To the Marine Biological Laboratory
of Woods Hole
from
Alfred G. Mayer
1911.

DEPARTMENT OF MARINE BIOLOGY
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ALFRED G. MAYER, DIRECTOR

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THE CONVERSE RELATION BETWEEN CILIARY AND NEURO-MUSCULAR MOVEMENTS.

By Alfred Goldsborough Mayer.

This research was commenced at Tortugas, Florida, during the summer of 1909, and was continued in the Marine Biological Laboratory at Woods Hole, Massachusetts, and also at the New York Aquarium. I am greatly indebted to Prof. Charles R. Stockard of Cornell Medical College, Prof. Frank R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, and Dr. Charles H. Townsend, Director of the New York Aquarium, for their kind aid in gathering and maintaining material alive for the research, and for granting to me the excellent facilities of the Woods Hole Laboratory and of the New York Aquarium. To Professors Carlton C. Curtis, of Columbia, and Roland Thaxter, of Harvard, I am also indebted for some highly appreciated advice upon the botanical side of the research.

Preliminary reports of the present research were published in the Biological Bulletin, Woods Hole, vol. 17, pp. 341, 342; in the Proceedings of the Society for Experimental Biology and Medicine, 1909, No. 7, pp. 19, 20; and in the Year Book of the Carnegie Institution of Washington for 1909, p. 152.

CONCLUSIONS NEW TO SCIENCE.

The effects of the several cations of the blood-salts, sodium, magnesium, calcium, ammonium, potassium, and hydrogen upon neuromuscular movements are in each case the exact opposite of their effects upon the ciliary movements of animals.

There is wide diversity in the reactions of various species of motile fungi and algæ to these ions, and the statement made in the preceding paragraph applies only to animals.

Ordinary ciliated epithelium such as that covering the external surface of ~~Trematodes~~ is not wholly under the control of the nervous or muscular system of the animal, and the cilia continue to beat even when the muscles underlying them contract. Neme

The more highly specialized cilia, however, such as those of the meridional combs of Ctenophores, the lobes of veliger larvæ, the peristomial ring of trochophores, or the longitudinal band of Semper's actinian larva, cease to beat when the muscles underlying them contract, and resume their rhythmic movement only when the muscles relax. Thus an electrical stimulus which causes the muscles to contract stops the cilia, but if the muscles be anesthetized with magnesium so that they

can not contract an electrical stimulus does not stop the cilia. The stopping of the cilia is therefore dependent upon the contraction of the muscles. It appears then that the stimulus which produces ciliary movement tends to cause muscular relaxation but is too weak to prevent muscular movement, but the stimulus which produces muscular contraction is of an opposite nature, and is more energetic than that required to maintain ciliary movement and completely overpowers it, stopping the cilia when the muscles contract. Considering all things which normally affect the animal, whatever stimulates the neuro-muscular system inhibits ciliary movement, and whatever stimulates cilia depresses neuro-muscular activity.

GENERAL CONCLUSIONS.

A research published in 1908 [1] leads me to believe that in Scyphomedusæ each pulsation is due to a stimulus produced by the constant setting free of ionic sodium in the marginal sense-clubs. Sodium oxalate appears to be constantly forming in the entoderm of the sense-club. This precipitates the calcium which constantly enters the sense-club from the surrounding sea-water, and thus insoluble crystals of CaC_2O_4 are formed while NaCl is set free. The Na ion is a powerful nervous stimulant, and being maintained thus in slight excess in the sense-clubs it acts as a constantly present minimal stimulus which produces periodic responses. It will be recalled that Romanes found that constantly present minimal electrical stimuli could cause periodically recurring pulsation in Scyphomedusæ.

Pulsation can not be maintained by the sense-organs unless calcium constantly enters them to form the crystals of calcium oxalate and to set free the ionic sodium, and all movement soon ceases in sea-water deprived of calcium.

In Cephalopods, Veligers, marine Annelids, Barnacles (*Lepas*), and Ctenophoræ, the sodium of the sea-water is a powerful neuro-muscular stimulant, whereas the magnesium, calcium, and potassium are inhibitors and counterbalance the stimulating effect of the sodium, giving a neutral or, more properly speaking, *balanced fluid*, thus permitting weak *internally engendered* stimuli to produce movements. This is very evident in the case of animals placed in distilled water, wherein they may move normally, although no direct stimulus to produce movement can come from the surrounding medium. Yet fresh-water animals react to sodium, magnesium, potassium, calcium, NH_4Cl, and CO_2 as do marine animals. Physiological conditions in vertebrates are often different from those in invertebrates, but it will be recalled that Martin, 1906,[2] concludes that the vertebrate heart is maintained in rhythmical activity by an inner stimulus due to the metabolic activity of the heart's own tissue, and its pulsation is not caused by an external stimulus due to the ions of the inorganic salts of the blood. Howell has also come to the conclusion that an inner and not an external stimulus causes the heart's activity,

[1] Papers from the Tortugas Laboratory, vol. 1, pp. 115–131; Publication No. 102, Carnegie Institution of Washington.

[2] Martin, 1906, American Journal of Physiol., vol 16, pp. 191–220.

and Terry's [1] studies lead to the conjecture that the pulsation of *Gonionemus* may possibly be due to an oxidative process within the tissues.

The calcium of sea-water combines with the sodium ion and assists the sodium to overcome the depressant effect of the magnesium. Calcium does not combine directly with magnesium, and in the *absence* of *sodium* it has no power to offset the depressant effect of magnesium. I first showed this in 1906, pp. 4, 46, and now present further experimental evidence to the same effect. It is also interesting to see that Joseph and Meltzer, 1910 (Proc. Soc. Experimental Biology and Medicine, vol. 7, p. 67), find that if the indirect irritability of the muscles of the frog be destroyed by perfusion with magnesium, the irritability can not be restored by calcium unless sodium be present.

It is remarkable that the effects of the ions sodium, magnesium, calcium, and potassium upon the ciliary movements of both marine and fresh-water animals are the *exact opposite* of their effects upon the neuro-muscular system. Thus for ciliary movement sodium is the most powerful inhibitor, while for the neuro-muscular system it is the most potent stimulant. Similarly, considering the ions magnesium, calcium, and potassium among themselves, magnesium is the most powerful stimulant for ciliary movement and the greatest depressant for neuro-muscular movements. Potassium in weak concentrations at first stimulates, but finally depresses the neuro-muscular activities, while it at first depresses and finally permits the movement of cilia. Calcium depresses neuro-muscular movement, but permits ciliary movement.

For neuro-muscular activities we find that the stimulating effect of Na is offset by the depressant action of Mg, K, and Ca; whereas in ciliary movements the depressant influence of Na is offset by the stimulation due to Mg, K, and Ca.

Thus marine invertebrates placed in 0.6 molecular NaCl have their muscular movements most highly accelerated, while their cilia cease to beat almost immediately after immersion in this solution. Also in 0.4 molecular $MgCl_2$ their neuro-muscular movements cease without initial stimulation soon after immersion, while their ciliary movements, although reduced in rate, are maintained for a long time. Ctenophores and ciliated Annelid larvæ illustrate these reactions very clearly.

R. S. Lillie, 1901–09, in an able series of papers in the American Journal of Physiology,[2] found that the muscular movements of marine animals were affected in a manner different from their ciliary movements by the ions Na, Ca, K, and Mg; but he did not observe that the action of these ions upon the neuro-muscular system is in each case the *exact opposite* of their effect upon cilia. I find, however, that this law holds not only for the above-mentioned ions of the blood-salts, but also for NH_4Cl, which at first stimulates but finally depresses the neuro-muscular movements of animals, while it at first checks and then permits of ciliary movement. Also weak concentrations of acids (hydrogen ion) are momentary stimulants but final depressants for the neuro-muscular

[1] Terry, 1909, American Journal Physiol., vol. 24, pp. 117–123.

[2] 1901, vol. 5, pp. 56–85; 1902, vol. 7, pp. 25–55; 1904, vol. 10, pp. 419–443; 1906, vol. 16, pp. 117–128; 1906, vol. 17, pp. 89–141; 1908, vol. 21, pp. 200–220, 1908, vol. 22, pp. 75–90; 1909, vol. 24, pp. 14–44; *Ibid.*, pp. 459–492.

system, while they are initial depressants for ciliary movement, but after a brief interval of cessation the movements of the cilia recover and become quite active.

It is probable that, in common with neuro-muscular movements, ciliary movement is not normally caused by stimuli originating from the outside, but is controlled by internal stimuli which in primitive cilia, such as those of ordinary epithelial surfaces, arise within each ciliated cell itself, and is only weakly under the control of the nervous or muscular system of the animal. Such ciliated cells may be separated one by one from the epithelium and each isolated cell still continues to lash its cilium in a normal manner. At best only a weak coördination exists between the constituent cells of such an epithelium, and impulses are only slowly transmitted by deep-seated cells lying under the cilia in the manner shown by Kraft, 1890, Archiv für gesammte Physiologie, Bd. 47, p. 196, or Gruetzner, 1882, Zur Physiologie des Flimmerepithels; Physiol. Studien, p. 1–32.

On the other hand, the more highly differentiated cilia, such as those of the combs of Ctenophores, which move in a coördinated rhythm, are certainly under the control of the nervous or muscular system of the animal. When this control is destroyed by placing the animal in magnesium chloride the cilia beat independently and incessantly and lose all coördination; thus reverting to the condition of the cilia of ordinary epithelia. It is therefore probable that for ciliary movement the combined stimulating effects of the magnesium, calcium, and potassium of the sea-water counterbalance the inhibiting effect of the sodium, for we find that in trochophores of marine annelids the cilia move with more than normal activity when the animals are placed in a solution lacking sodium but containing the proportions and amounts of magnesium, calcium, and potassium found in sea-water.

If, on the other hand, they be placed in a pure sodium solution, their cilia immediately decline and soon cease to beat and are so injured that recovery does not occur.

In experimenting upon the effects of the cations sodium, magnesium, calcium, and potassium upon unicellular motile fungi and algæ I find such great diversity in their reactions that one can not find any general law, this being in marked contrast to the uniform behavior of animals from the protozoa to the vertebrates. There is, however, an actively moving transparent *Spirillum* which lives in a culture of dead house-flies in fresh water containing algæ. This form reacts to the ions Na, Mg, Ca, and K as do the cilia of animals. It is therefore possible that the ciliary movements of animals were taken directly from their plant-like ancestors, while their muscular movements were developed only later and in a mode the exact converse of ciliary movement. This hypothesis must, however, be taken with the greatest reservation, and I present it only as a mere speculation calling for further study.

Hargitt, 1899 (Biological Bulletin, vol. 1, p. 42), succeeded in causing two individuals of *Gonionemus* to unite by their bell-rims, and whenever one of the individuals pulsated the other moved in coördination. The medusæ being joined rim to rim, it would seem, however, that if one

pulsated the bell of the other must necessarily be mechanically drawn together even though the pulsation-stimulus might not be able to pass from one to the other. In order to obviate this possible ambiguity, two individuals of *Cassiopea xamachana* were joined together so that their subumbrella surfaces came in contact by a narrow isthmus, as shown in fig. 1. It was an easy matter to cause the medusæ to unite in this manner, but the union was usually of such a nature that no nervous intercommunication was established and the two animals although joined together continued to pulsate independently. In two instances, however, a nervous union was established in from 5 to 7 days after the operation, and then the individual which had the fastest rate of pulsation assumed control of the other, which always followed its every contraction. In both cases the small medusa, whose natural rate was the more rapid, controlled the larger one, and caused it to follow its rate; but if one pinched the bell-rim of the larger medusa its rate suddenly *increased* so that it became faster than the small medusa, and then it controlled the smaller until its excitement had subsided, when the smaller one regained its control. This shows that the complex formed of the two grafted individuals always pulsated at the rate of its faster mem-

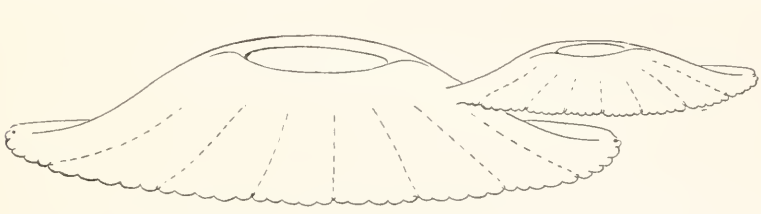

FIG. 1.—Two individuals of *Cassiopea xamachana* grafted one upon another.

ber. Prof. Jacques Loeb has called attention to the fact that in a coördinated series of organs the whole series must beat at the rate of its fastest component, and in this connection he cites the book-gills of *Limulus*, for he finds that the whole series of gills moves at the rate of its fastest member. Long before this Romanes demonstrated that *Aurellia* pulsates at the rate of its fastest working sense-organ.

I found that if the two medusæ of the complex were cut apart by severing the narrow isthmus of connecting tissue, the rate of each is lower than that of the two when beating together. Thus in one case the two medusæ joined and beating in unison had a rate of from 31 to 34 pulsations per minute, but when cut apart one of the medusæ had a rate of 26 to 29 and the other 18 to 25 per minute. It is evident, therefore, that the two medusæ together behaved, in so far as pulsation is concerned, as if they constituted a single individual, for Eimer and also Romanes long ago demonstrated that small pieces cut from a pulsating medusa beat slower than large ones.

A certain nervous *tonus* is imparted to the sense-organs if they be in nervous connection with a large area of subumbrella tissue. It is evident that this tonus is not of a trophic nature, for the lowering of rate occurs immediately when the medusæ are severed one from another, and the

isthmus of connecting tissue was far too narrow to permit of any considerable interchange of circulatory or nutrient fluids between the two medusæ.

In experimenting with circuit-waves in ring-shaped strips of subumbrella tissue of *Cassiopea* or *Stomolophus* it is often desirable to be able to direct the course along which the wave shall travel.

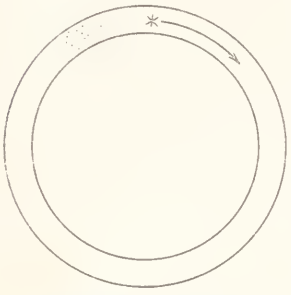

Fig. 2.—Method of determining direction of a wave of contraction.

This can be accomplished if a slight momentary pressure, such as the touch of a finger, be brought to bear upon the ring near the point of stimulation and opposite to the direction in which one desires the wave to travel. Thus in fig. 2 the star (*) marks the point at which the ring is to be stimulated, while the dotted area marks the pressed place, and the arrow shows the direction of the resulting wave which will continue to travel around and around the ring. That half of the initial wave which proceeds to the pressed place is so much dampened and reduced by the poorly conducting tissue of the pressed area that the wave in the opposite direction annuls it when they meet.

DETAILS OF EXPERIMENTS.

Part I.

NEURO-MUSCULAR MOVEMENTS.

An extensive series of observations was carried out upon the effects of the cations sodium, magnesium, potassium, and calcium upon the neuro-muscular movements of *Lepas* and of Annelid worms at Tortugas. The Annelids experimented upon were several common species of Eunicidæ found in the crevices of coral rock, among them being the Atlantic palolo worm *Eunice fucata*. I also made use of several forms of *Nereis*.

The sea-water at Tortugas is well imitated for physiological purposes by a van't Hoff's solution consisting of 100NaCl + 7.8$MgCl_2$ + 3.8$MgSO_4$ + 2.2KCl + 2.5$CaCl_2$, all of 0.625 molecular concentration. The Atlantic palolo *Eunice fucata* will live for over three weeks in this solution in an apparently normal condition. How much longer it might have survived I can not state, but the worms appeared to be normally active when the experiment was terminated; indeed, the worms and *Lepas* lived better in the artificial than in natural sea-water in aquaria under similar conditions in the laboratory. This I attribute to the purity of the Kahlbaum salts and of the distilled water used in making the artificial sea-water, and its consequent freedom from bacteria. It is remarkable that, contrary to Bethe's[1] experience in maintaining *Rhizostoma pulmo* alive in artificial sea-water at Naples, I found that the addition of a slight amount of $CaCO_3$ was not necessary and that it did not appear to improve the life-sustaining powers of the van't Hoff's solution. It produced, however, no injurious effect.

[1] Bethe, 1908, Pflüger's Archiv für ges. Physiol., Bd. 124, pp. 541–577.

Figure 3 gives a graphic illustration of the effects of various solutions containing from one to three of the four constituent cations of van't Hoff's sea-water[1] on *Lepas* (left-hand column), *Cassiopea* (middle), and Annelid worms (right-hand column). The drawings of *Lepas* and of the Annelids are from careful sketches made from actual cases and are quite true to nature, but those of the Scyphomedusa *Cassiopea* are wholly diagrammatic, for the mouth-arms are not shown and the flatten-

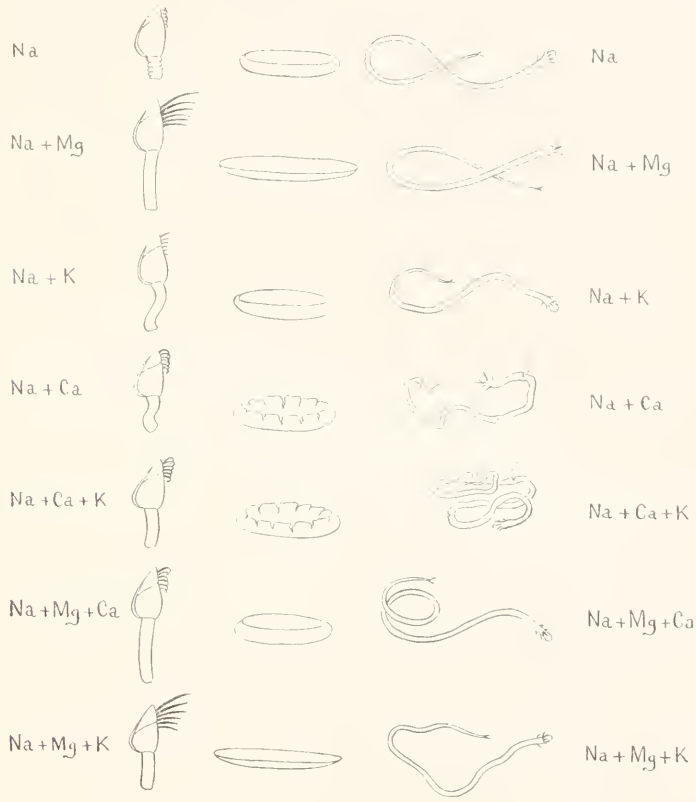

Fig. 3.

ing of the bell in magnesium is much exaggerated in order to make it more apparent.

The figures show that muscular relaxation is produced in solutions containing magnesium, and tetanus in solutions containing calcium; also that calcium and magnesium counteract each the other in these respects when both are present in a solution containing sodium. Potassium, on the other hand, has but little power to alter the effects of calcium or magnesium. For example, in 0.625 molecular ($100NaCl + 7.8MgCl_2 + 3.8MgSO_4$) *Lepas* comes to rest without initial stimulation in about half an hour, with its pedicel abnormally extended and its branchial

[1] The complete formula is 0.625 molecular ($100NaCl + 7.8MgCl_2 + 3.8MgSO_4 + 2.2KCl + 2.5CaCl_2$).

arms thrust straight outward without being recurved or crooked at the ends. *Cassiopea* soon flattens out, and the Eunicidæ and Nereidæ come to rest in relaxed, smoothly-flowing folds.

On the other hand, in solutions containing calcium but lacking magnesium we find that *Lepas* comes to rest with its branchial arms contracted and strongly recurved at their tips. *Cassiopea* tears its bell through muscular tetanus, and the annelid worms set themselves into rigid, distorted, sharply-twisted, "kinked" shapes, with spasmodic, local twitchings of the longitudinal muscles due to clonic tetanus, and often rents are torn through the cuticula, thus permitting the body-fluids to escape.

If, however, we have both magnesium and calcium in the solution they tend, in the presence of sodium, to offset one the other, the magnesium preventing tetanus and the calcium preventing complete relaxation. We see, however, that in the Na + Mg + Ca row the branchial arms of *Lepas* still tend to recurve at their ends as in calcium solutions, showing that the magnesium has not completely offset the calcium in this respect, although the pedicel is as fully relaxed as in magnesium solutions. It will be recalled that Meltzer and Auer, in a series of well-known and able papers have called prominent attention to the anesthetic (or asphyxiating[1]) influence of magnesium when introduced into the blood-system of vertebrates, and in 1908, in the American Journal of Physiology (vol. 21, p. 400), they announce that the effects of a lethal dose of magnesium upon a rabbit can be counteracted by a subsequent injection of calcium salt into its blood-system.

The drawings also show the strong stimulating effect of sodium, for in 0.625 molecular NaCl, *Lepas* comes to rest after abnormally active movements of its branchial arms, with its pedicel strongly contracted and usually with its shells shut and arms withdrawn; although in the specimen I have figured the arms are slightly projecting with their tips strongly recurved, this being the only solution save those containing calcium, which commonly causes a "crooking" of the tips of the arms. The medusa and the annelids move with abnormal rapidity in NaCl but come to rest without tetanus, although not in so completely relaxed a state as when in Na + Mg.

Thus the effect of ionic sodium, magnesium, potassium, and calcium is in each case of the same nature in all of these invertebrates. Sodium stimulates, magnesium depresses, potassium, after a momentary stimulation, depresses,[2] and calcium depresses and produces tetanus in each of these animals; as, indeed, they do also in their effect upon the ganglia that control the heart of *Limulus*, according to Carlson, 1906 (Amer. Jour-

[1] See Guthrie and Ryan, 1910, American Journ. Physiol., vol. 26, p. 329.

[2] In experimenting upon Annelids and *Cassiopea* I find that the initial stimulus caused by the addition of a slight concentration of potassium to the sea-water can be counteracted if we add together with the potassium about 5.5 times the amount of magnesium in a solution isotonic with the potassium. As this is about the ratio of potassium to magnesium in normal sea-water, it is probable that the primarily stimulating effect of the potassium is offset by the magnesium of the sea-water, and thus the potassium of the sea-water may be considered as a simple depressant.

nal Physiol., vol. 16, pp. 386–395);[1] Bethe's[2] studies on the effects of these ions upon *Rhizostoma pulmo* also show that this medusa behaves as does *Cassiopea*.

This similarity of behavior suggests that the stimulus which produces rhythmical or other neuro-muscular movements in all of these forms is of one and the same nature in each and all of them. It appears that a slight excess of sodium maintained at the ganglionic centers causes the nervous stimulus which produces pulsation in the medusa *Cassiopea*,[3] but I am unable to state what may be the normal cause of movements in the other animals experimented upon in this research. In both *Cassiopea* and the Annelids, however, 0.1 per cent oxalic acid in sea-water quickly produces a permanent paralysis of the central nervous system, leaving the muscles still capable of contracting locally under the influence of stimuli, such as the touch of a crystal of postassium sulphate, although the contraction spreads only in a slow myogenic manner from the stimulated point.

Moreover, in both the medusæ and the worms, if the animals be in sea-water and a small amount of a solution of 4 parts of sodium oxalate in 1,000 parts of sea-water be allowed to diffuse locally upon them from a pipette, movements wholly natural in appearance arise from the stimulated area and spread over their bodies. These experiments do not prove that the cause of movement is due to a slight excess of sodium at the ganglionic centers in these invertebrates, but they *suggest* that this is possible. It will indeed be remembered that Parker and Metcalf, 1906 (American Journal of Physiol., vol. 17, pp. 55–74), found that the earth-worm reacts vigorously to 0.002 molecular CaCl, responding to the cation, and that it is more sensitive to sodium than to ammonium, lithium, or potassium.

Also Carlson finds that sodium chloride isotonic with sea-water gives a primary augmentation to the ganglionic rhythm of the *Limulus* heart, and that the specific influence of sodium, ammonium, potassium, and magnesium on the heart's activity is similar or the same in *Limulus* and in the vertebrates. In *Limulus* the heart muscle is stimulated by sodium and depressed by magnesium, potassium, and calcium, as in *Cassiopea*.

In an interesting paper Mathews, 1907 (American Journal Physiol., vol. 19, pp. 5–13), finds that $MgSO_4$ depresses the heart action of all vertebrates, decreasing both amplitude and rate, but this effect is offset by $CaCl_2$, as is also the case in invertebrates.

We may conclude that while we can not prove that ionic sodium produces the nervous stimulus which results in muscular activity in invertebrates, we may logically entertain the suspicion that this is the

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[1] Mines, 1908 (Journal Physiol., Cambridge, vol. 37, pp. 408–444), finds that sodium, calcium, and potassium act upon pulsating skeletal muscles of vertebrates as described above for *Limulus*, *Cassiopea*, *Lepas*, and Annelids.

[2] Bethe, 1908 and 1909, Pflüger's Archiv für ges. Physiol., Bd. 124, pp. 541–577; Bd. 127, pp. 219–273.

[3] Papers from the Tortugas Laboratory of the Carnegie Institution of Washington, vol. 1, pp. 115–131, 1908.

case. Hering[1] was the first to publish the fact that vertebrate skeletal muscle may develop rhythmical contractions in a solution of sodium chloride, and Mines[2] has conducted a thorough study of this reaction, obtaining graphic records of the movements of amphibian skeletal muscles; he finds that under certain conditions a 6 or 7 per cent solution of NaCl will give rise to pulsations of the muscle which are comparable in regularity of rhythm to those of lymph-hearts or even of the heart itself. These movements may, however, be stopped by calcium chloride, and are, indeed, probably due to the abstraction of calcium from the muscle by the sodium solution. Hence calcium tends to inhibit the movements. Mines finds also that potassium chloride at first increases the rhythmical movements, but afterwards stops them entirely. It is evident that the action of sodium, potassium, and calcium is similar upon skeletal muscle and on Scyphomedusæ, Annelids, *Lepas* arms, and the heart of *Limulus*. (See Carlson.)

Indeed in all essential respects the Annelids behave as does the Scyphomedusa *Cassiopea* to ionic sodium, potassium, magnesium, calcium, ammonium (NH_4Cl), weak concentrations of acids (H), and ionic CO_3. For example in *Cassiopea* the pulsation stimulus is transmitted by the nerves and is independent of muscular contraction, and also in the Annelid *Eunice fucata* the mid-region of the worm may be rendered inert through immersion in molecular $MgSO_4$, and yet if the anterior end of the worm be stimulated in any manner the posterior end may respond at once while the middle region remains inert. Thus the nerve-chain of the middle region may still transmit the stimulus without causing the magnesiumized segments to respond to it by muscular movement. This middle region will, however, still be capable of transmitting slowly from segment to segment a wave of myogenic peristalsis in the manner demonstrated by Friedlaender[3] for the earthworm.

It is well known that neurogenic stimuli pass more vigorously from head to tail than from tail to head, and in killing worms if one wishes to avoid contraction one should cause the killing fluid to pass from tail to head.

Contractions are caused by 0.625 molecular NaCl in severed parts of Annelids and in *Lepas* from which the central nervous system has been removed, but I am not sure that such pieces can be said to contract in a wholly myogenic manner. Distilled water gives the same effect in Annelids, and indeed any marked change in osmotic pressure of the fluid surrounding them causes a vigorous series of contractions.

If any part of the length of a worm be immersed in an excess of calcium, clonic tetanus with a peculiar convulsive twitching of the longitudinal muscles appears in the part affected, but this twitching does not spread to other parts of the worm, which remain quiescent if in normal sea-water. In *Cassiopea* also the local tetanus caused by calcium does not spread to parts of the medusa not affected by the calcium. If we touch the calcium-affected part of the worm with a

[1] Hering, 1879, Sitzungs. Wien. Akad., Bd. 89, Abth. 3, p. 1.
[2] Mines, 1908, Journal Physiol. Cambridge, vol. 37, pp. 408–444.
[3] Friedlaender, 1894, Pflüger's Archiv ges. Physiol., Bd. 58, pp. 168–206.

crystal of NaCl, while the part is twitching in tetanus, it responds by a vigorous contraction which travels immediately throughout the length of the worm. This leads one to conclude that the calcium tetanus is myogenic, not neurogenic.

Calcium-tetanus, as is well known, can be counteracted by magnesium, but in order to stupefy marine animals without any trace of distortion, one should use a pure 0.4 molecular $MgCl_2$ or a molecular $MgSO_4$ solution, which are practically isotonic with the NaCl of sea-water and which produce a complete relaxation of the muscles without any considerable initial stimulation. In this respect they are more efficient and much quicker in action than a mere excess of magnesium in sea-water.

In solutions containing ionic sodium any calcium which may be present tends to counteract the depressant effects of magnesium; but if sodium be absent the calcium has no power to offset the inhibiting tendency of magnesium. It seems therefore that the calcium ion combines with the sodium and forms a compound which is capable of overcoming the inhibiting effects of the magnesium.

