

preserved vesicular nuclei. Thus, periosteum and young bone provided the best material for study (Figs. 1, 2). In these tissues the sex chromatin body could be recognized in about 75 percent of the cells (Table 1). In adult bone, approximately 50 percent of the nuclei contained an identifiable sex chromatin body. These figures compare favorably with the reported observations in other tissues of the dog and cat (2, 4). In sections of bone from male animals less than 10 percent of the cells contained a nuclear chromatin mass of a size, shape, and position comparable to that which is found in the female. Of the unlabeled coded sections from the 16 animals that were investigated, all were correctly identified according to their sex source.

Although the significance of the sex chromatin body has not been established, its recognition in tissues has led to many important clinical applications. Thus, it has been used extensively in prenatal sex determinations, in studies of infertility, and in distinguishing the chromosomal sex of individuals with congenital errors in sexual development (10, 14). Experimentally, the presence of a histologic sex difference in cells also provides a useful physiologic tag for studying grafted tissues (15). As a result of the findings in the present investigation, this technique is now being used to evaluate the fate of bone homografts.

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Acrylic Acid, an "Antibiotic"

Principle in *Phaeocystis* Blooms in Antarctic Waters

Abstract. An acidic algal substance has been isolated and characterized as the sodium salt. Synthetic sodium acrylate possesses chemical and antibacterial properties identical with those of the natural product. This observation identifies the algal substance in the stomach contents of euphausiids, the diet of pygoscelid penguins, which inhibits the penguin gastrointestinal microflora.

A substance which inhibited the gastrointestinal microflora of pygoscelid penguins (1) was traced to the phytoplankton-laden stomach contents of their euphausiid diet (*Euphausia superba*) and then to a green mucilaginous colonial alga (2). The purpose of this report is to describe the isolation of an antibacterial substance from the mucilaginous colonial alga, *Phaeocystis*, and the identification of this substance as acrylic acid (3).

The phytoplankton communities in the Gerlache and Bransfield straits off the west coast of Palmer Peninsula were studied in January and February 1959. Antibiotic assays and microscopic examination of concentrates of plankton gathered by net indicated that antibacterial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* is correlated with the predominance (40 to 90 percent) of a species of *Phaeocystis* (similar to *P. pouchetii*) in the blooms. Algal concentrates (5.1 percent dry wt.) of *Phaeocystis* were obtained with a number 2 net which retained these mucilaginous algae but permitted most of the diatoms to pass through. These preparations were stored in polyethylene bottles at -20°C aboard ship, transported in dry ice, and maintained in the laboratory at -55°C .

Air drying on glass plates, desiccation in a vacuum, and lyophilization of the algal concentrates caused an approximately 20-fold reduction in activity. Extraction of this dried material, as well as the wet algae, with acetone, ethers, and alcohols produced concentrates which lost their activity rapidly at 5°C . The formation of inactive gummy material usually occurred as a result of the numerous abortive attempts to concentrate and purify the antibacterial substance. Distillation of acidic algal supernatants produced antibacterial distillates which failed to leave an active residue after evaporation.

A virtually pure active solid has been obtained only by the following procedure. The supernatant fluid of thawed cells (pH 5.5) was adjusted to pH 3.0 with HCl or H_2SO_4 . The acidic substance readily volatilized under reduced pressure (in a Rinco rotating vacuum-type evaporator) to yield an active

condensate (pH 3.7) in the solvent recovery condenser. Barium chloride and silver nitrate tests on the condensate indicated that the mineral acids used for acidification were absent. After careful adjustment of the pH to the equivalence point (pH 7.5) with NaOH, evaporation in a vacuum yielded an active sodium salt. Recovery of the original activity was increased from 80 percent to virtually 100 percent by the use of additional ice traps in the vacuum line.

