SIDE PROJECT

Bacteriophage extraction from the microbial mat from Sippewissett MA.

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Microbial Diversity Course

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The aim of the present report was to obtain a crude extract of bacteriophages present in the microbial mat. The sample was not sorted out according to infected bacteria or free bacteriophages in order to obtain a great variety of viruses from the mat. The bacteriophage DNA was obtained as well as transmission electron micrographs of the intact viruses.

A sublibrary containing large bacteriophage DNA fragments digested with a six cutter restriction endonuclease could be cloned in a high copy plasmid able to produce single standed DNA for sequencing. Positive clones can be sorted out according to their restriction pattern. Several groups are expected due to the heterogenicity of the DNA sample. Clones containing viral-related genes can be used to prepare non-radiactive probes (e.g. dioxygenin dUTP labeled). These probes could be used to screen bacterial isolates from the salt marsh in order to find out if they contain related viral genes and hopefully, find the host-virus system each probe belongs to.

The present approach could be very helpful for future studies on the role of viral control of bacterial population in the Salt Marsh Microbial Mat, as well as to study the genetic exchange among the residents of this community. This is a long term proyect that could be combined with the bacterial isolation projects done by the students of the Microbial Diversity Course, Woods Hole MA, interested in this topic.

Some useful readings are listed below:

Bratbak G, Thingstad F, Heldal M (1994) Viruses and the Microbial Loop. Microb. Ecol. 28, 209-221.

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60-62.

Suttle CA (1994) The significance of viruses to martality in aquatic microbial communities. Microbial Ecol. 28, 237-243.

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Procedure

1) A sample of ~50 ml of microbial mat from Sippewissett MA was collected in sterile Falcon tubes on July, 1995. The sample was kept at 4°C and processed two days later.

2) The sample was splited in two Falcon tubes and resuspended with 100 ml of sterile TE buffer pH 8.0. The buffer was added stepwise, i.e 20-30 ml per tube each time until 100 ml of resuspended microbial population was obtained. The sample was vigorously shaken for about 10 min and let it stand for 15 min on ice.

3) Heat shock the resuspended sample 10 min at 70°C and place it on ice for 1 h.

4) Spin down at 6 800 x g, 4°C for 20 min. Transfer the clear supernatant into a sterile 250 ml beaker with magnetic bar without disturbing the bacteiral pellet.

5) Add 5 grams of NaCl (final concentration 5 % w/v) to the supernatant (~90 ml recovered), stepwise, and stir slowly on a ice-water bath. Let it stand on ice for one hour.

6) Spin down the sample at 11 000 x g, 10 min, 4°C and save the supernatant in a sterile beaker with magnetic bar.

7) Add 9 grams of polyethyleneglycol (PEG 8000, final concentration 10%) stepwise to the sample and stir at room temperature for 30 min once all the PEG is dissolved (about 10 min). Chill the sample on ice for 1 h.

8) Spin down the sample at 11 000 x g, 10 min, 4°C. Discard the supernatant and resuspend the brownish pellet in SM buffer, final volume 4.5 ml. This is the crude bacteriophage extract.

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9) Spin down the phage extract in 3 microfuge tubes for 10 min at room temperature. A visible brownish pellet is obtained, but the supernatant is still brownish. Keep both samples at 4°C.

10) Resuspend one of the microfuge pellets to a final volume of 500 µl in TE buffer and extract it with equal volumes of phenol, phenol-chloroform-isoamylic alcohol, and chloroform. For each organic solvent extraction, the sample should be vigorously vortexed for 1 min and spin down at 10 000 rpm in a microfuge at room temperature for 2-3 min until an interface is observed.

11) Precipitate the final aqueous phase with 1/10 vol of sodium acetate 3 M pH 7.0, two volumes of cold ethanol, and store it at -20°C for 18 h, approximately.

12) Spin down the sample for 10 min at highest speed in the microfuge, at room temperature. Wash the pellet with cold 70% ethanol, spin down again and let the pellet dry at 37°C.

13) Resuspend the final pellet in 10 µl of TE buffer pH 8.0, add 2 µl of 6x loading buffer and run all the sample on a 1% agarose/ethidium bromide/1x TBE buffer gel.