In order to demonstrate the inability of calcium to offset the inhibiting effect of magnesium in a solution lacking sodium, I cut ten *Cassiopea* medusæ into quadrants. One quadrant of each medusa was placed in $MgCl_2$, another in $MgCl_2 + CaCl_2$, another in $MgCl_2 + KCl$, and the fourth quadrant in $MgCl_2 + CaCl_2 + KCl$; the proportionate parts of the Mg, Ca, and K being those found in sea-water. The result is shown in the following table, wherein the medusæ are numbered from I to X and the number of pulsations given by each quadrant of each medusa before coming to final rest is shown in the vertical columns. It will be seen that adding Ca to solutions of Mg or of Mg + K does not overcome their depressant powers. The medusæ come to rest after a very few pulsations in Mg, or Mg + Ca, but the addition of potassium causes a characteristic initial stimulus which sustains movement for a longer time than it can endure in pure Mg or Mg + Ca. Weak concentrations of the potassium ion are always a primary stimulant for the neuromuscular system and an initial depressant for cilia, and the potassium does not combine with any of the other metallic ions to produce this effect, but is itself competent to produce it.

Table showing the number of pulsations given by ten Cassiopea xamachana medusæ.

[Each is cut into quadrants and each of the quadrants placed in one or the other of the 4 solutions shown below:—Medusæ numbered I to X.]

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	Sum.
130 c.c. $\frac{4}{10}$ *m* $MgCl_2$	0	2	1	1	4	9	0	3	2	1	23
100 c.c. $MgCl_2$ + 21 c.c. $\frac{2}{3}$ *m* $CaCl_2$	0	4	1	5	0	3	0	2	2	0	17
100 c.c. $MgCl_2$ + 19 c.c. $\frac{2}{3}$ *m* KCl	16	25	19	23	20	13	25	22	13	15	191
$MgCl_2 + CaCl_2 + KCl$	17	23	15	34	18	28	27	16	14	8	200

It may be of some interest to observe that the palolo worm, *Eunice fucata*, if in the dark, writhes vigorously when stimulated by the light of a match or an incandescent electric lamp. This reaction is not due

to heat rays, for the worm gives no response to the more intense radiant heat from a large dark area of heated iron. It is, however, sensitive to light rays between the red and violet, and will respond to the light of a two-candle-power incandescent electric lamp, the rays of which have passed through a layer of carbon bisulphide 60 mm. in thickness, thus filtering out the ultra-violet.

As was demonstrated by Hesse,[1] in other Annelids, the anterior end is most sensitive, the posterior end next, and the middle of the worm least sensitive to stimuli.

Solutions which consist of sodium, potassium, and calcium chlorides are powerful initial stimulants, but finally produce depression of movement and muscular tetanus in invertebrates. This deleterious effect can, however, be overcome by adding magnesium to the solution, although this destroys its stimulating properties.

Part II.

CILIARY MOVEMENTS.

Studies were made of the effects of various ions upon the ciliary movements of fresh-water infusoria, vertebrate spermatozoa, Annelid larvæ, Veligers, Actinian larvæ, and Ctenophoræ. Among Annelid worms I paid special attention to the trochophore larvæ of the Atlantic palolo worm, *Eunice fucata*, and of *Spirobranchus tricornis* and *Pomatostegus*

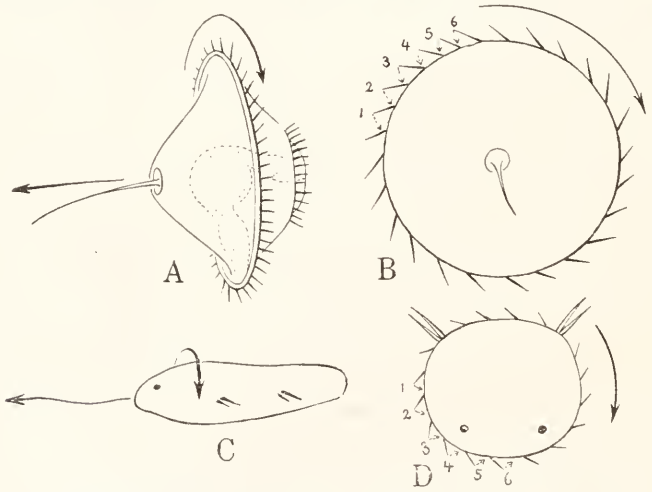

Fig. 4.—Above: *Spirobranchus* larvæ 48 hours old. Below: Palolo larvæ. A and C, side views. B and D, oral views. Arrows show direction of movement.

stellatus, all three of which may be obtained in abundance at Tortugas, Florida. The trochophores of both *Spirobranchus tricornis* and *Eunice fucata* rotate axially in the manner of a left-handed screw as they advance in a sinuous path through the water. The rotation is in each case due to the set of the cilia (see fig. 4). In stale sea-water or in magnesium

[1] Hesse, 1896, Zeit. für wissen. Zool., Bd. 61, pp. 393–419.

solutions the larvæ are often observed to swim in circles, as shown in fig. 5. When young, the trochophores of *Eunice fucata* often turn over end for end as they advance through the water. When placed in a flat Petri dish in the diffuse light of the laboratory the larvæ of both these worms swim in all directions, at random, as is shown in fig. 6, which represents observed paths. When, however, larvæ enter the light meniscus on the side toward or away from the window they exhibit the Jennings-reaction, as is shown within the dotted areas in fig. 6. They are thus trapped in locations of optimum light-intensity, not directed toward the light along the path of the rays. This applies not only to the larvæ of the palolo but to *Spirobranchus* and *Pomatostegus*.

FIG. 5.—Palolo larva "circling" in a magnesium solution.

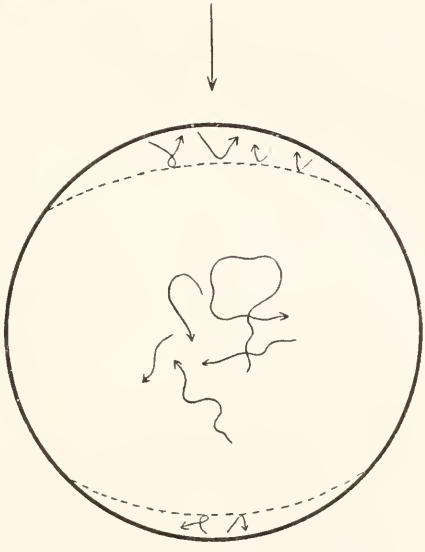

FIG. 6.—Paths of Palolo larvæ in a Petri dish.

It is remarkable that whatever the effects of the cations we are here considering may be upon the neuro-muscular system, their effect upon the movements of cilia are the exact opposite.

EFFECTS OF SODIUM.

A 0.6 molecular NaCl solution is a primary neuro-muscular stimulant, but its effect upon the ciliary movements of Infusoria, worm-larvæ, Veligers, Semper's Actinian larva, and Ctenophoræ is to derange and inhibit without initial stimulation, so that ciliary movement ceases usually in a few seconds, although in rare instances slight ciliary movement may continue in this solution for 20 minutes. In other cases, such as those observed by Prof. R. S. Lillie in *Arenicola* larvæ and in *Mytilus*, the cilia rapidly dissolve in NaCl. It is remarkable that while

the ciliary movements are most seriously depressed in NaCl the neuro-muscular system is highly stimulated, and long after ciliary activity has ceased the muscles continue to contract and move actively.

Thus a solution of 5 grams of $Na_2C_2O_4$ in 1000 c.c. of sea-water is a stimulant for neuro-muscular movements, but it is a primary depressant for ciliary activity. A similar statement may be made of the effects of NaOH in sea-water.

After ciliary movement has ceased in 0.6 molecular sodium chloride solution, activity may be revived by placing the animals in isotonic magnesium solutions such as molecular $MgSO_4$ or 0.4 molecular $MgCl_2$; provided the cilia have not already dissolved in the sodium solution. When so revived by magnesium the cilia beat incessantly at a rapid rate while the muscles subside into quiescence.

EFFECTS OF MAGNESIUM.

Magnesium is the most powerful depressant among ions of blood-salts for the neuro-muscular system, but among these salts it is the most efficient single ion for maintaining ciliary movements. Its effects are thus the exact opposite of those of sodium. For example, the cilia of the trochophore larva of the palolo worm continue to beat for nearly an hour in pure 0.4 molecular $MgCl_2$, whereas such a solution causes muscular activity to cease in a few minutes. In 0.625 molecular ($100NaCl + 11.6MgCl_2$) the trochophores of the palolo and *Spirobranchus* soon begin to circle, as shown in fig. 5. At the end of about half an hour, however, they recover from this circling and move forward slowly, but in a normal manner. Some of them cease to move at the end of an hour, but a few continue to vibrate their cilia for from 3 to 7 hours, after which they die.

In the Ctenophore *Bolinopsis vitrea* the muscles are relaxed and the cilia are highly stimulated in magnesium, so that the animal darts with abnormal rapidity through the water, but the stroke of the cilia is *reversed* and the coördination destroyed, so that the creature moves *backward* (mouth forward) sometimes for more than 45 minutes.

Fresh-water infusoria, such as *Paramœcium*, soon cease to move their cilia in 0.166 molecular NaCl, but remain active a very much longer time in an isotonic solution of $MgCl_2$ or $MgSO_4$, although their rate is never as fast as the normal in these solutions.

Spermatozoa of terrestrial vertebrates cease to move almost instantly in NaCl, but will continue to move for more than an hour in $MgCl_2$. In the worm larvæ, Veligers, Actinians, and Ctenophoræ it is noticeable that the cilia continue to beat in magnesium long after muscular activity has ceased.

The addition of potassium or calcium to solutions composed of sodium and magnesium greatly prolongs their power to sustain ciliary movement. Thus in 0.625 molecular ($100NaCl + 11.6MgSO_4 + 2.2KCl$) cilia of the trochophores of the palolo and *Spirobranchus* cease at once to beat, but in a few seconds they recover and the larvæ swim for from 18 to 48 hours at a rate nearly if not quite normal, reacting normally to light. This behavior is remarkable in contrast with that of neuro-muscular movements in the same solution, for in this solution *Cassiopea*

ceases to pulsate in about 6 minutes, Annelids cease to move in from 19 to 70 minutes, and the branchial arms of *Lepas* can not continue their rhythmical movement for more than 45 minutes.

Upon Ctenophoræ the above solution also causes a momentary cessation of ciliary movement followed by recovery, so that the cilia may continue to beat in a normal rate and manner for more than 9 hours. In some cases, however, the cilia, after having recovered their movement, *dissolve* and disappear in a few minutes—a most dramatic occurrence.

The body-movements of the Ctenophoræ cease in about half an hour in this solution. I am inclined to regard the sudden disappearance of the cilia in these Ctenophores as being possibly due to a nervous derangement, for I have seen it occur in *Cestum* in natural sea-water, the animal suddenly tearing itself into fragments and destroying its entire body without any apparent external cause.

It is evident that while potassium has but little ability to offset the inhibitory effect of magnesium upon neuro-muscular movements, it exerts a marked influence in sustaining ciliary movements in solutions containing magnesium.

A solution composed of sodium, magnesium, and calcium chlorides in the amounts and proportions of van't Hoff's solution[1] is also efficient in sustaining ciliary movement, for in this we find no preliminary checking of ciliary movement of the palolo trochophores, and the cilia continue to move for more than 48 hours, the rate continually *increasing* until it becomes fully that of the normal animal at the end of 24 hours, after which it declines. The *Spirobranchus* trochophores, however, behave somewhat differently, for their bodies degenerate into mere sacs (fig. 7), and finally, after from 18 to 24 hours, the cilia themselves are gradually absorbed, although still beating until they disappear. In fig. 7, the upper part of the figure shows the normal trochophore of *Spirobranchus;* the lower shows the effect of 18 hours' immersion in a solution containing amounts and proportions of sodium, magnesium, and calcium found in sea-water.

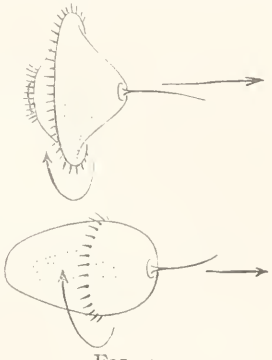

Fig. 7.

Those solutions which are most efficient in stimulating ciliary movement are the strongest depressants of neuro-muscular activity. For example, neuro-muscular movements cease almost immediately in 0.625 molecular $MgCl_2$ or 0.625 molecular ($12MgCl_2+2.5CaCl_2$) or in 0.625 molecular ($12MgCl_2+2.5CaCl_2+2.2KCl$). In the last-named solution the cilia exhibit a momentary pause or a lowering of rate for a few seconds after being placed in the solution, this effect being seen in all solutions containing potassium. After this, however, they quickly recover and beat at an abnormally rapid rate but without coördination, each cilium beating independently of its neighbors. This loss of coördination

[1] 0.625 molecular ($100NaCl+7.8MgCl_2+3.8MgSO_4+2.2KCl+2.5CaCl_2$).

is well illustrated in Veligers, Ctenophores, Trochophores, or Semper's Actinian larva, and is due to the fact that the neuro-muscular system is paralyzed by the magnesium and loses control of the cilia, which thus revert to the primitive condition in which they are not appreciably influenced by the neuro-muscular system but each cilium is controlled chiefly by its own cell.

After cilia have ceased to beat in a magnesium solution they may be restored to temporary activity by 0.625 molecular NaCl. In this case ciliary movement is regained in the sodium chloride before the muscles revive their activity, and the cilia cease to beat long before the muscles cease to contract. The cilia cease to beat, or are dissolved, in from 4 to 8 minutes after being immersed in the sodium chloride, while the muscles may continue to contract for half an hour or more. Often individual ciliated cells are cast off from the epithelium and set free with their cilia lashing vigorously, and if these freed cells be restored to sea-water they continue to beat actively for hours in a normal manner, thus showing that primitive ciliated cells are practically independent of the neuro-muscular system of the animal, and each cell contains within itself its own means of maintaining its movement.

EFFECTS OF POTASSIUM.

It will be recollected that potassium in weak concentration is a powerful but only momentary stimulant for neuro-muscular movements, this initial activity being followed quickly by pronounced depression and toxic effects. Upon cilia, however, its effects are the exact opposite. Thus the movements of the cilia of the worm-trochophores, Veligers, and Ctenophores are momentarily checked by a solution containing the amounts and proportions of potassium and sodium found in sea-water,[1] but after a few seconds of arrested movement recovery takes place and they beat slowly for about half an hour in the case of the Veligers or worm larvæ, and for about 4 hours in that of the Ctenophores.

The muscular movements in all of these forms are at first highly stimulated by this solution, but depression follows, so that the muscles cease to move long before ciliary activity dies out.

The movements of the cilia of the fresh-water *Paramœcium* are at first checked by a weak concentration of potassium chloride or sulphate, so that they cease to beat, or the animal reverses or spins in a circle. Soon, however, recovery takes place and the *Paramœcium* swims slowly but normally forward. Jennings[2] first observed that *Paramœcia* placed in a 1 per cent solution of potassium iodide at first swim backward, then spin around on the short axis of the body for half an hour or more, and finally recover and swim forward.

Parker[3] finds that 2.5KCl in 100 sea-water reverses the stroke of the cilia of *Metridium*, and the same effect is produced by meat-juice, but in this case probably not by the contained potassium, but by the creatine in the meat.

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[1] 0.625 *molecular* (100NaCl + 2.2KCl).
[2] Jennings, 1899, Amer. Jour. of Physiol., vol. 2, p. 319.
[3] Parker, G. H., 1905, Amer. Jour. Physiol., vol. 14, p. 5.

R. S. Lillie [1] found that pure solutions of potassium of 0.5 molecular concentration are capable of long maintaining the activity of the cilia of *Arenicola* larvæ and *Mytilus*, and it is of interest to observe that such strong concentrations of the potassium ion immediately depress neuro-muscular movements without primary stimulation, thus acting upon the neuro-muscular system in a manner the exact reverse of their effect upon cilia.

EFFECTS OF CALCIUM.

Strong concentrations of calcium such as 0.5 molecular $CaCl_2$ quickly check ciliary movement in worm larvæ, Veligers, and Ctenophores without destroying the cilia. In *Mytilus*, however, according to Lillie (1906, p. 130), ciliary movement may last for several hours.

In weak concentrations, however, or if combined with NaCl, ciliary activity continues much longer in calcium solutions than do the neuro-muscular movements. We may conclude that calcium in combination with sodium is a stimulant for the movement of cilia, for it prolongs the duration and increases the rate of movement over and above that caused by NaCl alone.

The larvæ of the palolo worm will swim slowly without an initial stop for about half an hour in 0.625 molecular ($100NaCl + 3CaCl_2$), while *Spirobranchus* larvæ will beat from 45 to 90 minutes, and Ctenophore cilia will vibrate with abnormal rapidity for from 6 to 30 minutes in this solution, the coördination being in many cases destroyed, so that each comb beats independently of its neighbors and the animal is unable to progress despite the fact that its cilia are abnormally active. In this connection it is interesting to observe that Lillie [2] finds that mechanical stimulation arrests the automatic activity of the swimming-plates of Ctenophores, but that this does not occur in the absence of calcium, and the effect decreases as the calcium is decreased.

Carlson, 1906,[3] concludes that calcium depresses both the ganglia and the muscles of the heart of *Limulus* without primary stimulation; and my studies of *Cassiopea* led me in 1908 [4] to a similar conclusion in respect to this animal. Authorities differ in respect to the rôle played by calcium in the pulsation of the vertebrate heart. We must remember that calcium combines with sodium, forming a compound which offsets the inhibitory effects of *magnesium*, and it appears to me that the so-called stimulating effect of calcium is due to this secondary action, for if sodium be absent calcium has no power to overcome the depressant action of magnesium. In the case of *Cassiopea*, calcium augments the rate of pulsation only if added to solutions containing sodium and magnesium, and it is evident that this effect is due to its combining with sodium, thus forming a compound which counteracts the depressant effects of magnesium, not to any direct stimulating action of its own.

[1] Lillie, R. S., 1902, Amer. Jour. of Physiol., vol. 7, pp. 46, 52; also, *Ibid.*, 1906, vol. 17, p. 93.

[2] Lillie, R. S., 1908, Amer. Jour. Physiol., vol. 21, pp. 200–220.

[3] Carlson, 1906, Amer. Jour. Physiol., vol. 16, pp. 390, 394.

[4] Papers from the Tortugas Laboratory of the Carnegie Institution of Washington, vol. 1, 1908, pp. 115–131.

For example, when calcium is added to a pure NaCl solution, the rate of pulsation in *Cassiopea*, *Lepas* (branchial arms), and in pulsating skeletal muscle is *lowered*, not increased, as we would expect were calcium a stimulant.[1] If, however, we add calcium to a solution containing *both* NaCl and magnesium, the rate is increased. I am led to conclude that in all of these animals, as in *Limulus*, calcium is a depressant. A more complete discussion of this question will be found in my paper of 1908.

In combination with sodium, however, calcium appears to be a stimulant for ciliary movement, for it greatly prolongs the duration of the activity of cilia if added to a pure sodium solution, and causes them to beat faster than in NaCl.

EFFECTS OF CARBON DIOXIDE.

In strong concentration carbon dioxide in sea-water quickly checks ciliary movement without initial stimulation. A weak concentration of the CO_3 ion in sea-water, however, at first stops all ciliary movement in trochophore larvæ, but after an interval of from 1 to 3 minutes the cilia begin to recover normal activity. This effect is not due to the escape of CO_2 from the water, for if we place a lot of larvæ in the solution and after they have regained their ciliary movements introduce a second lot of larvæ, these newly introduced animals at once lose their ciliary activity, but afterwards recover. This reaction occurs in sea-water at 82° F. which has been charged with CO_2 in a "sparklet" bottle and then exposed to the air for 8 hours in a shallow Petri dish.

EFFECTS OF WEAK CONCENTRATIONS OF ACIDS, AMMONIUM, ETC.

Weak concentrations of lactic or uric acid (H ion) are primary depressants for ciliary action but are initial neuro-muscular stimulants, although this momentary stimulation is quickly followed by depression.

The ammonium ion (0.625 molecular NH_4Cl) at once stops ciliary movement in *Spirobranchus* trochophores, but after being in the solution for about 3 minutes they begin to beat and recover fairly well, moving for about half an hour, but never with fully normal activity. It is interesting to observe that the muscular system of these larvæ is instantly stimulated as soon as they are introduced into the 0.625 molecular NH_4Cl, but depression soon follows; the effect of the solution upon the neuro-muscular system thus being the exact converse of its effect upon the cilia. NH_4Cl is also a powerful primary stimulant for the neuro-muscular system of *Cassiopea* or adult worms, but depression quickly follows.

A few crystals of urea, CH_4N_2O, added to sea-water cause an active initial stimulation of neuro-muscular movement followed by depression. The cilia, on the other hand, are stopped at first, but soon recover and beat at a rapid rate.

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[1] Bancroft, 1909, Journal Physiol., Cambridge, vol. 39, pp. 1–24, concludes in agreement with Loeb that stimulation must be associated with a decrease in concentration of calcium within skeletal muscles.

In a 0.625 molecular lithium chloride solution the cilia of *Spirobranchus* cease instantly, but do not dissolve, whereas they both cease at once and dissolve in a molecular dextrose solution in water.

SUMMARY.

Among the cations of sea-water sodium is the most potent inhibitor of ciliary activity and the most powerful neuro-muscular stimulant.

On the other hand, magnesium is the most potent in maintaining ciliary movement and the most powerful inhibitor of neuro-muscular movements.

Potassium in weak concentration is a primary depressant for cilia, but afterwards ciliary action recovers in its presence. For neuro-muscular movements, however, it is at first a stimulant and finally a depressant.

Calcium is a weak stimulant for ciliary movement, but a depressant for neuro-muscular activity.

Ammonium at first stops and finally permits the recovery of ciliary movement, but it at first stimulates and afterwards inhibits neuro-muscular movements.

Weak acids (H ion) at first depress and afterwards permit recovery of ciliary movement, but they at first stimulate and afterwards depress neuro-muscular movement.

In each case the effect of the solution is exerted through its cation, and among these cations whatever stimulates cilia depresses muscular activity and whatever inhibits muscular movement stimulates cilia. In nature the more highly specialized cilia, which are under the control of the neuro-muscular system, stop whenever the muscles contract, and beat only when the muscles are relaxed. This is well illustrated in the combs of Ctenophores, the cilia of the lobes of Veligers, or the ciliated bands of Trochophores, and Semper's larva.

At times the trochophore larva of *Spirobranchus* does not contract as a whole, but only a small sector of muscles underlying the peristomial ring of cilia contracts. In this case the cilia overlying the contracted sector immediately stop but the impulse which produces the "wheel movement" of the ring of cilia passes over the inert cilia without affecting them, so that the cilia of the uncontracted parts of the ring maintain their normal movement. This accords with Parker's[1] observation that cooling a part of the length of a row of ciliated plates in Ctenophores stops the combs over the cooled area, but does not inhibit the transmission of the wave impulse across this area. Kraft obtained similar results in his experiments upon the ciliated epithelium of vertebrates.[2]

We may present the results of this paper in a graphic manner if we represent a stimulus by a + sign and an inhibition of movement by a − sign. Successive effects may be represented by a succession of signs; thus, − + means a depression followed by recovery of movement and + − an initial stimulus followed by depression. Bearing this

[1] Parker, G. H., 1905, Journal Experimental Zool., vol. 2, p. 417.
[2] Kraft, H., 1899, Pflüger's Archiv für ges. Physiol., Bd. 47, pp. 196–235.

preamble in mind, the following table will illustrate the effects of the various cations:

Cation.	Effect on neuro-muscular movement.	Effect on movement of cilia of animals.
Sodium	+	−
Magnesium	−	+
Potassium	+ −	− +
Calcium	−	+
Ammonium	+ −	− +
Hydrogen	+ −	− +
Lithium	+	−

I am unable to interpret the meaning of this law of the converse effects of cations of blood-salts upon ciliary and upon the neuro-muscular movements of animals, yet any adequate explanation of the phenomena of animal movement must offer an explanation of this relation. Indeed the discovery of this converse relation makes very apparent the incompleteness of all existing explanations of the cause of animal movements.

The electrical stimulation obtained from an induction coil is peculiar in that it excites both the muscles and the cilia. Its effect is to cause the muscles to contract and this contraction stops the cilia in Ctenophores or Veligers, but if the muscles be paralyzed in magnesium so that they can not contract, or if the cilia be not underlaid by muscles, as in the auricles of *Bolinopsis vitrea*, they are excited when the electrical current passes through them. This leads one to suspect that, whereas a negative variation accompanies neuro-muscular contraction, a positive variation may accompany ciliary excitation, but this hypothesis I have not yet been able to test experimentally.

EFFECTS OF IONS UPON MOTILE PLANT-SPORES.

It is commonly supposed that ciliary movement is of a very primitive nature, and it naturally occurs to one that the ciliary movements of animals may be a heritage from their more or less plant-like ancestors. If this be true we would expect the motile spores of the lower plants to react to electrolytes as do the cilia of animals. I find, however, that there is wide diversity in the behavior of various motile plant-spores under the influence of ions, this being in marked contrast to the uniform reaction of animals, wherein one finds only differences of degree in the behavior of the cilia in forms so diverse as protozoa or spermatozoa, worm larvæ, ciliated epithelia of Molluscs, and the combs of Ctenophores. There is, however, a *Spirillum* which lives in a culture of dead house-flies in fresh water which reacts to ions somewhat as do the cilia of animals. This *Spirillum* is elongate, helically coiled, and of uniform transparency (fig. 8 A), and it thrives in the water containing

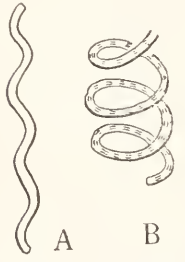

FIG. 8.—Two sorts of *Spirillum* living in a culture of dead flies in fresh water.

dead flies, provided green algæ, such as *Spirogyra*, be in the aquarium in which it lives. It is very active, darting rapidly through the water.

In 0.625 molecular sodium chloride it stops instantly, but in weak concentrations of sodium it may move for half an hour or more without initial stimulation and with gradual loss of movement.

In 0.375 molecular magnesium sulphate it continues to move slowly for about 90 minutes, gradually coming to rest.

In solutions of about 0.066 molecular potassium chloride we sometimes observe that the initial effect is to check or stop all movement, but after an interval of 2 or 3 minutes partial recovery takes place and the spirillum moves slowly forward in a normal manner. A slightly stronger concentration of potassium, however, checks movement without permitting recovery.

In 0.166 molecular calcium chloride it moves with normal activity for from 15 to 30 minutes and then suddenly ceases to move.

These reactions, it will be observed, are in each case identical in nature with those of animal cilia. In no other case, however, have I been able to find a plant-spore which reacts as do the cilia of animals. For example, another form of *Spirillum* living in this same dead-fly culture in fresh water will continue to swim for somewhat more than 3 hours in 0.625 molecular sodium chloride, being even abnormally active for the first few minutes in this solution. This spirillum is closely coiled and exhibits irregularly-spaced nodes of milky or ground-glass-like translucency, contrasting with its general transparency (fig. 8 B).

To cite another case, the actively motile zoöspores of the *Saprolegnia* which develops within the bodies of dead flies in fresh water will retain some movement even after they have been in a 0.625 molecular sodium chloride solution for 24 hours. In molecular magnesium sulphate they remain motile for more than 9 hours, but are never so active as the normal. In 0.625 molecular potassium chloride they are normally or even supernormally active for 4 or 5 hours, but after this their movements decline and die out after about 6 hours. In 0.166 molecular calcium chloride they move normally for about 2 hours and then stop.

The zygospores of *Fucus* illustrate another exceptional case. They move slowly for from 13 to 19 minutes in 0.625 molecular sodium chloride, gradually coming to rest. In 0.625 molecular magnesium sulphate they cease to move in from 4 to 8 minutes, and in solutions of potassium chloride they soon cease to move, strong solutions stopping them almost instantly. Altogether their reactions resemble those of the neuromuscular system of animals rather than animal cilia.

I have thus met with nothing but confusion in attempting to correlate the reactions of plant spores with those of animal cilia, but it is still possible that further study may demonstrate the existence of relationships in the reactions of certain special forms of plants to those of animals, but I doubt if any general significance can be attached to this even if it be a fact.

TABLE I.—*Neuro-muscular Movements.*

Effects of solutions composed of one or more of the constituents of van't Hoff's sea-water solution upon invertebrates. The complete solution is 0.625 *molecular* ($100NaCl+7.8MgCl_2+3.8MgSO_4+2.2KCl+2.5CaCl_2$).

