PURIFICATION OF WATER BY INFUSORIA.

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CONTENTS.

Introduction.

According to Hüntemüller (1905), Razetto (1907), Stokvis (1909) etc. flagellates contribute actively to the extermination of bacteria in water. River water, naturally polluted by bacteria or artificial bacterial emulsions (of *Bacillus typhosus*), could be cleared in four days if flagellates (*Bodo saltans* or *B. ovatus*) were added to it.

The object of our present research was: (1) to see whether other Protozoa especially Infusoria have the same bactericidal power, (2) to study some of the conditions favouring or inhibiting this power.

We made our experiments by adding Infusoria (*Colpoda cucullus*) to emulsions of bacteria (*B. typhosus* and *Vibrio cholerae*). The Infusoria were obtained from cultures in hay-infusion, where they were living together with *B. subtilis*, *B. megatherium* and *Spirilla*. The emulsions were obtained from pure cultures aged 24 hours and cultivated at a temperature of 37° C.

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Demonstration of the bactericidal power of Colpoda cucullus.

Our first experiment served to ascertain whether *C. cucullus* shows the same bactericidal power as the flagellates studied by our predecessors. We prepared four bottles, two of them containing an emulsion of *B. typhosus*, the two others an emulsion of *V. cholerae*. To each of the bottles containing the bacteria was added a loopful of a culture of *Colpoda*, then the bottles were placed at a temperature of 22° C. Table I shows the results of this experiment.

TABLE I.

Date	Emulsions of *B. typhosus* with *Colpoda*	Emulsions of *V. cholerae* with *Colpoda*	Emulsions of *B. typhosus*	Emulsions of *V. cholerae*
June 14	Fluid clouded ...	Fluid clouded ...	Fluid clouded	Fluid clouded
,, 15	Fluid clouded; few *Colpodae* present	Fluid clouded; few *Colpodae* present	,,	,,
,, 16	Fluid begins to clear; many *Colpodae* present	Fluid clouded; many *Colpodae* present	,,	,,
,, 17	Fluid almost cleared; many *Colpodae* present	Fluid begins to clear; many *Colpodae* present	,,	,,
,, 18	Fluid completely cleared; no *Colpodae* present	Fluid almost cleared; many *Colpodae* present	,,	,,
,, 19	Fluid completely cleared; no *Colpodae* present	Fluid completely cleared; no *Colpodae* present	,,	,,

This table shows, that the *Colpodae* are able to clear away emulsions containing *B. typhosus* and *V. cholerae* and that this clearage is preceded by an increase in the number of Infusoria. After the disappearance of the bacteria the number of *Colpodae* rapidly diminishes.

On repeating this experiment with *Vibrio Dunbar*, *V. El Tor*, a water-vibrio resembling *V. El Tor*, *B. megatherium* and *Spirillum volutans*, the emulsions containing these microbes were all cleared after 4–5 days when *Colpodae* were added to them.

The Infusoria used in these experiments were always mixed with bacteria, especially with *B. subtilis*. It might be asked whether the clearing of the emulsions is not due to some noxious influence which *B. subtilis* may exert on the bacteria present in the emulsions. This supposition is however untenable since we were unable to clear the emulsions with the aid of cultures of bacteria found together with the *Colpodae*.

Hüntemüller proved that the flagellates kill the bacteria by phagocytosis. Some authors think however that they do so by the production of toxic excretions. It is impossible to demonstrate bacilli within the Infusoria, because they are rapidly destroyed within the nutrient vacuoles. Consequently we do not know whether phagocytosis actually takes place. To ascertain whether the bacteria are killed by poisonous excretions of the Infusoria, a culture of *Colpodae* was filtered through a Chamberland-filter; 5 c.c. of the filtrate, which was completely clear and did not contain any traces of Infusoria, was added to an emulsion of *V. cholerae.*

After two weeks no sign of clearing was to be seen. It may be added that the same negative result was obtained when using the filtrate of a flagellate culture (*Monas* sp.), whereas this flagellate itself showed a marked bactericidal power. Consequently we may conclude that the bactericidal power of *C. cucullus* does not depend upon its excreting toxic substances.

Since the bactericidal action of Infusoria and Flagellates may be very effective in clearing river water it is of importance to determine the conditions influencing their power of destroying bacterial life. With this object we studied the influence of temperature, sunlight, oxygen and of some impurities found in river water upon the organisms in question.

Influence of temperature. Bottles containing emulsions of *B. typhosus* and *V. cholerae,* to which *Colpodae* were added, were kept at temperatures of 20° C. and 6° C. Table II shows the results of this experiment.

TABLE II.

Date	Emulsions of *B. typhosus* at 20° C.	Emulsions of *V. cholerae* at 20° C.	Emulsions of *B. typhosus* at 6° C.	Emulsions of *V. cholerae* at 6° C.
June 10	Fluid clouded ...	Fluid clouded ...	Clouded	Clouded
,, 12	Fluid begins to clear ...	,, ,, ...	,,	,,
,, 13	Fluid almost cleared ...	Fluid begins to clear ...	,,	,,
,, 17	Fluid completely cleared	Fluid almost cleared ...	,,	,,
,, 19	,, ,, ,,	Fluid completely cleared	,,	,,
,, 20	,, ,, ,,	,, ,, ,,	,,	,,

Consequently no bactericidal influence was to be observed at a temperature of 6° C. Similar negative results were obtained at a temperature of 37° C.