Solutions:

TE buffer Tris-HCl 10 mM, EDTA 1 mM pH 8.0

SM buffer NaCl 0.58%, $MgSO_4 \cdot 7H_2O$ 0.2%, Tris-HCl 50 mM pH 7.5, gelatin 0.01%

Acknowledgments

I want to thank Elizabeth Sherwood and Dr. Thomas Pitta for the transmission electron micrographs, Drs. Abigail Salyers and Edward Leadbetter for discussion and helpful hints.

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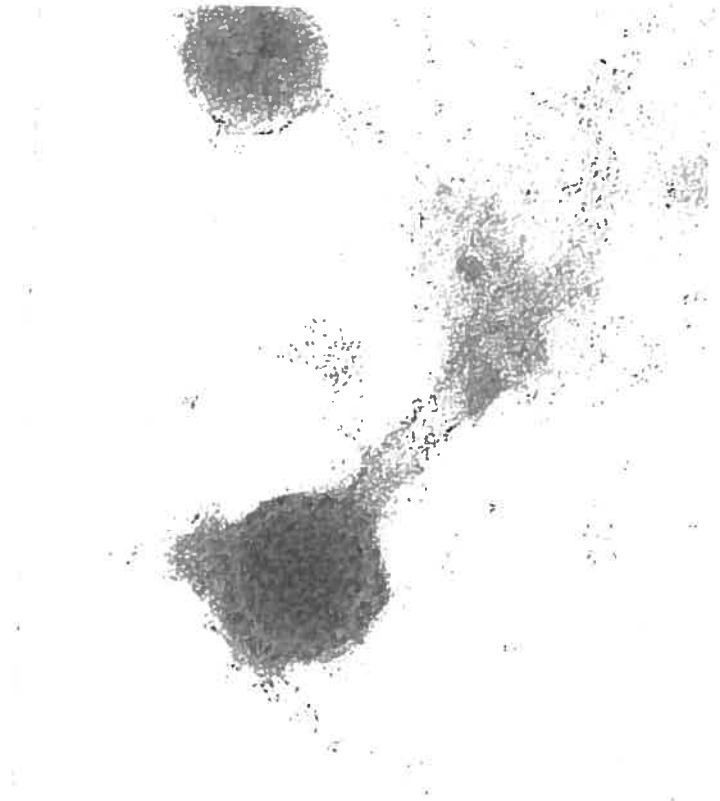

