sampling *E. coli* have been effective in the collecting of organisms in air ducts, rooms, and test boxes over a period of 11 years (Rentschler *et al.* [*9*]). Settling of organisms on Petri plates has been shown to give reproducible results. This has also been demonstrated by Robertson *et al.* (*10*). To obviate the effect of air currents, as many as 10 Petri plates, placed in various positions in a room, were used for one test. In the experiments described, Petri plates, the Hollaender Dallavalle sampler, and the Luckiesh-Holladay-Taylor electrostatic precipitator placed at the bottom of the test box gave similar results. This indicates that sampling could not account for the variance in the figures of Robertson *et al.* and those in our work.

The amount of glycol in the air could not have been a factor in our tests. Both propylene and triethylene glycol were calculated to give a saturated atmosphere, and in the case of a commercial vaporizer the vapors were visible in the room. According to Puck (*5*), the killing is always a direct function of the concentration of glycol in the air. It would be expected, therefore, that some indication of bactericidal effect would be seen even when conditions were not optimum. However, the tests indicate that the glycols increased the rate of fall of the organisms and showed no germicidal action. Twort used propylene glycol with hexylresorcinol but did not observe any bactericidal effect of the glycol per se. Mallmann and Churchill (*4*) found that spores of bacteria and of *Aspergillus niger* and *Penicillium italicum* were unaffected by glycol vapors or sprays. They concluded that these compounds were not effective in controlling microbial contamination of cold storage and food preparation rooms.

The apparent germicidal, viruscidal effect of glycol can be attributed to the precipitating of water droplets from air containing bacteria or viruses.

Puck (*5*) calculated that the weight of a droplet increases 20 times by adsorption of glycol vapors. According to Stokes' law,

$$v = \frac{2gr^2(d_1-d_2)}{9n},$$

where $v$ = velocity of fall in cm/sec, $g$ = gravity 980, $r$ = radius of particles, $n$ = coefficient of viscosity, $d_1$ = density of sphere, and $d_2$ = density of medium. The velocity of fall will be proportional to the square of the radius. If we take Robertson's (*12*) value of 3 μ as the diameter of a droplet, and the height of his experimental chamber as 15 in., it would take approximately 20 min for the organisms to settle. However, if the droplet increases in weight by 20 times and the diameter by three times, the rate of fall will be increased as the square of the radius so that the droplets will settle in 2.5 min. This is the value reported by Robertson (*11*) for the apparent germicidal and viruscidal action of glycols in his chamber. The Hollaender-Dallavalle (*2*) sampler in his apparatus would not have collected any more organisms after 2.5 min, whereas settling on Petri plates would have shown that all the organisms were precipitated, as in our tests. Using a larger box and the same size droplet, the length of time of settling would be proportional to the height, which accounts for the much longer settling time in our tests. The vapors do not condense on dust-borne organisms, and therefore the particles will remain suspended in air. It is conceivable that under practical conditions the glycol-precipitated organisms may lose their moisture and act as a reservoir for the reinfection of the air.

### References


1. Hamburger, M., Puck, T. T., and Robertson, O. H. *J. Infectious Diseases,* **76**, 208 (1945).
2. Hollaender, A., and Dallavalle, J. M. *Pub. Hlth. Rep.,* **54**, 574 (1939).
3. Luckiesh, M., Holladay, L. L., and Taylor, A. R. *Gen. Elec. Rev.,* **49**, 8 (1946).
4. Mallmann, W. L., and Churchill, E. S. *Refrig. Eng.,* June (1946).
5. Puck, T. T. *J. Exp. Med.,* **85**, 729 (1947).
6. Puck, T. T., and Chaney, A. L. *Ind. Hyg. Assoc. Quar.,* **7**(3) 10 (1946).
7. Puck, T. T., *et al. J. Infectious Diseases,* **76**, 216 (1945).
8. Puck, T. T., Robertson, O. H., and Lemon, H. M. *J. Exp. Med.,* **78**, 387 (1943).
9. Rentschler, H. C., and Nagy, R. *J. Bact.,* **44**, 85 (1941).
10. Robertson, O. H., Puck, T. T., and Wise, H. *J. Exp. Med.,* **84**, 559 (1946).
11. Robertson, O. H. *Wis. Med. J.,* **46**, 311 (1947).
12. Robertson, O. H., *et al. J. Exp. Med.,* **75**, 593 (1942).
13. Robertson, O. H., *et al. Science* **93**, 213 (1941).
14. Robertson, O. H., *et al. Science* **94**, 612 (1941).
15. Twort, C. C., *et al. J. Hyg.,* **40**, 253 (1940).
16. Wells, W. F. *Am. J. Hyg.,* **20**, 611 (1934).
17. Committee Sanitary Engineering, National Research Council, Div. of Medical Sciences. *Am. J. Pub. Health,* **37**, 189 (1947).


## Spade-Foot Toad Sperm as an Activating Agent in Producing Gynogenetic Haploid Embryos from *Rana* and *Pseudacris* Eggs


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Much interest has been directed toward the study of the mode of development of haploid frog embryos by experimental embryologists and geneticists in their search for a better understanding of nucleocytoplasmic relationships. Gynogenetic haploid embryos, resulting from eggs activated by the penetration of spermatozoa and developing with egg-chromatin alone, have been produced by one of two methods: (*a*) eggs inseminated by sperm which had been moderately irradiated beforehand (*1*, *2*, *3*) or had been treated with certain chemicals (*4*, *5*); or (*b*) by the use of foreign sperm as found by G. Hertwig (*6*) and Tchou (*7*) in their hybridization experiments with certain European species of anurans.