Influence of oxygen. Emulsions of *B. typhosus* and *V. cholerae* + *Colpodae* were kept under aerobic and anaerobic conditions.

The following results were obtained (Table III):

TABLE III.

Date	Emulsions of *B. typhosus*, aerobic cultures	Emulsions of *V. cholerae*, aerobic cultures	Emulsions of *B. typhosus*, anaerobic cultures	Emulsions of *V. cholerae*, anaerobic cultures
June 16	Fluid clouded ...	Fluid clouded ...	Clouded	Clouded
,, 19	Fluid almost cleared ...	Fluid begins to clear ...	,,	,,
,, 20	Fluid completely cleared	Fluid almost cleared ...	,,	,,
,, 27	,, ,, ,,	Fluid completely cleared	,,	,,

Consequently no bactericidal effect was observable under anaerobic conditions.

Influence of sunlight. Emulsions of *B. typhosus* and *V. cholerae* + *Colpodae* were exposed to the influence of direct sunlight for $1\frac{1}{2}$ hours.

The following results were obtained (Table IV):

TABLE IV.

Date	Emulsions of *B. typhosus* in the dark	Emulsions of *V. cholerae* in the dark	Emulsions of *B. typhosus* in sunlight	Emulsions of *V. cholerae* in sunlight
June 14	Fluid clouded ...	Fluid clouded ...	Fluid clouded ...	Fluid clouded
,, 16	Fluid begins to clear	Fluid almost cleared	Fluid almost cleared	Fluid begins to clear
,, 17	Fluid almost cleared	Fluid completely cleared	Fluid completely cleared	Fluid almost cleared
,, 19	Fluid completely cleared	Fluid completely cleared	Fluid completely cleared	Fluid completely cleared

Consequently direct sunlight has no influence on the bactericidal power of the Infusoria. It is interesting, that in this experiment the emulsion of *V. cholerae* was cleared sooner than that of *B. typhosus*. Generally the latter emulsion is the first to be cleared.

Influence of impurities in water. Emulsions of *B. typhosus* and *V. cholerae* were made with water from the canals running through Amsterdam. This water is very impure as it receives the contents of the sewers, moreover it is brackish. Before using it to make the emulsions it was filtered through a Chamberland-filter. Table V shows the results of this experiment.

TABLE V.

Date	Emulsions of *B. typhosus* in tapwater	Emulsions of *V. cholerae* in tapwater	Emulsions of *B. typhosus* in canal-water	Emulsions of *V. cholerae* in canal-water
June 14	Fluid clouded ...	Fluid clouded ...	Fluid clouded ...	Fluid clouded
,, 16	Fluid begins to clear	,, ,, ...	,, ,, ...	,, ,,
,, 17	Fluid almost cleared	Fluid begins to clear	Fluid begins to clear	,, ,,
,, 19	Fluid completely cleared	Fluid completely cleared	Fluid completely cleared	Fluid completely cleared

Consequently we note a slight delay (of about 24 hours) in the clearage of the emulsions made with canal-water.

The sewage of starch manufactories, containing a considerable quantity of amylum, has a markedly unfavourable influence on the bactericidal power of Infusoria. We imitated this sewage by using an infusion of potatoes. With this infusion, filtered before use, we prepared emulsions of *B. typhosus* and *V. cholerae*. The emulsion to which *Colpodae* were added was not cleared after two weeks.

A similar unfavourable influence was observed by preparing the emulsions with water containing ammonium chloride and other ammonium salts such as occur in sewage derived from gas factories.

SUMMARY.

Infusoria have the same bactericidal power as flagellates. Emulsions containing *Bacillus typhosus, Vibrio cholerae, V. Dunbar, V. El Tor, B. megatherium* and *Spirillum volutans,* to which *Colpoda cucullus* is added, are soon cleared. Before the clearage the *Colpodae* multiply actively.

This bactericidal effect does not depend upon the production of toxic substances by the Infusoria. The fluid obtained from filtered cultures of the Infusoria exerts no bactericidal effect. Only living *Colpodae* are able to clear the emulsions.

Direct sunlight does not prevent the clearing of the emulsions by the Infusoria, but temperatures below 10° C. and above 30° C. and absence of oxygen (anaerobic culture) are unfavourable to their exerting a bactericidal effect.

The polluted water of the canals of Amsterdam slightly delayed the clearage of the emulsions; the sewage of starch factories and gas factories completely prevented this clearage.

Consequently under natural conditions Infusoria will only play a part in purifying river water (1) if the temperature is above 10° and under 30° C., (2) if the aquatic vegetation is rich enough to supply the necessary quantity of oxygen, (3) if the water is not highly polluted by adjoining factories.

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