into the laboratory and induced to spawn. After spawning they were returned to the harbor. Samples of the gonads taken approximately once a month for histological studies showed that by the middle of September the oysters had almost recovered from the summer spawning.

Toward the end of November the oysters began to hibernate. Late in December 1947 they were brought from the harbor, which at that time was covered with a layer of ice, and placed in the laboratory in running sea water at a temperature of about 4° C. Using our method for conditioning mollusks for winter spawning (*2*), the temperature was gradually increased a few degrees every two or three days until it was slightly over 20° C, after which the oyster became ripe within three to four weeks. They were induced to spawn in January and February 1948, discharging normal gametes.

Following spawning, and partial recovery of the oysters, the temperature of the water was gradually decreased. This process resembled fundamentally the condition occurring in our natural waters in late summer and fall. In March 1948 the oysters were returned to Milford Harbor, where the water temperature was only about 4° C—i.e., cold enough to make them hibernate.

Some of the oysters were returned to the harbor while their gonads were still in the process of active resorption and while they contained undischarged eggs. When examined early in May, at which time the water temperature was still below 10° C, the oysters remained in about the same condition. Apparently the temperature between the dates of return to the harbor in March and the examination was too low to permit active resorption of the remaining gonad material. However, with the increase of temperature, which reached 16° C by the end of May, the resorption proceeded more rapidly, and was soon completed.

Between the end of May and the middle of June 1948 the oysters again underwent and completed gametogenesis and were easily induced to spawn when brought into the laboratory. After the spawning they were once more returned to the harbor and went through the normal process of gonad resorption and glycogen accumulation, eventually entering hibernation late in the fall. Thus the condition and behavior of the oysters in the summer and fall of 1948 were basically the same as during the corresponding period the previous year, regardless of their unusual extra reproductive activities during the winter.

In January 1949 the oysters were again brought into the laboratory, conditioned, and induced to spawn. Then, following the procedure described for the preceding year, they were once more returned to the harbor. They again spawned in the summer of 1949. Thus within a period of two years, June 1947–June 1949, the oysters were made to develop gonads and discharge normal spawn on five occasions at approximately six-month intervals.

The experiments have shown that the processes of gonad development and spawning of these oysters are not of the endogenous type; i.e., there is nothing in their physical pattern that will not permit reproduction oftener than once a year, provided the ecological conditions are favorable for all aspects of the physiological activities involved in this complex process.

The experiments have also demonstrated that the oysters, in developing gonads, showed no dependency on seasonal changes in such factors as light, tidal rhythm, precipitation, small variations in salinity, or other changes that usually occur during the spring and early summer when the gonads of oysters are rapidly developing. It has also been shown that gonad development of oysters is not dependent on certain types of plankton organisms that are present in the water only during the spring and summer, the time of normal gametogenesis and spawning.

Although the observations reported here concern oysters only, we think, nevertheless, that the conclusions may also apply to some other lamellibranchs. For example, in our work with the clam *Venus mercenaria*, several individuals have been spawned in the January-July-January pattern. This ability of some Northern lamellibranchs to be conditioned for spawning at more frequent intervals than under natural conditions offers an interesting and practical method for studies of physiological ecology, genetics, and other aspects of their behavior.

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Enzymatic Reduction of Cystine by Coenzyme I (DPNH)[1,2]

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The occurrence of glutathione reductase, catalyzing the reduction of oxidized glutathione (GSSG) by reduced triphosphopyridine nucleotide (TPNH), has been demonstrated in pea seeds by Mapson and Goddard (*1*) and in wheat germ by Conn and Vennesland (*2,3*). The latter authors (*3*) stated that their purified preparations of glutathione reductase are without activity toward cystine. Meldrum and Tarr (*4*) had previously supplied considerable circumstantial evidence for the occurrence, in rat blood and in yeast, of an enzymatic process utilizing TPNH for the reduction of GSSG. The property of reducing cystine has, for a long time, been attributed to plant and ani-

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[3] Standard Brands Research Fellow in Microbiology.

