# CARNEGIE INSTITUTION
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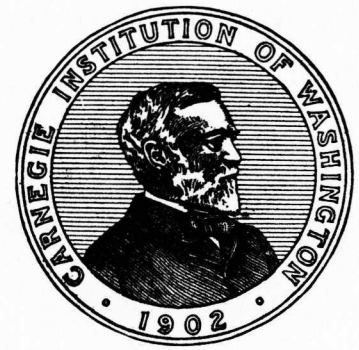


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arms, which serve as a net for capturing food. As daylight approaches they withdraw their arms and retreat to shaded regions.

These animals can best be collected at night by the use of flashlights or submerged lamps. If nets are used, the animals are usually injured in removing them from their attachment, but if the sea fans to which they are attached are also taken they can be collected uninjured.

Contrary to the general impression, basket stars are quite common on coral reefs especially where sea fans occur.

## Studies on Tumors in Cold-blooded Vertebrates

Balduin Lucké

The comparative study of neoplastic growths has in the past dealt chiefly with tumors in mammals and birds, neglecting the more primitive cold-blooded vertebrates. This restriction has been due largely to the belief that among amphibians, reptiles, and fish, neoplasms are rare and difficult to obtain for study. Information concerning tumors in these classes has been based upon chance observations, rather than upon systematic investigations of particular kinds of tumors in certain species. It seems not unlikely, however, that such study would add much to our knowledge concerning the nature of neoplastic growths in general.

The aim of the present work was to investigate the occurrence, distribution, and nature of tumors among the fish and turtles available in the waters around the Dry Tortugas. The search has yielded much additional information about tumors of nerves in fish of the snapper family (the first account of which was given in last year's report); in addition, two new varieties of tumors were found, one in the green turtle, another in the slippery dick, a small reef fish. These several kinds of tumors are common and occur in easily available species, and all of them appear to be suitable for a more detailed experimental investigation.

*Nerve sheath tumors (neurilemmoma, neurofibroma, schwannoma) in fish of the snapper family (Lutianidæ).* This investigation is a continuation of the study reported in the Year Book for 1936–1937. The total number of tumors which have been obtained to the present is 76. This relatively large number permits a more precise analysis of the anatomical distribution and habits of the growths. It is found that most of the tumors are situated along the course of the larger subcutaneous nerves, particularly those of the head and the dorsal regions. Their peculiar distribution, together with their characteristic histological appearance, now makes it very probable that these growths have their origin in the sheath of the nerves. They bear a striking similarity to tumors of the nerve sheath in man. Like them they form two main types, in one of which the component cells are oriented in such a manner as to form rows or so-called palisades; the other type has a very loose edematous structure, and no particular arrangement of its component cells is demonstrable. It is estimated that approximately 1 per cent of gray snappers *(Lutianus griseus)* are affected with this neoplasm; the incidence in the other varieties of snappers is not known.



Transplantation experiments were made using a technique which had proved successful with an amphibian tumor studied by the writer. Small fragments were inoculated by means of a hollow needle into the anterior chamber or the vitreus of the eyes of a number of snappers. After from 8 to 10 weeks the inoculated fish were killed and the eyes excised and prepared for histological examination. The results of these transplantation experiments will be reported on completion of the study.

*Multiple papillomas of the skin and the eye in the green turtle (Chelonia mydas).* The newgrowths which have been observed in reptiles are even more limited in number than is the case in amphibians and fish. Hence it is of particular interest to record that green turtles not infrequently suffer from papillomatous neoplasms which may attain so great a size as seriously to interfere with their locomotion.

The tumors occurred in a large female green turtle caught off Cape Sable. They were located on the edges of both anterior flippers, in the axillary regions, the neck, on the eyelids, the corneal surfaces, and on the tail. In shape they were hemispherical or globular, and had rough, warty surfaces covered with dry, coarse, cornified epiderm, which in some areas was superficially ulcerated. Some of the tumors were sessile, others had a broad pedunculated base. In some areas, particularly in the axillary regions, several were crowded together, elsewhere they were solitary. In size, the individual tumors ranged from small warts a few millimeters in greatest diameter to large masses nearly 5 cm. in diameter. All were of tough consistency, and had a dense white fibrous, bloodless cut surface.

Histologically, the neoplasms are typical papillomas, consisting of a fibrous core covered with many layers of epiderm the surface of which has undergone extensive keratinization. All stages of the papillomatous growth are represented in the animal; the smaller show a relative preponderance of epithelial over mesodermal components; as the tumors increase in size the fibrous components become more prominent, in the largest growths they greatly predominate. Richly cellular areas, frequently encountered, indicate that the fibrous portions of the tumors are actively proliferating.

The papillomas of the turtle correspond very closely to epidermal papillomas of man and other mammals. Several of these have been shown to be caused by viruses. It would be of great interest if a similar etiologic factor could be demonstrated in these papillomas of a very different group of animals.