Realizing the convenience of this latter method, the writers have applied it in crossing a number of species of anurans occurring in the United States. We found that, when eggs of *Rana pipiens* and *Pseudacris nigrita triseriata* were inseminated with sperm of the spade-foot toad, *Scaphiopus holbrookii holbrookii*, the embryos ob-

tained were almost all haploid, as shown by their chromosome number studied cytologically. Only on two occasions among many crosses were a few gynogenetic diploid individuals found. The percentage of developing eggs in such crosses was always high, usually greater than that of the control group inseminated with sperm of its own species. Serial sections of the inseminated eggs show that the sperm head lies along the first cleavage spindle, but does not fuse with the female pronucleus. It is eliminated after the first division. A study of the chromosomes of the spade-foot toad shows that they are smaller in size than either those of *R. pipiens* or *P. nigrita triseriata*, and their diploid number is 26. The chromosome number of *R. pipiens* is 26, whereas that of *P. nigrita triseriata* is 24.

The above method is very convenient and requires little time to prepare the material when a large number of gynogenetic haploid embryos of *R. pipiens* or *P. nigrita triseriata* is needed for study. The toad can be kept alive in the laboratory in a container filled with damp loose soil for 2–3 months without feeding. A pair of adult testes is enough to inseminate several hundred eggs.

### References


1. HERTWIG, G. *Arch. mikroskop. Anat.*, **81**, Abt. II, 87 (1913).
2. HERTWIG, O. *Ibid.* **77**, Abt. II, 1 (1911).
3. *Ibid.* **82**, Abt. II, 1 (1913).
4. HERTWIG, G. *Anat. Anz.*, **58**, Erg. Heft, 223 (1924).
5. DALCQ, A. *Arch. Biol.*, **43**, 343 (1932).
6. HERTWIG, G. *Arch. mikroskop. Anat.*, **91**, Abt. II, 203 (1918).
7. TCHOU-SU. *Arch. d'anat. micr.*, **27**, 1 (1931).


## Studies on Algal Epiphytes


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Studies have been undertaken to determine if certain algal epiphytes possess parasitic characteristics. Light on this problem can only be obtained through a careful study of the physiology of the host and the epiphyte, as well as a careful study of the histology of their attachment areas.

Obligate epiphytes and mild or partial parasites among the algae have been observed and discussed by many writers and investigators (*1–8*), but the literature does not contain thorough reports concerning their physiology and morphology. Many investigators postulated that some of these algae were parasitic simply because they showed preference for certain hosts. Knight (*3, 4*), in describing *Pylaiella* on *Fucus vesiculosus*, stated that the epiphyte shows exclusive preference for fucoids. This, she pointed out, suggests selective epiphytism or a parasitic relationship not of the obligate type. Chapman (*1*) pointed out that, since *Ectocarpus siliculosus* often penetrates the host tissue, the relationship is possibly an example of mild parasitism. Kylin (*5, 6*), as well as Fritsch (*2*), also mentioned similar conditions among the algae. In all cases, however, the writers and investigators have shown some doubt about the physiological and histological relationship between the epiphyte and the plant upon which it lives.

TABLE 1

SUMMARY OF ALGAE STUDIED

| Symbiotic relationships | Degree of penetration |
|---|---|
| *Polysophonia lanosa* on *Ascophyllum nodosum* | +++ |
| *Elachistea fucicola* on *A. nodosum* | + |
| *E. fucicola* on *Fucus vesiculosus* | + |
| *Spermothamnion turneri* on *Chondrus crispus* | ++ |
| *S. turneri* on *F. vesiculosus* | – |
| *Calothrix* sp. on *Fucus* sp. | – |
| *Calothrix* sp. on *Cystoclonium* sp. | + |
| *Acrochaetium moniliforme* on *Dasya pedicellata* | + |
| *Ceramium rubrum* on *Chondrus crispus* | – |
| *Ectocarpus siliculosus* on *Laminaria agardhii* | – |
| *E. siliculosus* on *Zostera marina* | ++ |
| *Polysiphonia variegata* on *Chorda filum* | + |
| *Lithothamnion turneri* on *F. vesiculosus* | – |
| *Bangia ciliaris* on *Gelidium crinale* | – |
| *Erythrotricia* sp. on *Cladophora* sp. | – |
| *Acrochaetium* sp. on *Grinnellia* sp. | +++ |

+++ Cells of epiphyte penetrate deeply into tissue of host.
++ Cells of epiphyte penetrate just below the superficial cells of host.
– Cells of epiphyte do not penetrate tissue of host. host.
– Cells of epiphyte do not penetrate tissue of host.

During the summer of 1949 a number of algae epiphytes were collected in the vicinity of Woods Hole, Massachusetts, specifically on Penikese Island, Nobska Point, Martha's Vineyard, No Mans Land, and the Elizabeth Islands. Many microslides of the attachment areas of these epiphytes were made, and some of the slides were prepared by freehand sectioning, thereby making it possible to observe the living cells. Most of the slides, however, were prepared by the paraffin method, which made possible a more careful histological study.

Of the algae studied thus far, the attachment areas fall into four categories: those in which the cells of the epiphyte deeply penetrate the host; those in which they penetrate just below the superficial cells of the host; those in which they are wedged between the superficial cells of the host but go no further; and those in which they do not enter the host. At the present stage of this investigation, it is not the intention of the writer to imply conclusively that the degree of penetration of the epiphyte correlates with the degree of parasitism. Before such a conclusion can be drawn, studies must be made to determine whether the attachment areas observed represent a stage in the growth of the epiphyte or its ultimate growth.

Sufficient data have been obtained, however, to suggest that many of these so-called algal epiphytes may be symbionts of a nutritive antagonistic type, or of a nutritive reciprocal type. It can be observed from Table 1 that some of the algae deeply penetrated the tissues of the plant upon which they were growing. One that is of particular interest is *Polysiphonia lanosa* on *Ascophyllum*