lism. Obviously the activity of these two enzyme systems was not influenced by 2,4-D in the direction that might have been anticipated from observed changes in distribution of protein in leaves, stems, and roots following treatment of plants with 2,4-D (*2*). Possible interpretation of these findings will be presented elsewhere with further analyses of these plants.

References

1. CORNS, W. G. *Can. J. Research,* **28**, 393 (1950).
2. FREIBERG, S. R., and CLARK, H. E. *Botan. Gaz.,* **113**, 322 (1952).
3. SELL, H. M., *et al. Plant Physiol.,* **24**, 295 (1949).
4. SMITH, F. G., HAMMER, C. L., and CARLSON, R. F. *Ibid.,* **22**, 58 (1947).
5. WELLER, E. L., *et al. Ibid.,* **25**, 292 (1950).
6. WORT, D. J. *Am. J. Botany,* **36**, 673 (1949).
7. BLAGOWESTSCHENSKI, A. W., and MELAMED, R. M. *Biochem. Z.,* **273**, 435 (1934).
8. LAUFFER, S., TAUBER, H., and DAVIS, C. H. *Cereal Chem.,* **21**, 267 (1944).
9. MOUNDFIELD, J. D. *Biochem. J.,* **130**, 549 (1936).

Manuscript received January 21, 1952.

Chlorogenic Acid a Possible Metabolite in the Terminal Oxidase System of the White Potato[1]

Gestur Johnson

Colorado Agricultural Experiment Station, Fort Collins, Colorado

Since chlorogenic acid occurs in a significant amount in the white potato (*Solanum tuberosum*), it may be involved in other functions aside from the protective action against invasion by *Streptomyces scabies* and other types of injury as reported by Johnson and Schaal (*1*). Boswell and Whiting (*2*) were among the first to demonstrate experimentally that polyphenolase is involved in the respiration of the potato tuber. They were able to concentrate a natural tyrosinase substrate from potato tuber. This substance gave a green color with $FeCl_3$ which is characteristic of ortho-dihydroxy phenols. Upon adding it to respiring potato slices, they found increased rates of oxygen uptake and of carbon dioxide evolution. They were, however, not able to identify this substance. On the basis of the work of Johnson and Schaal, the indications are that it is chlorogenic acid.

Robinson and Nelson (*3*) contend that the active principle in potato juice which increases respiration is tyrosine. According to their view, tyrosine is oxidized to 3,4-dihydroxyphenylalanine (DOPA), which is the respiratory carrier. They estimated that 85% or more of the oxygen uptake may pass through this system.

The presence of chlorogenic acid in potatoes has been demonstrated by use of paper chromatography (*1*). However, no DOPA could be detected in potatoes by this method. Minute quantities of DOPA can be detected on a paper chromatogram by the use of Folin-Denis reagent or Pauly reagent (diazotized sulfanilic acid). The former gives a blue, and the latter a reddish-brown, color with DOPA. These reagents failed to show any DOPA in concentrated extracts prepared from potato flesh.

Rudskin and Nelson (*4*) found the natural polyphenolase substrate in the sweet potato (*Ipomoea batatas*) to be chlorogenic acid, and they concluded that chlorogenic acid and polyphenolase are involved in the terminal oxidase system of the sweet potato.

In view of the fact that chlorogenic acid is a natural substrate for tyrosinase (polyphenolase), and is present in greater quantities in the potato than DOPA, the author suggests that chlorogenic acid rather than DOPA is involved in the terminal oxidase system of the white potato.

References

1. JOHNSON, G., and SCHAAL, L. A. *Science,* **115**, 627 (1952).
2. BOSWELL, J. G., and WHITING, G. C. *Ann. Botany,* N. S., **2**, 847 (1938).
3. ROBINSON, E. S., and NELSON, J. S. *Arch. Biochem.,* **4**, 111 (1944).
4. RUDSKIN, G. O., and NELSON, J. M. *J. Am. Chem. Soc.,* **69**, 1470 (1947).

Manuscript received January 3, 1952.

Repeated Semiannual Spawning of Northern Oysters

V. L. Loosanoff and H. C. Davis

U. S. Fish and Wildlife Service, Milford, Connecticut

In Long Island Sound, as well as in ecologically similar areas, the spawning season of oysters, *Crassostrea virginica,* is confined to the period extending approximately from the last week of June until the beginning or middle of September. Thus the season is comparatively short and occurs once a year. It is the latter circumstance that suggested studies designed to determine whether the gonad development and spawning of the oysters were of the exogenous type—initiated and regulated by periodical seasonal changes of environment—or of the endogenous type—controlled by a pattern confined within the organism itself.

The question has been answered in part by experiments which showed that ripening of gonads in oysters could be achieved even in midwinter by placing the oysters for several weeks in warm water of about 20° C (*1*). It still remained uncertain, however, that this was not merely a case of precocious development of gonads which would make the oysters unable to undergo normal gametogenesis the following summer. To settle this an experiment was devised to find whether the oysters are able, under certain conditions, to accumulate and discharge spawn in a normal way at least twice a year, at intervals of about six months, and to do so for two or three successive years.

The experiment began in the spring of 1947 when a group of approximately 250 adult, individually numbered oysters was suspended on a float in Milford Harbor, Connecticut. By the middle of June, when the oysters reached ripeness, they were brought

[1] Published with the approval of the director, Colorado Agricultural Experiment Station, as Scientific Journal Series Article No. 375.

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.

References

1. LOOSANOFF, V. L. *Science*, **102**, 124 (1945).
2. LOOSANOFF, V. L., and DAVIS, H. C. *Biol. Bull.*, **98**, 60 (1950).

Manuscript received January 9, 1952.

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

Walter J. Nickerson and Antonio H. Romano[3]

Department of Microbiology, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick

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-

[1] Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University—The State University of New Jersey, Department of Microbiology.

[2] This investigation was supported (in part) by a research grant from the National Institutes of Health, U. S. Public Health Service.

[3] Standard Brands Research Fellow in Microbiology.