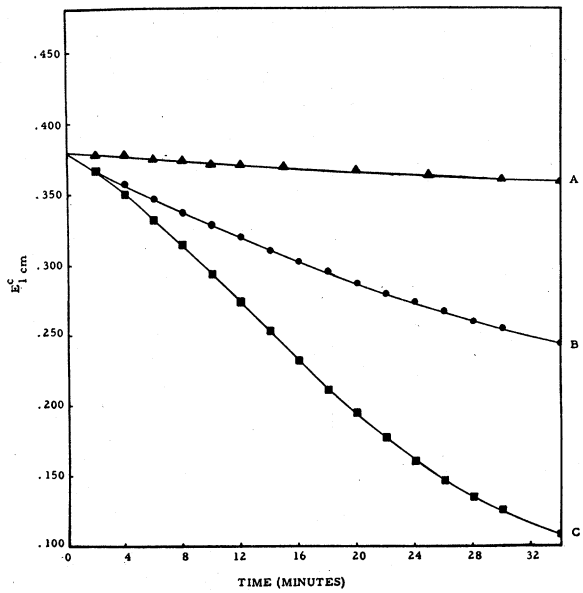

FIG. 1. Rate of oxidation of DPNH by a heat-labile cystine reductase system: Curve *A*, 1 ml heated cell-free extract of baker's yeast (in *M*/40 phosphate buffer, pH 6.2) + 1 ml DPNH solution (280 μg) + 1 ml saturated cystine solution in *M*/40 phosphate buffer; Curve *B*, 1 ml cell-free extract of baker's yeast + 1 ml DPNH solution (280 μg) + 1 ml buffer; Curve *C*, 1 ml cell-free extract of baker's yeast + 1 ml DPNH solution (280 μg) + 1 ml saturated cystine solution in buffer.

mal tissues, but the mechanism of this reduction has not been elucidated. Tunnicliffe (*5*) reported the existence, in tissue residues, of a thermostable substance capable of reducing disulfides. The statements of Abderhalden and Wertheimer (*6*) that frog muscle can be washed until it shows no power of reducing cystine, and that the reducing power can be restored by the addition of *"Kochsaft"* suggest the participation of a thermostable coenzyme.

After grinding washed cells of baker's yeast (starch-free pound cakes of Fleischmann's yeast) at 2° with powdered glass in a microhomogenizer designed by Brendler (*7*) (employing a Lucite rotor and made by E. Machlett & Son), we have succeeded in obtaining a cell-free extract containing a heat-labile enzyme catalyzing the reduction of cystine to cysteine by reduced disphosphopyridine nucleotide (DPNH). The rate of oxidation of DPNH in the presence of cystine and the enzyme (which may be termed *cystine reductase*) has been followed in a Beckman Model DU Quartz Spectrophotometer at 340 mμ. The DPN (Schwarz Laboratories, 65% pure, TPN-free) was reduced by the method of Hogeboom and Barry (*8*). As shown in Fig. 1, a slow rate of oxidation of DPNH in the absence of cystine is occasioned by substances contained in the enzyme preparation; in the presence of cystine the rate of oxidation of DPNH is greatly accelerated. Heating at 100° C for 10 min completely destroys this activity. The apparent reaction may be written:

$$\text{Cystine} + \text{DPNH} \xrightarrow{\text{reductase}} 2\ \text{cysteine} + \text{DPN}^+.$$

Reduced coenzyme II (TPNH) is without activity in this system. We have, however, observed glutathione reductase activity in cell-free extracts from baker's yeast; and, confirming observations of others (*1–3*), find that TPNH is the hydrogen-donating agent whereas DPNH is not active in the glutathione reductase system.[4]

The appearance of —SH from the enzymatic reduction of cystine has been followed by iodine titration and by the phospho-18-tungstic acid method of Shinohara (*9*); the data obtained by the two methods showed excellent agreement. Cystine reductase activity has been found in acetone powder preparations of ungerminated peas (Topper variety) and in two strains of the pathogenic yeast *Candida albicans*, as well as in baker's yeast.

If acetone powder preparations of baker's yeast are washed, the ability to reduce cystine to cysteine is lost. This activity is restored to the washed residue by the addition of DPNH, as is shown in Fig. 2.

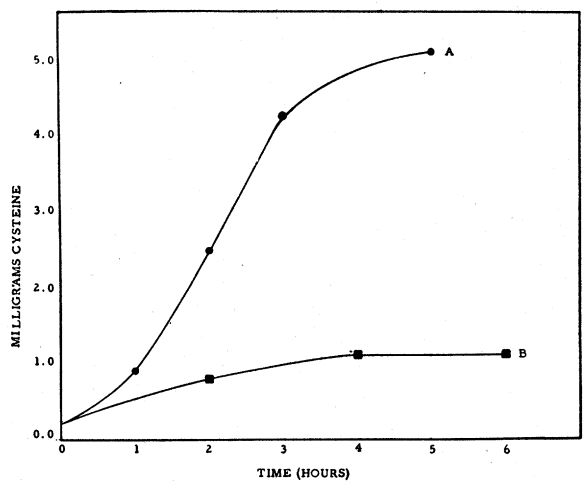

FIG. 2. Effect of DPNH on cystine reduction as measured by iodine titration: Curve *A*, 2 g washed acetone-dried baker's yeast + 90 mg cystine + 780 μg DPNH; Curve *B*, 2 g washed acetone-dried baker's yeast + 90 mg cystine.

