THE RÔLE OF FUNGI IN THE DIET OF TERMITES

TERMITES of the species *Zootermopsis angusticollis* (Hagen) were fed individually and in groups on various fungus-containing and fungus-free diets.

When fed on rotten, fungus-containing Monterey pine, individually isolated termites, while suffering an initial loss in average dry weight and milligrams of nitrogen per termite, possibly due to a lag in adjustment to the condition of isolation, showed finally significant increase in weight and in nitrogen per termite and exhibited normal appearance and activity. Individually isolated termites on sound, fungus-free Monterey pine, on the other hand, showed an even greater initial loss in weight and nitrogen per termite and failed to make any significant gain in weight or nitrogen during the course of the experiment. They were sluggish in activity, abnormal in appearance, and those which died showed a reduced intestinal protozoan fauna.

Termites kept in groups on rotten, fungus-containing Douglas fir also showed better growth than those on sound, fungus-free Douglas fir, as evidenced by the greater increase in average dry weight per termite and the more rapid passage through successive instars of the termites on rotten wood. On rotten wood the termites were healthy and exhibited apparently normal viability, while on fungus-free wood there was high mortality during the second week of the experiment, cannibalistic feeding was excessive, and the increase in weight and nitrogen of surviving individuals was accomplished at the expense of the group as a whole.

Termites fed on sound Douglas fir on which there was a recent, superficial growth of the fungus *Trichoderma lignorum* (Tode) Harz showed good viability at a time when mortality was high on fungus-free, sound wood. Viability was good on sound Monterey pine, also, when fungi introduced by the termites were allowed to grow on the wood. In the latter case, it is significant that increase in average weight of termites occurred only after a visible growth of fungi had developed on the wood.

Rotten Douglas fir, however, proved to be a better diet for the termites than the wood which had only the superficial growth of *Trichoderma lignorum*. This was shown by the greater increase in average weight per termite and by the maintenance of higher group weight by termites on the rotten wood. Other fungi present in the rotten wood may have had greater nutritive value or more favorable enzymatic action on the wood than *T. lignorum*. Furthermore, it seems probable that the extensive chemical changes brought about in wood through the actual production of rot are important in preparing the wood to be a suitable diet for *Zootermopsis*.

Individually isolated termites when fed on fungus-free filter paper failed to make significant gain in weight. Termites kept together in groups on fungus-free filter paper showed greater decrease in group weight, higher mortality and less increase in average weight per termite than did those on their natural diet of rotten, fungus-containing wood. These results are in agreement with the observation by Cook and Scott (1933)[1] that termites are unable to live and grow normally on a diet of purified cellulose.

During the course of these feeding experiments better growth and higher viability have been observed among termites on fungus-containing wood than on fungus-free wood. It is evident, then, that fungi play an essential rôle in the natural diet of *Zootermopsis angusticollis*. The fungi offer a source of proteins. They probably supply vitamins which are essential to the normal growth and development of termites. Through the secretion of extracellular enzymes they may render the wood itself more nutritious. It is not known what effect the fungi may have on harmful extractives in the wood. On fungus-free, sound wood mortality of termites was even higher during the early part of the experimental period than on the more deficient diet of filter paper, while on sound wood on which a growth of fungus had developed viability was good. Whether the termites survived because they were better nourished or because the fungi had rendered some toxic factor in the wood harmless or because of both conditions is uncertain. It is certain, however, that the differential factor was the presence of the fungus.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PHYSIOLOGICAL MEDIA FOR FRESH-WATER AND MARINE PROTOZOA

THE cultivation of Protozoa for investigation involves principally three factors: medium, food and bacteria. Bacteria play a very important part when an organic medium is used, but their effect may be eliminated, or practically eliminated, if the medium is an inorganic one. The question of food is comparatively simple, because the food of Protozoa is in most cases not so specific. Any nutritive material

[1] S. F. Cook and K. G. Scott, "The Nutritional Requirements of *Zootermopsis* (*Termopsis*) *angusticollis*," *Jour. Cell. Comp. Physiol.*, 4: 95–110, 1933.

admissible to the feeding apparatus of the organism concerned is usually satisfactory. The most important factor is, however, the medium. On account of the selective-permeability of the cell membrane, the constitution and changes of the medium have immediate and direct influence on the life processes of the organism.