Composition of solution.	Initial effect of solution.	Duration of movement in minutes.			Condition of muscles when movement has ceased.	Movement may be restored after it has ceased by addition of—
		Cassiopea.	Lepas.	Annelids.		
NaCl.....	The most powerful stimulant, causing marked initial increase in rate and vigor of movements.	45—	1 to 12	30 ±	Muscular tonus practically normal, being neither in tetanus nor relaxed.	Calcium readily restores temporary movements; potassium also does this but far less efficiently.
$NaCl+MgCl_2+MgSO_4$	Immediate and continued depression without initial stimulation.	0.75 to 6	20—	4 to 45	Muscles relaxed....	Calcium.
NaCl+KCl....	Stimulating, but to a lesser degree than a pure NaCl solution; though movement endures longer than in pure NaCl it is neither so rapid nor powerful.	120+	8 to 32	45 ±	As in NaCl.....	Calcium.
$NaCl+CaCl_2$...	Stimulating, but not so actively as in NaCl, though movement lasts longer than in NaCl.	90—	9 to 75	40 to 300	Muscles in strong tetanus, which is clonic in case of Annelids.	Magnesium removes tetanus and restores movement.
$NaCl+MgCl_2+MgSO_4+CaCl_2$	Very slightly stimulating; nearly normal.	40—	50 to 220	Not recorded. About 300 minutes or more.	*Lepas* shows a slight tetanus. *Cassiopea* and Annelids come to rest, neither relaxed nor in tetanus.	Potassium exhibits a slight and only momentary restorative power.
$NaCl+MgCl_2+MgSO_4+KCl$	Movements decline steadily, but not so rapidly as in Na+Mg.	6—	20 to 45	19 to 70	Muscular relaxation.	Calcium.
$NaCl+CaCl_2+KCl$	Stimulating, but not so actively as in a pure NaCl solution, though movements endure longer.	250+	240 to 360+	240 to 420+	Tetanus, resulting in tearing apart of muscles of *Cassiopea*, strong contraction of muscles of *Lepas*, and tearing open of cuticula of Annelids, whose bodies come to rest in kinked and contorted folds.	Magnesium removes muscular tetanus and restores movement. This is more efficacious in case of medusa than in higher forms.

TABLE II.—*Ciliary Movements.*

Reactions of the cilia of Annelid worm larvæ and Veligers in solutions composed of one or more constituents of van't Hoff's sea-water solution. The complete solution is 0.625 *molecular* ($100NaCl+7.8MgCl_2+3.8MgSO_4+2.2KCl+2.5CaCl_2$).

Composition of the solution.	Initial effect on cilia.	Duration of movement in minutes.	General behavior.
NaCl.....	Strongly depressed.....	0.5 to 20	An immediate derangement of ciliary movement. The cilia may even dissolve.
$NaCl+MgCl_2+MgSO_4$	Slower than normal, but faster and better sustained than in any other combination of two cations.	60 to 400+	At first the Annelid larvæ tend to swim in circles, but soon they recover and progress normally but slowly forward.
$NaCl+KCl$....	All ciliary movement stops immediately, but after 5 to 20 seconds recovery takes place and slow ciliary movement is regained.	30 to 60	Initial checking of ciliary movement is a characteristic of all solutions containing potassium. After recovery the cilia move *slowly*.
$NaCl+CaCl_2$...	Movement continues, slowly dying out, without an initial arrest.	30 to 90	Larvæ swim slowly but in a normal manner. Cilia never dissolve in solutions containing calcium.
$NaCl+MgCl_2+MgSO_4+CaCl_2$	There is no initial arrest of movement. Ciliary activity is slow at first but it continually augments until its rate is fully that of the normal.	1000 to 3000+	In *Spirobranchus*, body of larva degenerates while cilia continue to beat, but in palolo this does not occur and cilia beat normally.
$NaCl+MgCl_2+MgSO_4+KCl$	Ciliary movement stops at once, but after a few minutes movement is regained and finally becomes normal in rate.	1080 to 3000+	After initial arrest of movement, ciliary activity continually augments and becomes normal in rate, finally, however, declining, so that larvæ swim in circles shortly before they die.
$NaCl+CaCl_2+KCl$	In many larvæ ciliary movement is completely arrested for a few seconds, but recovery soon takes place and the larvæ move slowly.	40 to 2000	A few larvæ move in circles but most of them progress normally but slowly. In some cases ciliary movement is not wholly checked but merely reduced at start.
0.4 molecular $MgCl_2$	There is no initial arrest of movement. The cilia begin at once to beat at about a normal rate. After cilia have ceased to beat temporary activity may be restored by 0.625 molecular NaCl.	30 to 300	The muscular movements cease in less than 7 minutes and the cilia beat incessantly, independent of the control of the neuro-muscular system.
$MgCl_2 + CaCl_2$	As in $MgCl_2$.....	25 to 270	As in $\frac{4}{10}$ molecular $MgCl_2$.
$MgCl_2 + KCl$	The cilia stop instantly but recover after about 2 minutes and beat at about a normal rate.	40 to 180	The muscles give a few initial contractions and then stop, and the cilia then beat incessantly and without being controlled by the neuro-muscular system.
$MgCl_2 + CaCl_2 + KCl$	Ciliary movement is checked at first as in all solutions containing potassium, but after a brief initial pause the cilia begin to beat at an *abnormally rapid* rate.	50 to 350	As in $MgCl_2 + KCl$.
$CaCl_2 + KCl$...	The cilia are checked or stopped at first but revive and beat more slowly than the normal.	90 ±	The neuro-muscular system is quickly inhibited, so that the cilia beat independent of its control.

II.

EFFECT OF DIFFERENT TEMPERATURES ON THE MEDUSA CASSIOPEA, WITH SPECIAL REFERENCE TO THE RATE OF CONDUCTION OF THE NERVE IMPULSE.

By E. NEWTON HARVEY
Of Columbia University

5 text figures

THE EFFECT OF DIFFERENT TEMPERATURES ON THE MEDUSA CASSIOPEA, WITH SPECIAL REFERENCE TO THE RATE OF CONDUCTION OF THE NERVE IMPULSE.

By E. Newton Harvey.

During the summer of 1909 a partial study was made of the effects of temperature on the nerve and muscle tissues of the scyphomedusa *Cassiopea xamachana*. The excellence of this jelly-fish for experimental work has already been commented on by several writers. It lives in great numbers in the bottom of the moat surrounding Fort Jefferson, Tortugas. The water in the moat varies in depth from 3 to 5 feet at low tide with a difference between high and low water of less than 3 feet. The temperature of the water at the bottom, where the jelly-fish live, after a 2-days storm, was 27° C. on July 18, 7 a.m., the coolest weather this summer. I think this is the lowest temperature attained in the summer. Next day at 1 p.m. the temperature had risen to 30°, and next day, also at 1 p.m., it was 29.5°. The temperature on the very hottest days was not taken, but I think it may become as high as 32° to 33°, as the surface-water in the moat on such days is very warm to the touch. The average normal summer temperature of *Cassiopea* may therefore be placed at about 29°.

Since the temperature to which tropical animals are exposed is so uniform, it is not surprising to find that they are quite sensitive to even slight changes of temperature. However, *Cassiopea* offers such exceptional advantages in other respects that the experiments reported below were undertaken, even though the effects of a wide range of temperature could not be investigated.

I wish to express my sincerest thanks to Dr. Mayer for valuable suggestions and for the many opportunities which he gave me of carrying out the work described herein. I am also indebted to Dr. J. H. Hilderbrand, of the University of Pennsylvania, for assistance, especially in calculating the diffusion rates of $MgSO_4$ and CH_3COOH.

The investigations may be arranged under two heads: (1) The temperature limits of activity and thermal death-points of muscle and nerve. (2) The temperature-coefficients of pulsation and nerve conduction.

A third series of observations was made on the effects of inhibiting electrolytes at different temperatures, 10° apart. If the ions enter into combination with any substance in the muscle and the cessation of contraction is connected with the formation of some compound, for instance an ion-proteid, then we should expect contraction in the inhibit-

ing solution to cease two to three times more rapidly with every 10° rise in temperature, according to van't Hoff's empirical rule for the increase in velocity of chemical reactions with rise of temperature.

There is one possible source of error in an experiment of this kind. We may not be able to distinguish between the time it takes for the ions to diffuse into the nerve-muscle tissue and the time for them to stop contraction, when present in sufficient concentration. It may be said, however, that the diffusion time is very small. The nerve-muscle layer of *Cassiopea* is thin, certainly less than 0.2 mm. A calculation of the time required for a 0.375 molecular $MgCl_2$ solution to diffuse across a distance of 0.3 mm. and reach 0.9 of the original (0.375 molecular) concentration on the other side gave a value $<$ 1 minute. The diffusion constant for $MgSO_4$ in pure water was used, as none other was available. Acetic acid would diffuse through the same distance even more rapidly. The above calculation is very rough and is only of value in showing how short the diffusion time may be provided there is no resistance at the cell boundary.

There is positive evidence that considerable time is required for $MgCl_2$ and CH_3COOH to stop conduction in the nervous network. This network is epithelial in nature, external to the muscles, and directly in contact with the sea-water, yet *the muscles always cease to contract before the nerves cease to conduct.* Conduction continues about twice as long as contraction. We see, then, that diffusion of the electrolyte must play a very small part in the stoppage of nerve conduction in *Cassiopea.*

This is also true for the muscles, as is shown below. Let us compare the times required for different concentrations of CH_3COOH to inhibit contraction and conduction. The following is a table of inhibition times:

cc. $\frac{n}{10}$ CH_3COOH to 100 cc. sea-water.	Molecular concentration.[1]	Contraction ceases, in minutes.	Conduction ceases, in minutes.
6	n/166	0.25	0.25
5	n/200	0.75	1.25
4.5	n/222	1	1.5
4	n/250	2	3.5
3.5	n/285	1.75	5
3	n/333	4	7
2.5	n/400	9	Not lost in 15 min.
2	n/500	Weak but not lost.	Not lost in 15 min.

[1] A correction must be made for the alkalinity of the sea-water, which is very high at Tortugas. Red litmus paper is quickly turned blue. Phenolphtalein becomes faint pink.

We assume that CH_3COOH actually enters the muscle cells and that the presence of a definite concentration within makes any further contraction an impossibility. The problem is, in what time would this concentration be reached from varying concentrations on the outside. It takes nine minutes for $\frac{n}{400}$ CH_3COOH to stop contraction, yet less than one minute for $\frac{n}{200}$ CH_3COOH. Velocity of diffusion is proportional to the degree of concentration. The time values, one and nine, could not possibly be accounted for by the above law, so that the difference in time must be attributed to the concentration of the acid in affecting the tissue, and not to the diffusion rates for the respective concentrations.

So much for a vindication of the experimental method. As for the results, they have been in part stated in my preliminary report.[1] Using the sense-organs as the source of stimulus and a temperature interval of 24° to 34° C.[2] there is without doubt a very marked increased efficiency of both $MgCl_2$ and CH_3COOH in stopping both contraction and conduction at the higher temperature (34°). At 34° functioning ceases 2 to 2.5 times as soon as at 24°. This value is identical with van't Hoff's coefficient (2 to 3) for increase in velocity of chemical reactions with 10° rise of temperature.

Inasmuch as a similar value was not obtained with 20° to 30° as the 10° interval, and the stimulus given out by the sense-organs may have varied in strength at different temperatures, it has seemed better not to publish any data until further experiments can be made. It is hoped that they will afford sufficient evidence to decide the question.

TEMPERATURE LIMITS OF ACTIVITY AND THERMAL DEATH-POINTS OF MUSCLE AND NERVE.

METHOD.

The muscles and nerves of *Cassiopea* form a layer almost as thin as paper over the whole of the subumbrella surface, the nerves outermost. Although intimately connected and impossible to separate by dissection, the greater resistance of the nerves to temperature extremes renders possible a study of their properties apart from the muscles. This is done in the following way, first described by Mayer[3] in studying the effects of salts. A long strip of disk-tissue with several sense-organs on one end is laid across three dishes filled with sea-water (A, B, and C, fig. 1).

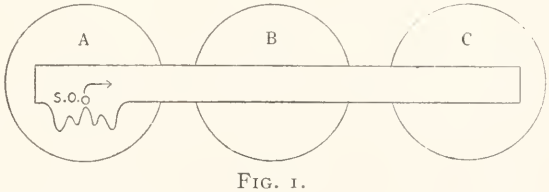

FIG. 1.

Stimuli are constantly arising in the sense-organs in dish A, and pass along the strip of tissue stimulating the muscles as they go. By slowly raising or lowering the temperature of the sea-water in B, a temperature can be found where contraction of the muscles ceases, yet the impulse is still transmitted. A cessation of nerve[4] conduction is indicated by the muscles in C, which can then no longer contract, except, of course, on direct stimulation.

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[1] Carnegie Institution of Washington, Year Book, No. 8, p. 117, 1909.

[2] 5° either side of the average summer temperature of the water at Tortugas.

[3] Mayer, A. G., Carnegie Institution of Washington, Pub. No. 102, p. 128.

[4] Conduction is assumed to take place always through the nervous network, inasmuch as it has not been shown for *Cassiopea* that conduction can take place through muscles independently of nerves.

The influence of temperature on the sense-organs apart from other tissues can be studied by the method indicated in fig. 2. The sense-organs are in A and the sea-water is slowly warmed. Cessation of contraction in B indicates that the stimuli are no longer given out by the sense-organs.

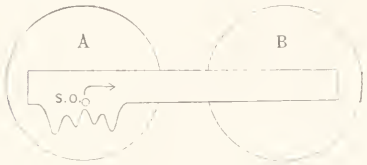

FIG. 2.

The thermometer used had been standardized and was graduated to 0.1° C. The rate of change of temperature was kept as constant as possible, 1.0° in 2 minutes.

LOWERING THE TEMPERATURE.

Cooling of the sea-water containing normal medusæ produces at first a rapid pulsation, but the rate per minute gradually decreases at lower temperatures. At about 18° *Cassiopea* begins to turn inside out, the subumbrella surface becoming convex and the exumbrella concave. This relaxation, as it may be called, is also characteristic of diseased (by parasites) and dying (in stale or constantly agitated sea-water) jelly-fish. The fact that it begins at 18° to 19° shows how sensitive the animal is to a decrease in temperature. The summer temperature of the sea-water at Woods Hole is about 18°, whereas on the California coast Loeb [1] reports the temperature of the water to be about 10° and the eggs of *Strongylocentrotus* fail to develop above 23°.

Using the methods illustrated in figs. 1 and 2, it was found that the functioning of three different tissues ceases at the following temperatures: sense-organs (pulsation), 14° C.; muscles (contraction), 9.5° to 10.6°; nerves (conduction), 8.8° to 9.5°.

If cooled to 9.5° and immediately returned to sea-water at 29° there is complete recovery, but there is no recovery if cooled to 7° to 8° C. The tissues disintegrate on warming, whether warmed suddenly or slowly. This fact is especially interesting, as, with the exception of warm-blooded animals, few forms are killed by exposure to low temperatures, but above the point at which ice crystals form.[2] Irreversible changes do not take place in all jelly-fish, however, as Romanes [3] reports freezing *Aurelia aurita* solid in a block of ice, yet there was complete recovery on thawing.

RAISING THE TEMPERATURE.

A sudden rise of temperature brings about a sudden increased pulsation of the normal medusa, but this increase is not so marked as with lowering. Relaxation begins about 36°. The tissues cease to function at about the following temperatures: muscles,[4] 39.5°; sense-organs, 42.6°; nerves, 44.0°.

[1] Loeb, J., Arch. f. d. ges. Physiol., 124, p. 417, 1908.

[2] See Loeb's discussion in Dynamics of Living Matter, p. 110.

[3] Romanes, J. J., Jelly-fish, Star-fish, and Sea-urchins, International Scientific Series, New York, 1885, vol. XLIX, p. 167.

[4] The circular muscles cease perhaps 0.5° before the radial muscles, but this difference is not always constant.

If returned to sea-water at 29° from 44°, the nerves may partially recover at first, but later lose their power of conduction, while the muscles can still contract when stimulated by induced shocks. On returning to sea-water at 29° from 44.5° neither the nerves nor muscles recover. The strips eventually disintegrated. The heat stand-still of the sense-organs is also reversible until 44°, above which there is no way of telling whether recovery would occur or not.

At 39.5° the muscles are perfectly relaxed. Although unable to contract they do not pass into heat rigor. On still further raising the temperature the muscles remain in the same relaxed condition until about 55°, when they slowly contract. A large amount of whitish slime is given off during the heating. If this temperature corresponds to that of heat rigor in other animals it is exceptionally high. In skeletal muscles of the frog heat rigor occurs at 39°, in the mammal at 47°, and in the heart of Limulus at 48.5°.

If the tissues of *Cassiopea* are kept near their temperature limit for longer times, both contraction and conduction stop at lower temperatures. The upper temperature limit is therefore a function of time. In this connection two very interesting recent papers may be mentioned:

A. Meyer [1] has shown that the killing time for bacteria above the temperature at which life continues indefinitely may be calculated from the following equation:

$$x = aq^{n-1}$$

where

x = time of exposure

q = a constant for a given bacterium

a = the killing time at 80° C., the first of a series of terms 10° apart (80°, 90°, 100°, etc.).

n = the term in the series (80 = 1st term, 90° = 2d term, etc. For 100° $n - 1 = 3 - 1$).

Calculated as Q_{10}, the temperature coefficient [2] is 5 for *Bacillus subtilis* and 4 for *Bacillus robur*. The reader is referred to the original paper for the values of q and a, which vary with each bacterium.

Loeb [3] has studied the temperature coefficient for the length of life of sea-urchin eggs at temperatures above normal. If the length of life for T degrees is known and is represented by D, then the length of life for a temperature $T - n$ degrees is $2^n D$. Q_{10} was found to be about 1000. This is true for the unfertilized as well as the fertilized eggs. In both of these cases it will be seen that Q_{10} is constant for all 10° intervals. I mention them especially because there is another class of vital temperature coefficients in which Q_{10} varies according to the 10° interval. I shall speak of this and its meaning later.

It may be of interest to compare the temperature limits of activity of a tropical animal with those of a northern form. As the analogy

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[1] Meyer, A., Berichte, d. deutsch. Bot. Gesell., XXIV, p. 340, 1906.

[2] Q_{10} is the ratio of a constant at T + 10 degrees to a constant at T degrees $C.$, or $Q_{10} = \frac{k_{t+10}}{k_t}$

[3] Loeb, J., Arch. f. d. ges. Physiol., 124, p. 417, 1908.

between a heart and a medusa is very close, the temperature limits of an invertebrate heart have been selected.

Carlson [1] gives the following temperatures at which activity ceases in the heart of *Limulus* from the region of Woods Hole. The effect of temperature on the heart ganglion, muscle, and nerves can be readily studied separately in this form:

Muscle	Contractions cease	0 to − 1° C.
	Optimum	10 to 14°
	Contractions cease	32°
	Tonus or heat rigor	47 to 50°
Ganglion	Stimuli cease	0 to −1° C.
	Stimuli cease	42°
	Ganglion killed	Above 47 to 50°
Nerves	Conduction ceases	−2° C.
	Conduction ceases	43°
	Nerves killed	Above 47°

It will be noticed that, although the normal temperatures of the two animals are very different (more than 10°) yet their upper temperature limits are nearly the same. *Cassiopea* is an animal living constantly within about 15° of its death-point, yet is not adapted to withstand higher temperatures than the heart of a northern animal living 25° to 30° from its death-point. The *Limulus* heart can withstand much lower temperatures than *Cassiopea*, however. This is what we might expect.

It would be interesting to determine the temperature limits for the heart of the *Limulus* which occurs around the Marquesas Keys, near Tortugas, and whose normal temperature is very different from that of the *Limulus* on which Carlson worked.

THE TEMPERATURE-COEFFICIENTS OF PULSATION AND NERVE CONDUCTION.

PULSATION.

Cassiopea is not a favorable object for a study of the influence of temperature on pulsation, because it is so readily excited to rapid beating, by handling, by currents of water, and by sudden though slight changes in temperature, both higher and lower than the normal summer temperature (29°). After some time this initial excitation caused by a change in temperature passes off and the beats become fairly constant. On account of the difficulty of obtaining and keeping ice at Tortugas, only a few observations on the pulsation rate were made.

An average of 6 counts gave the following as the number of pulsations per minute for 4 temperatures: 30°, − 33 per minute: 25°, − 26 per minute; 20°, − 17 per minute; 16°, − 8 per minute.

$$Q_{10}\ (20°-30°) = \text{ca.}2 \qquad Q_{10}\ (16°-25°) = 3$$

The coefficient is of the same magnitude as that for the increase in the velocity of chemical reactions per 10° rise in temperature. All

[1] Carlson, Am. Journ. Physiol., 15, p. 215, 1906.

observations thus far indicate that Q_{10} for the rate of increase of the heart beat, both of vertebrates and invertebrates, is about 2 to 3 for normal temperatures. We may conclude that in the medusa, as in the heart, the origination of stimuli in the sense-organs is dependent on the progressing of some chemical reaction.

Mayer [1] believes that the pulsation is due to a constant formation of sodium oxalate in the sense-organs. This precipitates the $CaCl_2$ and $CaSO_4$ diffusing it from the sea-water, thus forming a slight excess of NaCl and Na_2SO_4, which act as stimulants. The rate of formation of Na oxalate (probably from carbohydrates) conditions the rate of pulsation. This, then, appears to be, in part at least, the reaction whose increase in velocity with rise of temperature quickens the pulsation rate at the same time.

NERVE CONDUCTION.

With the exception of Maxwell's paper on the pedal nerves of *Ariolimax*, previous work on the influence of temperature on nerve conduction has been confined to vertebrates.

Snyder [2] calculated from Helmholtz's [3] observation on the frog a temperature-coefficient (Q_{10}) of 3.16; from Nicolai's [4] observations on the olfactory nerve of the pike a value for $Q_{10}=2.6$; and from von Miriam on the frog's ischiadicus, $Q_{10}=1.95$. In his own experiments, reported in the same paper, Snyder also determined Q_{10} for conduction rate in the frog's sciatic to lie for the most part between 2 and 3.

Lucas [5] finds in the leg nerves (sciatic+tibial+sural) of the frog Q_{10} for 8° to 18° and 9° to 19°=1.64 to 2.08 with an average of 1.79.

Maxwell,[6] in 1907, working on the pedal nerves of *Ariolimax*, in which the conduction rate is slow, found Q_{10} to equal on the average 1.78.

In this connection it may be of interest to mention Wolley's [7] paper on the rate of conduction of a contraction wave in the frog's sartorius. The latent period is also recorded. His results are as follows:

	Rate of conduction of contraction wave.			Latent period of contraction.		
	Max.	*Min.*	*Aver.*	*Max.*	*Min.*	*Aver.*
Q_{10}(5°–15°).....	2.37	1.47	2.01	3.94	2.65	3.34
Q_{10}(10°–20°).....	2.03	1.46	1.79	4.47	2.78	3.51

The rate of conduction in muscle appears to be influenced by temperature to the same degree as nerve.

In *Cassiopea* the rate of nerve conduction is very much more uniform than that of pulsation. It is not affected by currents in the sea-water or by slightly disturbing the piece of tissue. Transferring from

[1] Mayer, A. G., Carnegie Institution of Washington, Pub. No. 102, p. 129.
[2] Snyder, C. D., Am. Journ. Physiol., 22, p. 179, 1908.
[3] Helmholtz, Müller's Archiv, 1850, pp. 345, 358.
[4] Nicolai, Arch. f. d. ges. Physiol., 85, p. 113, 1901, and Snyder, Arch. f. Anat. u. Physiol., Phys. Abt., p. 113, 1907.
[5] Lucas, K., Journ. Physiol., 37, p. 112, 1908.
[6] Maxwell, S. S., Journ. Biol. Chem., 3, p. 359, 1907.
[7] Wolley, W. J., Journ. Physiol., 37, p. 112, 1908.

one dish of sea-water to another slows the conduction while in the air, but nearly the original rate is regained again in sea-water. A more favorable object for the study of nerve conduction could not be desired.

For the following experiments ring-shaped pieces of tissue, without sense-organs, are cut from the disk, as has been described by Mayer.[1] If these are stimulated strongly at one point (S) by induced shocks, a nerve impulse passes around each side of the ring and the two block on the opposite side (fig. 3). If one impulse is itself blocked (fig. 4) by

FIG. 3.

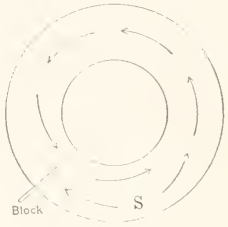

FIG. 4.

pressing on the nerves, for a moment, with a glass rod near the point of stimulation, the impulse of the opposite side will travel around the ring (stimulating the muscles as it goes) indefinitely, since it meets no counter impulse to stop it. Mayer has recorded rings of *Cassiopea* started in this way conducting for several days with a practically constant rate.

Below 18° it was found difficult to start impulses that would keep going. Even when the ring was first cooled and then stimulated, the impulse stopped suddenly after a few seconds. Between 18° to 38°, however, an accurate temperature conduction rate curve could be plotted. All the readings on curve *A* (fig. 5) have been obtained from one ring which was started at 17° and then gradually warmed at an average rate of 1.0° in 4 minutes.

Temperatures are plotted as abscissæ; conduction rate (*i.e.*, the number of times per minute the nerve impulse passes around the ring of tissue) as ordinates. The outside diameter of the ring was 104 mm. and its inside diameter 61 mm. The following table gives actual velocities of propagation for a few temperatures in millimeters per second:

	18°	20°	25°	30°	33° (max.)	35°	38°
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Velocity about inner portion	136	178	276	371	401	391	324
Velocity about outer portion	234	304	469	635	707	669	553

It is interesting to note that the conduction wave, to pass around the ring regularly, must move much more rapidly on the outer than the inner side. There is apparently some coördinating mechanism regulating the velocity of the impulse in different regions of the subumbrella. If a conducting ring be cut in two the original velocity is not maintained in each new ring, *i.e.*, the new rings do not remain synchronous.

[1] Carnegie Institution of Washington, Pub. No. 102, p. 117.

Curve B was plotted from data obtained with a ring whose outside diameter = 75 mm.; inside diameter = 30 mm. Its form is essentially that of A, but it is less regular. This must be attributed to experimental errors, as my apparatus for changing the temperature uniformly was not as perfect as in the experiment from which A was plotted, and the thermometer used was only graduated to degrees instead of 0.1 degree.

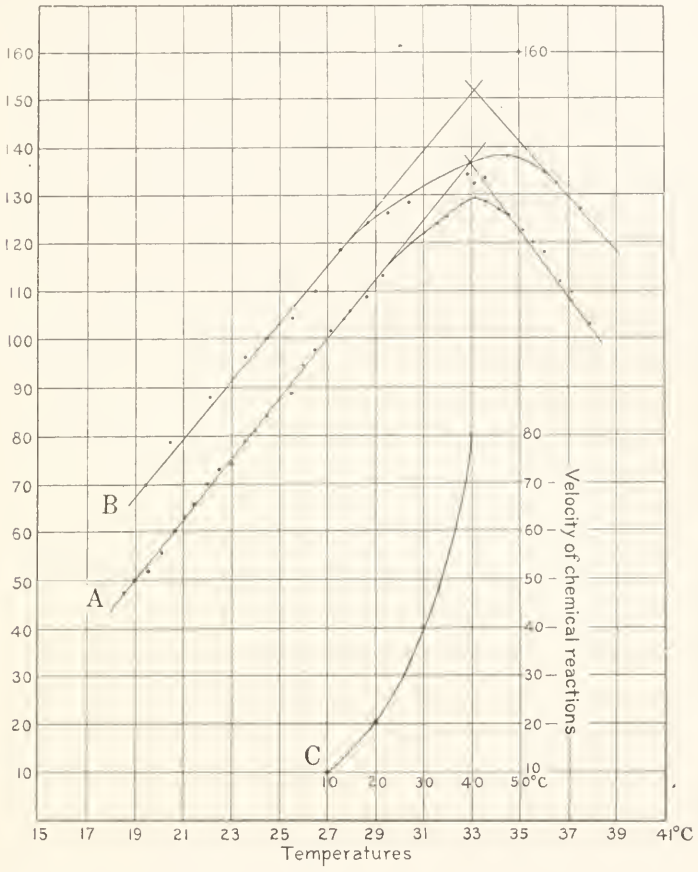

FIG. 5.

