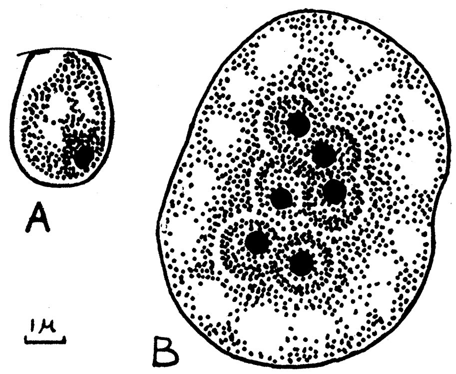

Fig. 1. *Haplosporidium costale. A*, Mature spore; *B*, early plasmodium.

May and stopped dying early in July. Numerous dead and dying oysters were collected and fixed in formol–acetic acid–alcohol for subsequent microscopic examination. Sections were stained with Harris or iron-alum hematoxylin and eosin.

As evidence of a previously unrecognized disease accumulated, it also became apparent that there was an undescribed parasite associated with the mortality. For convenience the parasite was first given the name SSO (for Seaside organism). The presence of SSO in live oysters prior to the mortality and in a large proportion of dead oysters implicated it as the etiological agent, here described as a new species of *Haplosporidium*.

Haplosporidium costale n. sp.: Endoparasite of connective tissues of *Crassostrea virginica*. Spores obovate, truncate, operculate, uninucleate, 2.42 to 4.20 μ (mean 3.09 μ) by 2.14 to 3.26 μ (mean 2.58 μ), lacking projections (Fig. 1*A*). Sporocysts: 7 to 14 μ (mean 9 μ) in diameter, containing 20 to 50 spores per cyst. These measurements, made on stained sections, are approximately 25 percent less than those made on spores in fixed material mounted in lactophenol (that is, before dehydration). Host: *Crassostrea virginica* (Gmelin). Type locality: Hog Island Bay, Virginia. Range: Bays and inlets of Seaside, Virginia, from Chincoteague to Cape Charles, occasionally in Chincoteague Bay and on Bayside in The Gulf and Cherrystone Inlet. The species name refers to its coastal distribution.

The earliest stage observed, cytozoic and histozoic in connective tissue, is a small, multinucleate (4 to 12 usually) plasmodium, 6.1 by 7.8 μ in size; essentially isodiametric, at times irregular in outline; cell membrane at first definite, later inconspicuous; nucleus vesicular, essentially isodiametric with a rather indefinite nuclear membrane and tiny but readily apparent endosome; nucleus 1.6 μ in diameter; endosome 0.6 μ in diameter (Fig. 1*B*).

The early plasmodia (trophozoites) enlarge, the nuclei multiply in number and the cytoplasm becomes vacuolated. A definite central vacuole is sometimes visible. The multinucleate plasmodium finally cleaves into uninucleate portions, each of which becomes a characteristic operculate spore (Fig. 1*A*).

The order *Haplosporidia* Caullery and Mesnil is an artificial assemblage of spore-producing organisms that are not assignable to other groups. The characteristics of the genus are: a large plasmodium which divides into uninucleate bodies, each of which develops into a truncate spore with a lid at one end; envelope may be prolonged into processes; in aquatic annelids and mollusks.

Haplosporidium costale falls into the group of species characterized by spores with an overhanging lid and no appendages, and it has the smallest spores in the group. Division of the sporoblast nucleus and fusion of nuclei or binucleate spores have been described for some species (*3*). The spores of *H. costale* are uninucleate, and in the sporoblasts neither division nor fusion of nuclei was observed.

The fate of ripe spores is not known and it is not clear how oysters become infected with this organism. Uninucleate amoebulae are described for certain species of *Haplosporidium* and not for others (*3*). We have not observed uninucleate trophozoites in the new species.

