with a six months' survival time, give a negative reaction in the degenerating area. This finding, in contrast to the monkey, possibly has its explanation in its being either a more chronic preparation or more likely on the basis of an augmented metabolic rate which results in the complete assimilation of the degenerating neuron.

In conclusion, we feel that studies on acid phosphatase in the nervous system open up possibilities in the field of neuronal metabolism and function and that an additional method is offered for demonstrating both normal and degenerated nerve tissue.

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PRECOCIOUS GONAD DEVELOPMENT IN OYSTERS INDUCED IN MIDWINTER BY HIGH TEMPERATURE

SPAWNING of the American oyster, *Ostrea virginica*, of Long Island Sound and the adjacent geographical areas of the North Atlantic Coast begins late in June or early July and is usually completed late in August or early in September.[1] After resorption of the undischarged sex cells the gonads of the oysters pass through the indifferent stage, when the sexes are undistinguishable, the sex differentiation stage, when slight gametogenic activities occur, and then enter into the winter, or inactive stage, which persists until April or, in some individuals, until May.[2] During the inactive stage the gonad follicles are small, containing only the cells of the early stages of gametogenesis. Seasonal gonadal changes of many other pelecypods closely resemble those of *O. virginica*.[3]

Since the period during which the oysters contain morphologically and physiologically ripe gametes is relatively short in northern localities, the time which could be devoted to the study of these cells or to observations on the development, growth and physiology of the larvae is correspondingly limited. Therefore, if oysters could be induced to develop gametes at periods other than the summer time, additional opportunities would be available for investigations in the field of embryology and in that of the biology of oyster larvae.

The literature contains numerous references on precocious gonad development in many species of animals induced by artificial changes in the external or internal environment. This out-of-season gonad development may be caused by changing the physical factors, such as the relative length and the intensity of illumination, changes in temperature, or by employing chemical methods, such as injection of hormones, or changes in food. A review of the literature on these subjects is not, however, in the scope of this article, the purpose of which is merely to suggest a method which would provide laboratory workers with active spermatozoa and fertilizable oyster eggs during the winter and early spring months.

The present experiments were conducted from the end of November to the middle of March. The oysters were taken from the outdoor tanks, where the water temperature was near the freezing point and usually a layer of ice was formed over the surface. Examination of samples of the tank oysters revealed that their gonads were in a typical winter or inactive stage.

To avoid the effects of sharp changes in temperature, which would occur if the hibernating oysters were changed directly from ice-cold water to that of 20° C. or above, the necessary precautions were observed. The animals brought in from outside were placed in the aquaria filled with water the temperature of which was the same as that at which the oysters were kept before being taken into the laboratory. Within 24 hours the water in the aquaria usually reached room temperature. After keeping the animals under such a condition for 48 or 72 hours the temperature was slowly brought to the desired level and maintained there by thermostats throughout the experiment. The temperatures to which the different groups of oysters were exposed were 20°, 25° and 30° C. The fluctuations in the temperatures were within ±1.5° C. of the above given figures. A direct transfer from cold water to that of 30° C. appeared to injure some of the oysters and sometimes caused their death.

Examination of the oysters kept at a temperature of 20° C. for 20 days showed that some of them had developed a large number of eggs during that period. Active spermatozoa were also observed in a few individuals. Nevertheless, the majority of the oysters appeared unripe. After 30 days, however, the animals were in a much more advanced condition and many individuals possessed well-developed eggs or active spermatozoa.

Oysters kept for one month at temperatures of 25° or 30° C. formed a gonadal layer the thickness of which in some cases was 3.5 mm, thus comparing favorably with the animals developing their gonads in the summer time under natural conditions.[1] The eggs of the experimental oysters were carefully removed from the follicles and placed in sea water to which spermatozoa were added. Fertilization occurred and zygotes proceeded to develop into larvae, which were kept alive for several days and appeared normal in their form and behavior.

[1] V. L. Loosanoff and J. B. Engle, *Biol. Bull.*, 82: 413–422, 1942.
[2] V. L. Loosanoff, *Biol. Bull.*, 82: 195–206, 1942.
[3] W. R. Coe, *Quarterly Review of Biology*, 18: 154–164, 1943.