The values of Q_{10} for 10° intervals along curve A are as follows:

18 to 28 degrees	$2.40Q_{10}$		24 to 34 degrees	$1.58Q_{10}$	
19 29	2.24		25 35	1.41	
20 30	2.08		26 36	1.25	
21 31	1.93		27 37	1.10	
22 32	1.82		28 38	0.96	
23 33	1.73				

So long as the temperature-velocity curve is a straight line, the value of Q_{10} will not be constant for all 10° temperature-intervals, but will gradually become less and less, the higher the temperature. This is not the case (as a rule) for temperature-reaction velocity curves. Q_{10}

is very much more nearly a constant for all 10° temperature-intervals,[1] although it does vary somewhat, and differently for different reactions.[2] Curve C (fig. 5) may be taken as the type for increase in reaction velocity with rise of temperature. It has been so plotted as to be easily compared with A and B. For this curve $Q_{10} = 2$, constant at all temperatures.

If the rate of nerve conduction depends on the velocity of some chemical reaction in the nerve, the above-mentioned difference in its temperature curve remains to be explained. It is possible, indeed probable, that yet another factor than reaction velocity determines conduction rate, and the resultant curve of the two factors is the one actually observed. The velocity of enzyme actions, which show similar characteristics, will be spoken of later.

Q_{10} for the rate of heart beat of the Pacific terrapin, as given by Snyder,[3] also decreases rapidly, the higher the 10° temperature interval. The curve as given by Wolley for the velocity of a contraction wave in the frog's sartorius is only slightly curved and the average value of Q_{10} for 5° to 15° is 2.01 as compared with 1.79 for 10° to 20°.

Snyder's observations on the frog's sciatic nerve, before mentioned, also point to a right-line temperature curve of nerve conduction in this animal. He gives the following as a typical case (p. 196).

Temp.....	0°	10°	20°	30°	
Velocities.....	2.9(X)	11.3(Y)	28.3(Y)	44(X)	m. per sec.
Q_{10}.....	3.9	2.5	1.5		

Snyder explains the above as follows:

The velocity of the nerve impulse is assumed to depend on the velocity of more than one reaction in the nerve, let us say *X* and *Y*. If reaction *Y* proceeded throughout the range of temperatures (0° to 30°) we might have corresponding nerve-impulse velocities of 4.5, 11.3, 28.3 and 70 meters per second. Its temperature coefficient would be 2.5. With reaction *X* proceeding, we might have nerve-impulse velocities of 2.9, 7.2, 18, and 44, corresponding to the four temperatures. The coefficient for *X* is also 2.5. Now if reaction *X* only proceeded at 0° and 30° and reaction *Y* at 10° and 20°, the result indicated above would be attained.

It is hard to see, however, why *X* should function at 0°, then cease, and then begin again at 30°, and it seems highly improbable that any such change would occur. The decrease in Q_{10} with increasing temperature is inevitable so long as the temperature-conduction curve is a straight line (as it is between 17° to 29° in *Cassiopea*). There is no way of combining the velocities of two reactions having the same temperature-coefficient, and obtaining a straight line. It is also noteworthy that there are no critical points between 17° to 29°, such as we might expect were a radical change in the reaction at the basis of nerve conduction to take place.

[1] See the velocities of reactions as given by Snyder in Univ. Cal. Pub. Physiol., 2, p. 136, 1905.

[2] As a rule the ratio of $\frac{K_{t+10}}{K_t}$ falls off very slightly with rise of temperature. See van't Hoff, Lectures on Theoretical and Physical Chemistry, London, p. 228.

[3] Snyder, C. D., loc. cit., p. 141.

The characteristic maxima, at an optimum temperature, exhibited by curves *A* and *B* (fig. 5) require a word of comment. Maxima occur in all temperature curves of vital processes. A maximum is also exhibited by temperature curves of enzyme action. In fact the curves for enzyme action as given in Cohen's Physical Chemistry [1] are very similar to curves *A* and *B*. *There is the same falling off in calculated (if Q_{10} were a constant) velocity, the higher the temperature until a maximum is reached.*

Different enzymes exhibit maxima at different temperatures. Most of these are rather high, much higher than the maximum for nerve-conduction, which lies at about 33° C. The same ferment obtained from different sources may exhibit different maxima. For instance, the indigo enzyme obtained from *Indigofera heptostacha* has a maximum at 61° C.; from *Polygonum tinctorium*, 53° C.; from *Phajus grandiflorus*, 42°; and from *Saccharomyces sphæricus*, 44°. Cohen explains this as meaning that the optimum depends on the medium containing the ferment. If we may assume that conditions in the nerve are such as to give a very low optimum, then we may say that the propagation of the nerve impulse is not only dependent on the velocity of a chemical reaction, *but that the reaction is further accelerated by the presence of an enzyme.* Thus the characteristic difference in the form of curve from that of a simple chemical reaction.

The maximum for enzyme actions is generally interpreted as the point beyond which the enzyme begins to undergo decomposition with consequent falling off in the reaction velocity, even though the temperature is continually increased.[2] Taylor [3] regards all reactions as having an optimum temperature at which the velocity is a maximum, only in enzyme action this temperature is low. Blackman points out that in a process proceeding at a certain rate (*e.g.*, a reaction) and dependent on several factors, any one of the factors may become a limiting factor for the process in question.

Whatever the explanation may be, it is interesting to find that nerve conduction exhibits a falling off in rate with rise of temperature to a definite maximum, similar to that for enzyme action and for other life processes.

A literature list of the effect of temperature on vital processes interpreted with respect to van't Hoff's coefficient (Q_{10}) is given by Loeb, Robertson, Maxwell, and Burnett in Science, N. S., 28, p. 647, 1908. References to literature on this subject are also given in Cohen's Physical Chemistry, translated by Fischer, New York, 1903, pp. 50–67.

Cases of vital processes exhibiting chemical temperature-coefficients have been collected by Kanitz, A., in Zeit. für Elektrochemie, 13, p. 707, 1907, and Snyder, C. D., in Am. Jour. Physiol., 22, p. 309, 1908. The reader is referred to the above four papers for the literature on this subject.

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[1] Translated by Fischer, p. 56.

[2] For a discussion of the meaning of an optimum temperature, see Blackman, Annals of Botany, 19, p. 281, 1905, and Am. Nat., 42, p. 659, 1908. Also Bayliss, Nature of Enzyme Action, in Monographs on Biochemistry, p. 52, 1908.

[3] Taylor on Fermentation in Univ. of Cal. Pub. Pathology I.

III.

THE INFLUENCE OF REGENERATING TISSUE ON THE ANIMAL BODY.

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3 text figures.

THE INFLUENCE OF REGENERATING TISSUE ON THE ANIMAL BODY.

By Charles R. Stockard.

When the adult animal body begins to regenerate new tissue in order to replace a lost part, or when abnormal secondary growths arise, the condition of growth-equilibrium is disturbed and such a disturbance is followed by changes which affect the usual physiological condition of the body. The question arises whether the changes following or accompanying normal regenerative growth are in any way similar to those effects resulting from malignant or abnormal secondary growths. If one believe, with many pathologists, that cancerous formations are growths of a secondary nature induced by some derangement in the normal growth states, and are not of infectious origin, then normal secondary growths, in some stages at least, should affect the body in a manner somewhat similar to that resulting from an active tumor growth. The emaciated or cachectic conditions of the body resulting from the effects of cancerous growths are not always thought to be attributable to toxins or substances taken into the circulation from the cancer, but at times seem to be due to the excessive appropriation of nutriment and energy by the rapidly growing cancer itself. A malignant tumor continues to grow and so finally kills the body, while on the other hand the regenerating part, although rapidly growing at first, gradually decreases in growth rate and begins to differentiate and function, thus diverting the energy previously used in the growth processes.

I showed in the second of my "Studies of Tissue Growth" (Jour. Exp. Zool., vol. VI, 1909) that the medusa disk of *Cassiopea xamachana* decreased rapidly in size while regenerating new oral-arms, and that the rate of decrease was faster in those specimens regenerating the greater number of parts. In these experiments a source of error was realized, since those specimens with 6 or 8 oral-arms removed might have been deprived of more reserve food held in the oral-arms than had the individuals which lost fewer arms. I determined to control this condition by operating on medusæ so as to remove the same number of oral-arms from all and to increase the amount of new regenerating tissue in some individuals by also removing a part of the disk. The specimens were kept under identical conditions and were not fed during the time of the experiment. Thus any difference in their responses is due only to the additional amount of regeneration imposed upon the individuals with the cut disks.

Emmel (36th Ann. Rep., Inland Fisheries of Rhode Island, 1906) has contributed an observation which is most interesting in connection with these experiments. He found that when larval lobsters were regenerating new legs they molted after longer intervals than normal individuals, and grew in size at a rate sometimes 24 per cent slower than the non-regenerating specimens. Most important was his observation that

when the removal of legs was *not* followed by regeneration such specimens grew in size faster than the regenerating individuals, and in most instances actually faster than the control. These observations clearly show that the process of regeneration itself, and not the injury inflicted, is responsible for the retardation of growth in the regenerating lobsters.

The experiments here recorded were performed upon the Scyphomedusa *Cassiopea xamachana*, which may be so readily obtained at the Tortugas Islands. Healthy individuals of medium size were selected and operated upon as described below.

The first experiment consisted of two groups of 20 individuals of the same average size. Group A had 5 of the 8 oral-arms cut from each specimen (fig. 1). Group B also had 5 oral-arms cut in a similar manner from each of the 20 individuals, and in addition each medusa had a

FIG. 1.—Medusa with 5 oral-arms cut away at their bases.
FIG. 2.—Medusa with 5 oral-arms cut away and a peripheral strip cut from the disk.
FIG. 3.—Medusa disk after all of the oral-arms and the stomach have been removed.

peripheral strip cut from its body disk which included one-third of the circumference and in width extended in beyond the oral, zigzag, muscular layer shown in fig. 2. The specimens were then allowed to regenerate for 34 days, their disk diameters being measured at intervals so as to determine the differences in decrease of body size in the two groups.

TABLE I.—*Record of disk diameters and regeneration of oral-arms in Cassiopea, when 5 arms are removed (in millimeters).*

Disk diameter May 15.	Disk diameter May 27.	Length of arm-buds May 27.	Disk diameter June 4.	Length of arm-buds June 4.	Disk diameter June 12.	Length of arm-buds June 12.	Disk diameter June 18.	Length of arm-buds June 18.
95	80	3–3–4 –4–4	73	4–4–4–5–5	65	5–5–6–6–6	60	6–6–6– 6– 7
85	75	3–3–4 –4–5	67	5–5–5–6–6	61	5–6–6–6–7	57	6–7–7– 8– 9
82	68	4–4–4.5–5–5	60	4–5–6–6–7	55	5–6–6–6–7	51	6–7–7– 7– 8
90	75	4–5–5 –5–5	64	5–5–5–5–6	58	6–7–7–7–8	54	6–7–7– 8– 9
90	73	4–4–4 –5–5	64	5–5–6–6–6	58	5–6–6–6–7	55	6–7–7– 7– 8
75	63	4–4–4 –4–4	53	4–5–5–5–5	47	5–5–5–6–6	44	5–5–6– 6– 6
80	68	3–4–4 –5–5	60	5–5–5–5–6	53	5–5–5–6–6	48	5 5 5– 6– 6
75	63	4–4–5 –5–5	55	5–5–5–5–6	49	5–5–5–5–6	45	5–5–6– 6– 6
75	59	4–5–5 –5–5	52	5–5–6–6–6	47	4–5–5–6–7	44	5–6–6– 6– 7
80	72	4–4–4 –4–4	64	5–5–6–6–6	57	6–6–6–7–7	54	6–6–7– 7– 7
75	61	4–5–5 –6–6	54	5–6–6–6–6	49	5–5–6–6–6	44	4–5–5– 6– 6
72	60	5–5–5 –5–6	53	4–5–5–5–5	48	5–6–6–6–6	44	6–6–6– 7– 7
80	64	5–5–5 –6–6	56	6–6–7–7–7	51	8–8–8–8–9	47	9–9 9– 9–10
80	63	5–5–6 –6–6	57	7–7–7–7–8	53	8–8–8–8–9	49	9–9–9– 9–10
75	60	5–5–5 –6–6	50	5–5–5–5–5	44	7–7–7–7–8	41	7–8–8– 8– 8
85	71	4–4–5 –5–5	64	5–5–5–6–6	58	5–6–6–7–7	54	6 7 7– 8– 8
75	61	3–5–5 –5–6	54	5–5–5–5–6	47	5–6–6 6–6	44	5–6–6– 6– 6
90	76	5–5–5–5.5–5	66	5–6–6–6–7	61	6–6–7–8–8	57	7–8–8– 9– 9
70	58	4–5–5 –5–6	49	5–5–6–6–7	44	6–6–6–7–7	40	6–6–6– 7– 7
100	80	4–5–5 –5–5	70	5–6–6–6–6	66	6–7–7–8–9	63	8–9–9–10–10
Av. 81.5	67.5	4.7	59.3	5.5	53.5	6.3	49.7	6.9

Table 1 shows the records for group A, the first column giving the original disk diameters, the second column the diameters after 12 days, the third column the length of the individual new arm-buds regenerated during the 12 days. The fourth column gives the diameters after 20 days and the fifth column the length of the new arm-buds at this time. Columns six and seven show the same after 28 days and columns eight and nine after 34 days when the experiment ceased. A line of averages at the foot of the table shows the general result.

Table II gives the same data for Group B and a comparison of the tables is facilitated by table III of averages.

The individuals of both groups averaged 81.5 mm. in diameter at the beginning of the experiment, and after 12 days the specimens of Group A were 67.5 mm. in diameter, while those in Group B, which were regenerating the disk-tissue in addition to the 5 oral-arms, were only 64.3 mm. in average diameter. In other words they averaged 3.2 mm. smaller than the ones growing only the 5 arms. After this time, however, Group B did not decrease so rapidly, since the disk injury had almost completely regenerated: thus after 20 days the A group was only 1.7 mm. larger than B, after 28 days only 1.2 mm. larger, and after 34 days there was still only 1.3 mm. difference in average size.

TABLE II.—*Record of disk diameters and regeneration of oral-arms in Cassiopea, when 5 arms and a part of the disk are removed (in millimeters).*

Disk diameter May 15.	Disk diameter May 27.	Length of arm-buds May 27.	Disk diameter June 4.	Length of arm-buds June 4.	Disk diameter June 12.	Length of arm-buds June 12.	Disk diameter June 18.	Length of arm-buds June 18.
95	78	3–3–4–4–4	70	4–5–5–6–6	63	7–8–8–8–8	59	9–9–9–9–9
85	70	3–3–3–3–4	62	5–5–6–6–6	56	6–6–6–7–9	51	8–8–8–9–9
82	65	4–4–4–5–5	58	5–5–6–6–6	52	5–6–7–7–8	47	7–8–8–8–8
90	69	4–4–3–3–3	59	4–5–5–5–5	53	4–5–5–5–5	48	4–4–4–5–5
90	73	3–3–3–4–5	65	3–3–3–4–4	59	4–4–4–5–6	57	4–4–4–5–5
75	59	3–3–4–4–5	54	6–6–6–6–6	50	5–5–5–6–6	46	6–6–6–7–7
80	60	2–4–4–4–5	55	4–5–6–6–6	49	5–5–6–7–7	45	6–6–7–7–7
75	60	4–4–5–5–6	54	5–6–6–6–6	48	5–5–6–6–6	44	6–7–7–7–8
75	55	3–3–4–4–4	50	5–5–6–6–6	45	5–6–6–6–7	41	5–5–6–6–6
80	63	4–4–4–5–6	57	5–5–5–6–6	54	5–6–7–7–7	49	7–7–7–7–7
75	60	3–3–4–4–5	56	6–6–6–6–6	51	6–7–7–7–8	47	7–7–7–7–8
72	55	5–5–5–5–5	50	6–6–6–6–7	46	6–7–7–7–7	42	6–8–8–8–8
80	66	4–4–5–5–5	60	6–6–7–8–8	54	6–6–7–7–8	49	6–7–7–8–8
80	57	5–5–5–5–5	50	6–6–6–6–7	45	6–6–6–7–7	41	5–5–6–6–7
78	57	4–5–5–5–6	51	5–6–6–6–6	45	7–7–7–7–7	42	7–7–7–7–7
87	70	2–3–4–4–5	62	3–4–5–5–6	56	3–5–6–6–7	52	5–7–7–8–8
72	59	3–5–5–5–6	52	5–6–6–7–7	48	7–8–8–9–9	45	6–9–9–9–9
90	70	3–3–4–4–5	62	3–3–3–4–4	58	2–3–4–5–5	53	2–4–5–6–7
70	57	4–5–5–5–5	50	5–5–6–6–6	44	7–7–8–8–8	42	7–7–8–8–8
100	84	0–3–3–4–4	76	*0–4–4–5–6	71	*0–5–6–6–7	67	*0–8–8–9–9
Av. 81.5	64.3	4.1	57.6	5.4	52.3	6.25	48.4	*f* 6.9

* Not included in the average.
f Oral-arms now branching so that the linear measurement does not indicate the entire amount of growth.

TABLE III.—*Summary of Tables I and II for comparison.*

Group.	Original diameter.	After 12 days.	After 20 days.	After 28 days.	After 34 days.
Diameters in mm.:					
A	81.5	67.5	59.3	53.5	49.7
B	81.5	64.3	57.6	52.3	48.4
Length of arm-buds in mm.:					
A	81.5	4.7	5.5	6.3	6.9
B	81.5	4.1	5.4	6.25	6.9

The experiment clearly shows that, while the B group was regenerating the cut disk part in addition to the 5 oral-arms, the individuals of B were decreasing in body size, as a result of this additional regeneration, more rapidly than the specimens in A which were regenerating only the 5 oral-arms. The regenerating tissue, through an excessive capacity for the absorption of nutriment, draws upon the old body tissues and causes them to decrease in size very much as one may suppose the rapidly growing tumor to impose upon the substances of the surrounding body. It is certainly clear that in both cases the growing tissue causes the old body to become weak and emaciated while the growth itself continues in a vigorous manner.

The above experiment is of further value in regard to the influence of the degree of injury on the rate of regeneration. I have previously shown (*loc. cit.*) that the rate of oral-arm regeneration in this medusa is independent of the degree of injury, as is also the case in the brittle-star, *Ophiocoma riisei*, while *O. echinata* regenerates each arm the slower the greater the number of removed arms. These results are contrary to Zeleny's idea that the greater amount of injury will be followed by a more rapid regeneration.

The two groups of individuals A and B are each regenerating 5 oral-arms, but the group B is the more extensively injured since a portion of the disks was also cut away. If the additional injury or regeneration imposed upon the B group exercises any influence on the rate of regeneration, it should be shown by comparing the rates of growth of the arm-buds in the two groups. The lower lines of table III give this comparison. Those specimens with the disk uncut, or the least-injured ones, regenerated slightly more rapidly during the first 12 days, but after this time the rates were practically equal. These facts show that an increased injury to the medusa fails to give an increase in the subsequent regeneration rates.

A second experiment differed somewhat in manner of operation from the one just described, yet the results are in perfect accord. Twenty-eight healthy medusæ were arranged in two groups of 14 individuals each and operated upon as follows: The specimens of Group I had all of their oral-arms and the central stomach mass entirely removed, leaving only the medusa disk (fig. 3). Such a preparation lives and pulsates in a normal manner and regenerates new tissue to cover over the central stomach space. Then new oral-arms begin to bud from this tissue, until finally the medusa regains its normal organs and parts. The central space is first covered by a thin veil of tissue which tears repeatedly and reforms until it begins to thicken, and then the new arm-buds first appear. The regenerative growth is therefore very vigorous from such specimens during the early part of the experiment, and later becomes much less. Group II was operated upon in the same manner as the specimens of Group B in the above experiment, 5 oral-arms and a part of the medusa disk were cut away (fig. 2).

Tables IV and V contain the data from these specimens and table VI facilitates a ready comparison of the averages. The original diameters of each group averaged 88 mm.; after 14 days Group I was only 62 mm.

in diameter while Group II was 69 mm., or 7 mm. larger. After 22 days they were 55.3 mm. and 63 mm., and after 28 days 51 mm. and 59 mm. It will be noted, however, that Group I ceased to decrease rapidly after the first 14 days, when its rapid regeneration also ceased, and from this time on it decreased almost as slowly as Group II, since in the last 6 days of the experiment it lost only 4.3 mm., while Group II lost 4 mm.

The rate of growth for the arm-buds in table v is practically the same as from the specimens similarly injured in the previous experiment.

Groups I and II again show that when a specimen regenerates a certain amount of tissue in a given time such a specimen suffers a loss in body size which is greater than the loss from other specimens regenerating a less amount of tissue. Regenerating tissue, therefore, consumes the old body-substance and has an effect which would finally so weaken the body as to cause death should the regeneration continue for a sufficient time. A method which could eliminate the factors that cause growth to cease when an organ has attained a certain size would allow the organ to grow at the expense of the other body parts until death would follow in a manner closely similar to that by which a malignant tumor growth finally kills the body containing it. The absence of certain of the growth-inhibiting substances in the body may be responsible for the indefinite cancer growths, and experiments that in any way lead to a determination of the controlling factors in normal, primary, or secondary growths are of great importance in this regard.

TABLE IV.—*Record of disk diameters and regeneration from Cassiopea, when all of the oral-arms and the stomach are removed (in millimeters).*

Disk diameter May 20.	Disk diameter June 3.	Regeneration June 3.	Disk diameter June 11.	Regeneration June 11.	Disk diameter June 17.	Regeneration June 17.
90	65	Central space covered.	58	Small arm-buds.	53	Well-formed buds.
85	60	Covered, hole in aboral center.	55	Well-formed arm-buds.	51	Arm-buds 4 mm. long.
95	66	Covered central space.	56	No arm-buds.	51	Small arm-buds.
85	59	Covered central space.	54	No arm-buds.	50	Small arm-buds.
90	62	Covered central space.	55	No arm-buds.	49	Arm-buds 3 mm. long.
100	72	Covered central space.	65	No arm-buds.	61	No arm-buds.
80	53	Covered central space.	48	Well-formed buds.	44	Arm-buds 3 mm. long.
72	52	Covered, arms budding.	46	Arm-buds 4 mm. long.	42	Arm-buds 5 mm. long, new stomach, etc.
92	64	Covered central space.	56	No arm-buds.	52	No arm-buds.
92	69	Covered central space.	61	Well-formed buds.	56	Well-formed arm-buds.
85	55	Covered central space.	51	Small arm-buds.	47	Small arm-buds.
90	61	Covered central space.	55	No arm-buds.	51	Small buds and stomach.
93	62	Hole in aboral center.	55	Small arm-buds.	52	Small arm-buds.
92	65	Hole in aboral center.	59	No arm-buds.	56	No arm-buds.
Av. 88.6	62		55.3		51	

TABLE V.—*Record of disk diameters and regeneration of oral-arms in Cassiopea, when 5 arms and a part of the disk are removed (in millimeters).*

Disk diameter May 20.	Disk diameter June 3.	Length of arm-buds June 3.	Disk diameter June 11.	Length of arm-buds June 11.	Disk diameter June 17.	Length of arm-buds June 17.
90	70	3–4–4–5–5	65	4–4–5–5–6	59	6–6–6–7–7
85	65	4–4–4–5–5	61	4–5–5–6–6	58	6–6–6–7–8
95	78	2–3–3–4–4	70	3–4–4–5–5	65	4–5–5–6–6
80	63	3–4–4–5–5	56	4–6–6–6–7	53	6–7–7–8–8
90	69	4–4–5–5–6	63	5–6–6–6–7	60	6–7–7–8–9
100	75	3–3–5–5–5	71	3–4–5–6–6	67	5–6–6–7–8
80	61	2–3–5–5–5	56	2–5–6–6–7	52	3–5–6–8–8
72	59	3–5–5–6–6	52	5–5–6–6–6	47	5–5–5–5–5
92	73	3–3–4–5–*	66	2–3–5–6–*	61	3–3–5–6–*
92	75	3–3–3–4–5	66	4–5–5–6–6	62	4–5–5–5–6
82	65	3–3–3–5–5	61	5–5–5–6–6	59	5–6–6–7–7
90	70	*f* 0–0–2–4–4	64	*f* 0–0–5–5–5	60	*f* 0–0–5–6–6
93	70	3–3–4–4–5	64	5–5–5–6–7	60	5–6–6–7–8
92	74	4–4–5–5–6	67	5–5–6–6–6	62	6–6–6–7–8
88	69	4.1	63	5.2	59	6

* Not regenerated. *f* Not included in the average.

TABLE VI.—*Summary of Tables IV and V for comparison.*

	Group I.	Group II.
Original diameter in millimeters	88.6	88
Diameter after 14 days.....	62	69
Diameter after 22 days.....	55.3	63
Diameter after 28 days.....	51	59

IV.

CRADACTIS VARIABILIS: AN APPARENTLY NEW TORTUGAN ACTINIAN.

By CHARLES W. HARGITT,
Of Syracuse University.

1 plate.

CRADACTIS VARIABILIS: AN APPARENTLY NEW TORTUGAN ACTINIAN.

By Charles W. Hargitt.

Dr. J. F. McClendon, during his stay at the Tortugas Laboratory of the Carnegie Institution of Washington, collected a few actinians for some experiments on behavior, which he afterward preserved and later turned over to the writer for identification. This I was very glad to undertake, but found the task somewhat more difficult than had been anticipated, owing chiefly to lack of some of the older literature pertaining to the region and to the fact that the specimens were in a rather poor state of preservation. Fortunately, however, Dr. McClendon had made photographs of the specimens in the living conditions of his aquaria, a few of which appear in the accompanying plate. Details of internal morphology I shall defer till a later time, hoping that additional material may enable me to make it more accurate and adequate.

There seems to be no doubt as to the place of the species in the family Phyllactidæ; concerning the generic relations I find myself more or less uncertain. There are phases of likeness with several genera, *e.g.*, *Lebrunia* Duch. and Mich., *Oulactis* M. Edw., and *Cradactis* McMurrich. But with none of these is there such close correspondence as to induce any considerable assurance. The 6 to 8 dichotomously branched fronds and its habitat in holes and crevices in coral rocks, which Verrill emphasizes of *Lebrunia*, have much in common with the species under consideration. On the other hand, when he says, "The species examined by me has, on these fronds, at the forks, many more or less spherical bodies having the structure of acrorhagi," there is at once lack of correspondence. And a further comparison of McMurrich's description of *Lebrunia neglecta* (Journ. Morph., vol. III, p. 33) makes it almost certain that the present species can hardly belong under *Lebrunia*.

The characters of *Oulactis*, especially the longitudinal rows of verrucæ and the more or less circumscribed sphincter, are rather sharply in contrast with the very smooth walls and diffused sphincter of the present species and would seem to preclude this genus from serious consideration.

As to the genus *Cradactis* as defined by McMurrich,[1] there are also several difficulties. For example, the fronds of *Cradactis* are defined as "bunches of tentacle-like structures," the walls are dotted with verrucæ, and the sphincter is circumscribed. None of these characters is distinctive of the present species. But in view of the fact that McMurrich him-

[1] Proc. U. S. Nat. Mus., vol. XVI, p. 197.

self has defined his genus in a somewhat tentative way, and admits the lack of conformity as to the sphincter character, I am disposed to refer the species provisionally to the genus *Cradactis*, no representative of which I have seen, till such time as further facts may call for other adjustment.

So far as I have been able to ascertain, the species has not been hitherto described. I propose for it the specific name *variabilis*, as indicative of the extremely variable features exhibited. The following may be regarded as fairly diagnostic, so far as one may make up a definitive account from poorly preserved material. It may be said, however, that I have had the advantage of such verbal account as Dr. McClendon was able to give from memory.

Column low, in preserved specimens (McClendon gives it as his impression that in expansion it is about twice the diameter), smooth, with broader base than oral disk, the latter concave, with raised mouth which is oval in shape and as usual diglyphic. Tentacles somewhat finger-like, but in extension tapering to a delicate tip, about 30 to 40 in number or more in the largest specimens. The most remarkable feature is the peculiar frond-like organs situated about the margin of the oral disk and just outside the outer cycle of tentacles. There are usually 6 of these organs, more or less symmetrically arranged, though the number varies considerably, being frequently but 5 and sometimes as many as 7. Typically these are dichotomously forked once or occasionally twice, and the tips usually knobbed, as shown in some of the figures in the plate. Upon the upper surface of these organs there is usually a whitish disk or pad, sometimes several. These are shown in section to be glandular organs, and possibly secrete a substance, probably of an adhesive nature, such as might aid the creature in capturing prey. They are also provided with several large nematocysts, and the production and discharge of these may be an important, perhaps the most important, function they serve.

