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ISOLATION OF ENDONUCLEAR SYMBIONT HOLOSPORA
OBTUSA FROM MASS CULTURES OF PARAMECIUM
CAUDATUM.

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The gram-negative bacterium Holospora obtusa is a macronucleus specific symbiont of ciliate Paramecium caudatum, which invades into the host cells via the food vacuoles, infects its macronucleus exclusively and grows in the nucleus. The bacterium never infects the micronucleus. To know what kinds of differences between the macro- and the micronucleus, of common genetic origin, can be recognized by the bacterium, we have aimed to establish in vitro infection system between the isolated bacteria and the isolated nuclei. In the present work, we succeeded to isolate the infectious bacteria from Paramecium homogenates with Percoll density gradient centrifugation. Yields averaged 85 %, and the preparations were essentially free from contaminants such as mitochondria, cilia and food bacteria. The isolated bacteria infected the macronucleus within 15 min after adding the bacteria into an external medium of Holospora-free paramecia.

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DISCRIMINATION OF FOOD IN PARAMECIUM
MULTIMICRONUCLEATUM.

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It is known that when starved Paramecium is transferred to a medium rich in bacteria, the first vacuole formed is always huge. On the other hand, Paramecium in a medium containing polystyrene latex particles (PLP) forms only normal sized vacuoles. This fact implies that Paramecium discriminates between food and indigestible material.

The volume of vacuoles formed during the first 1 min was measured after giving either bacteria or PLP of nearly the same size as bacteria. The ingestion rate of bacteria was several times large as that of PLP giving at the same concentration in the low concentration range.

In the present study, both particles were given simultaneously in a medium. The volume of the vacuoles then formed was found to be the sum of those vacuoles formed when each kind of particle was given separately. This suggests that both particles are ingested independently without affecting each other and that Paramecium may have a mechanism of selectively ingesting the digestible particle, bacterium, over the indigestible one, PLP.

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EXTRACELLULAR PHOTOSYNTHETIC PRODUCTS OF
THE APOSYMBIOTIC ALGAE FROM A CILIATE,
STENTOR AMETHYSTINUS.

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An endosymbiotic alga of Stentor amethystinus was isolated immediately after the release from the host ciliate which was collected in large number from the natural habitat. The isolated endosymbiotic alga of S. amethystinus from another collection was cultivated on an agar slant and then grown in a liquid modified Bristol medium for 1 year in sunlight. The isolated endosymbiotic alga and the cultivated one were incubated in a medium constituted of an inorganic salt solution, acetate buffer (pH 5.0) and NaHCO₃ under 400 lux light at 25°C. At 0, 1, 2, 3, and 4 hr after incubation, the detection for sugar and amino acid in the extracellular photosynthate was carried out. Maltose and alanine were produced in the incubation medium of the isolated endosymbiotic alga and the quantity of the products increased with the prolonged incubation time. In the case of the cultivated endosymbiotic alga, the photosynthetic products were small in quantity. 1 year culture medium of the cultivated endosymbiotic alga contained a large amount of the extracellular photosynthate. It was suggested that the endosymbiotic alga was competent to supply the photosynthetic products to the host and that the quantity of the photosynthate released from the aposymbiotic alga decreased with time after the isolation.

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CULTIVATION OF INTESTINAL PROTOZOA OF THE
TERMITE (RETICULITERMES SPERATUS). 1.

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Several conditions for the cultivation of intestinal protozoa of the termite were examined. Solution U (Trager, 1934) was used as a basal saline. An optimum pH value for the cultivation was pH 7.0-7.5. A suitable O₂ concentration was below 20% in the solution U. A survival ratio in this condition fell to below 5% at 12 hr. A high survival ratio was obtained by the cultivation in a conditioned medium which was prepared as follows: 10 ml of the solution U contained the hindgut and its contents of 50 individuals of termite (worker-caste) was incubated at 25°C for 3 days. Twenty-four hours after the inoculation the survival ratios of Pyrsonympha modesta, P. grandis and Trichonympha agilis were about 45, 35 and 20%. By the addition of a cellulose powder the high survival ratio was able to hold for 48 hr or more time in three species of protozoa described above and in Dinenympha leidy. When a filtrated conditioned medium (membrane filter, 0.25 μ m) was used instead of the conditioned medium the survival ratio was reduced to that of first condition. These results suggested that a suitable condition for the cultivation of the intestinal protozoa of the termite was prepared by bacteria.