The writer has for a long time devoted himself to the finding of inorganic physiological media for his investigations on various unicellular forms, and has been able to develop two media, one for fresh-water and the other for marine Protozoa. The formulae of these media are as follows:

MEDIUM A FOR FRESH-WATER FORMS

$CaCl_2$	0.0008 N	
$NaNO_3$	0.0003 N	
$MgSO_4$	0.0002 N	
K_2HPO_4	0.0001 N	
KH_2PO_4	0.0001 N	
NH_4NO_3	0.0008 N	(for green forms only)

MEDIUM B FOR MARINE FORMS

NaCl	0.1335 N	
$CaCl_2$	0.0112 N	
KCl	0.0084 N	
$NaNO_3$	0.0055 N	
$NaHCO_3$	0.0048 N	
$MgSO_4$	0.0040 N	
KH_2PO_4	0.0005 N	
Na_2SiO_3	trace	
NH_4NO_3	0.0125 N	(for green forms only)
$FeCl_3$	trace	(for green forms only)

In developing Medium A, extensive experimental work was done on Pleurotricha and Chlorogonium, which is also used as food for the former. In developing Medium B, experiments were performed with Kerenopsis and Dunaliella, which also serves as food for Kerenopsis. For other organisms, the total salt concentration and the hydrogen-ion concentration of both media may be slightly modified.

The working basis in formulating Medium A are: (1) The chemical analysis of fresh water; (2) the toxicity and antagonism of salts; (3) the buffering reaction of salts.

The formula of Medium B is based for anions on the chemical analysis of the salt content of the blood of certain marine organisms and for cations on the buffering properties of salts. In case of green forms, the chemical requirements for photosynthesis have also been taken into consideration.

These media are less toxic than natural fresh water or sea water and many other physiological media tested. They are satisfactory for most unicellular organisms the writer has tried to cultivate, but they are fatal to a few others. With frequent transferring to fresh media, organisms grow very well. By using these media for experimentation, surprisingly uniform results are obtained. In case of Pleurotricha and Kerenopsis, variation in size and fission rate are extremely small. Rhythmic variation in fission rate, such as that obtained by Jennings and others on Paramecium, has not been found.

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MICRO-METHODS FOR THE DETECTION OF PROTEASES AND AMYLASES

DURING the past two years the authors have been engaged in independent investigations of the digestive enzymes of amphibian embryos (F. D.) and of spiders (G. E. P.), respectively. The results of these investigations will be published separately elsewhere but we wish jointly to record two simple methods which proved convenient for the detection of proteases and amylases, respectively, when extremely small amounts of the extract or fluid to be tested were available. The method used for proteolytic enzymes is similar in principle to the more accurate quantitative method suggested by Gates;[1] the method used for amylases is based on that described by Bond,[2] but instead of using a thick layer of starch suspension in agar in the bacteriological manner a very thin dry film is substituted.

PROTEASES

A drop of the fluid or extract to be tested is mixed with several drops of an appropriate buffer solution; a drop of the resultant mixture is withdrawn and placed on the gelatin surface of an unexposed Eastman lantern slide plate which has been previously cleared in 20 per cent. sodium thiosulfate, thoroughly washed and dried. The slide should be placed in a moist chamber or otherwise protected from evaporation during the period of digestion. If enough of the original sample is available, it is convenient to run a series of drops at different pH values so that the approximate pH range of the enzyme is determined in the first experiment. The slide may be allowed to stand at room temperature if this does not exceed 20° C.; at temperatures of about 26° C. control drops of buffer solution dissolve the gelatin in the acid range, while at 30° C. the buffers dissolve the gelatin throughout the pH range used. In warm weather it is therefore necessary to perform the experiment in a thermostat. Gilman and Cowgill[3] used Gates's

[1] F. L. Gates, *Proc. Soc. Exp. Biol. Med.*, 24: 936, 1926–27.

[2] R. M. Bond, *Bull.* Bingham Oceanographic Coll., Yale Univ., 4: art. 4, p. 1, 1933.

[3] A. Gilman and G. R. Cowgill, *Jour. Biol. Chem.*, 88: 3, 743, 1930.