The specimens seem to have the capacity to move about more or less freely, and it seemed to me these organs might aid in such movement; but McClendon is of the opinion that the tentacles are used for this purpose. It will be observed that several of the figures of the plate show specimens inverted, that is, adhering to the bottom of the aquarium by the oral end, the pedal disk being uppermost at the time the photograph was made. It may be stated that the photograph was taken of a series of specimens just as they happened to be disposed in the dish, and shows in a very striking way the remarkably variable character of the creatures.

Color pale olivaceous-green to brownish; tentacles somewhat lighter; foliose organs darker, even brownish, with flake-white pads, sometimes with a darker center, and with whitish lines extending along the upper side, especially in the region of the pads and towards the tips.

The body seems to be rather highly contractile, but there is only a weak or diffused sphincter, and none of the specimens showed any considerable contraction of the oral disk, or the retraction of the tentacles. The mesenteries are numerous, apparently hexamerously arranged.

The reproductive season seems to be in the spring and early summer. Early development takes place within the enteron of the parent, ciliated embryos being found rather commonly in the capacious cavities of the fronds, as well as in the gastric spaces among the mesenteries. Free swimming planulæ finally emerge and after a period of freedom settle down by attaching themselves after the usual method of planulæ. The first organ to appear is as usual the mouth, followed by the tentacles. The foliar organs would seem to appear rather late in the history of development, none having yet appeared in embryos reared in the aquaria.

The habitat seems to be chiefly in holes, crevices, or similar secluded places in the coral reefs or about the shoals where suitable conditions are afforded for their protection. In these places they expand and extend the tentacles and fronds beyond the opening, and in this attitude fish for appropriate prey. McClendon has expressed the opinion that the fronds may serve as lures for attracting prey. This may be extremely doubtful. I am disposed to regard them rather as organs which may aid in capturing and holding prey. The bifurcated tips armed with glandular pads bristling with nematocysts would seem to point to some such function as that herein suggested.

Photograph from live specimens in various aspects of posture, expansion, etc. C, D, and E show specimens in inverted position; the last seen from the side. At * are shown pads of nematocysts on tips of fronds.

V.

ON ADAPTATIONS IN STRUCTURE AND HABITS OF SOME MARINE ANIMALS OF TORTUGAS, FLORIDA.

By J. F. McCLENDON,
Instructor in Histology, Cornell University Medical College, New York City.

2 plates, 1 text figure.

ON ADAPTATIONS IN STRUCTURE AND HABITS OF SOME MARINE ANIMALS OF TORTUGAS, FLORIDA.

By J. F. McClendon.

In June, 1908, at the laboratory of the Carnegie Institution of Washington, at Tortugas, Florida, I began the study of the habits of some reef animals with a view to some comparative studies of behavior. The results were written up in the Zoological Laboratory of the University of Missouri.

It was found that many of these animals were thigmotatic and remained in glass tubes rather than in the open. They also learned to find the tubes when removed from them. Such was the case with five species of the Alpheidæ, one of the Pontoniidæ, *Typton tortugæ* Rathbun, and *Gonodactylus œrstedii*. All the anemones were thigmotactic on their bases. These same animals were heliotropic. The Crustaceans were negatively heliotropic and the anemones kept their bases from the light, while *Cradactis variabilis* Hargitt hid all but the tips of the fronds and tentacles from the light. In removing its base from the light, *Stoichactis helianthus*, which lives on coral heads, makes snail-like movements similar to *Metridium*,[1] while *Cradactis*, which lives in holes in decayed coral heads, crawls on its tentacles.

ON ADAPTATIONS OF SYNALPHEUS BROOKSI AND TYPTON TORTUGÆ.

In lagoons between the reefs is found the loggerhead sponge, *Hircinia acuta*, which grows to 3 feet or more in diameter, but is of no commercial value. The passages in this sponge are thickly populated by *Synalpheus brooksi* Coutière. These Alpheids are thigmotactic and negatively heliotropic and seldom come outside the sponge, which they do only at night and then rarely leave its surface. The only other animals seen in the interior of the sponge were a small species of Amphipod and a Pontoniid. The Alpheids were several hundred times as numerous as the Amphipods or Pontoniids. Near or at the surface crabs and worms were sometimes found.

Both Alpheid and the Pontoniid, *Typton tortugæ*, have the fourth and fifth pairs of thoracic appendages pincer-like (plate 1, figs. 1 and 3). In the Alpheid the fourth and in the Pontoniid the fifth pair are asymmetrically hypertrophied. In the Alpheid the asymmetry is very great, and the large chela can be snapped with such vigor as to produce a loud, clicking sound. When this claw is removed its mate grows to replace

[1] McClendon, 1906, On the Locomotion of a Sea Anemone, Biol. Bull. 10.

it and the asymmetry is reversed, as first shown by Przibram. It is not known on which side the large claw develops first. I interpret Herrick's records as demonstrating that the large claw develops first on the left side in *Synalpheus minus* (*Alpheus saulcyi*).[1] It was found in my specimens about as frequently on the right as left side in both large and small individuals. Of 50 taken at random, 22 had the large claw on the left and 28 on the right. In another species Przibram found 40 individuals with the large claw on the left and 47 on the right.

The Pontoniid *Typton tortugæ*, as was stated above, has the pincer-like appendages of the fifth thoracic segment well developed. One of these claws is much larger than the other, but the asymmetry is not as great as in the Alpheids. Both of these claws are snapped with a sharp, clicking sound. When the large claw is removed the small one grows to take its place, as in the Alpheids.

The two animals do not perhaps resemble one another as much in general coloration as in general form, though the color varies so much in both animals that these differences are not at first noticeable. The color darkens with age. The Alpheid varies from the color shown in plate 1, fig. 1, to a light brown. Specimens with a claw like fig. 2 may be a dull cream or light brown in general color. The nerve cord and some other organs may be surrounded by red pigment cells. Yellowish, brownish, or reddish glands in thorax or abdomen may show through.

The Pontoniid *Typton tortugæ* varies from the color shown in fig. 3 to an almost colorless condition, or to a light red or a pale bluish. The large claw of the pale specimens is often paler than the small claw in fig. 3. After the large claw has been removed the small one grows to take its place, but for some time retains more or less its general form and color. Often yellow, brown, or green glands show through in the thorax and abdomen.

As these animals pass their entire adult existence in the dark or dim light, it is improbable that their color is of much significance in their struggle for existence; hence it would not be fixed by natural selection. The fact that their eyes are not degenerate might indicate that they sometimes come near the mouths of the passages in the sponge. Perhaps they are forced out when the sponge becomes overcrowded, but I doubt that many of the larger ones would find another sponge before they were eaten by fish. Neither form was found in any other habitat, though Herrick records the Alpheid from reef rocks as well as loggerhead sponges in the Bahamas.

The Alpheid has large eggs, few in number, attached to the swimmerets of the female. The metamorphosis is abbreviated, and in some cases omitted. The young remain attached for a time to the mother, but perhaps always leave the sponge and live a short pelagic life before finding another sponge. The female Pontoniid deposits numerous small eggs on the swimmerets. These hatch into small larvæ which lead a comparatively long pelagic life before acquiring the form and habits of the adult.

[1] Brooks and Herrick: The Embryology and Metamorphosis of the Macroura. Mem. Nat'l Acad. Sci., 5, 1891.

I studied the habits of these animals as well as I could in dim light in the cavities of pieces cut from the sponge. They appeared to behave normally, whereas in glass tubes in brighter light they remained motionless. Both animals explore the cavities of the sponge with cautious movements unless disturbed, in which case they snap their claws. The Alpheid advances, using its large claw as an antenna and protector. Its antennæ can be extended about as far forward as the large claw. When meeting an Alpheid or a Pontoniid it may try to squeeze past or it may snap its claw. When placed in glass dishes Alpheids cut one another to pieces, but this is seldom if ever done in the narrow passages of the sponge.

The Pontoniid *Typton tortugæ* advances, using both claws as antennæ, the antennæ being very much shorter than the smaller claw. It spreads the claws apart and waves them about, thus exploring the cavity in front of it. On meeting another it behaves as the Alpheid, except that it may snap either or both claws. Both animals try to squeeze through small openings. The chelæ of *Typton* sometimes show what appear to be claw marks. As their claws are more slender, hence more easily grasped and less powerful than those of the Alpheids, it is to be expected that they would show claw marks first in case both species snapped with equal frequency.

Both animals appeared to eat from the walls of the cavities in the sponge, but I did not determine whether they ate the sponge itself or a sediment deposited on it. I did not determine whether they ate one another in the sponge, but they were so numerous that it seems strange that they received sufficient oxygen. The Alpheids are sometimes infested with a parasitic isopod, *Bopyrus*, in the gill cavity.

I do not intend to discuss here the origin of the form or habits of these animals, but it seems to me that we have here a convergence both in form and habit. It is probable that similarity in form and habits made both animals better suited to living in the same habitat, *i.e.*, the sponge, and that accidentally finding the sponge they remained there. However, this does not explain why the young at the end of pelagic life always (or at least usually) select the loggerhead sponge. There are numerous Alpheids living in holes in the reef rocks, and certainly they are more closely related in form and general habits to *Synalpheus brooksi* than is *Typton*.

This *Synalpheus* and the *Typton* select the sponge not because it has holes in it in which they can hide, but on account of some more specific quality, such as taste (smell), color, or outward form. Or when some individuals of these species have established themselves in a sponge the others may be attracted to it by a social instinct (which may not be disproved by the fact that they destroy one another when placed under unnatural conditions). The isolation of these animals in the loggerhead sponge is an example of what Gulick calls habitudinal segregation and may have been a factor in the evolution of the species.

Since the Alpheids occur in far greater numbers than *Typton* we might suppose the former to be much better adapted to living in the sponge than the latter. However, although *Typton* produces more

eggs, it has a much longer pelagic life than the Alpheid and is much more likely to be eaten or swept out to sea by the tides, where it can not find a sponge when the proper time comes.

The smallest loggerhead sponges I found would not live in a large aquarium with running sea-water more than 2 days before the water began to get foul within the passages in the sponge and the Alpheids and *Typton* began to die. Field observations were very limited. These and other difficulties restricted the investigation to its present limits.

ON ADAPTATIONS OF THE REEF ANEMONE, CRADACTIS VARIABILIS.

Cradactis variabilis Hargitt is an anemone about an inch or two in length when expanded, living in holes in old coral heads or reef rocks. Besides the tentacles, which are few in number and arranged as in *Sagartia*, long outgrowths called fronds extend from the region bearing the tentacles (plate 1, figs. 4, 5; plate 2, figs. 8, 9, 10). The animals may be a moss-green or brown in general color, but the tentacles are always paler and often colorless and transparent at their tips. The fronds may or may not be branched, and may end simply or in pale knobs, as in plate 1, fig. 4, or in curious "eyes," as in fig. 5.

These anemones are usually found in cavities in old coral heads that communicate with the exterior by a number of passages about half an inch or more in diameter. The anemones are attached near enough to these passages to extend the tips of the fronds to the exterior (plate 2, fig. 7). This extension is caused by heliotropism of the fronds. One mistakes them at first for sea-weed, although they do not resemble any particular kind of sea-weed that I have found growing on the reefs. The tentacles are extended about as far as and sometimes a little farther than the fronds, but the fronds tend to conceal the tentacles. At night the fronds are contracted and the tentacles remain extended; therefore it is probable that the fronds are not necessary as breathing organs.

If a bit of crab meat is held near the passage through which the *Cradactis* is extended no response is obtained. But if one of the fronds is touched with the meat the tentacles are extended toward it, while the frond touched may contract slightly. In order to observe the food-taking more minutely, some of the anemones were taken from the rock and allowed to attach themselves to the bottom of an opaque dish filled with sea-water. When a bit of crab meat is placed on the end of a tentacle it adheres and the tentacle and one or more adjacent ones are bent down and the food placed on the mouth and pressed there. Immediately many or all of the tentacles are pressed on the food, hiding it from view until it is swallowed. The fronds may contract more or less during the process. *Cradactis* sometimes swallows filter paper placed firmly on the mid-region of a tentacle or on the disk, but not when placed on the end of a tentacle. This may be a question of degree or extent of stimulation. It disgorges the paper within 10 minutes. It rejects bits of shell, etc., placed on the disk or tentacles.

India ink placed in the water near the anemone showed ciliary currents running towards the tips of the tentacles and fronds, and on

the disk running towards the mouth. A secretion sticks the particles together. These currents are useful on both fronds and tentacles in the rejection of particles, and on the tentacles in the placing of food in the mouth, the food being carried to the tip of the tentacle before it is placed in the mouth.

When disturbed by light falling on the base, it sometimes moves with snail-like motion (like *Metridium*) a short distance, but the tentacles catch hold of the substratum on all sides. The tentacles and column sometimes perform writhing movements. More often the animal bends over to one side and catches hold of the substratum with the tentacles, with or without previously elongating the column, the fronds contracting slowly all the while. It then loosens the base, walks on its tentacles to a new place (plate 2, figs. 11, 12), bends over and attaches the base, and lets go its hold with the tentacles. This method of locomotion is much more rapid than that of *Metridium*, but could not be used if the *Cradactis* did not live in holes, as it might otherwise be washed away by the currents that constantly sweep over the reefs.

The resemblance of the fronds to sea-weed leads one to suppose that they act as lures or in hiding the *Cradactis* from its prey (anemones being unpalatable are usually not in need of protection). The fact that the fronds are heliotropic and contracted completely at night is in harmony with this view. I did not cut them off to see whether the anemone would live and reproduce as well without them. The cavities containing the *Cradactis* are inhabited by other animals, especially a small black crab, and one might suppose that the fronds protected the tentacles of the anemone from the legs of the crabs that crawled over it. The crabs are active at night in the least light in which they can be seen (their black color making them hard to see in the holes in the rock). In case they are normally active at night the fronds would serve as a protection from the crabs only half of the time. The anemones sometimes grasp the crabs and hold them until they wrench themselves loose, which they invariably do in a short time. Perhaps the anemone gets part of its food as particles dropped from the crabs' mouths.

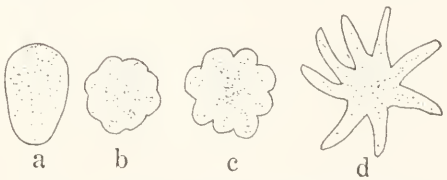


FIG. 1.—*Cradactis variabilis*. *a*, Planula just escaped from cœlentric cavity of mother, oral (pigmented) side uppermost. *b*, The same, second day, seen from oral side; pigment arranged radially. *c*, The same, third day. *d*, The same, fourth day; tentacles elongating and septa becoming distinct. The mouth should be elongated in the plane of symmetry.

Cradactis develops to the planula stage in the cœlenteron of the mother. On being released, the planula swims around for a few hours (text fig. 1, *a*) and attaches itself (*b*) by the smaller end. It gradually develops a mouth and tentacles (*b-d*). When first liberated, the planula has 8 mesenteries, and 8 tentacles develop soon after. Individuals were seen with 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and more tentacles. From this one might conclude that the tentacles (and mesenteries) appear in pairs, but they were often observed to appear in sets of four, symmetrical in relation to the oral plane. The first pair of fronds appear

in the 20-tentacle stage as outgrowths of the body-wall just beneath the tentacles and with their axis perpendicular to the oral plane. The second pair of fronds appear in the 28-tentacle stage or later.

SUMMARY.

(1) Convergence in structure and habitat is the cause of commensalism between an Alpheid and a Pontoniid living in the loggerhead sponge.

(2) Abbreviation of its pelagic life accounts for the numerical supersedence of the Alpheid.

(3) The weed-like outgrowths or fronds of a reef anemone, *Cradactis*, probably hide it from its prey.

(4) *Cradactis* is kept just within the mouths of cavities in reef rocks by the combined action of negative heliotropism of its base and positive heliotropism of the fronds. The fronds are entirely contracted in the absence of light.

(5) The fronds possess the sense of taste but do not carry food to the mouth.

(6) *Cradactis* moves from place to place by walking on its tentacles, a phenomenon sometimes seen in *Hydra*.

DESCRIPTION OF PLATES.

(Figures 4 and 5 were redrawn by Mr. Kline, fig. 6, from life, by K. Morita, otherwise the drawings and photographs are the author's.)

Plate 1.

1. *Synalpheus brooksi* Coutière.
2. Chela of same species to show different coloration.
3. *Typton tortugæ* Rathbun commensal with the above.
4. *Cradactis variabilis* Hargitt.
5. The same, showing another variety in color and shape of fronds.
6. *Cradactis variabilis* Hargitt, × 2.

Plate 2.

7. A portion of an old coral head showing the fronds (f) of *Cradactis* protruding from the cavities.

8–10. *Cradactis variabilis*, showing varieties in shape of fronds.

11, 12. *Cradactis variabilis*, walking on its tentacles, with detached base toward the observer.

1. Synalphens brooksi Coutière.
2. Chela of same species to show different colorations.
3. The Pontoniid commensal with the above.
4. Cradactis variabilis Hargitt.
5. Cradactis variabilis Hargitt.
6. The same, showing a third variation.

Fig. 7. A portion of an old coral head showing fronds (f) of Cradactis protruding from the cavities.
Figs. 8-10. Cradactis variabilis showing varieties in shape of fronds.
Figs. 11-12. Cradactis variabilis walking on its tentacles with detached base toward the observer.

VI.

BEHAVIOR OF THE LOGGERHEAD TURTLE IN DEPOSITING ITS EGGS.

By S. O. MAST,
Of Goucher College, Baltimore, Maryland.

BEHAVIOR OF THE LOGGERHEAD TURTLE IN DEPOSITING ITS EGGS.

By S. O. Mast.

The loggerhead turtle ordinarily comes out of the sea in the early part of the night and lays its eggs in the sand on the beach above high-tide water-mark. On July 11, 1910, I was fortunate enough to be present when a turtle came out to lay on Loggerhead Key, Florida, while it was still daylight (7^h5^m p. m.). This individual was about 3 feet long and 2 feet wide. She came out at right angles to the water line and proceeded directly up the beach 50 to 60 feet, where she immediately began to make her nest. There was no indication whatever of a process of selection of the place for the nest, as some have asserted in describing the breeding habits of this turtle. When the turtle reached the nesting-place she stopped and began at once to move the posterior end from side to side, throwing the sand out sidewise and forward alternately, with the two hind flippers, to a distance of 5 to 6 feet. Thus a crescent-shaped trench was made, wide and deep in the middle and narrow and shallow at either end. This trench was over 4 feet long and nearly 10 inches deep in the middle. The lateral movement of the turtle during this process of digging was largely due to the action of the muscles connected with the front flippers, which remained stationary as the body turned on them.

After the trench was finished the turtle took a position so that the right hind leg was very nearly over the middle of the bottom of it. This flipper was then thrust vertically down into the sand (the flat surface being nearly parallel with the long axis of the body) and the end turned in under the sand so as to form a cup much like one formed by a human hand partly closed. The posterior end of the animal was then raised by the action of the left leg and pushed to the right. During this process the right flipper, containing a fair-sized handful of sand, was of course raised and as the posterior end of the body moved to the right the flipper gradually rotated so as to face backward; it was then thrust out to the side and inverted so as to empty the sand in a heap, just in front of which the foot was placed on the ground in the customary position. The left flipper was now directly over the hole made by the right one and used in removing sand just as described, except that it took the sand from the right side of the hole while the right flipper took it from the left side. Before the body was pushed back to the left by the right leg it made a sudden movement forward and threw out a considerable bit of sand, making a hole just in front of the place where the sand taken from the nest had been deposited. This sand was pushed into the hole in front of it when the turtle moved back to the right again and thrown out just before it moved to the left the following time. Thus the two

hind flippers alternated in scooping the sand from the nest until a cylindrical hole was dug nearly as deep as their length. The alternation from right to left was perfectly regular. Neither flipper ever took sand from the hole twice in succession.

After the hole was completed the turtle assumed a position so that the cloaca was very nearly over the center of it and began to lay at once. The cloaca projected fully 2 inches during the process of laying. The head was well extended and flat on the ground. The anterior end of the body was raised so that the ventral surface made an angle of about 20° with the horizontal. There was no arrangement of the eggs in the nest as fishermen sometimes assert. The eggs were dropped from the cloaca into the hole in a series of one or two at a time at intervals of from 4 to 8 seconds. Two were deposited together about every fourth time. During the discharge of the eggs the hind flippers were slightly raised, and in one case (witnessed at night earlier in the summer) there was heavy breathing which was very distinctly heard. In the turtle under present consideration, however, not the slightest sound was detected.

Fishermen often say that after a turtle begins to lay it will continue even if it is turned on its back. I did not try this, but I did strike the turtle a sound blow on the head with a heavy stick, using both hands, at two different times while she was laying. She withdrew her head, moved slightly to one side and stopped laying, but only for a few moments. Noise and gentle contact did not appear to affect her in the least.

It is commonly thought that the loggerhead turtle ordinarily lays three times during each summer, about 150 eggs the first time, fewer the second time, and about 80 the third. I did not ascertain precisely how many eggs were laid by the turtle under observation. It is almost impossible to remove the eggs from the nest without killing the embryos, and, since there have been many trustworthy observations on the number of eggs laid, it seemed unnecessary to destroy the young for the sake of learning the exact number in this particular nest.

Immediately after the eggs were discharged the turtle began to cover them. In doing this she moved the posterior end back and forth much as she did in digging the hole. As this end proceeded to the right the left flipper was thrust backward into the sand and then suddenly moved inward so as to throw and scrape the sand on to the eggs immediately back of it. As it proceeded to the left the right flipper acted in the same way, but of course it threw the sand in the opposite direction. Thus the turtle filled the trench as well as the hole, stopping frequently to pack the sand, especially that over the eggs. This she did by placing the posterior pointed end of the body on the sand and elevating the anterior end so as to bring her full weight to bear upon it. After the trench was nearly filled she turned about over the region several times and threw and scattered the sand in every direction with all four flippers so as to conceal the place, especially that where the eggs were laid. This completed she returned to the sea and entered only a few feet from the spot where she came out. On the way down the beach I stood on her back and she carried me (165 lbs.) apparently with but little effort. From what has been said regarding the concealment of the nest of the

loggerhead turtle it must not be assumed that the nesting-place is difficult to find—quite the contrary, for the turtle-tracks leading to and from it are very conspicuous and can not be mistaken. The place where the eggs are buried is, however, not easy to find. In case of the nest described I had considerable difficulty in finding the eggs, even after carefully watching the whole process of laying and noting the position of the turtle in detail; and this is quite in harmony with the experience related to me by several fishermen who collect the eggs for food.

The eggs in this nest were 11 inches below the surface, and they occupied a space 6.5 inches in depth and 9 inches in diameter, making the bottom of the nest 17.5 inches from the surface. The turtle under observation was out of water 42 minutes, approximately 3 of which were required to come from the water to the nest, 4 to make the trench, 8 to dig the hole, 12 to lay the eggs, and 15 to fill the hole and trench, smooth off the place and get back to the sea. The rate of locomotion on land is about half a mile an hour.

VII.

CERTAIN REACTIONS TO COLOR IN THE YOUNG LOGGERHEAD TURTLE.

By DAVENPORT HOOKER,
Of Yale University.

2 plates, 1 text figure.

CERTAIN REACTIONS TO COLOR IN THE YOUNG LOGGERHEAD TURTLE.

By Davenport Hooker.

During the summer of 1907 I made some observations of a very preliminary and incomplete nature on the general habits and early instincts of the young loggerhead turtle. A series of experiments was performed with a view to determining the cause of the newly hatched turtles reaching the water and the results obtained led me, at the time, to believe that photophilism and negative geotropism were the elements at work. In the summer of 1908 I repeated my experiments in a much more thorough manner and extended the research greatly. From the newer and more complete data obtained, I am convinced that I overlooked certain very important factors in the environment and that reactions to color and geotropism are the determining factors. I therefore take this occasion to correct what was said last year about phototrophism as such an all-important factor, though it certainly does play some part, as my more detailed account will show.

An idea of the locale of the experiments may be obtained from fig. 1. Pit *A* is on the northwest side of the island, some 30 feet from the water's edge, and is overhung on the more easterly side by bay-cedar bushes. Pit *B*, on the center of the point, is 10 feet in diameter and about 4 feet deep, with sloping sides and the floor entirely out of sight of any bushes or the ocean. The floor and walls are of sand. The shore of the island is of coarse coral sand and free of vegetation. The deeply shaded area shows where the bay-cedar bushes extend along the central ridge of the island.

The material used was afforded by three nests totaling about 300 turtles. These nests were surrounded by a high wooden pen which prevented the escape of the young turtles and also prevented them from seeing the ocean, the bushes, and the directive rays of the sun. The young turtles were used as soon as possible after they had reached the surface of the ground. In no case was the interval over 10 hours, while in some instances they were helped out of the sand by gently raking it with the fingers. There was absolutely no noticeable difference in the response of the turtles kept 10 hours from those used immediately.

EXPERIMENTS.

When I was working on the young turtles in 1907, all my experiments were performed in Pit *A*, and in the evening. At that time the sun was in the west and the turtles all went west without exception. As

they were distinctly photophilous when swimming in the aquarium, I very carelessly neglected the overhanging bushes on the eastern side of the pit and seized upon their apparent tendency to go toward the light as the cause of their movements. On thinking the results over during the following winter, however, it seemed improbable that the sun's rays should be the governing factor in the young turtles reaching the sea, for what would happen if they were hatched on the eastern side of the island when the sun was in the west? It was with the purpose of finding the explanation to this question that the work was again undertaken in 1908, in a much more thorough manner. I found that if several young turtles were tried in Pit *A* when the sun was in the east, they still went west. Obviously, then, they were not responding to the sun in this case. Was it possible that the green bushes were the determining factor? If they went away from the bushes on the western side of the island irrespective of whether the sun was in the east or in the west, they should do so on the eastern side. Consequently, four turtles were given four trials each, under the following conditions: Sun to the west, bushes on the west within 8 feet of the place chosen for the experiment (fig. 1, E), which was a small, level place on the beach with a semicircular barrier of sand just high enough to shut out sight of the ocean about 15 feet to the east. The turtles were tried, one at a time, by being set down on the land side (west) of the barrier, headed successively to west, north, south, and east. In every case, the turtles crossed the barrier directly towards the sea.

The results show conclusively that the position of the sun had nothing to do with the response, but the question arose as to whether or not the odor of the ocean or the sound of its waves might not have been influencing factors. To obviate this I selected a spot as nearly in the center of the island as possible, about an eighth of a mile from either shore, where there was a natural semicircle of bushes inclosing a white, sandy space with a definite opening into a lane, also paved with white sand, on one side. At varying times of the day turtles were, one by one, placed in this inclosure, headed away from the opening, and in all cases (the operator, of course, being entirely out of sight) they came through the opening into the space beyond with little or no hesitation.

These experiments were further supplemented by placing green glass on one side of Pit *B* and a number of turtles tried there, one by one. In all cases they definitely and decidedly turned away from the glass and climbed out of the pit on the opposite side, while in the control experiments, where the turtles were placed in the pit when there was nothing but its sandy walls for an environment, they crawled out at random and went in no definite direction.

Under ordinary circumstances the young turtles are negatively geotropic, but if the possible descents have been exhausted, they become positively geotropic. As the green bushes apparently drove them down hill to the water, a series of experiments was performed to see if their negative geotropism and any stimuli (if there were such) received through the senses of smell and hearing from the ocean could be overcome by their avoiding reaction to the green bushes. The series of experiments

may be divided into three parts, *A*, *B*, and *C*, with the following conditions for each:

Series A.—East side of island (fig. 1, c), bushes cut from the interior of the island were stuck into the sand in an upright position to form a semicircle with a diameter of about 5 feet. The water line was tangent to the mid-point of the arc's circumference, about 2 inches from it. A sand barrier, 6 to 8 inches high, was erected just inside the bushes to prevent immediate sight of the ocean. There was a fairly heavy surf and the sun was in the west.

Series B.—The same conditions prevailed as for series *A*, except that the sun was in the east.

Series C.—West side of island (fig. 1, d). The conditions of series *A* were duplicated. The sun was in the east.