The disease has been followed closely for 3 years now, and its sharp seasonality is noteworthy. *Haplosporidium costale* is first evident in live oysters in February. Prevalence of infections in mid-May may run as high as 39 percent. During 2 weeks in June 1960, the death rate caused by the organism exceeded anything ever experienced in Chesapeake Bay with other pathogens. The mortality is sharp but of short duration, and the parasite lapses into obscurity for another year. Certain other species of *Haplosporidium* exhibit a similar seasonality (*3, 4*).

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4. We acknowledge the invaluable aid and criticism of Dr. Harold H. Haskin, Rutgers University, Dr. John G. Mackin, Texas A.&M. College, and Dr. Victor Sprague, Chesapeake Biological Laboratory. Mrs. Dorothy K. Emory, with the assistance of Miss Patricia Turner, prepared the slides. This report is contribution No. 102 from the Virginia Institute of Marine Science. Detailed results of our study of the mortality are in preparation.

21 December 1961

Cell Membrane Fusion and the Fertilization Mechanism in Plants and Animals

Abstract. Plasmatic fusion in both plant and animal fertilization seems to start with the coalescence of cell membranes of the gametes. In male gametes, the area of fusion is predetermined: in some algae it is the flagellar tip, while in animal organisms it is the acrosome filament. These organelles thus fulfill comparable roles in the mechanism of fertilization.

Recent publications on animal fertilization in *Hydroides* and the rat (*1, 2*) have revealed the central role of the coalescence of egg cell and spermium membranes during the fusion of gametes. This mechanism of fertilization resembles strikingly the oogamous fertilization of the green alga *Prasiola stipitata*, as described by Friedmann (*3*) and Manton and Friedmann (*4*). It seems appropriate, therefore, to point out some facts which seem to be analogous in these phenomena and which might well be characteristic of the fertilization process in both plants and animals.

A diagrammatic representation of some aspects of the fertilization in *Prasiola* is given (Fig. 1), based on light- and electron-microscopic observations already published (*3, 4*).

The process of plasmatic fusion in *Prasiola* can be summarized as follows. The spermatozoid bears two equal flagella. The fibrillar core of the flagellum is ensheathed by a membrane which is continuous with the cell body membrane (Fig. 1*A*). The basal bodies of the flagella are held close to the spermatozoid nucleus by fibrous roots. (The roots and the pointed flagellar tip are not represented in the diagram.) During the first step in fusion, the membrane of one spermatozoid flagellum coalesces with the membrane of the egg and the fibrillar core of the flagellum is engulfed by the egg protoplast. Subsequent stages of fusion are also represented in Fig. 1 (*B*, *C*, and *D*). The stage represented in *C* might not occur in each fusion although it has been observed frequently. When it does occur, the absorbed flagellum forms a "bridge" with an unfused indentation beneath and often penetrates deep into the zygote protoplast during comparatively late stages of fusion, near the time when the zygote begins to round up.

