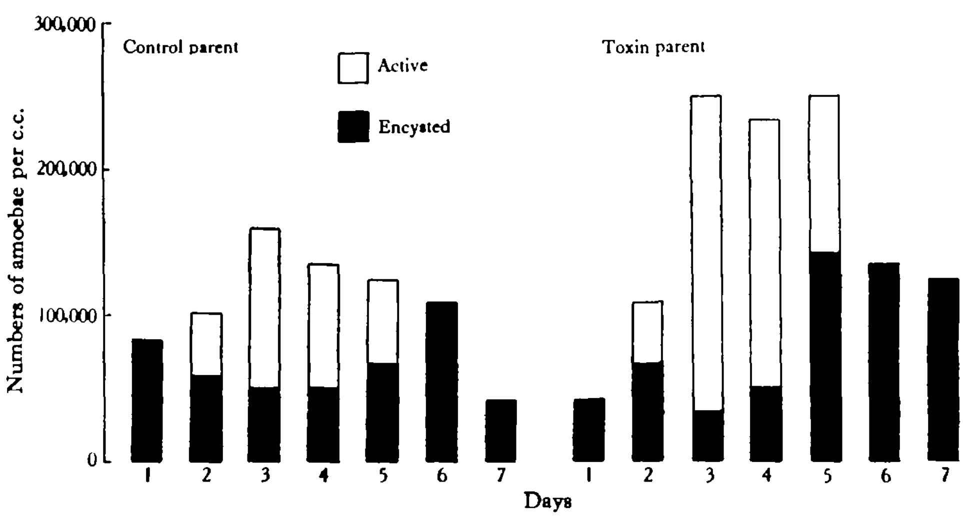

Fig. 4. Cultures prepared after second centrifuging and boiling by inoculating the cysts from the control and toxin cultures into fresh hay infusion.

determining causes of encystation is often encountered, but various considerations make this untenable.

In certain Protozoa it is known that the formation of cysts plays a regular part in the life cycle of the animal. For example, in *Oicomonas termo* when two individuals conjugate the result is the formation of a cyst, from which a single active animal emerges; while in *Colpoda cucullus* and many other ciliates true reproductive cysts occur as part of the life cycle, the encystation of one animal results in the production of four or more others. Another type of cyst also occurs as a regular episode in the life history of *Amoeba diploidea* where, instead of two individuals conjugating, fusion takes place between the two halves of an animal which has itself just divided within the cyst.

Table IV. *Average daily number of bacteria and three species of Protozoa for each month in the year* 1922. (*Protozoa given to the nearest hundred per gramme of soil.*)

Month	Bacteria in millions per gm. of soil	Dimastigamoeba		Oicomonas		Heteromita		Cercomonas	
		Total	% Active	Total	% Active	Total	% Active	Total	% Active
January	26	2319	63	879	52	3299	60	8	77
February	24	1514	57	1142	54	2080	64	5	69
March	23	1311	62	1208	51	2218	59	1	68
April	30	2010	66	503	53	3315	66	1	65
May	32	1869	67	961	55	3092	62	1	69
June	37	952	44	1101	45	3243	58	1	70
July	19	169	33	530	50	1079	56	58	69
August	17	121	32	896	63	1253	57	186	69
September	18	344	53	1153	51	1681	49	83	71
October	18	1010	41	733	47	1696	51	71	74
November	41	2111	65	1232	56	3000	57	102	78
December	34	2004	44	902	50	2787	56	38	68

Indirect evidence that cyst formation is a regular occurrence in the life history of free-living Protozoa is afforded by their behaviour in soil, where there are a number of species of amoebae and flagellates continually altering in numbers and playing their part in the soil economy (1). That they are successful members of the population and largely independent of the changing soil conditions is evidenced by the large numbers which are present throughout the year (Tables IV and V). It is impossible to imagine that such an average percentage of cysts, built up as it is from records of daily observations, when the numbers of cysts may fluctuate from 0 to 100 per cent. of the total numbers, can be explained on any assumption but that encystment is a normal stage in the animal's life history, though certain external conditions will undoubtedly help to determine the time at which the cysts are produced. The truth of this can be seen in the *B. mycoides* culture where the cysts were produced at an earlier date than in the control. Further, a consideration of Figs. 1, 2, 3 and 4 suggests that during an amoeba's career it may at any moment be faced with conditions both internal and external which make further reproduction impossible and compel it to encyst or die; such occasions are seen in Fig. 1 on the third and fourth days, and in Fig. 4 on the fifth day of the toxin culture.

Encystment cannot be an easy process because if this were the case one would expect the whole of a culture to form cysts, without any appreciable drop in numbers; but since death nearly always accompanies encystment it seems more likely that it is a matter of delicate adjustment; and unless the animal's internal condition, either physiological or morphological, coincides with the appropriate condition of the medium the cyst cannot be formed and death inevitably follows.

Table V. *Average daily percentage of cysts of* Naegleria gruberi *for each month compared with the average daily temperature, moisture and rainfall.*

Month	Cysts	Minimum soil temperature in °F. 12 in. thermometer	Percentage moisture	Rainfall in inches
January	36	43	23	0·007
February	42	39	22	0·006
March	38	43	19	0·031
April	34	46	19	0·049
May	33	54	17	0·004
June	55	59	14	0·006
July	64	59	21	0·013
August	68	58	21	0·004
September	47	56	20	0·007
October	55	51	22	0·004
November	39	43	22	0·056
December	58	40	23	0·077

SUMMARY.

1. Filtrates prepared from young cultures of "YB" bacteria and of *B. prodigiosus* do not depress the rate of growth of *Hartmanella hyalina* appreciably.

2. A filtrate prepared from disintegrated cells of *B. mycoides* inhibits reproduction in *Naegleria gruberi*, and also hastens the onset of cyst formation.

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