Twenty-four turtles were used in these three series, each one given four trials, headed successively north, west, south, and east. Without exception or even hesitation, all turned away from the bushes and went

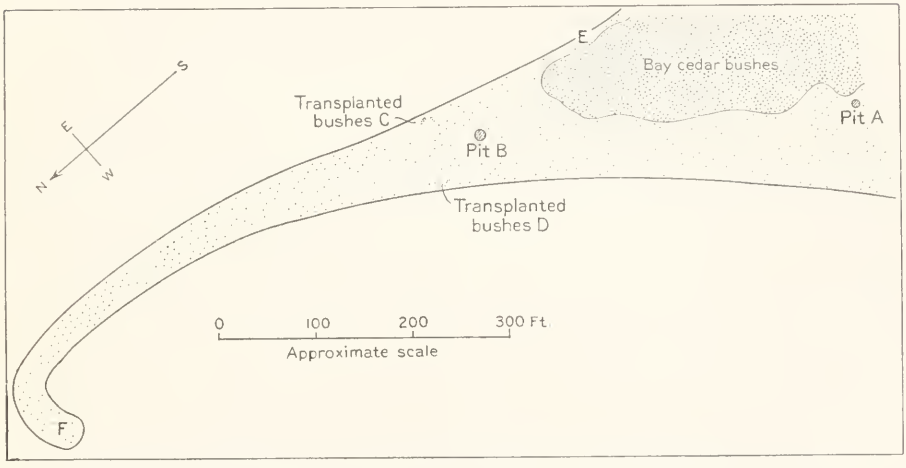


Fig. 1.—Northern end of Loggerhead Key, Tortugas.

up an incline of 30° away from the water. Their negative response was continued long after the ocean was in sight, but all the turtles, after going from 8 to 10 feet directly up the slope, made a wide detour and started for the ocean, giving the bushes a wide berth. The position of the operator was an entirely negligible quantity so long as he remained perfectly quiet.

The next series of experiments was performed in location fig. 1, c. The bushes were removed, and the barrier moved back so that it was washed by the waves. A piece of colored glass, 2 feet 6 inches long by 18 inches high, was forced into the sand in an opening in the middle of the barrier in such a manner that the sandy floor inside was on a level with the water outside and separated from it only by the glass. The barrier around the ends of the glass and up the slope of the shore for a few feet kept the water out of the semicircular inclosure. The young

turtles could see the ocean and the surf through the glass, but it was, of course, a colored ocean when thus viewed. The other conditions were the same as for series *A* in the experiment with the transplanted bushes. Three glasses were used, differing in color, green, orange-red, and blue. They were tried in the order named. Five turtles were tried in each experiment, each one giving four trials, facing in turn north, west, east, and south. Although they could see a green ocean perfectly well when the green glass was used, their avoiding reaction to green took them up the bank and away from the water in every instance. When the orange-red glass was used, there was a similar avoiding response, but when the blue glass was used, all the individuals in all their trials were strongly attracted to it. I had no red glass, so a red sheet of cardboard was used instead. To this they all responded with the avoiding reaction.

A series of experiments was made in Pit *B* by setting up blue glass or blue cardboard on one side and another colored glass or cardboard (red, green, or orange) opposite, and placing the turtles between the two. In every case they went to the blue. Controls with blue alone showed the same result, while a negative response was always obtained when blue was absent and one of the other colors present.

A series of experiments was tried at night with 9 turtles on the northern end of the key just beyond Pit *B*. It was a bright, moonlight night and the bushes and other objects on the point, as well as the sea on the east and west, were plainly visible. The turtles were placed one at a time at about 6 feet north of Pit *B*. The operator retired as far as possible, still keeping the subject in sight, and the turtle was allowed to go at will. All the turtles turned north at first until they reached a point on the central ridge of the sandspit from which the ocean was visible in either direction. Then the course was changed toward the west in 5 cases and toward the east in 4. The route to the water was not a direct one, but was either northwest or northeast, the point of entering the water varying from 75 feet (in one case) to 250 feet from the starting-point. The position of the operator was again an entirely negligible quantity. This series of experiments is of particular interest, as it seems to be a combination of or a transition reaction between the response to colors and the purely photophilous response which they do give.

All of these experiments recorded above were performed in the open air, under surroundings as nearly natural as possible. In addition to these, certain experiments were performed under a rigidly restricted environment to test their phototropic response.

Dr. L. J. Cole,[1] of the University of Wisconsin, found that "animals having image-forming eyes and which are positively phototropic, respond to a large area of light of comparatively low intensity rather than to an illuminated point of high intensity." The apparatus which he used was highly complex and entirely beyond the facilities at my disposal. Nevertheless, I made a much cruder and simpler form on essentially the same plan and tested several young loggerheads. While they are

[1] Cole, Leon Jacob, An Experimental Study of the Image-forming Powers of Various Types of Eyes. Proc. Am. Acad. Arts and Sci., vol. XLII, No. 16, 1907.

very slow in responding under such artificial conditions, their reactions bear out the expectations for a positively phototropic animal. When the light of a lantern is thrown at night into one of the pens containing from 50 to 75 turtles, all orient and crawl toward the lantern and remain in the area lighted by it. When in a large aquarium, all the turtles coming within the area lighted by a lantern remain there and orient toward the light.

In 1907, I thought that the young turtles followed the sun in swimming out to sea, but my more recent work has changed my opinion. About 50 young turtles were let loose on the point (fig. 1, F). All went into the sea and swam 100 yards in their initial direction, then paused, turned abruptly toward deep water and swam to it. By placing a simple type of spectroscope in a water-glass and covering my head and the bucket with a dark cloth, I was able to get very satisfactory "reflection spectra," if they may be so called, of the sea-water at different depths. This is a spectrum taken below the surface of the water and at a slight angle below the horizontal. The results obtained were interesting in that the deeper the water the greater the blue content of the light reflected and vice versa. As the turtle swims with its head below the surface of the water, it is quite possible that the greater blueness of the deeper water attracts it.

As a final test of the rôle played by any odor of the ocean water, a glass bowl 18 inches in diameter was sunk to its rim in Pit *B* and filled with sea-water. Turtles were placed one by one on its rim, some of them having been immersed in it before being set down. While some went into the water, many more turned away. They demonstrated no definite tendency to go to it. The turtles behaved in exactly the same manner that they did when there was nothing in the pit. This would seem to indicate that there is no odor in sea-water that attracts the young turtles.

DISCUSSION.

The general results obtained may be summed up as follows:

(1) The newly-hatched loggerhead turtle moves away from transparent and opaque red, orange, and green, and from green bay-cedar bushes, and moves toward transparent or opaque blue.

(2) After entering the water, the animal swims out to sea, apparently attracted by the darker blue of the deeper water. The position of the sun is an entirely negligible quantity.

(3) When on the beach in a large sand-pit with level floor, from which pit sight of the bushes and the ocean is excluded, but into which the sun's rays shine directly, there is exhibited no definite tendency to move in any definite direction.

(4) Young loggerhead turtles are negatively geotropic, but when all possible downward inclines have been exhausted they become positively geotropic.

(5) Under a restricted environment the young turtle is photophilous and responds to a large surface of light of low intensity rather than to an illuminated point of high intensity.

(6) The reactions of the young turtle are not modified by sound or odor of the sea, nor by a tank of sea-water in which there is not sufficient quantity to give color.

It might be well to state here that the colors used, both glass and paints, were far from monochromatic, so that their exact values are not known, but at the same time it should be remembered that most or all of the colors in nature are polychromatic. An excess of any particular color seems to give the effect. That we are dealing here with a case of true chromotropism is, perhaps, somewhat doubtful. Indeed, true chromotropism is so rarely met with and so many other factors, as intensity, the "color-blindness" of lower eyes, etc., contribute to it or to startlingly similar reactions, that I have studiously avoided the term. I must also confess myself unable to satisfactorily explain the cause of the response obtained at night on the beach. While the moon shone brightly, the differences in colors were much reduced to the human eye, so much so that, as far as color was concerned, the bushes and the water were practically the same. However, the sea was shiny and the bushes were not. This reaction seems to me to be, as I have before stated, a transitional stage between the color reaction and the photophilous response, but this helps us little in explanation of its ultimate factors.

In regard to the responses in the daytime, we may speak with a little more certainty, although here, too, the intensity of the colors is a bothering element. Practically all the investigators who have worked on color responses have found that blue or the blue end of the spectrum acts as white light and the red end of the spectrum as shadows would in phototropic responses. In the case of these loggerhead turtles we find blue chosen in preference to the directive rays of the sun. Whether this indicates a true chromotropism or not we can not be entirely sure.

The application of the bare facts obtained to their biological significance in the life of the animal is, I think, clear. Bushes, green in color, grow on all islands of any size, above the high-water mark. The shores of all islands slope down to the water. All turtles' nests are laid just above the high-tide mark, at or near the bush line. The young turtle, hatching out and crawling up to the surface of the sand, avoids the bushes, goes down the shore, and easily finds the water. Once in, the darker blue of the deeper water attracts it out of the dangerous fish-infested shoals of the reefs.

1.

2.

3.

Figs. 1 and 2. Young Loggerheads taking a breath of air. Note the flippers moved to preserve balance.

Fig. 3. Young Loggerhead Swimming.

1.

2.

3.

Fig. 1. Young Loggerhead Swimming.
Fig. 2. Young Loggerhead Floating in the Water (side view).
Fig. 3. Young Loggerhead Floating (from above).

VIII.

A CONTRIBUTION TO THE ANATOMY AND DEVELOPMENT OF THE POSTERIOR LYMPH HEARTS OF THE TURTLES.

By FRANK A. STROMSTEN,
Assistant Professor of Animal Biology, State University of Iowa.

2 plates, 5 text figures.

A CONTRIBUTION TO THE ANATOMY AND DEVELOPMENT OF THE POSTERIOR LYMPH HEARTS OF THE TURTLES.

By Frank A. Stromsten.

This paper is submitted as a preliminary report of the results obtained by the writer in the study of the lymphatic system of the turtles. While the anatomy of the lymph hearts has been so carefully and accurately described in the sea turtle by Müller (1839) and in the large land turtles by Fritsch (1874), their development, as far as is known to the writer, has never been studied.

Recently, the most general interest has been attached to the development of the lymphatics in general. But the results obtained have been by no means in accord. On the contrary, the most divergent conceptions have arisen through the study of the mammalian type alone. It has therefore seemed to the writer desirable to refer to a more generalized type of vertebrate, in the hope that some key to the divergent results noted might be found. Accordingly, studies have been carried on for several years upon reptilian forms, particularly the *Chelonia*. In the present paper it is proposed to consider only the posterior lymph hearts of the turtle, reserving the fuller treatment of the entire lymphatic system for a later paper.

Several years ago,[1] while investigating the fate of the subcardinal and postcardinal veins in the box turtles, the writer was interested in noting the formation of a pair of rather large, muscular-walled sacs in the subcutaneous mesenchymal tissue of the postiliac regions. These spaces, which proved to be the posterior lymph hearts, seemed to be intimately related in their development with certain changes in the first two or three coccygeal branches of the postcardinal veins. It was not possible at that time to complete the study of these interesting organs. The work was again taken up, however, in the fall of 1908, and has been carried on, as class-room duties permitted, ever since.

METHODS AND MATERIALS.

The work for the most part has been done in the laboratories of animal biology of the State University of Iowa. I wish here to express my thanks to Prof. Gilbert L. Houser, director, and to other members of the Laboratory staff for suggestions and aid in collecting material for the anatomical studies. The embryos of the loggerhead turtle (*Thalassochelys caretta*), used for the study of the development, were collected by the writer during the summer of 1907 at the Marine Laboratory of the Carnegie Institution of Washington at the Dry Tortugas, Florida.

[1] American Journal of Anatomy, vol. 4, 1905.

It has not been possible to dissect the adult lymphatic system of the loggerhead turtle in time for this preliminary paper. The anatomical description will, therefore, be based on the excellent work of Müller on the green turtle, supplemented by Fritsch's work on the land turtle, and by such dissections as the writer has made on the mud turtles found in the vicinity of Iowa City. The specimens dissected were mostly *Chrysemys marginata*, although specimens of *Chelydra serpentina* and of *Amyda mutica* were also studied. The turtles were killed with chloral hydrate or with chloroform. The blood-vessels were injected with red and with blue gelatin, while the lymphatics were injected with a thin yellow gelatin mass. The melting-point of the gelatin was lowered by adding potassium iodid to the melted solution. The posterior lymph hearts were studied by exposing them after the animal had been chloroformed. As they continue to beat for some time after they have been exposed, they are very easily found and studied. Their connections with adjacent veins can readily be demonstrated by injecting India ink into their cavity.

For the preparation of the embryonic material a large number of fixing agents were used. Corrosive sublimate solutions were, as a rule, not satisfactory, although Zenker's fluid gave fairly good results. Gilson's fluid proved very unsatisfactory, especially with large embryos. The mesenchymal tissues were poorly fixed and the material did not keep well. By far the best results for embryos of all stages were obtained by using mixtures of chromic and acetic acids with pure formaldehyde. These mixtures seem to penetrate the thick shell more readily and to fix the internal organs more thoroughly and with less distortion than any other combination tried. The following mixture for embryos up to about 30 days of development was found satisfactory:

Chrom-aceto-Formaldehyde:[1] Chromic acid, 1 per cent aq. solution, 64 parts; glacial acetic acid, 4 parts; formaldehyde, pure 40 per cent, 32 parts.

The strength of the chromic-acid solution may be varied somewhat to suit conditions. The solution should be allowed to stand until it assumes a greenish or "chrom-alum" color before using. Embryos are fixed from 15 minutes to 6 hours according to size, and washed thoroughly in running water. Although Lee refuses to recognize chromic-formalin fixing agents in the later editions of "The Microtomist's Vade-mecum," the writer is compelled to confess that, after using the mixture mentioned above for a number of years, none of the standard fixing agents have given such uniformly good results as has this one. If the material is not left in the fixing solution entirely too long, and is thoroughly washed out, there is no difficulty whatever in obtaining beautiful stains with any of the standard methods.

Various methods of staining were employed. The most satisfactory results were obtained with Delafield's hæmatoxylin or iron hæmatoxylin followed by slightly acidulated orange G. or eosin-aurantia-orange G.

[1] Lee "The Microtomist's Vade-mecum." 4th ed., page 55. Norris, Proceedings of the Iowa Academy of Science, vol. 8, page 78 (1900).

Mallory's connective-tissue stain is also very good. Many of the older embryos had the blood-vessels injected with India ink.

Embryos up to 26 days were imbedded in paraffin and cut 10μ to 20μ thick. Older embryos were cut by the celloidin method, about 25μ in thickness and mounted on lantern slides. One series of embryos was cut into 50μ sections, and another series of older stages was cut into sections 100μ in thickness. Wax and blotting paper, as well as graphic reconstructions, were made whenever necessary.

ANATOMY OF THE POSTERIOR LYMPH HEARTS OF TURTLES.

Lymph hearts were discovered in turtles by Joh. Müller in 1839. He describes them in the green turtle (*Chelonia mydas*) as a pair of rounded organs, more or less flattened dorsoventrally, lying just caudad of the upper end of the ilium, one on each side of the body. They rest upon the origin of the semitendinosus muscle of each side and are bordered laterally by the biceps and caudally by the semimembranosus muscles. Lymph channels from the posterior extremities and caudal portion of the body open into their posterior ends. Their pulsations are irregular, occurring at the rate of 3 or 4 times per minute, and are not necessarily synchronous for the two hearts. Their inner wall is smooth and their cavity is free and not broken by trabeculæ or septa, as it is in the land turtle. The openings of the afferent and of the efferent ducts are guarded by valves. They open, by means of a short duct, into the vein that runs forward along their mesial border to become the posterior renal advehent vein of each side.

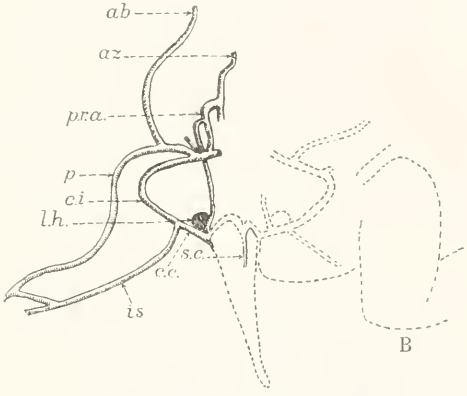


FIG. 1.—Diagram showing relations of posterior lymph hearts to veins of pelvic regions of *Chrysemys marginata*. *ab*, abdominal vein; *az*, azygos; *p.r.a.*, posterior renal advehent; *p*, peroneal; *c.i.*, circumflex iliac; *l.h.*, lymph heart; *is*, ischiadic; *c.c.*, common coccygeal; *s.c.*, superior coccygeal.

In the large land turtle (*Testudo elephantina*), described by Fritsch (1874), the lymph hearts are ovoid in form with a longitudinal diameter of 38 mm. and a width of 20 mm. in the anterior half, and 15 mm. in the posterior half. Their walls contain striated muscle fibers and are about 1 to 2 mm. in thickness. Their central cavity, especially in the posterior region, is broken up by septa and trabeculæ. They open directly into the ischiadic vein, and in the right heart a second opening communicates with a small vein on the inner border of the heart.

In the mud turtle, *Chrysemys marginata*, studied by the writer, the posterior lymph hearts are found just beneath the carapace, immediately caudad of the upper end of the iliac bones. They are somewhat elliptical in form, but are slightly flattened dorsoventrally.

In a specimen about 15 cm. in length, they measure about 6 mm. in length and 4 mm. in breadth in their widest part. They occupy the angle formed by the junction of the common coccygeal with a branch of the posterior renal advehent vein of each side (fig. 1). They do not lie exactly parallel with the long axis of the body, but diverge laterally at their anterior ends. The central cavity is broken up, more or less, into communicating alveoli, especially in the posterior half. The walls are made up of loose connective tissue and striated muscle fibers. The hearts open into a vein connecting the common coccygeal with the posterior renal advehent vein, or sometimes directly into the common coccygeal vein. In the specimens studied, the rate of pulsation, shortly after the animal had been chloroformed, was about four times per minute.

A more detailed description of the anatomy of the lymph hearts, together with their histological structure, is reserved for a later paper on the complete anatomy of the lymphatic system of the loggerhead turtle.

DEVELOPMENT OF THE POSTERIOR LYMPH HEARTS.

The first definite indications of the posterior lymph hearts appear toward the close of the third week of development. But as early as the end of the second week a noticeable vacuolation of the mesenchyme takes place in the region immediately caudad of the anlagen of the posterior limbs, giving it a spongy appearance. About the beginning of the fourteenth day this spongy area becomes invaded by small capillaries from the first three dorsolateral branches of the caudal portion of the postcardinal veins. These minute capillaries can be readily distinguished from the mesenchymal spaces among which they meander, by their endothelial walls. The increased activity of the mesenchyme cells due to the rapid growth of the posterior limbs causes a greater accumulation of lymph in the intercellular spaces, causing them to enlarge and become confluent with each other and, to some extent, with the invading capillaries. The exact relation of the tissue spaces to the capillaries is very difficult to determine. However, after studying a large number of thin sections taken through this region, under the oil-immersion lens, I feel sure that these spaces do open directly into the capillaries.

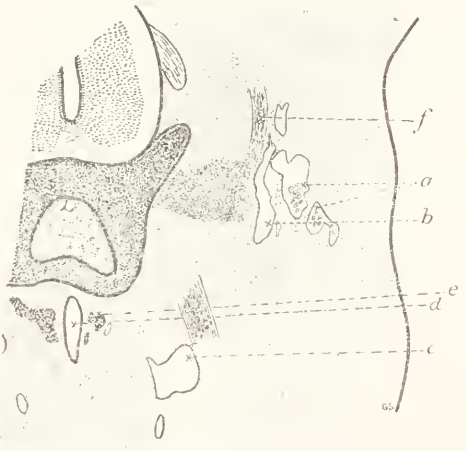


FIG. 2.—Transverse section through lymph heart region of Loggerhead Turtle embryo No. 230. (20 days, 14 mm.) ×100. *a*, veno-lymphatic channels; *b*, vein; *c*, postcardinal vein; *d*, aorta; *e*, sympathetic system; *f*, nerve.

A careful study of such a series of sections can not fail to convince the observer that these spaces play a far greater part in the development of the lymphatics and even of the lymph hearts than is now usually supposed. By the sixteenth day another change takes place which greatly accelerates the development of the lymph hearts. This is brought about by the longitudinal anastomosis of the segmental branches of the postcardinal veins caudad of the opening of the iliac veins and outside of the muscle plates. The veins thus formed continue cranially as the most important branches of the posterior renal advehent veins, and caudally as the lateral coccygeal veins of the tail. The formation of these veins together with the tapping of the mesenchymal spaces allows a much more complete and rapid drainage of the lymph from posterior limbs and tail than was hitherto possible. The capillaries of the region subsequently to be occupied by the lymph hearts increase enormously in size and fuse with each other to form several large, anastomosing spaces, the veno-lymphatic channels. These channels extend parallel with the pair of newly-formed veins mentioned above, and communicate with them at two or three points.

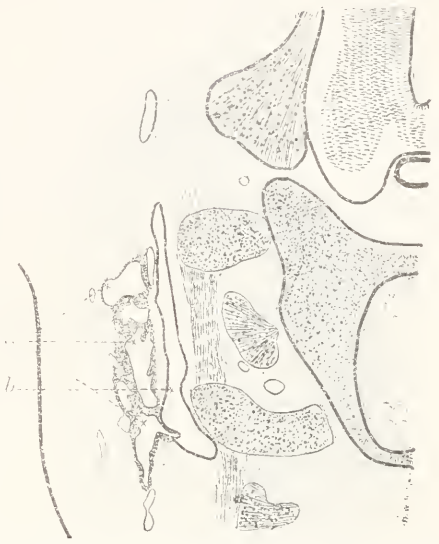


FIG. 3.—Transverse section through lymph heart region of Loggerhead Turtle embryo No. 232. (22 days, 15 mm.) ×100. *a*, veno-lymphatic channels; *b*, vein.

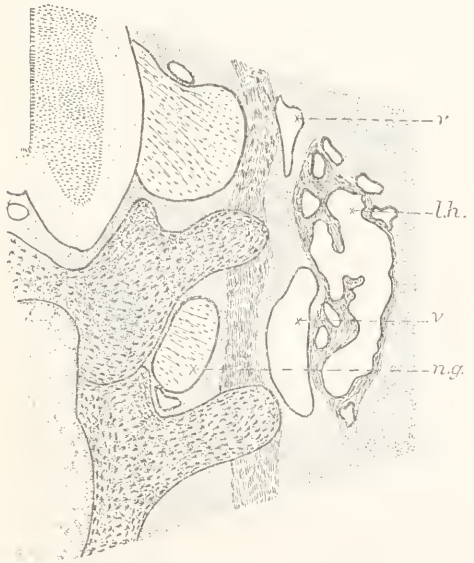


FIG. 4.—Transverse section through left lymph heart of Loggerhead Turtle embryo No. 236. (25 days, 17 mm.) *v*, vein; *l.h.*, lymph heart; *n.g.*, nerve ganglion.

In an embryo 20 days old (fig. 2) the segmental connections between the postcardinals and the recently formed common coccygeal veins are still retained. The anlagen of the lymph hearts are scarcely distinguishable from the veins in this stage. At 21 days of development, the mesenchyme cells begin to condense around these veno-lymphatic channels to form definite walls of considerable thickness and density. Muscle cells also wander in from the adjacent muscle plates and become involved in the formation of these

walls. These channels continue to increase in size and complexity through the twenty-second and twenty-third days. Figure 3 represents a section taken through the region of the posterior lymph hearts of an embryo 22 days old. The lower lymph space opens into the vein by a valve-like opening. From the 24-day stage on, the partitions between the several veno-lymphatic channels begin to break away in the anterior part of the heart region, forming fewer and larger spaces. Figure 4, from an embryo of 25 days, shows a section taken through the middle portion of the lymph heart.

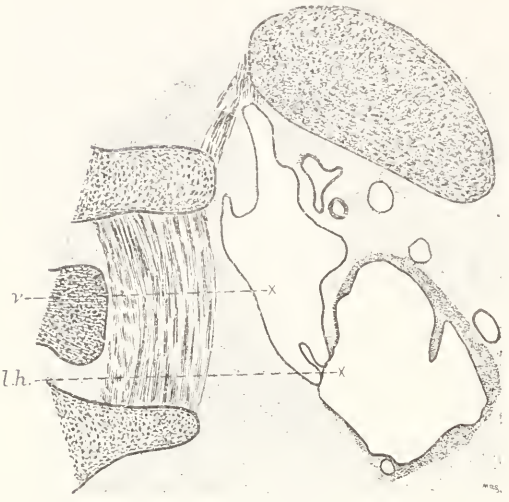


FIG. 5.—Transverse section through left lymph heart of Loggerhead Turtle embryo No. 228. (32 days, 40 mm.) *v*, vein; *l.h.*, lymph heart.

The formation of the lymph hearts is well advanced in embryos of 32 days and older. Figure 5 shows the relation of the lymph heart to the vein and lymph spaces in a 32-day embryo. The process of the atrophy of the walls separating the several lymph heart anlagen continues from before backward until at the time of hatching there seems to be a single cavity present for each heart. In this, as well as in several other points, the development of the lymph hearts of the sea turtles differs from that of fresh-water turtles. In the mud turtles the spongy character of the hearts is retained, to some extent, in the adult animal.

GENERAL CONCLUSIONS.

Recent investigations of the ontogeny of the lymphatic system of mammals have given rise to three general theories. Two of these agree in deriving the lymphatic system entirely from the venous system, while the third assigns to it, in part at least, an independent origin. For purposes of comparison with the results obtained by the writer from a study of the development of the posterior lymph hearts of the loggerhead turtle, a very brief résumé of these several theories will be given at this point.

(1) *The theory of the confluence of independent spaces to form continuous channels.*—This view, which is the one formerly held by most embryologists, is now held in a modified form by Huntington (1908–10). According to this writer:

> The peripheral general lymphatic channels appear to be developed by the confluence of spaces independent of the venous system, although closely associated with the same. * * * They begin as minute extra-venous vacuoles, closely applied to the surface of the veins which they accompany. They enlarge as the lumen of the vein diminishes. They become confluent with each other, but never contain red blood-cells, nor do they communicate with the blood channels.

Huntington believes, however, that the jugular lymph sacs are "direct derivatives of the early, redundant, embryonal, venous pathways of the precardinal and postcardinal regions, adjacent to and involving their point of confluence to form the duct of Cuvier." [1]

(2) *The theory of the continuous centrifugal outgrowth of four or more venous buds.*—By improving the methods of Ranvier, Miss Sabin (1902, 1904, 1908) was able to inject the lymphatics of very young pig embryos. By injecting India ink into the subcutaneous tissue of the neck and inguinal regions of pig embryos of 18 mm. and over, she obtained a beautiful series of pictures showing the peripheral growth of the superficial lymphatics. Her investigations convince her that the lymphatic ducts are budded off from the venous system. She says: "The lymphatic ducts bud off from the veins in four places: two in the neck, at the junction of the jugular and subclavian veins; and two in the posterior part of the body, from the vein which enters the Wolffian body and which is formed by the union of the femoral and sciatic veins." From these four points of origin the lymphatics grow first along the veins toward the skin to form the superficial lymphatic, and secondly along the aorta and its branches to form the thoracic duct and the deeper lymphatic system.[2]

(3) *The theory of the splitting off of the lymphatic from the venous system by a process of fenestration.*—McClure (1908) describes the formation of the lymph sacs and lymph channels in the cat as follows: The anterior lymph sacs are formed "by the separation of parallel venous channels from the embryonic veins by a process of fenestration, and the subsequent conversion of these channels into definite lymph sacs by a process of growth and fusion." The thoracic ducts are formed by "a series of independent outgrowths which first appear along the common jugular and innominate, and then along the azygos veins exactly in the line subsequently followed by these ducts; these outgrowths are subsequently split off from the veins by a process of fenestration, in a series of isolated, more or less spindle-shaped spaces, which later become confluent with each other and with a process of the jugular lymph sac of the corresponding side to form a continuous system disconnected with the veins, except through the mediation of the jugular lymph sac." [3]

The explanation for these divergent results seems to be found partly in the methods of investigation and partly in the material investigated. After examining a few series of cat and pig embryos, it seems to the writer that the earlier, critical stages of lymphatic development are too masked or passed through too rapidly to warrant us in basing our conclusions upon the study of the mammalian type alone. The phylogenetic history of the lymphatic system and its relation to the blood vascular system needs more careful study. Huntington (1910) has well said: "Any theory of lymphatic development must agree in its postulates with the phylogenetic facts, as far as they have been definitely established." Our knowledge, however, of the lymphatic system of the lower

[1] The Anatomical Record, vol. 2, page 25.
[2] American Journal of Anatomy, vol. 3, p. 183.
[3] Anatomischer Anzeiger, Band 32, S. 533 and 542.

vertebrates is very incomplete. Favaro (1906) and Allen (1907, 1908) have done much to clarify our knowledge concerning the relations between the lymphatic and the blood vascular system in fishes. Much more work of this kind is greatly needed to fill up the many gaps still existing in our knowledge of the lymphatics of lower vertebrates. The literature of the ontogeny of the lymphatic system of lower vertebrates is still more scanty. Outside of the mammals, it is limited almost entirely to the paper by Sala (1900) on the lymphatics of the chick, and by Knower (1908) and others on the frog.