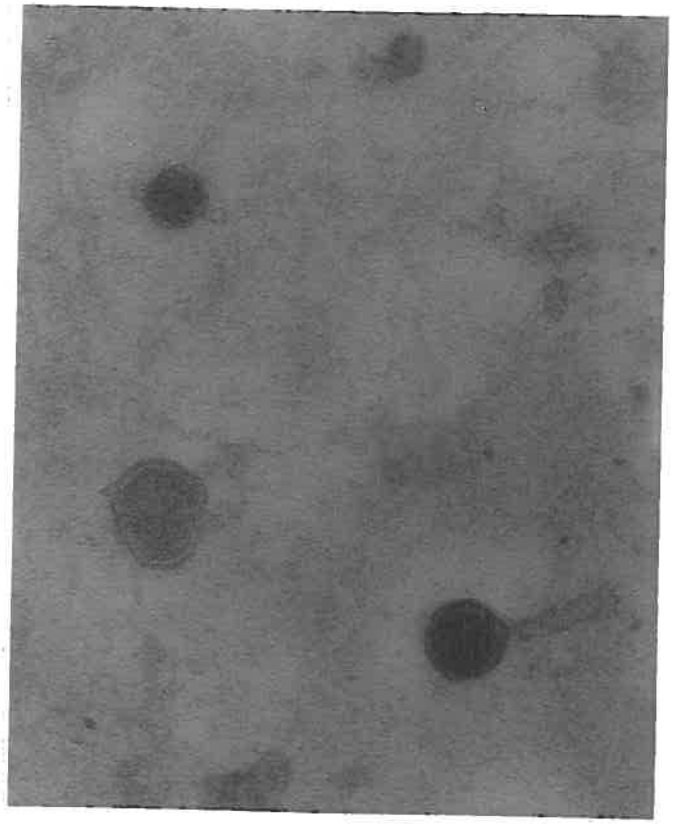

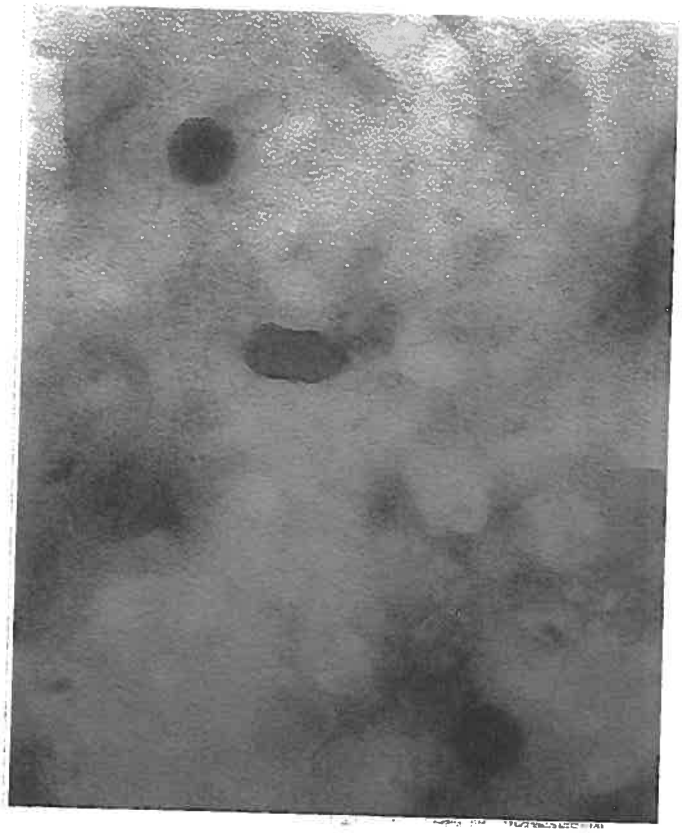

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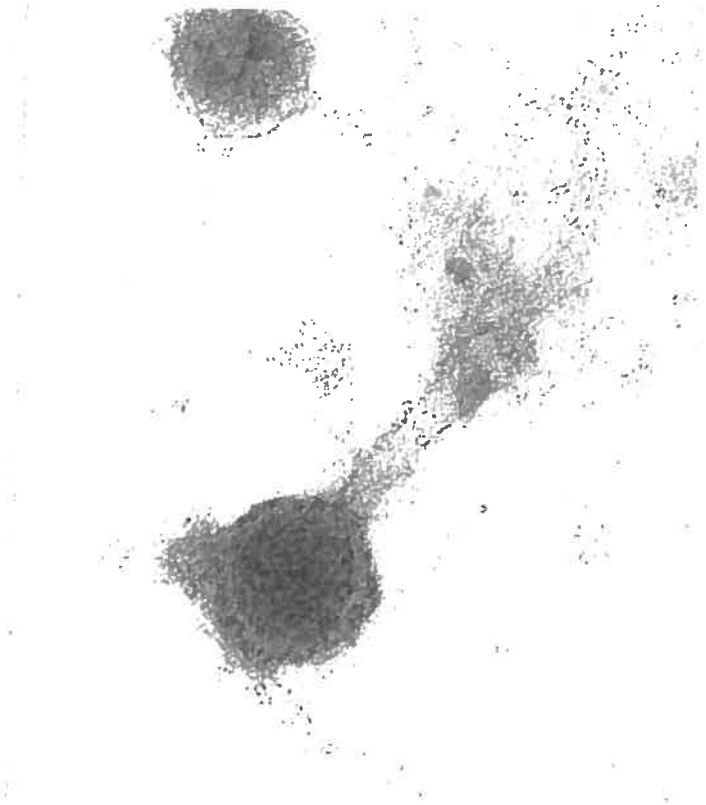

