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

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[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.)

obtained from 10 ml of broth is added ½ ml of sterile skim milk. One-tenth ml quantities of this milk suspension are added to sterile, cotton-plugged, straight-sided specimen vials (10 mm × 42 mm). A narrow strip of cellulose tape is placed over the cotton plug to assure its remaining in position. The vials are then placed in a desiccator containing Drierite (anhydrous $CaSO_4$) and lyophilized by holding under vacuum over night. If a pump such as the Cenco Hyvac is used, the contents are quickly frozen and after desiccation yield a white fluffy powder which readily forms a suspension upon the addition of liquid. With a lower vacuum a dark, resinous mass occurs which forms a suspension only after a considerably longer period of time. Although cultures of *L. arabinosus* dried under low vacuum have given results after three months that were comparable to

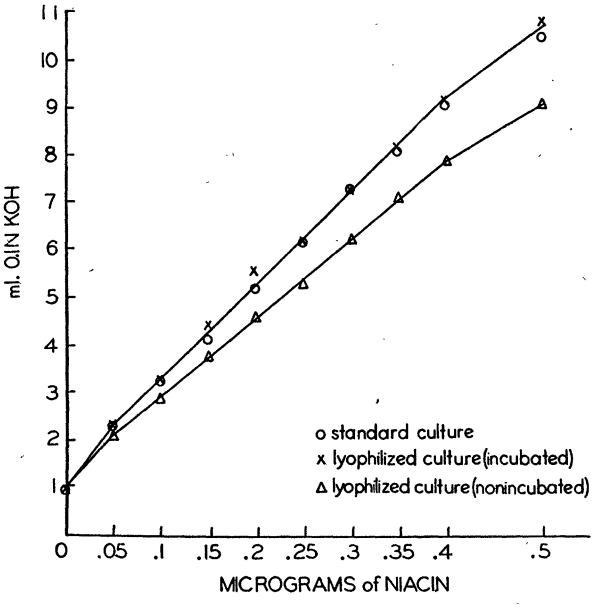

FIG. 1. A comparison of the 72-hour acid production of standard cultures and incubated and nonincubated lyophilized cultures of *Lactobacillus arabinosus* 17–5 in response to added niacin, employing the basal medium of Krehl, Strong and Elvehjem (*Ind. Eng. Chem., Anal. Ed.*, 15: 471, 1943). The lyophilized cultures had been stored two months.

cultures lyophilized by high vacuum, cultures of *L. casei* did not seem as viable when the lower vacuum was used. For best results one should employ high vacuum.

After the contents of the vials are dry (12–18 hours under vacuum), the vials are placed in ¾ inch test-tubes, which are then constricted in an oxygen flame. The tubes are evacuated by means of a high vacuum pump and sealed by rotating in a Fisher burner. The cultures are stored at room temperature away from the light.

For the preparation of the inoculum a desiccated culture is suspended in about one ml of sterile saline or basal medium, and after being stirred by means of a sterile inoculation needle the entire content of the vial is added to 20 ml of diluted basal medium containing the vitamin or amino acid to be assayed (for example, 0.3 μg niacin or riboflavin) and is incubated the customary 24 to 36 hours prior to use. Lyophilized cultures of *L. arabinosus* suspended in 10 ml of saline and used directly as inoculum for assay procedure did not produce as much acid in 72 hours as did the inoculum incubated before use. The response was as linear, however. A comparison of the acid production of standard cultures and of incubated and non-incubated lyophilized cultures of *L. arabinosus* stored two months is shown in Fig. 1.

Further storage studies are in progress and will be reported at a later date. It is felt that the lyophile process as applied to these microbiological assay organisms is saving of time, labor and culture materials, since it obviates the preparation of agar stabs and broths and also the frequent transfer of the cultures necessary to maintain highly viable cells. It also improves the reproducibility of the assay curves in so far as they are influenced by the bacterial cultures.

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ISOLATION OF *TRICHOMONAS VAGINALIS* WITH PENICILLIN[1]

FOLLOWING the initial isolation of a bacteria-free strain of *Trichomonas vaginalis* by Trussell[2] in June, 1939, various investigations of the pathogenicity,[3] physiology[4,5,6,7,8,9,10,11,12] and chemotherapy[13,14] of

[1] Aided by a grant from The Ortho Research Foundation.

[2] Ray E. Trussell, *Jour. Iowa State Med. Soc.*, 30: 66, 1940.

[3] Ray E. Trussell and E. D. Plass, *Am. Jour. Obst. and Gynec.*, 40: 883, 1940.

[4] Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 45: 567, 1940.

[5] Alfred B. Kupferberg, *Proc. Soc. Exp. Biol. and Med.*, 45: 220, 1940.

[6] S. H. McNutt and Ray E. Trussell, *Proc. Soc. Exp. Biol. and Med.*, 46: 489, 1941.

[7] Ray E. Trussell and Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 47: 176, 1941.

[8] Ray E. Trussell and S. H. McNutt, *Jour. Inf. Dis.*, 69: 18, 1941.

[9] A. B. Kupferberg and Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 48: 516, 1941.