The procedure employed was as follows: 20 g acetone powder was washed twice with 200 ml distilled water, and suspended in 100 ml *M*/40 phosphate buffer, pH 6.2. Ten ml of this suspension (2 g acetone powder) was added with 90 mg cystine and 780 μg DPNH to a 250-ml filter flask. The volume was made up to 40 ml with *M*/40 phosphate buffer, pH 6.2; 1 ml toluene was added to prevent microbial activity. The flask was stoppered, evacuated through the side arm, and filled with nitrogen. The reaction was stopped by the addition of 2 ml 10% phospho-24-tungstic acid in 5% HCl. After filtration, the volume of the filtrate was made up to 50 ml. A 10-ml aliquot was removed for cysteine determination by the Shinohara method, and the remaining 40 ml was titrated with 0.005 *N* iodine.

[4] TPN was generously provided by the Armour Laboratories, Chicago, through the courtesy of Lawrence L. Lachat.

Since it is known that the action of many antibiotics can be reversed by cysteine, it was thought that this enzyme system might be affected. However, cystine reduction was not inhibited by streptomycin, fradicin (*10*), or penicillin.

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Veralbidine, a New Alkaloid from *Veratrum album*

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After cautious extraction of *Veratrum album* and working up of the alkaloids, it was possible to isolate the already known bases protoveratrine, jervine, and rubijervine. From the mother liquors, by crystallization from ether, we were able to separate a new alkaloid for which we propose the name "veralbidine." Pure veralbidine crystallizes from dilute acetone in pentagonal plates, from dilute methanol in prisms, and from ether in bunches of fine needles. The crystals melt between 181° and 183° C and exhibit a specific rotation of $[\alpha]_D^{20} = -11.7°$ in pyridine and $[\alpha]_D^{20} = +5.4°$ in chloroform. In 84% sulfuric acid, veralbidine gives a colorless solution. It is sparingly soluble in ether, alcohol, and acetone and insoluble in water. It dissolves readily in chloroform. Veralbidine is irritating to the nasal mucosa, causing sneezing.

The empirical formula of the new alkaloid, as determined by chemical analysis, is $C_{37}H_{61}O_{12}N$. For analytical purposes the alkaloid was dried at 110°.

Required: C, 62.44%; H, 8.57%; N, 1.97%.
Found: C, 62.21%; H, 8.53%; N, 2.14%.

Veralbidine yields a crystalline thiocyanate which melts at 235°–236° with decomposition and frothing. It is readily soluble in methanol and acetone, but sparingly soluble in water. The analytical figures obtained after drying at 110° agreed with the empirical formula $C_{37}H_{61}O_{12}N \cdot HNCS$.

Required: C, 59.26%; H, 8.05%; N, 3.64%; S, 4.16%.
Found: C, 59.04%; H, 8.09%; N, 3.59%; S, 4.09%.
C, 59.10%; H, 8.17%; N, 3.54%.

Veralbidine also yields a crystalline hydrochloride which is readily soluble in alcohol and water. The hydrochloride melts at 250°–251° with decomposition and frothing. Empirical formula, $C_{37}H_{61}O_{12}N \cdot HCl$.

Required: C, 59.43%; H, 8.29%; Cl, 4.73%.
Found: C, 59.32%; H, 8.45%; Cl, 4.61%.

It is intended to give a more detailed report on the constitution and pharmacological action of veralbidine at a later date.

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The Effect of Experimental Stress upon the Photically Activated EEG[1]

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In the search for neurophysiological concomitants of mental processes it has seemed worth while to augment electroencephalographic investigation with the use of the stimulus of intermittently flashing light (*1*). The ability of such photic stimulation to drive the brain waves was described in 1934 by Adrian and Matthews (*2*). Such stimulation produces visual sensations (Prevost-Fechner-Benham effect) and a variable dysphoria. Walter (*3*) has made the observation that the type of brain response produced seems at times to vary in a complex manner with alterations in the subject's mood and that EEG responses appearing at a harmonic of the stimulus frequency might increase at the expense of the primary response. The ability of photic stimulation itself to produce mood changes (*1, 3*), however, renders such isolated disclosures difficult of interpretation, and hence prompted our investigation of changes in the photically stimulated EEG in subjects whose mental state was deliberately altered under laboratory conditions.

Ninety-six subjects 18–35 years of age were used in this procedure. They were divided into three groups. Groups I and II were selected from a larger sample studied for "anxiety-proneness" by psychiatric and psychological examination (*4*). Group I consisted of 30 subjects judged least likely to develop symptoms of anxiety under stress. Group II was composed of 25 psychiatric patients with diagnoses of psychoneurosis or character disorder in which anxiety was the predominant symptom. These two groups were placed under an experimental anxiety-producing situation in an attempt to determine whether such stress could affect the photically driven EEG and whether the two groups might react differently.

Group III (41 subjects) was the control group, consisting of experimentally sophisticated medical

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[2] The technical assistance of Rosemary Baessler and Ruth Ellerman is gratefully acknowledged.