The injection of the lymphatics with India ink gives very beautiful demonstrations of the spread of the lymphatic ducts from their point of origin. But this method alone does not prove conclusively that they are the continuous peripheral outgrowths of certain venous buds; since if the ducts are formed by the confluence of independent mesenchymal spaces it is more than likely that this process proceeds peripherally from the several primary foci; hence the injections would merely show the several stages of progress. A few cat embryos were successfully injected by this method, but have not been studied in sections. The writer hopes to prepare a series of turtle embryos by this method during the present summer for comparison with the reconstructions prepared from serial sections.

The choice of a suitable fixing agent is a very important factor to be taken into account. In turtle embryos, where the integument is so thick and the tissue composing it is so very compact, this becomes very apparent. In embryos fixed with corrosive sublimate mixtures, it was impossible to study the intercellular mesenchymal spaces with any degree of satisfaction.

The results of the investigation of the development of the posterior lymph hearts of the loggerhead turtle by the means of serial sections seem to indicate that the mesenchymal spaces play a much greater part in the development of the lymphatics in general than is usually supposed. It appears as though these spaces had captured, as it were, certain capillaries and had converted them into the anlagen of the lymph hearts. This process takes place very rapidly, even in the turtle, so that it might easily be overlooked. As soon as the mesenchyme begins to condense around the veno-lymphatic spaces the process is so masked that, if it still continues, it is no longer recognizable.

SUMMARY.

(1) The development of the posterior lymph hearts of turtles is initiated by the vacuolation of the postiliac mesenchymal tissue during the middle and latter part of the second week of development.

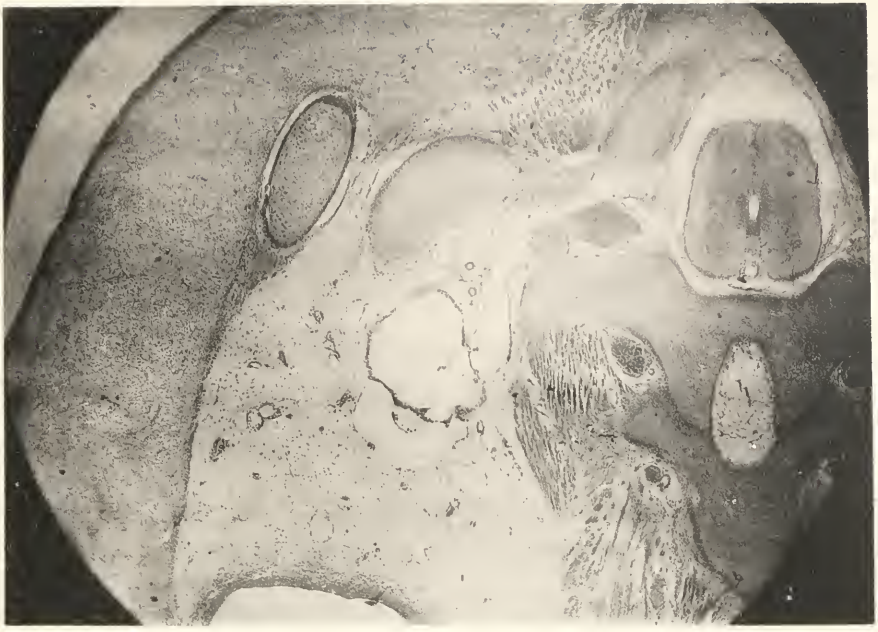
(2) The spongy tissue thus formed is then invaded by capillaries from the first two or three dorsolateral branches of the caudal portion of the postcardinal veins.

(3) Near the close of the third week, parallel veno-lymphatic channels are formed in this spongy area by the confluence of the mesenchymal spaces with each other and with the invading capillaries. These channels

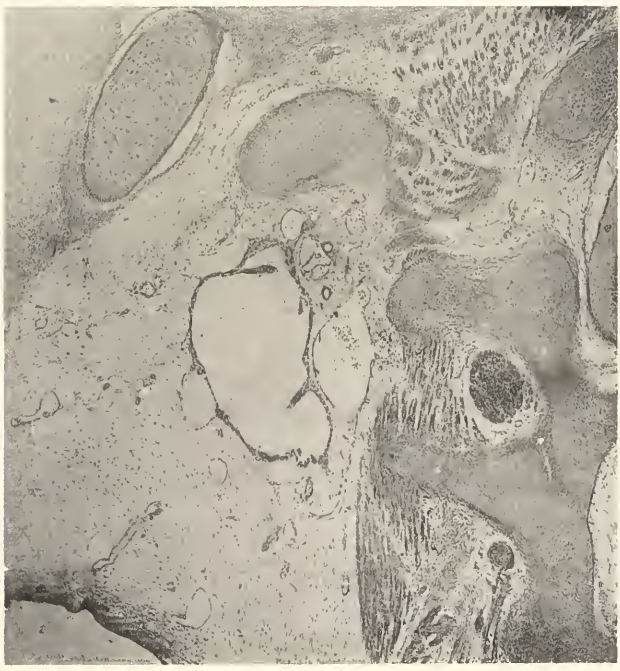
anastomose freely with each other and communicate, by means of two or three openings, with the vein running along their mesial borders.

(4) At the beginning of the fourth week, the mesenchymal tissue condenses about the veno-lymphatic channels to form definite walls. These cardiac walls are then invaded by muscle cells from the adjacent muscle plates.

(5) The final stage in the development of the posterior lymph hearts is reached by the dilation and confluence of these veno-lymphatic sinuses, from before backward, forming a pair of sac-like organs, each with a single central cavity.



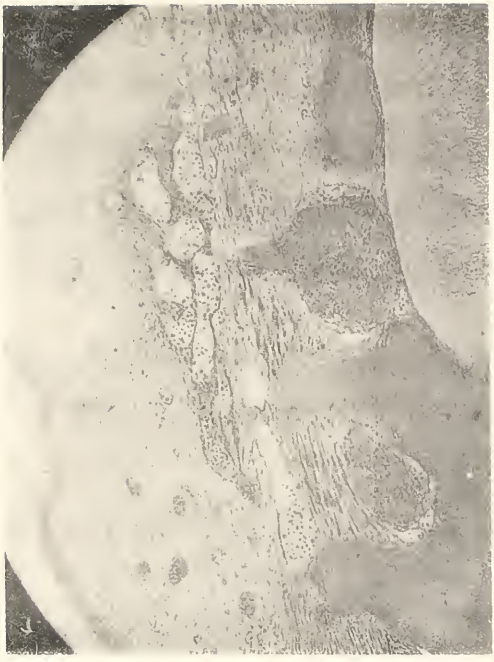
A.



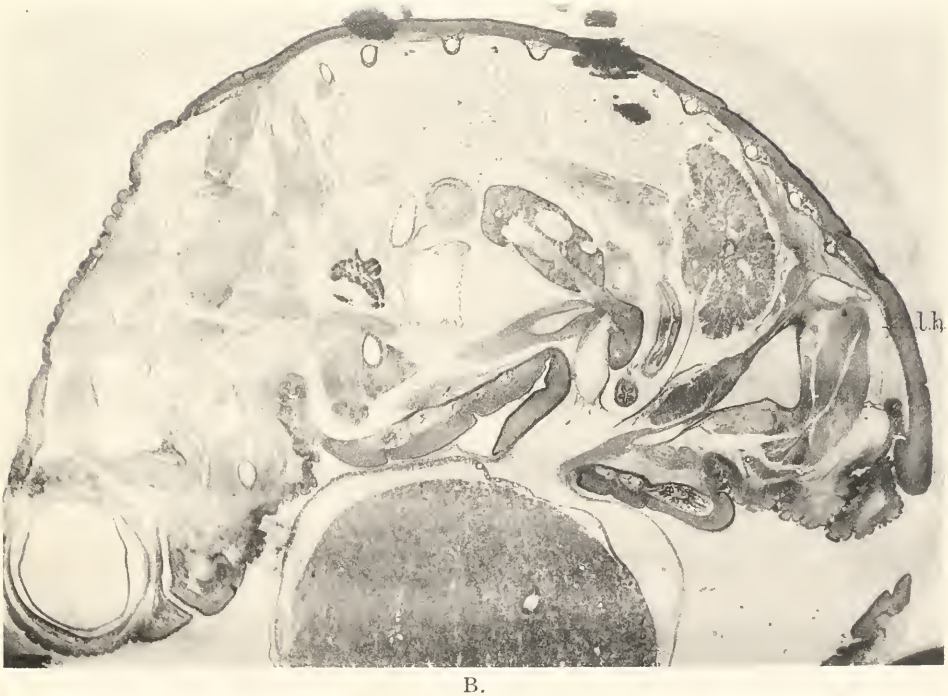
B.

A, Transverse section through Lymph Heart of Loggerhead Turtle Embryo No. 228 (32 days), showing relation of Lymph Heart to Vein and Lymph Channels. (Photo-micrograph.)

B, Photo-micrograph of transverse section taken through Anterior Portion of Posterior Lymph Heart of Loggerhead Turtle Embryo No. 228 (32 days).



A.



B.

A, Transverse section through Lymph Heart of Loggerhead Turtle Embryo, No. 236 (25 days). (Photo-micrograph.)

B, Photo-micrograph of a Sagittal Section taken through Loggerhead Turtle Embryo, No. 80 (38 days), showing position of Posterior Lymph Hearts in Body of Embryo.

IX.

POLYCITOR (EUDISTOMA) MAYERI NOV. SP., FROM THE TORTUGAS.

By Dr. R. HARTMEYER, Ph.D.
Of the Berlin Zoological Museum.

1 plate.

POLYCITOR (EUDISTOMA) MAYERI NOV. SP., FROM THE TORTUGAS.

By R. Hartmeyer.

Among the ascidians I collected in 1907 at the Tortugas, and concerning which I published a preliminary note in 1908 (Year Book Carnegie Institution of Washington, No. 6, p. 110), there is a new and very interesting species of *Polycitor*, a genus not hitherto known, from the West Indies. I name this new species *Polycitor* (*Eudistoma*) *mayeri*, in honor of Dr. Alfred Goldsborough Mayer, director of the Department of Marine Biology of the Carnegie Institution of Washington.

Polycitor mayeri is the largest and most beautiful ascidian of the Tortugas. It was collected in the deeper water of the Southwest Channel near Loggerhead Key, on sandy or muddy bottoms, where it seems to be fairly abundant and where it lives together with many other species of ascidians, most of which are wanting on the reefs or in the shallow water inside of the reefs.

The colony varies in shape. It usually consists of a more or less club-shaped mass (fig. 1), supported by a short peduncle. There is no distinct constriction at the (upper) distal end of the peduncle. Sometimes the colony forms an irregularly rounded or ellipsoidal mass, laterally little compressed, the area of attachment being broad. In one case, the lower end is prolonged on one side to form a thin expansion by which the colony probably was attached. In some cases two or three masses of different sizes are united and attached by a common base. In its variable shape *Polycitor mayeri* resembles *Macroclinum pomum* (Sars). The length of the largest club-shaped colony is 70 mm., the greatest breadth 35 mm., and the greatest thickness 27 mm. A smaller one is 52 mm. in length, 27 mm. in greatest breadth, and 21 mm. in greatest thickness. On the other hand, one of the rounded, broadly attached colonies measures 30 mm. in length, 44 mm. in greatest breadth, and 23 mm. in greatest thickness. There are some much smaller club-shaped colonies in the collection, not over 20 mm. in length.

The lower part of the colony, especially the peduncle, is incrusted with sand grains, fragments of shells, small stones, serpula-tubes, etc. The upper part of the colony is free from any foreign bodies, the surface being perfectly smooth and glistening.

The color is pale yellowish with a reddish or violet tint. Specimens preserved in formalin have become milky-white; alcoholic specimens are more transparent, so that the ascidiozooids can be more or less distinctly seen as yellow spots.

The test is firm and cartilaginous; small sand grains are included in the interior of the test, but not in great quantity.

Each colony contains several parasitic amphipods.

In some parts of the colony the ascidiozooids are arranged in regular, circular systems of 5 to 6. In other parts no distinct systems are visible. The ascidiozooids are large, reaching 5 to 6 mm. in length. They are rather closely placed and lie at right angles to the surface.

The preserved specimens are of course more or less contracted. In one colony, I found an abdomen apparently very little if at all contracted, measuring 9 mm. (fig. 3). In the living condition ascidiozooids may therefore reach 12 mm. or more in length.

The abdomen is about three or four times as long as the thorax. From the posterior end of the abdomen or from the ventral side near the posterior end one or, more rarely, two ectodermal processes (vascular appendages) are usually given off (fig. 5), which vary in length and sometimes are wholly wanting. The siphons are well developed, provided with strong musculature, and both 6-lobed. They are of equal length or the atrial siphon is a little longer than the branchial siphon. The branchial sac was very much contracted. There are only three rows of long, narrow stigmata.

The alimentary canal forms a very long and narrow loop and is not twisted (fig. 2). The œsophagus and intestine are both very long and straight. The stomach is smooth-walled and situated near the posterior end of the abdomen in the long axis of the body. In somewhat contracted specimens it is heart-shaped or nearly globular in outline (figs. 2, 4); in others which were not contracted it is of ellipsoidal or fusiform shape (fig. 3). Leaving the stomach the intestine bends dorsally for a short length and then extends straight anteriorly. The rectum lies along the œsophagus on the left side, and is partly covered by it (fig. 2). In some ascidiozooids the course of the alimentary canal was somewhat different. The stomach was situated on the dorsal side; the intestine leaving the stomach turned first ventrally and then backward dorsally, passing the stomach on the left side (fig. 4). Intestine and rectum contained several fecal pellets.

The testes were developed in only some of the ascidiozooids. They are pyriform and very numerous, 30 to 40 in number, situated near the posterior part of the stomach (fig. 4) or covering the right side of the stomach (fig. 6). None of the specimens examined contained eggs or embryos.

Polycitor mayeri is one of the largest and most beautiful species of the genus *Polycitor*. On account of the number of the rows of stigmata and the smooth-walled stomach it belongs to the subgenus *Eudistoma* Caull. It seems to come nearer to *Polycitor* (*Eudistoma*) *mucosum* Drasche, from the Mediterranean, than to any other species of the genus, but differs from it in shape and color of the colony as well as in the length and some other anatomical details of the ascidiozooids, so that it is doubtless a distinct and well-marked species. It is not only the first-known *Polycitor* from the West Indies, but from the whole eastern coast of the American continent.

From the western Atlantic only five species of this genus have been described, and all of these are mentioned by Van Name from the Ber-

mudas, but all these species have four rows of stigmata in the branchial sac and are in many other respects quite different from our species.

DESCRIPTION OF PLATE.

a, atrial aperture;
b, branchial aperture;
e, endostyle;
ep, ectodermal processes;
fp, fecal pellets;
i, intestine;
oe, œsophagus;
r, rectum;
s, stomach;
t, testes;
vd, vas deferens.

1. Largest colony of the collection; natural size.
2. *Ascidiozooid*, from the right side; magnified (ca. 16×).
3. Part of abdomen of another ascidiozooid, very little contracted; magnified (ca. 10×).
4. Part of abdomen of an ascidiozooid, with stomach situated dorsally; magnified.
5. Posterior end of the abdomen of an ascidiozooid with two ectodermal processes; magnified.
6. Posterior end of abdomen of an ascidiozooid with fully developed testes; magnified.

Polycitor (Eudistoma) mayeri nov. sp.

X.

REACTION TO LIGHT AND OTHER POINTS IN THE BEHAVIOR OF THE STARFISH.

By R. P. COWLES,
Associate in Biology in Johns Hopkins University.

6 text figures.

REACTION TO LIGHT AND OTHER POINTS IN THE BEHAVIOR OF THE STARFISH.

By R. P. Cowles.

The experiments described below deal largely with the reactions of starfishes to light. Two species are used, namely: *Astropecten duplicatus* and *Echinaster crassispina*, both of which occur near the Tortugas group off Key West, Florida.

Echinaster crassispina is rather common in the latter part of June (1900) on the sandy and flat rocky bottom at the south side of Boca Grande, one of the keys lying between Key West and Tortugas. In this region the bottom is fairly level and much of it is of white sand; in some places, however, flat stretches of rock extend horizontally above the level of the sand and these are often covered with algæ, corallines, gorgonia, etc. The echinasters usually live in about 2 feet of water at low tide and they seem to be distributed irrespective of the character of the bottom, about as many being found on the white sand as on the rocky places. They are decidedly migratory and use their tube feet in moving about over the sand in much the same manner as human beings use their legs. This characteristic was observed by Romanes and Ewart (1881) and by Preyer (1886–1887). Recently Jennings (1907), in his usual clear manner, has studied and described the behavior of the tube feet of *Asterias forreri* de Loriol, confirming the observations of the earlier workers. Unlike *A. forreri*, the suckers of *E. crassispina* and *A. duplicatus* do not adhere tightly to the substratum as a rule, nor do these two species usually occur on the under side of rocks.

The rather level character of the bottom on which *Echinaster* lives leads one to believe that the animal is seldom turned over and therefore seldom finds it necessary to right itself. I can hardly believe that this turning over occurs so often that an echinaster would have a chance to form a habit of righting itself on any one pair of rays, but there might be some structural peculiarity which would determine the pair to be used.

Echinaster lives in the open and seems always to be found in regions exposed to the brightest light. In this respect it differs from *A. forreri*. So, as would naturally be expected, we find that in experiments in the laboratory *Echinaster crassispina* reacts positively to bright light and *Asterias forreri* reacts negatively.

Astropecten duplicatus has somewhat similar habits to those of *Echinaster*. It lives usually in deeper water, being found at a depth of 1 or 2 fathoms. It is migratory, but suckers on the tube feet seem to be practically absent. This form also lives on the sandy bottom and reacts positively to bright light.

Note.—I wish to express my thanks to the Carnegie Institution and to Dr. Alfred G. Mayer for the privilege of working in the Tortugas Laboratory.

DOES ONE RAY HAVE GREATER FUNCTIONAL VALUE IN LOCOMOTION THAN ANOTHER?

The experiments of Preyer (1886–1887), in which he tested several species of starfish to see if one ray had greater functional value than another in locomotion, seem to answer the question in the negative except in the case of a specimen of *Astropecten pentacanthus*, which showed a decided tendency to use ray 4 (*d* of Jennings's paper and my own) as director. In general, however, Preyer concluded that one ray has no greater functional value in locomotion than another (fig. 1).

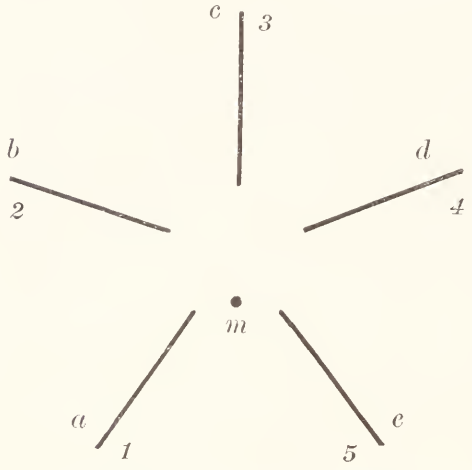


FIG. 1.—Diagram of a starfish in the normal position, i.e., with aboral surface uppermost. The labeling of the rays by numerals is that used by Preyer and Bohn; the labeling of the rays by letters is that used by Jennings, Cole, and the writer. The madreporic plate is indicated by the black spot and is labeled *m*.

While Jennings (1907) in his work on the starfish has not taken up this particular problem, he has made a rather extensive study in order to determine if *Asterias forreri* de Loriol tends to right itself on any special pair of rays and he concludes that the rays lying close to the madreporic plate are more often used than any others.

Bohn (1908) finds that specimens of *Asterias rubens* show "une sorte de préférence pour certains bras," especially when the individuals are of a large size. The rays *3* (*c*) and *5* (*e*) of one individual were used most frequently as directors in locomotion. Bohn, however, does not draw the conclusion that *A. rubens* shows any very definite habit in this respect.

Recently, in a preliminary report (1909), I stated that *Echinaster crassispina* does not show any tendency to use a special ray or pair of rays as a director. This conclusion was based on a considerable number of observations with directive light excluded, but the work was not a careful statistical study such as that undertaken by Cole (1910). The latter finds that *Asterias forbesii*, in the absence of directive stimuli, moves most often with the ray (*e*) in advance and it is possible that if *Echinaster crassispina* is subjected to similar tests a tendency to move with a certain ray in the lead may be shown.

CLIMBING OF VERTICAL WALLS.

Most naturalists who have observed starfishes in their natural habitat have seen them attached to the vertical walls of rocks, masonry, or the piles supporting docks, and those who have studied these interesting animals in the laboratory have been struck by what seems to be their general tendency to climb up on the more or less vertical side of the aquarium, often reaching the surface of the water and apparently attempting even to crawl on this surface with the oral side uppermost. It seems to me the "tendency of these animals to move upward" is more evident in laboratory aquaria than under natural conditions.

Preyer (1886–1887) was one of the first to call our attention to this phenomenon and he saw in the behavior of starfish and brittle-stars a strong tendency to move upward. Strange to say, he ruled out such factors as lack of air, lack of food, changes in temperature or currents of water, and desire for light, suggesting that parasites sometimes found in the ambulacral furrows might be the cause of the upward movement.

Loeb (1900) very justly pointed out the insufficiency of Preyer's suggestion and also objected to the latter's generalization as to the factors that do not influence the behavior. Loeb states that *Asterina tenuispina* is attracted upward by the light; on the other hand, he seems to be inclined to believe, from his study of *Asterina gibbosa*, that certain starfish move vertically upward on account "of the force of gravity"; that they are "driven there by negative geotropism."

Romanes (1885) makes no attempt to discover any factors that might account for the upward movement of certain starfish, but simply describes their behavior in a tank. He finds that they crawl in a determinate direction and that when a starfish happens to touch a solid body it may continue its direction unchanged or may turn toward the body which it has touched. If while crawling along the floor of the tank a ray happens to touch the perpendicular side of the tank, the starfish may continue its direction of advance unchanged on the floor, feeling the perpendicular side with the end of its rays, perhaps the whole way around the tank; yet it may not ascend or it may go directly up the perpendicular wall.[1]

The starfish *Echinaster crassispina*, which I used in experiments, has well-developed suckers on its tube feet and is able to ascend vertical walls without any difficulty. It lives as a rule on rather level bottoms and is migratory in its habits. When several healthy specimens of this species are put in a medium-sized circular glass aquarium filled with sea-water they begin to move after a short time, usually in a determinate direction, and are soon seen climbing up the vertical sides of the aquarium until they reach the surface. If, instead of the small aquarium, a large one holding 200 or 300 gallons is used, some specimens will climb the walls, others will move along the bottom with one or more rays in contact with the walls; while the ascent of some individuals nearly always occurs, the phenomenon is not so striking as when a small aquarium is used.

[1] Bohn's (1908) experiments show that light has much to do with "geotropic" reactions; that the same lighting is sometimes followed by an ascent and sometimes by a descent of a vertical surface. Assuming that there is a geotropic factor whose action results in the ascent or descent of vertical surface by a starfish, his observations leave the impression that it is a very variable one.

Several series of experiments were undertaken in order to determine the behavior of *Echinaster* on inclined surfaces in the presence or in the absence of light. In these tests a very simple piece of apparatus was used (fig. 2).

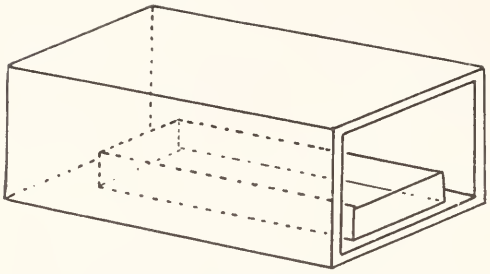


FIG. 2.—Apparatus used to test the effect of great differences of light intensity on starfishes. A glass experimenting dish (36×27×7 cm.) lined with dead-black paper is placed, as shown, in a wooden box (50×32×19 cm.) which is painted dead-black inside and open at one end. The glass dish is filled with sea-water and the whole apparatus is so adjusted that intense sunlight is reflected from a white sand bank into the open end of the box. The top of the box can be removed when starfishes are introduced into the dish.

Before beginning each series the starfish to be used was tested in the apparatus to observe the starfish's reaction to light. The specimen was placed in the dish of sea-water, care being taken to have the bottom of the dish level. The method of handling and the position of the rays with reference to the open end of the box were varied, but almost always the starfish moved toward the light. Rarely the specimen moved to the darker end, but even then it soon returned to the bright end of the apparatus. When the same kind of tests were made, the apparatus being covered with a thick piece of velvet so that no light could enter the open end of the box, the starfish behaved differently, moving more slowly and without reference to any end of the apparatus. Every *Echinaster* and every *Astropecten* experimented with showed a decided tendency to react positively to bright light.

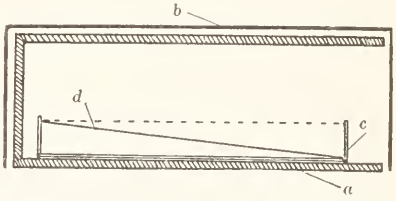


FIG. 3.—Vertical section through apparatus shown in fig. 2, with the addition of the false bottom inclined at 10°, and the light-tight velvet cover. *a*, wooden box; *b*, velvet cover; *c*, glass dish; *d*, inclined false bottom.

After observing the starfish's behavior toward light, as described above, a false bottom inclined at an angle of 10° was placed in the glass dish and so arranged that the base of the incline was at the open end of the apparatus (fig. 3). The results of these tests are as follows:

TABLE 1.

Test.	Orientation.	Direction of locomotion.
1	*a* + *e* toward light.....	To base of incline.
2	*c* + *d* " "	" " " "
3	*a* + *b* " "	" " " "
4	*d* + *c* " "	" " " "
5	*b* + *c* " "	" " " "

The starfish, it will be seen, moved to the lightest end of the apparatus and in so doing climbed down the incline. In order to observe the behavior without the light stimulus the apparatus was now covered with a thick piece of velvet, so that it was light-tight. The results were as follows:

TABLE 2.

Test.	Orientation.	Direction of locomotion.	Test.	Orientation.	Direction of locomotion.
1	*a* + *e* toward base...	To base.	9	*a* + *b* toward base...	To base.
2	*c* + *d* " "	To top.	10	*b* + *c* " "	To top.
3	*d* + *e* " "	To base.	11	*a* + *e* " "	To base.
4	*a* + *b* " "	To base.	12	*c* + *d* " "	To base.
5	*b* + *c* " "	To top.	13	*a* + *b* " "	To top.
6	*a* + *e* " "	To base.	14	*d* + *e* " "	To base.
7	*c* + *d* " "	To top.	15	*b* + *c* " "	To base.
8	*d* + *e* " "	To base.			

The results of these 15 tests show that the direction of locomotion is quite variable. There is no strong tendency to ascend or descend the incline, although it is true that the descent was made 10 times out of the 15. Whatever this may mean, there is surely no strong tendency to climb a floor tilted at 10°; there is no indication of "negative geotropism"; they are not "driven there by negative geotropism."

Light is undoubtedly a very strong stimulus in the tests listed in table 1, and one might claim that it resulted in the formation of a habit in which the descent of the incline was associated with strong light entering the open end of the box and that this habit was not overcome by the negative geotropic stimulus. For this reason a series of tests was undertaken like those given in tables 1 and 2 except that the false bottom was so placed that the top of the incline was at the open end of the apparatus and the base at the other. The results of the tests with light as a factor are indicated in the following table:

TABLE 3.

Test.	Orientation.	Direction of locomotion.
1	*a* + *e* toward light.....	To top of incline.
2	*b* + *c* " "	" " " "
3	*d* + *c* " "	" " " "
4	*a* + *b* " "	" " " "
5	*c* + *d* " "	" " " "

It will be seen that the starfish moved to the lighted end of the apparatus in every case, and in so doing mounted the incline.

Light was now excluded by covering the apparatus with velvet. The results of the tests were as follows:

TABLE 4.

Test.	Orientation.	Direction of locomotion.	Test.	Orientation.	Direction of locomotion.
1	*b* + *c* toward top....	To base.	9	*c* + *d* toward top....	To base.
2	*d* + *e* " "	To top.	10	*a* + *e* " "	To top.
3	*a* + *b* " "	To base.	11	*b* + *c* " "	To base.
4	*c* + *d* " "	To top.	12	*a* + *b* " "	To base.
5	*a* + *e* " "	To top.	13	*d* + *e* " "	To top.
6	*b* + *c* " "	To base.	14	*c* + *d* " "	To top.
7	*d* + *e* " "	To top.	15	*a* + *e* " "	To side.
8	*a* + *b* " "	To top.			