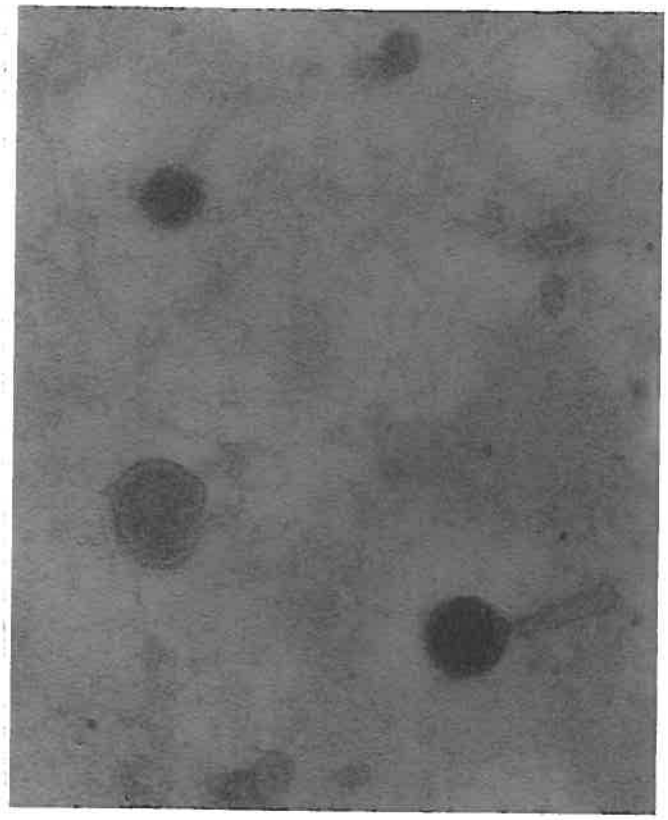

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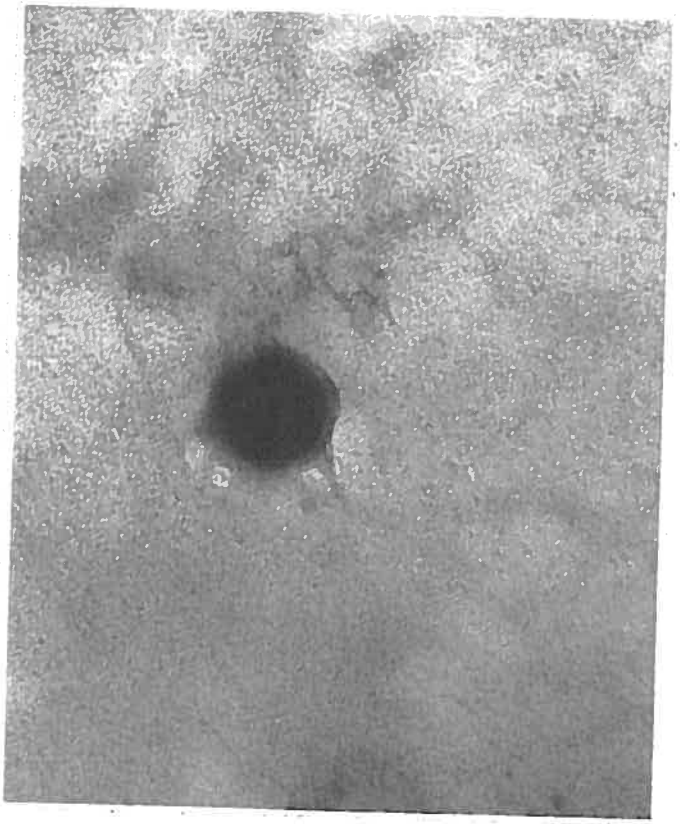