*Epithelial growths of the skin in the slippery dick (Halichœres radiatus).* There is no sharp dividing line between "true" neoplasms and certain exaggerated growths due to irritations of one kind or other. Indeed many of the latter have been found to merge by ill-defined stages with the former. Their study is the more indicated because the transition stages may furnish information as to the nature of neoplastic growths in general. A tumorous condition which seems to belong in this border-line group occurs as a rather common disease in the little reef fish, slippery dick. Thirty examples were observed among approximately 6000 of these fish. The tumors generally are circumscribed, flat, somewhat nodular elevations of the skin tending to undergo ulceration. They have a grayish, dull appearance and a soft con-

sistency. The scales in affected areas are elevated or have apparently been destroyed entirely. The disease is distributed over many parts of the surface, but is most commonly encountered in the caudal region; destruction of fins is common. The growths often attain very large size. Histologically they are composed of masses of epidermal cells, arranged in alveolar groupings; stroma and vessels are scanty. The corium is infiltrated but no extension into the subjacent musculature is observed in the present series. In many tumors striking cytological changes have taken place; the cytoplasm is ballooned and partly occupied by a small deep staining chromatic body surrounded by faintly acidophilic material; the nature of these inclusions is still a matter of uncertainty.

Fish affected with these tumors can readily be kept in indoor aquaria. They should prove excellent material for the experimental investigation of this disease.

## Further Studies on the Electrical Behavior of Valonia ventricosa

Gordon Marsh

By means of a rotating sector disk giving two equal light-dark periods per revolution, intermittent and continuous light were compared in their effect upon the inherent E.M.F. of *Valonia*. At frequencies of about 8 to 10 per second and intensities up to 1600 foot-candles no consistent difference was found, the effect of intermittent light approximating that of continuous light of half the intensity.

The effect of KCN upon the potential was determined over the range of concentrations from $2 \times 10^{-8}$M to $5 \times 10^{-3}$ both in light and in darkness. The cyanide was dissolved in sea water and the latter restored to its original pH with HCl, using thymol blue as indicator. When the steady potential in the presence of cyanide is plotted against concentration, a curve results which drops rapidly to around $1 \times 10^{-4}$M and but slightly more for higher concentrations. All depressions in potential were reversible during the time limits involved. Recovery of the original potential upon removal to sea was slower the higher the concentration of cyanide used. In the dark the maximum depression obtained varied from 40 to 75 per cent. In the light the depression was much greater both as a percentage and as an absolute figure; the potential level in high concentration of KCN was approximately the same in light as in darkness. This is consistent with the known effects of cyanide, which normally depresses respiration reversibly to the above amounts for many materials and at high concentrations completely inhibits photosynthesis. NaCN yielded substantially the same results as KCN.

A similar study was made with ether between the concentrations 0.01 and 2.5 per cent by volume. The curve of potential *vs.* concentration resembles roughly that obtained with cyanide. Reversible depression of the potential was obtained with concentrations up to 1.0 per cent; 1.5 per cent and higher coagulated the protoplast. The individual variability of behavior of the cells was high, as is typical of the effect of ether. Concentrations between 0.5 and 0.1 per cent produced a preliminary increase in potential which in extreme cases reached eight times the value of the potential in sea water for cells in the dark and three times the value for cells exposed to light, and required about three hours to descend to a steady level.

A preliminary survey of the influence of the pH of the surrounding sea water on the protoplasmic potential difference was made, using sulfonphthalein indicators. The potential increased with pH 4—11, the effect of equal pH steps being less near the pH of sea water. Differences were observed between the effects of KOH and NaOH and between the effects of illumination at low and at high pH's.



## The Response of Ascidian Larvæ Toward Certain Hormones

Paul A. Nicoll

The immediate stimulus of ascidian metamorphosis, regardless of the ultimate cause, may be regarded as a state of condition incompatible with continuance of larval life. The subsequent differentiation, development, and growth of the primitive cells from which the adult organism arises follows this breakdown of the larval action system more or less rapidly depending on the species under consideration. Grave and Nicoll (in press) have suggested several paths by which this condition could be reached. This summer a start was made in the study of possible factors which would prevent the development of this state of condition that terminates larval existence and so permit the more highly organized prochordate type of individual to survive.

The possibility of a deficiency or complete lack of some hormone essential for completion and survival of the prochordate animal was investigated. Using the same technical procedures described by Grave, larvæ of *Ascidia nigra* were subjected to various concentrations of theelin, theelol, adrenalin, thyroxine, and testosterone, as well as a cortical extract of proved potency for survival of adrenalectomized dogs. The first three hormones were supplied through the kindness of Parke, Davis and Company. Of these only theelol and adrenalin had any influence on the larvæ, which in both cases amounted to lengthening the larval life period but without allowing any development of a digestive or circulatory system, which would seem to be necessary if the prochordate type were to have a separate existence. Technical difficulties prevented adequate tests with testosterone but the failure of crystalline thyroxine to influence metamorphosis in either direction was definite.

In addition to the hormone experiments the larvæ were treated with two crystalline vitamins, vitamin C and vitamin $B_1$, on the theory that possible deficiencies of basic food essentials might lead to the breakdown of the larval action system. However, neither of the vitamins, using a wide range of concentrations in both cases, was found to influence in any way the length of larval life.

Besides the experimental work considerable time was devoted to the collection and study of various species of ascidians that may be found in the waters near the Tortugas Laboratory. Two regions previously uninvestigated which proved rich in ascidians of all species were located on the east sides of Sand and East Keys.

Of the many species collected, some of which will undoubtedly prove to be undescribed when thoroughly studied, one may be mentioned at this time.