

fully adapted to salinities below blood levels showed changes characteristic of the fresh water condition (loss of the apical pits in the chloride cells). The dye binding capacity of the mucoid substances of the gills was not affected by changes in environmental salinity.

- 3) Maternal and extra-embryonic blood vessels in *Squalus* were prepared for electron microscopic examination in view of their special uterine environment.

1962 #10

#### HYPOXIA ON DEVELOPING SAND DOLLAR EGGS

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In the course of studies designed to test the permeability of sand dollar eggs to dyes, it became necessary to view simultaneously the successive stages in the development of the fertilized egg. This was accomplished by transferring a large quantity of fertilized eggs to a deep flask of sea water (250 ml), and allowing them to remain at the bottom, undisturbed, for 24-36 hours at the temperature of ambient sea water. At that time, all stages of development could be seen, including the first divisions into two and four cells. That this retarding effect on cell development was attributable to hypoxia was shown in several different ways: 1) The same effect could be produced by bubbling a mixture of 5 per cent oxygen in nitrogen through the sea water surrounding the eggs, 2) it could be prevented either by agitating the flask, by bubbling oxygen through the sea water, or by placing the fertilized eggs in a flat dish instead of at the bottom of a deep flask and, 3) retardation of development did not occur when 5 per cent carbon dioxide was bubbled through the sea water. These results suggest that the metabolic activity of the developing eggs depletes the stagnant water in their vicinity of oxygen, thereby retarding their normal development.

1962 #11

#### FURTHER STUDIES ON THE SOURCE OF AMMONIA EXCRETED BY THE GILLS OF MARINE FISHES

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Previously we showed that the combined 'activities' of glutaminase I and glutamic acid dehydrogenase in gill tissue could more than account for all the measured ammonia excreted branchially by the shorthorn sculpin, Myoxocephalus scorpius. Current investigations, which were undertaken to identify possible precursors of ammonia and to develop techniques for the determination of branchial blood flow rates by application of the "Fick Principle," suggest a modification, however, of a second argument which favored peripheral formation at the gills rather than diffusion of preformed ammonia. This presumed evidence was based on experiments in which the ammonia concentration of afferent gill blood drawn from the heart was compared with what was thought to be efferent gill blood drawn from the tail, and it appeared that the gills extracted only 10 per cent of the blood ammonia. In the current series of experiments, blood

obtained from the tail, previously considered arterial (see, for example: Smith, H. W. From Fish to Philosopher. Ciba Edition, 1959, p. 56) was compared with blood taken directly from the dorsal aorta where it emerges as the confluence of the efferent branchial vessels. The latter, in contrast to caudal blood, was found to be almost completely cleared of ammonia. This observation prompted a functional examination of the general plan of circulation in the sculpin.

The chief feature that emerged from these anatomical investigations was that a large venous sinus lies alongside the much smaller caudal artery, and, in addition to the relatively high resistance renal portal and hepatic portal routes, it conveys blood directly to the heart via a large valveless thoroughfare, the posterior cardinal vein. Suction applied after a syringe needle has been inserted midventrally into the caudal vessels results almost invariably in contaminating drawn arterial blood with large volumes of venous blood which, moving retrograde, can empty both the Cuvierian duct reservoir and the entire contents of the posterior cardinal vein. This blood, taken in effect from directly behind the heart, is, of course, essentially similar to blood taken from the ventricle.

This investigation was supported by a research grant, HE-04457-04, from the Heart Institute, Public Health Service.

1962 #12

PATTERNS OF PIGMENT CELL REPOPULATION DURING WOUND HEALING IN  
fundulus heteroclitus\*

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Integumentary melanophores in *F. heteroclitus* constitute an expanding population of cells with definite lifespans in turnover rates. Represented by a graded series of cell sizes and by corresponding increments in the number of nuclei, these cells differentiate from impigmented precursors, become increasingly multinucleate as their cytoplasmic mass grows, and eventually die to be replaced by another.

On the theory that excessive destruction of pigment cells might accelerate the rate of renewal, their response to epidermal injury has been investigated. Circular wounds 0.5 mm in diameter were inflicted in the median head scales of fish maintained at 28°C in running sea water. Within one day, as epidermal migration proceeded, the mature pigment cells at the periphery of the wound extended their processes centripetally but did not otherwise move into the wound area proper. After 24 hours, small attenuated melanophores appeared de novo in the outer regions of the healed area and later repopulated the entire surface of the wound. Originally mononucleate, these cells eventually increased in size and nuclear number until a normal population of cells was reestablished. Xanthophore regeneration was not evident until about the fifth day. Observations of healing wounds in fish kept in constant light or in a completely dark environment, revealed that melanophore differentiation and repopulation was accelerated in dark-adapted fish with maximally expanded pigment cells.

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\*This investigation was supported by a research grant (B-923) from the National Institutes of Health.