Titration curves on the acid condensate as well as the weight of the sodium salt formed at equivalence were used to calculate neutralization equivalents of 92 to 99. Analysis of the sodium salt was 35.39 percent carbon and 3.6 percent hydrogen. Sodium fusion elemental analyses were negative for sulfur, nitrogen, and halogens. Qualitative tests indicated the absence of carbonyl and hydroxy groups, while the rapid color changes in bromine and potassium permanganate solutions indicated unsaturation. These data suggested that acrylic acid ($\text{CH}_2=\text{CH}-\text{COOH}$), the sodium salt of which has a neutralization equivalent of 94 and a composition of 38.3 percent carbon and 3.2 percent hydrogen, was a likely possibility.

The monomer was extracted from a partially polymerized commercial preparation of acrylic acid and used to prepare sodium and calcium salts in the same manner as the algal material. The infrared spectra of the natural and synthetic preparations of sodium acrylate and calcium acrylate, determined by a Beckman IR-5 infrared spectrophotometer, were found to be identical. Filter paper disk antibacterial assays (on pH 6.5 heart infusion agar, Difco) of the natural and synthetic salts against 12 microorganisms were used to calculate the minimum inhibitory concentrations. The results presented in Table 1 indicate identical spectra of activity. Ascending filter paper chromatograms and x-ray diffraction analyses also

Table 1. Antibacterial spectrum of natural sodium acrylate from *Phaeocystis* and the synthetic salt.

Test organism	Minimum inhibitory concn. (mg/ml)	
	Synthetic	Natural
<i>Pasteurella multocida</i>	0.025	0.030
<i>Corynebacterium pseudodiphtheriticum</i>	0.21	0.21
<i>Mycobacterium smegmatis</i>	0.35	0.35
<i>Streptococcus pyogenes</i>	0.6	1.3
<i>Staphylococcus aureus</i>	0.8	1.6
<i>Proteus vulgaris</i>	2.5	2.5
<i>Candida albicans</i>	4.5	9.0
<i>Escherichia coli</i>	4.8	5.0
<i>Sarcina lutea</i>	5.0	5.0
<i>Klebsiella pneumoniae</i>	25.0	38.0
<i>Torula lactosa</i>	> 50.0	> 50.0
<i>Aspergillus fumigatus</i>	> 50.0	> 50.0

yielded identical data for both the natural and synthetic preparations.

Acrylic acid, which is known as a toxic chemical, is apparently nontoxic to at least certain animal tissues in dilute solutions. The free acrylic acid content of the *Phaeocystis* preparations was calculated to be 0.38 percent (7.4 percent dry wt.). This level was apparently nontoxic for euphausiids which were grazing on *Phaeocystis* blooms and whose stomach contents had antibacterial activity identical with that of the algae upon which they were feeding. Concentrations of acrylic acid sufficient to cause inhibition of the penguin gastrointestinal microflora were apparently nontoxic to the penguins. Preliminary tests in mice indicated that 700 mg of sodium acrylate per kilogram of body weight, injected intramuscularly, were well tolerated. However, *Phaeocystis* blooms which foul herring nets in the North Atlantic Ocean were believed to exclude herring (4), and the mucilaginous colonial form of *P. pouchetii* in culture has been observed to be toxic for herring fry (5).

The natural occurrence of appreciable amounts of acrylic acid in *Phaeocystis* raises some very interesting questions about its precursor, its function in the algae, its mode of antibacterial action, and its effect on the physiological ecology of the marine habitat. Apparently the only reports on the natural occurrence of acrylic acid in marine plants concern investigations of the precursor of dimethyl sulfide (6) in the epiphytic intertidal alga, *Polysiphonia lanosa*. Dimethylpropiothetin, which occurred in considerable amounts, was easily cleaved with heat or alkali (7) or by an enzyme (8) to form dimethyl sulfide and acrylic acid. Preliminary attempts to show the presence of this precursor or dimethyl sulfide in *Phaeocystis* were inconclusive. The mucilaginous substances in the colonial form of *Phaeocystis* may be acrylic polymers. Although substituted acrylates have been tested for their antibacterial activity (9) the activity of the parent compound has evidently not been reported. Possible modes of antibacterial action may be the inhibition of D-amino acid oxidase (10) or propionate oxidation (11). Acrylic acid may have anti-algal properties, since *Phaeocystis* replaced the diatoms in certain areas to become the predominant form. Acrylic acid apparently affects green plants (12) and may inhibit photosynthesis. Studies are in progress to further elucidate the role of acrylates in the physiological ecology of the Antarctic marine habitat (13).