An additional interesting observation made in the course of these studies was that the oysters had developed gametes regardless of the fact that the mollusks were kept under comparatively unfavorable conditions. The water in the aquaria, although aerated, was changed only once every 7 or 10 days. At the time of the change a small quantity of food was added. It was quickly consumed by the oysters; thus, with the exception of brief periods, the animals were virtually deprived of food. These observations may indicate, therefore, that the apparently normal development of the oyster gonads may proceed when the quantity of food is rather limited.

Undoubtedly, the method described in this article, or its modifications, may be successfully applied to certain pelecypods other than *O. virginica.* Through recent personal communication with Mr. D. L. McKernan and Dr. Vance Tartar, of the State of Washington Oyster Laboratory at Gig Harbor, Washington, I was advised that they also repeatedly succeeded in inducing a larviporous species of oyster, *O. lurida,* to release larvae in midwinter by keeping the animals in warm sea water.

I wish to express my appreciation to Mr. Charles Nomejko and Miss Frances Tommers for their assistance in this work.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPLICATION OF THE LYOPHILE PROCESS TO THE MAINTENANCE OF CULTURES FOR MICROBIOLOGICAL ASSAY[1]

WHILE the lyophile process of desiccation from the frozen state has long been employed by bacteriologists for the preservation of cultures, there appears to have been no application of this method to the maintenance of highly viable cultures for use in the microbiological assay of vitamins and amino acids. It is a common experience in many laboratories that there is a gradual departure in linearity of response and activity of cultures of *Lactobacillus arabinosus* and *Lactobacillus casei* transferred at monthly intervals from one agar stab to another. In a search for a means of obtaining more constant standard curves and of obviating the frequent transfer of the culture organisms in order to maintain them in optimum condition for acid production, experiments were carried out to determine whether or not this could be successfully accomplished by lyophilizing the cultures.

Thus far, results have shown that *L. arabinosus* 17-5 and *L. casei* ε cultures maintain their activity when lyophilized by a simple procedure (*cf.* Table 1 and Fig. 1). After three months' storage such cultures gave equally as good response as did the standard culture maintained by weekly transfer through broth[2] to liver tryptone agar. Since these cultures have been stored without decreased activity for three months and are completely desiccated and sealed under high vacuum, they presumably will maintain their activity for a much longer period. It should be feasible then to lyophilize a six months' or year's supply of cultures at one time. This procedure would be especially advantageous for laboratories where microbiological assays are carried out only infrequently or on a seasonal schedule.

TABLE 1

A COMPARISON OF THE ACID PRODUCTION OF STANDARD AND LYOPHILIZED CULTURES OF *Lactobacillus casei* ε*

Micrograms riboflavin	72-hour lactic acid production (ml N/10 KOH)			
	One month		Two months	
	Standard culture	Lyophilized culture	Standard culture	Lyophilized culture
0	0.99	1.02	1.00	0.99
0.05	4.95	4.99	4.82	4.84
0.1	8.11	8.44	8.02	8.41
0.15	10.32	10.81	10.53	10.76
0.2	12.25	12.90	11.75	13.17
0.25	13.84	14.16	13.08	13.84
0.3	14.52	14.94	13.23	15.08

* Basal medium of Snell and Strong (*Ind. Eng. Chem., Anal. Ed.,* 11: 346, 1939) modified to contain 2 per cent. glucose and 2 per cent. sodium acetate.

The lyophile process used in our laboratories requires no special equipment and could be accomplished in any laboratory at very little cost. The procedure is as follows: Cultures of *L. arabinosus* and *L. casei* are grown for 24 to 36 hours at 30° C. and 37° C., respectively, in a nutrient-rich medium.[2] These cultures are then centrifuged, and to the cells

[1] Aided by a grant from the Consolidated Edison Company of New York, Inc. Received for publication, February 19, 1945.

[2] We have used the following medium: 1 per cent tryptone; 0.5 per cent K_2HPO_4; 0.2 per cent glucose; 0.2 per cent yeast extract (Difco); 10 per cent fresh liver extract (1 pound ground liver per 2 l. water. Steam 60 minutes. Filter through cheesecloth. Neutralize to pH 7.0. Heat 15 minutes. Filter through coarse filter paper.)