Gram-negative bacteria, would, in the presence of 1:28,000 methylene blue or brilliant cresyl blue, completely inactivate 10 million cells of a 24-hour culture of *E. coli* in 10 cc of nutrient broth buffered at pH 6.8 and containing a final concentration of 1:14,000 sulfapyridine, sulfathiazole or sodium sulfathiazole.

TABLE 1

THE CONCENTRATIONS OF INDIVIDUAL DYES AND SULFONAMIDES REQUIRED TO KILL 10^7 CELLS OF *E. coli* OR *Staph. aureus* IN 10 CC NUTRIENT BROTH BUFFERED AT PH 6.8, INCUBATED AT 37° C.

Reagent	Concentration *Staph. aureus*	Concentration *E. coli*
Methylene blue	1:100,000	1:13,000
Brilliant cresyl blue	1:130,000	1:20,000
Sulfathiazole	1:100	complete bacteriostasis not apparent at saturation levels
Sulfapyridine	1:100	
Sulfanilamide	1:100	
Sodium sulfathiazole	1:100	

TABLE 2

THE CONCENTRATION OF METHYLENE BLUE OR BRILLIANT CRESYL BLUE REQUIRED TO KILL 10^7 CELLS OF *E. coli* IN 10 CC NUTRIENT BROTH BUFFERED AT PH 6.8 IN THE PRESENCE OF SULFONAMIDES AT 1:14,000

Sulfonamide	Methylene blue	Brilliant cresyl blue
Sulfathiazole	1:28,000	1:32,000
Sulfapyridine	1:28,000	1:28,000
Sulfanilamide	1:18,000	1:18,000
Sodium sulfathiazole	1:28,000	1:32,000

Clinical studies undertaken in cooperation with Dr. J. T. MacLean at the Ste. Anne de Bellevue Military Hospital indicate a promising therapeutical value for the combination of sulfathiazole and methylene blue in the treatment of chronic genito-urinary infections by Gram-negative bacteria.

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ACID PHOSPHATASE IN GROWING AXONS AND DEGENERATED NERVE TISSUE

WOLF, Kabat and Newman[1] have reported the presence of acid phosphatase in the axis-cylinders of both the peripheral and central nervous system. The myelin sheaths appear to be devoid of the enzyme. Since phosphatase has been implicated in the metabolism of both phospholipids and sugars, it would appear that studies on this enzyme might give an indication of some of the metabolic processes going on within the nerve fiber and specifically in the axonal portion. This preliminary report deals with the results obtained from the study of (1) the growing nerve fibers of the cat from birth to neurologic maturity and (2) the behavior of the enzyme in the degenerating neuron.

It was found necessary to make considerable change in the existing technics for demonstration of acid phosphatase as applied to the tissue of the central nervous system. The method used, as well as a more complete description of our observations, will be published elsewhere.

Studies of the growing neuron have been made on cats of the following ages: 1, 2, 5, 10, 15, 20, 70 days and adult. The evidence from this series indicates that variations in the time of appearance of the enzyme in the axons are to be correlated with the phylogenetic background of the several nerve tracts as well as the ontogenetic age of the animal. Thus, at birth the ventral and dorsal roots, the sensory tracts within the cord, the medial longitudinal fasciculus and tecto-spinal tracts in the brain stem all give a marked positive reaction. At this age, the higher brain centers, as well as the great motor bundle, the pyramidal tract, show comparatively little or no phosphatase. Likewise, the tracts which react positively are known to be the first to become myelinated. In the later postnatal stages, a progressive increase in the reaction occurs in the higher brain centers. Comparatively, the pyramidal tract gives an incomplete reaction until some time after the 70th day. Thus, if the presence of acid phosphatase indicates a functional state in nerve conduction it would appear that the neuronal elements concerned with the cord and brain-stem reflexes are the first to show the presence of the enzyme.

Studies on the degenerating neuron have been made on the pyramidal tract in cats and monkeys following removal of the motor cortex. This bundle, in two monkeys with a post-operative survival time of four and five months, can be traced with ease from the cortex to the sacral cord segments. The entire degenerating field gives a marked acid phosphatase reaction. Since, in this breakdown, the affected axis-cylinders have disappeared the phosphatase must be associated with the glial tissue and possibly with chemical remnants of the myelin sheaths. One of the fundamental and unanswered questions in the field of neurology and neuropathology is why the medullated membrane should degenerate concurrently with the axis-cylinder when the cell body or nerve fiber is seriously damaged. There is the distinct possibility, on the basis of the present study, that the axon may liberate a substance (enzyme) which acts on the myelin sheath in a manner to cause its disintegration. Studies are in progress to determine the immediate or acute secondary and retrograde changes in phosphatase following injury to the neuron.

Two cats, similarly treated as the monkeys, but

[1] A. Wolf, E. A. Kabat and W. Newman, *Am. Jour. Path.*, 19: 423, 1943.

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.