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Completion of Meiosis in Uninseminated Eggs of *Drosophila melanogaster*

Abstract. Contrary to earlier statements, meiosis goes to completion in "unfertilized" eggs of *Drosophila melanogaster*. Evidence suggests that this is not only characteristic of the strain examined but of the species as a whole and of other *Drosophila* species as well.

The earliest reference to meiotic figures in the "unfertilized" (that is, uninseminated) eggs of *Drosophila melanogaster* is that of Huettner, who stated, "... maturation does not take place unless the egg is fertilized" (1). No evidence was presented in support of this statement, and yet the idea that meiosis does not continue beyond metaphase of the first division unless sperm enter the egg has become incorporated into the literature (2). The situation has been examined by analysis of eggs laid by virgin females (3, for preliminary report).

Eggs collected at half-hour intervals from individual virgins of an Oregon-R strain were aged from ½ to 2 hours at 25°C, sectioned, and stained with Heidenhain's hematoxylin. As an extra precaution, egg samples were collected prior to and after those used for sectioning and were incubated to make certain that none of them hatched.

Cytological details were studied in 93 sectioned eggs. In every case meiosis had gone to completion, contrary to views expressed in the literature. In 20 of these eggs, four interphase or

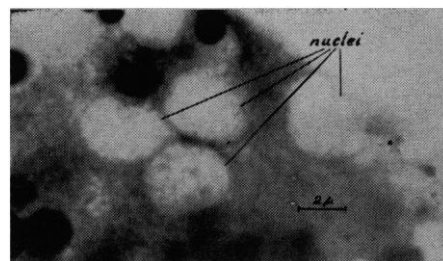


Fig. 1. Cytoplasmic island from dorsal anterior region of uninseminated egg with the four nuclear products of meiosis.

prophase nuclei were found and identified as the products of maturation (Fig. 1). The remaining eggs contained one, two, or three groups of metaphase chromosomes whose appearance often resembled the polar body chromosomal groups described in fertilized eggs (4), with spindle elements usually discernible.

The sequence of events following maturation varies, depending upon which of the meiotic products fuse with one another. Counts made of the number of chromosomes in various groups indicate that two, three, or even four nuclei may come together and fuse. The innermost or outermost chromosomal group, or both, appear to be omitted from the fusion product when it involves only two or three nuclei. With the completion of meiosis, a cytoplasmic extension forms, along which the female pronucleus, or metaphase fusion product, may or may not pass to the center of the egg, even though sperm have not entered. It seems probable that the fusion product in older degenerating eggs is the figure assumed to represent the first meiotic division in previous papers.

The possibility exists that the completion of meiosis in uninseminated eggs is a feature peculiar to the Oregon-R strain examined. However, other evidence supports the view that it is a more general phenomenon and probably represents the rule rather than the exception in *Drosophila*. First, a somewhat similar description of "unfertilized" eggs has been given for *D. funebris* (5), although no systematic investigation of the situation was reported. Second, Guyénot and Naville (6) noted an occasional first meiotic division anaphase spindle, as well as second division spindles in "unfertilized" eggs of *D. melanogaster*. Further support comes from recent studies of parthenogenesis in *Drosophila*. Genetic (7) and cytological (8) evidence has been presented for the fusion of haploid nuclei after the completion of meiosis in *D. parthenogenetica*. In this species, continuation of meiosis beyond Meiosis I has been described (8) in "unfertilized" eggs of a uniparental parthenogenetic line and also in some eggs from a biparental line. Cytologi-