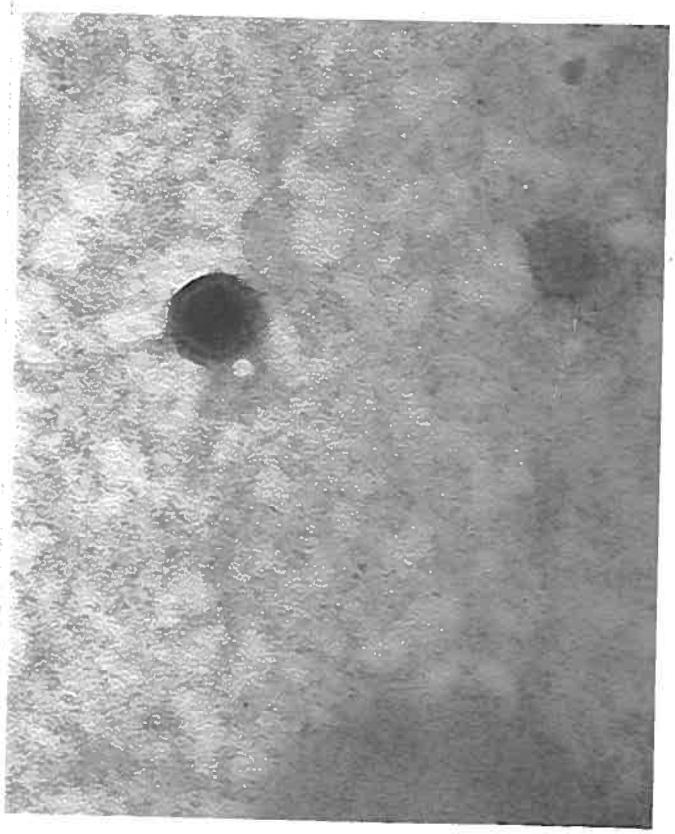

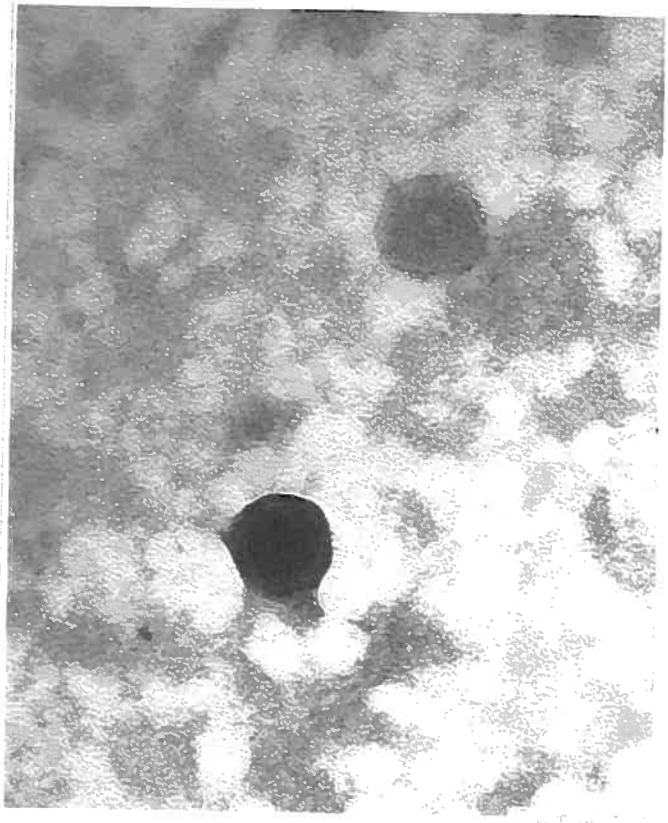

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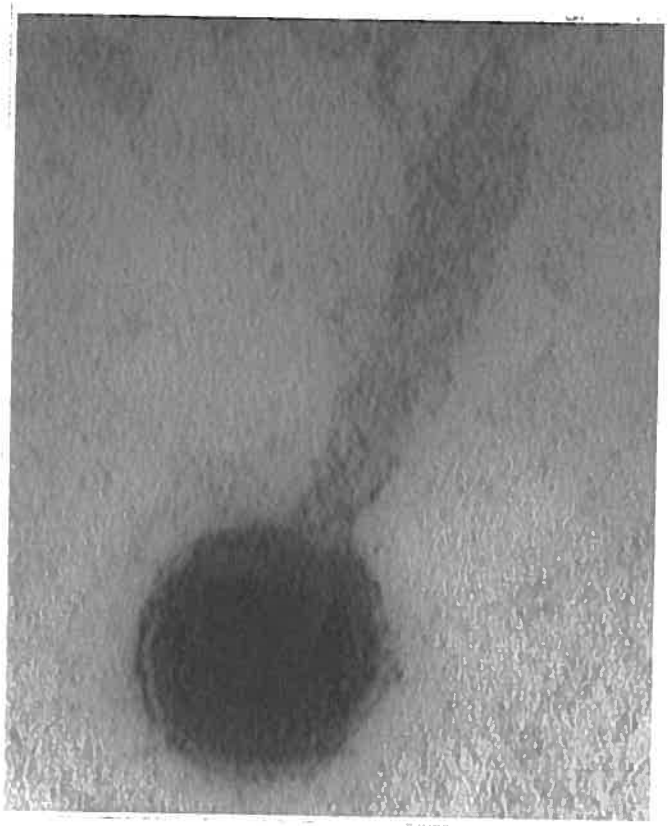