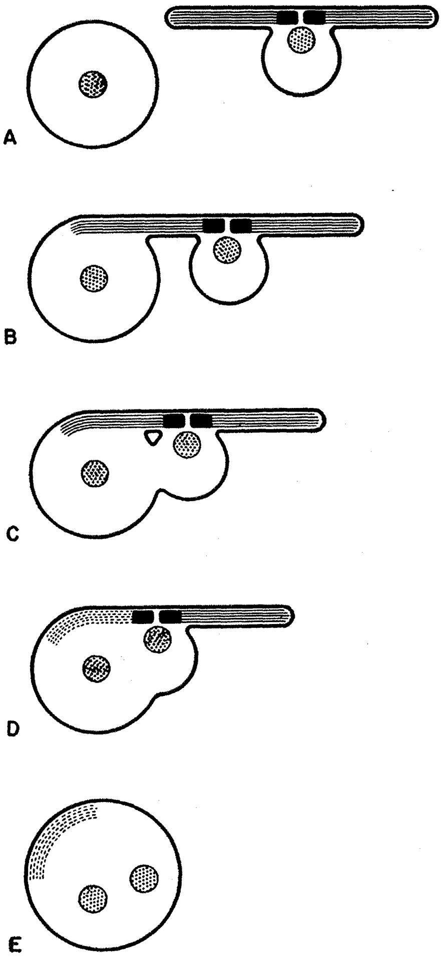

Fig. 1. Plasmatic fusion in the fertilization of the green alga *Prasiola stipitata* Suhr, based on the investigations of Friedmann (*3*) and Manton and Friedmann (*4*). *A*, Egg cell and biflagellate spermatozoid. *B*, Beginning of fusion. Coalescence of the membranes of the egg cell and of the tip of one of the spermatozoid flagella. Engulfing of the fibrillar core of the fusing flagellum by the egg cell protoplast. *C*, Later stage in fusion with a "bridge" and an unfused depression between the gametes. *D*, Motile stage of the zygote. Disintegration of the fibrils of the engulfed flagellum. *E*, Immobile stage of the zygote after "rounding up." Withdrawal of the motile flagellum and disintegration of its fibrils.

The pear-shaped zygote in Fig. 1*D* carries in its protoplasm the engulfed flagellum, which later disintegrates. The second, free flagellum remains motile at this stage but it is withdrawn when the zygote rounds up (Fig. 1*E*). The nucleus is then no longer attached to the flagellum, and the flagellum disintegrates.

There are several points of interest which emerge from the comparison of the fertilization processes in *Prasiola*, *Hydroides*, and the rat. The actual fusion of the protoplasts is preceded and apparently initiated by the coalescence of the male and female cell membranes. The ability to fuse is certainly a very specific state of the cell surface membrane.

In the egg, there is no morphological evidence that a particular area of the cell surface is predetermined to be the point of fusion. On the contrary, in the male gamete, the potentiality of the surface membrane to fuse is restricted to a distinct area. In animal sperms, this area seems to be the membrane of the acrosome (when this organelle is apparent). In *Prasiola*, the distinct area which can fuse is the membrane of the apical region of the flagellum, which thus assumes a function apparently analogous with the function of the acrosome. In spite of their very different physiological behavior, there is no structural difference between the fusing and nonfusing flagellum of the *Prasiola* spermatozoids and there is even some evidence that each of the flagella is potentially capable of functioning in either way (*5*).

In the brown algae (Phaeophyta), however, where the two flagella of the spermatozoid are morphologically different, only one flagellar type seems to participate in fusion (*6*).

The strict comparability of "bridges" and unfused depressions in *Prasiola*, "vesiculation" in *Hydroides* (*1*), or the "fold" which is present during the fertilization in rat (*2*) remains to be clarified by further investigations. The fact that all these structures appear at the area of actual fusion of the male and female cell membranes is suggestive.

There is indication that some analogous biophysical or biochemical mechanisms might have a basic role in both plant and animal fertilization. Some of the elastic properties of the cell membranes of flagellated plant cells were discussed to some extent by Manton and Friedmann (*3*). Similar analysis might also be considered in connection with the fertilization mechanism in animals.

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14 November 1961

Conditioning in Fish: Effects of X-irradiation

Abstract. Fish were subjected to different levels of high intensity x-irradiation to ascertain the effects of x-rays on acquisition and retention of conditioning. Lethal dosages were also determined. Light was the conditioned stimulus, and electric shock the unconditioned stimulus. Responses were forward darting and backward swimming movements accompanied by increased gill movement. Acquisition of conditioned responses was suppressed by higher dose levels.

Conditioned responses in various mammals are affected by x-irradiation. The general effects are transitory or gradual decrements in responses (*1*). However, Furchgott (*2*), in summarizing a number of ionizing radiation experiments, concluded that general learning functions are relatively unaffected by high doses of x-irradiation, even in the lethal ranges. Any decrements in learning were considered to be due to motivational and perceptual factors.

Table 1. Comparison of overall mean and standard deviation (S.D.) of conditioned response frequency of x-irradiated and nonirradiated fish.

Av. x-irradiation (r)	No.	Mean	S.D.
0	14	77.2	8.8
7,200	12	76.7	10.2
10,100	32	66.8	11.6
18,400	10	51.6	18.0