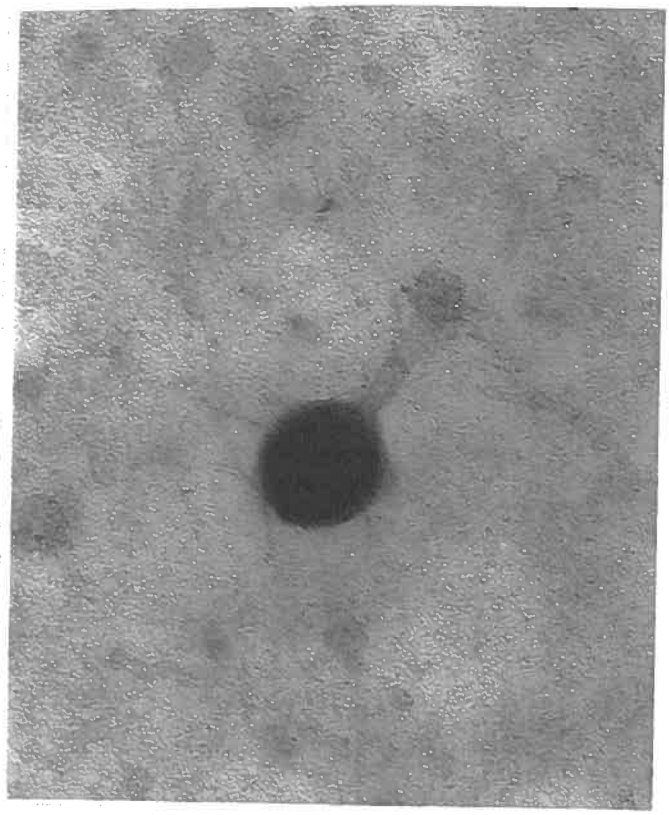

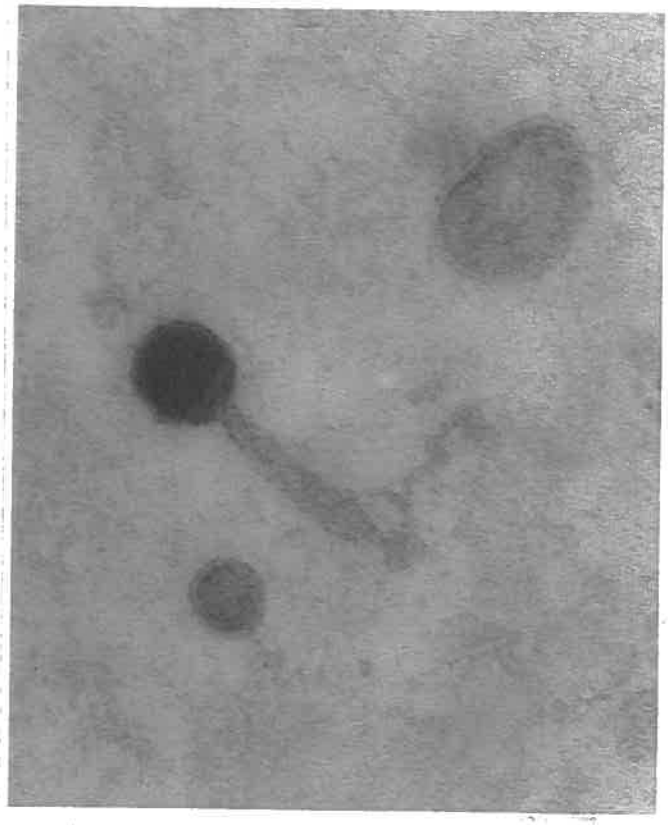

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