In these 15 tests (table 4) it will be seen that the starfish moved to the top of the incline 8 times, to the base 6 times, and to the side once. Although the ratio is slightly in favor of movement up the incline, yet we are not justified in considering that a case of "negative geotropism" is demonstrated. Surely the direction of locomotion is not stereotyped. While it would be desirable to have more data, I think one may draw the conclusion that when *Echinaster crassispina, which usually reacts positively to bright daylight and which usually crawls to the top in a small aquarium exposed to light, is placed on a surface tilted at an angle of* 10° *(light being excluded), there is no decided tendency for it to move either negatively or positively to the attraction of gravitation.*

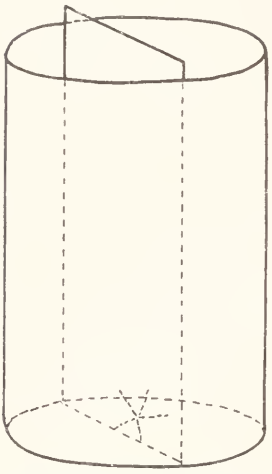


FIG. 4.—Glass cylindrical aquarium with vertical glass plate set inside. Used for observing the behavior in climbing tests.

In order to test further the behavior of *Echinaster* on vertical walls, several series of tests were made, using a very large cylindrical glass aquarium filled with sea-water. Inside the aquarium was fixed a flat piece of glass in a vertical position (fig. 4). A specimen which had been tested and found positive in its reaction to light was then allowed to attach itself to the vertical plate, with one or two of its rays resting on the bottom. The apparatus was then covered with a thick velvet cloth, so as to be light-tight. The specimen climbed to the top. 10 tests were made with the following results:

TABLE 5.

Test.	Orientation.	Direction of locomotion.	Test.	Orientation.	Direction of locomotion.
1	*d* + *e* toward top ..	To top.	6	*a* toward top ..	To top.
2	*a* + *b* " "	To top.	7	*b* " "	To bottom.
3	*c* + *d* " "	To top.	8	*c* " "	To top.
4	*a* + *e* " "	To top.	9	*e* " "	To top.
5	*b* + *c* " "	To top.	10	*d* " "	To top.

In 9 tests out of the 10 the starfish ascended the vertical wall and in 1 test descended to the bottom. The results seemed to indicate a strong tendency to ascend, even with light excluded. In these tests, however, the starfish was exposed to light while it was being allowed to attach itself, and it occurred to the writer that during that time the light stimulus might have resulted in the formation of an impulse to move toward the light and that in the attempt to do so it climbed the wall. Accordingly a series of tests was made in a photographic dark room where no light was allowed to reach the starfish except the brief flash from an electric hand-lamp when the creature had completed its test. The results of 20 trials, 8 with the starfish used in table 5 and 12 with another specimen, in which the orientation and handling were varied, show a descent of the vertical wall in 14 tests and an ascent in 6 tests. These trials were made at intervals of 3 minutes. The temperature of the water in the ocean was 29° C. and that in the aquarium 30° C.

It is certain that many starfish show a decided tendency to climb the vertical walls of a small aquarium when exposed to light; it is also true that starfish while climbing a wall are under the influence of the attraction of gravitation; there is no doubt that in going up a wall they move in an opposite direction to that of the attraction of gravitation; but it does not follow that the behavior in question is caused by "negative geotropism," i.e., by the "action of the force of gravity" (Loeb, 1900, p. 71). It does not seem to me that starfish in an aquarium show this decided tendency because of the influence the attraction of gravitation has over them, but that *the climbing is simply incidental, the result of the surroundings.* The explanation of this behavior must be sought in the stimuli received by the starfish before it was placed in the aquarium, in the stimuli received while in the aquarium, and in the physiological state of the animal. These factors together finally result in the "impulse" (Jennings) to move in a determinate direction. *If there is light of much intensity coming from a certain direction the impulse will be such that in the majority of cases the starfish will move toward the source. In so doing if in an aquarium it will soon come to the vertical wall. A characteristic of the starfish is that when once the impulse to move in a certain direction is formed, the starfish is quite persistent in its behavior and continues to move in that direction; so when the creature reaches the wall it ascends owing to the persistence of the impulse strengthened by the continued stimulus produced by the light.*[1]

REACTION TO LIGHT.

Echinaster crassispina is a starfish which lives on a more or less flat sea-bottom and which does not crawl under stones and attach itself as does *Asterias forreri*. It lives in the open and is exposed to the sunlight throughout the day.

We find *Echinaster* reacting positively to bright light while *Asteria* reacts negatively. *Echinaster* is very sensitive to light and, as we shall see, light perception is not confined to the "eye-spots" at the tips of the rays. A large part of the aboral surface at least seems to be sensitive to light stimuli. Not only does *Echinaster*, as a rule, react positively to intense light, but it is also sensitive to a light of 2 to 3 candle-power, or even to a light of somewhat less intensity when placed in a dark room.

Light is undoubtedly a very important factor in determining the movements of *Echinaster*. The dependence of this starfish on light stimuli is shown in its behavior when placed in the dark. The rate of locomotion usually becomes reduced and the tips of the rays turn upward as if in search of the customary light stimulus. Sometimes a specimen is found lying perfectly still on the bottom of an aquarium with all five rays curled upward. *Echinaster* usually assumes an exceptional attitude in the absence of light or in light of very reduced intensity, just as *Asterias forreri* does in the presence of bright light.

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[1] Jennings (1907) has recognized the persistence in behavior after an impulse is formed in the starfish, and Morgulis (1910) has recently drawn attention to a neglected factor in the movement of the earthworm, namely, the tendency to move in a straight line and the obstinate maintenance of a path when once assumed.

While *Echinaster* is very sensitive to light and usually reacts positively to it, such is not always the case. I have described above the reaction of this starfish when placed in a black-lined box with bright light entering one end. Under these conditions the specimen usually moves almost directly toward the lighted end of the apparatus. Making a few tests of this sort, one might draw the conclusion that the starfish's behavior is invariable, that it is stereotyped; but if enough series of tests are made it is found that occasionally the starfish does not react positively to the light. Furthermore, it frequently occurs that when a starfish reaches the lighted end of the dish, instead of staying there it returns again to the dark end, especially when it follows the base of the vertical walls of the dish. Bohn (1908) and, above all, Jennings (1907) lay stress on the importance of not neglecting the internal factors while studying the behavior of the starfish and they recognize the variability of the reactions.

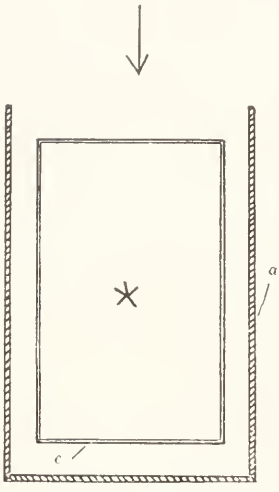

FIG. 5.—Plan of apparatus used in test 1, table 6. The arrow indicates the direction of light. *a*, wooden box; *c*, glass dish.

REACTION TO BRIGHT LIGHT.

Using the apparatus shown in fig. 2, many echinasters were tested to determine their reaction to the intense light reflected from a white sand-bank. The starfish was placed in the experimenting dish, with a single ray or with an interradius directed toward the lighted end. The method of handling was varied. Many series of tests were made and in the large majority of cases the starfish reacted positively and moved to the brightly lighted end of the dish. Under the influence of the intensely lighted region at the open end of the apparatus the specimens seldom failed to react positively, but the reaction was not invariable.

The results of these experiments show that *Echinaster*, as a rule, reacts positively to regions of increased light intensity, that it does this irrespective of the ray or rays directed toward the bright light, and that its locomotion is somewhat more accurate when a ray is pointed toward the light than when an interradius is directed in that direction. Fig. 5 is a plan of the apparatus as used in tables 6 and 7. Table 6 records a series

TABLE 6.

Test.	Orientation.	Direction of locomotion.
1	*c* toward light.....	Directly to light end.
2	*e* " "	" " " "
3	*a* " "	" " " "
4	*b* " "	" " " "
5	*d* " "	" " " "

of tests with a single ray directed toward the bright end. Table 7 records tests with an interradius directed toward the bright end.

While I suppose it will not be doubted that light has a directive influence on the direction of locomotion of *Echinaster*, yet it may be well to state that series of tests were made in which the entrance of the light

TABLE 7.

Test.	Orientation.	Direction of locomotion.
1	*e* + *a* toward light	Directly to lighted end.
2	*a* + *b* " "	" " " "
3	*b* + *c* " "	To lighted end with same rays forward but moved sideways.
4	*c* + *d* " "	To lighted end with same rays forward but moved sideways.
5	*d* + *e* " "	To lighted end with same rays forward but moved sideways.

into the apparatus was regulated by black screens. Sometimes the light was only allowed to enter by a vertical slit at one corner, sometimes at the other corner, and sometimes in the middle of the open end of the apparatus. As a rule, the starfish moved directly toward the slit irrespective of the latter's position.

PARTS SENSITIVE TO LIGHT.

Tiedemann (1815), who was one of the first to study the starfish, came to the conclusion that the whole surface was sensitive to light. This was before the discovery of the eye-spots in the starfish. After these organs had been found, however, they came to be considered as the light-receiving organs. Both Romanes (1885) and Preyer (1886–1887) consider the presence of eye-spots necessary to produce a reaction to light. While Bohn (1908) does not state that the eye-spots are the only organs sensitive to light in the starfish he studied, yet he does not mention any other parts as being sensitive. Uexkull in his numerous papers has not taken up the reaction of starfishes to light, but his experiments with sea-urchins show that these creatures react to differences in intensity of light, even though they have no eye-spots.

In order to determine the effect of the removal of the eye-spots on the reactions of *Echinaster* to light, several individuals were used and each was first tested before the operation in the apparatus shown in figs. 2 and 5. All specimens reacted positively to the light in almost every test and moved to the lighted end of the dish. The tips of the rays of these starfish were then cut off about 1 cm. from the end, so that all the individuals were without eye-spots. The operated starfish were then allowed to recover from the shock under as favorable conditions as possible. Two hours after the amputation the starfish were again tested. The results obtained with one individual are shown below.

TABLE 8.

Test.	Orientation.	Direction of locomotion.	Test.	Orientation.	Direction of locomotion.
1	?	Directly to lighted end.	10	*b* toward light	Directly to lighted end.
2	*c* toward light	Directly to lighted end.	11	*e* " "	Directly to lighted end.
3	*a* " "	Directly to lighted end.	12	*c* " "	Directly to lighted end.
4	*b* " "	To side, then to lighted end.	13	*a* " "	Directly to darkened end.
5	*c* " "	To side, then to lighted end.	14	*b* " "	Directly to lighted end.
6	*c* " "	Directly to lighted end.	15	*d* " "	Directly to lighted end.
7	*d* " "	Directly to lighted end.	16	*c* " "	Directly to lighted end.
8	*e* " "	Directly to lighted end.	17	*e* " "	Directly to lighted end.
9	*a* " "	Directly to darkened end.	18	*a* " "	Directly to lighted end.

The first five of the above tests were made 2 hours after the operation and the balance about 24 hours later. The method of handling and the orientation were varied, but there is every indication that the starfish reacted positively toward the brightly lighted end of the dish. In every test but 2 of the 18, the echinaster moved to the light end. On the second day after the operation the behavior was more direct. There can be no doubt that the eye-spots as well as 1 centimeter of the ray are not necessary in the reaction of *Echinaster crassispina* to the light. The rest of the surface of the starfish must be sensitive to light (Jennings's work indicates this) to such a degree that the direction of movement is influenced by it. In fact, since this work was done I have tested the sensitiveness of the tube feet and the gills of *Pentaceros reticulatus* and have found that they react definitely to changes in intensity of light.

Besides the series of tests recorded in table 8, other series equally convincing were undertaken with different individuals; furthermore, we shall see in some later experiments additional proof that an echinaster without eye-spots is sensitive to light; while there is no doubt that eye-spots are not necessary, yet it is true that echinasters with these organs removed do not react as quickly as normal individuals. Whether this is the result of the injured condition or the lack of the eye-spots I can not say.

DIFFERENCE IN INTENSITY OF ILLUMINATION AND RAY DIRECTION AS FACTORS IN DETERMINING THE DIRECTION OF LOCOMOTION.

So far as I know, Jennings (1907) has been the first to attempt to ascertain whether the direction of the rays of light or the differences in the intensity of illumination on the different parts of the body determine the direction of locomotion of the starfish. By manipulating a screen in various ways, so as to cut out the direct action of the sun's rays on the surface of the starfish, Jennings obtained results which point strongly to the view that the "relative intensity of illumination" is the important factor in the determination of the direction of locomotion.

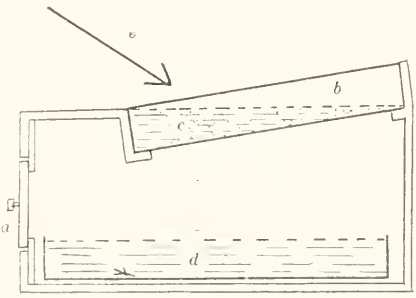

FIG. 6.—Section through the apparatus used to obtain a field of light of graded intensity. *a*, light-tight door; *b*, glass container; *c*, prism of water mixed with a little Higgins waterproof ink; *d*, rectangular glass dish containing sea-water and starfish; *e*, direction of sun's rays.

While many of my experiments led me to believe that the starfish (*Echinaster*) and the brittle-star (*Ophiocoma*) are influenced more by the relative intensity of illumination of the surface of the animal than by the direction of the rays, yet I undertook the following experiments to confirm my belief. A brief preliminary notice of these experiments has been published by the writer (1909). The apparatus (fig. 6) described in that paper consisted essentially of a tight wooden box open above and painted dead black inside. Into this opening was fitted a glass container partly filled with water mixed with a little Higgins waterproof ink, so that when the container was in position there was formed

a prism of the solution, thick at one end and thin at the other. A rectangular glass dish lined with dead-black paper and almost filled with sea-water was used for testing the echinasters. This was put within the black-lined box and the whole apparatus was then placed so that the direct rays of the sun entered the prism, thus producing a lighted area in the dish which was of graded intensity.

Four series of tests were made with the sun's rays entering the apparatus in the general direction shown in fig. 6. In each series a different starfish was used; two of the specimens were large and two were small; one of the larger ones had all of its eye-spots removed. Without a heliostat it was impossible to have the light rays enter the apparatus at the same angle in each experiment, but this should make no difference in the results. In one of the four series there were ten tests made; the angle the sun's rays made with the floor of the apparatus varied in each case as follows: 22°, 24°, 25°, 33°, 44°, 45°, 46°, 47°, 50°, 57°. A test consisted of placing an echinaster in about the middle of the dish of sea-water and of observing its direction of locomotion. In every case the starfish moved apparently without hesitation to the bright end of the graded field and remained there for some time. A series of ten tests with the specimen whose eye-spots had been removed gave similar results, except that the reaction was slower.

After testing echinaster with the sun's rays entering as shown in fig. 6, the apparatus was turned around so that they entered from the opposite direction. The results were the same as in the test made above; the starfish moved to the light end of the dish.

A few tests were made with the sun directly overhead and again in every case the starfish moved to the end most intensely lighted.

Finally the apparatus was altered so that the layer of ink and water no longer made a prism. The container was set level, so that the layer of water and ink was of uniform thickness throughout. The result was that the sun's rays produced an area of light of equal intensity in the experimenting dish. Echinasters tested under these conditions moved about "aimlessly," sometimes in the direction of the rays, sometimes opposite to the direction of the rays, and often from side to side.

These experiments, it seems to me, make it evident that *Echinaster crassispina* tends to move from the region of least intensity to that of greater intensity without reference to the direction of the sun's rays.

There has been an objection raised to the use of a prism containing india-ink particles in suspension. It is claimed that the particles of ink disperse the light before it reaches the starfish, so that parallel rays do not strike the starfish. Such is undoubtedly the case, for, as Mast (1907) states: "reflection and refraction can not be entirely eliminated, even with the utmost precaution." The writer does not hold that the rays impinging on the surface of the starfish are parallel. Since the rays are not parallel it may be claimed that the particles at the thin end of the prism reflect the rays and that these rays act as a directive influence resulting in movement toward the bright end of the field; but it is also true that the particles at the thick end of the prism disperse the sun's rays and that many of these reflected rays impinging on the surface of

the starfish come from a direction opposite to which the creature is moving. To the writer, it seems impossible that the rays reflected from the extremely minute particles of india ink in the mixture have any directive effect, since it has been found that the behavior is the same, i.e., the starfish moves to the bright end of the field, whether the sun's rays enter the prism as shown in the figure, whether they enter in the opposite direction, or whether they enter vertically.

EFFECT OF TEMPERATURE ON REACTION TO LIGHT.

It has been shown above that *Echinaster crassispina* usually reacts positively to brightly lighted regions, although occasionally the opposite is found to be the case. Several investigators, including Loeb (1893), Massart (1891), and Strasburger (1878), have stated that the sign of reaction of some lower organisms is reversed when the temperature changes from a certain degree to another. On the other hand, Parker (1901), Yerkes (1903), and Mast (1907) find no change in the sign of the reaction in the case of copepods, *Daphnia pulex* and *Volvox globator.*

The writer tested the reaction to light of the starfish *Echinaster crassispina* at different degrees of temperature. The apparatus used is shown in figure 2. It was found that when the temperature of the water was from 29° to 30° C. the reaction to light was at its optimum and that the movement toward the most intensely lighted end of the dish was very definite. A series of tests was made in which the temperature of the water was gradually decreased and also one in which it was gradually increased. The range of temperatures was as follows: 17.8°, 18.4°, 20°, 21.1°, 21.7°, 22.2°, 24.4°, 25.6°, 26.7°, 28.9°, 29.5°, 32.7°, 33.3°, 34.4° C. As the temperature was reduced from 28.9° C. to 17.8° C. the rate of movement toward the brighter end of the dish decreased until locomotion was entirely inhibited at the latter degree. The starfish settled down closely on the bottom of the dish and the tube feet stuck so tightly to the glass that it was impossible to remove them without tearing. This condition is exceptional for *Echinaster crassispina* at ordinary temperatures. On the other hand, when the temperature was increased from 28.9° C. to 34.4° C. the starfish did not adhere to the dish, but its activity was very much reduced and although it continued to react positively the movement was very slow. Above 34.4° C. the starfish became almost lifeless. In no case was there any indication of a reversal of the sign of reaction. The starfish continued to move from the region of least intensity to that of greater intensity.

Bohn (1908) describes an experiment which he considers as an example of change in sign of "phototropisme." He placed an *Asterina gibbosa*, which usually reacts negatively to bright light, in a lighted field. The starfish began to move in a certain direction and was then turned around through 180°; the result was that the specimen then moved in the opposite direction. In other words, a starfish which reacted negatively to bright light by moving away from it, reacted positively to bright light by moving toward it when rotated through 180°. This behavior Bohn interprets as a change in sign of "phototropisme." It is true that *Asterina* changed its direction of movement with reference to the more intensely lighted region, but this was undoubtedly not due

to any change in the stimulus produced by light. Such behavior, it seems to me, should be explained by the persistence of the "coördinated impulse" (Jennings, 1907). The starfish had been placed in the lighted field; some stimulus (either the light or other external or internal stimuli) so affected the creature that the tube feet began to move coördinately, resulting in locomotion with a certain ray of interradius directed forward; the asterina moved in a definite direction, namely, *away* from the light; when this impulse was firmly established and the starfish was moving away from the brightly lighted region it was turned through 180°; the established impulse to move with a certain ray or interradius forward persisted and the starfish then moved *toward* the light.

Although I have offered this interpretation of Bohn's experiment, I do not wish to leave the impression that a starfish may not change its sign of reaction to light, as a result, for example, of being subjected to intense light or to darkness for a considerable time. While I believe that the behavior of the starfish with reference to light is largely a matter of the intensity to which it has been accustomed, I have not tried any experiments along this line.

EFFECT OF LIGHT RAYS OF DIFFERENT QUALITIES ON THE REACTION TO LIGHT.

Several investigators have attempted to show that some of the lower organisms exhibit a "preference" or "aversion" for light rays of different qualities. It has been held that animals which react positively to light show an "aversion" to the red and a "preference" for the ultra-violet; and that animals which react negatively to light show a "preference" for the red and an "aversion" for the ultra-violet. It has also been shown that *Daphnia*, which is decidedly positive in its reaction to light, repeatedly collected in the yellow-green region of the spectrum. On the other hand, Bert (1869), Merejkowsky (1881), and Yerkes (1900) have offered evidence to show that what often appears to be "preference" or "aversion" for rays of different qualities is probably nothing more than a difference in reaction to lights of different intensities.

Later I hope to take up the quality-intensity problem, but have here simply recorded the sign of reaction to light of different qualities without reference to the intensity.

Color screens were used, and although these were not monochromatic the transmission was tested by photographic methods so that the quality of the light used was known exactly. The tests were made in an apparatus like that shown in fig. 2, except that the open end of the box was closed. In the closed end was cut an opening into which were fitted the various color-screens. Sunlight was used as a source of light. The results obtained were rather meager, showing only that no one kind of ray is necessary to stimulate *Echinaster*. The following 4 screens cut out: (*a*) ultra-violet; (*b*) ultra-violet and violet; (*c*) violet and blue; (*d*) green, yellow, orange, and red. A series of 10 tests was made with each screen, 17 in all; the orientation and handling were varied. In every series the reaction was decidedly positive, i.e., in nearly every test the starfish moved toward the light without hesitation.

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XI.

THE ANATOMY OF PENTACEROS RETICULATUS.

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3 plates, 2 text cuts.

THE ANATOMY OF PENTACEROS RETICULATUS.

By D. H. Tennent and V. H. Keiller.

The following account does not presume to be a complete description of the anatomy of *Pentaceros reticulatus*. Its chief value lies in the figures which are used in its illustration.

During my stay at Tortugas in 1909 I made some use of *Pentaceros* eggs and sperm in experimental work and noted, in making the dissections necessary for obtaining these products, some points in the anatomy which were of unusual interest. These were concerned with the organs of reproduction and with the intestinal cæca.

I preserved a number of these starfish and sent them to Bryn Mawr, where I suggested to one of my students, Miss V. H. Keiller, that she make a study and careful drawings of their structure. Although done under my supervision, full credit for the work must be given Miss Keiller. During the summer of 1910 I again examined fresh material as the basis for this account and in order to make sure that none of the structures shown in the drawings were due to the effect of preservation.

Pentaceros reticulatus (Linck, Linnæus) is the common West Indian starfish which is so familiar in the dried form in which it is exhibited as a curio in the sea-shore shops. Linck (1733) in his "De stellis marinis" gives two figures of *Pentaceros reticulatus*, an aboral view on his plate 41 and an oral view on plate 42.

Müller and Troschel (1842) use the generic name *Oreaster* instead of *Pentaceros*. Perrier (1875) and later systematists have accepted the Linnæan and pre-Linnæan name. Agassiz (1877) and Viguier (1878) have contributed to our knowledge of the skeleton of this form, Agassiz's plate XVI, figs. 1, 2, 4, and 5, and Viguier's plate X, figs. 4 and 5, giving excellent representations of the hard parts of the body.

Clark (1901, p. 237) notes for this species an observation by Mr. George Gray, of the Marine Biological Laboratory at Woods Hole, "that there are two well-marked varieties of this starfish, so different from each other that, were connecting links wanting, they would easily pass for distinct species. One has the rays acuminate, the disk very high, the skeleton comparatively light, and the oral surface quite spiny, while the other has the rays shorter and more rounded, the disk lower, the skeleton very solid and covered with large tubercles; oral surface more granular and less spiny."

Studer (1880, p. 545) mentions differences in the color and form of *Pentaceros turritus* Linck and correlates these with sex. With *Pentaceros reticulatus*, although I was able to verify the observation made by Clark, I could not convince myself that these were sexual differences.

The ground color of the aboral surface of *Pentaceros reticulatus* varies from light yellow to a very deep reddish-brown. The reticulation, on the nodes of which the spines are situated, is brought into sharp contrast with this ground by its usually lighter color. In all of the specimens examined the aboral surface (fig. 6) was light in color with strong, glistening spines bordering the ambulacral grooves.

The body integument is extremely hard and tough. Removal of any part of the wall is best accomplished by the use of a heavy, sharp scalpel.

THE DIGESTIVE SYSTEM.

The anus is large and distinct and may be seen without difficulty, slightly to the left of a line which might be drawn through the madreporic plate to the tip of the opposite arm.

Upon removing the aboral wall one sees the short, cone-like rectum (fig. 2, an.) which arises from the center of the broad, five-sided, pyramidal intestine. Into this large intestine open interradially the ducts of the intestinal cæca (resp.), each of these ducts arising by the union of the single ducts of the cæca of adjacent arms at the inner termination of the interradial septa (i.p.).

The intestinal cæca, two of which lie in each arm and whose ducts reach the intestine as above described, are attached to the aboral body-wall by strong muscular connections. The cæca consist of a single main duct from which arise numerous bladder-like diverticula which are capable of great distention. Upon opening some specimens the cæca were found to be greatly distended. Upon stimulation they slowly contracted, the entire organ shrinking to about one-third of its former size. The contents were watery, although the inner wall of the cæca was found to be slimy.

Beneath the intestine, upon the surface of the stomach in each radius, is what appears at first sight to be a second set of five cæca, each made up of two parts. Further examination shows that these are merely pouches formed by the folding of the upper wall of the pyloric portion of the stomach. They involve the regions into which the ducts of the pyloric cæca open and have a narrow slit-like connection with the stomach. This connection may be greatly widened by straightening out the folds. The pyloric cæca (pyl. cæc.) are large and greatly branched. In color they are brownish-green.

The cardiac portion of the stomach (fig. 5, card. st.) is large and its muscles, both those which are attached along the sides of the ambulacral ridge (retr.) and those attached at the oral ends of the ridges, are powerful.

THE WATER VASCULAR SYSTEM.

The madreporic plate (figs. 1 and 2, madr.) is inconspicuous but may be recognized readily by its color which is lighter than the surrounding ground color, From the plate the stone canal (mad. can.) leads downward to the ring canal (r. c.). From the ring canal are given off the Polian vesicles, the Tiedemann bodies, the ampullæ of the first tube feet, and the radial canals. A large-stalked Polian vesicle

(pol. ves.) is present in each interradius except the madreporic. Two Tiedemann bodies are present in each interradius, including that in which the stone canal is situated. The ampullæ (amp.) of the first tube feet arise directly from the ring canal. The radial canal (rad.) runs directly

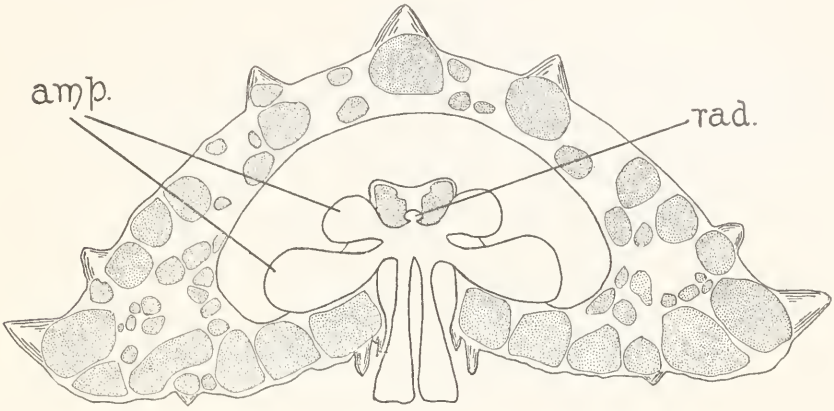

FIG. 7.—Diagram transverse section of arm through ampullæ and tube feet.

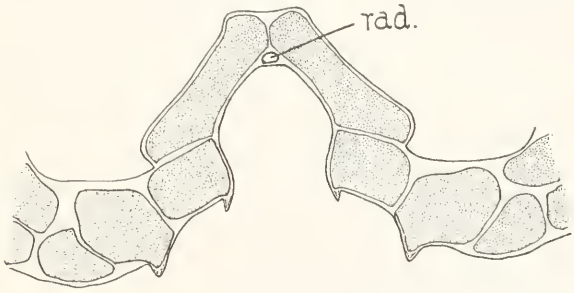

FIG. 8.—Diagram ambulacral groove between ampullæ and tube feet.

to the tip of the arm. The tube feet lie in a single row on each side of the ambulacral groove, but the ampullæ lie in a double row, an upper and a lower, each tube foot being connected with two ampullæ (fig. 7).

THE ORGANS OF REPRODUCTION.

The ovaries (fig. 3, ov.) or the testes (fig. 5, ts.) are in the form of bunches or clusters of short, tubular bodies, each cluster having a separate duct to the aboral surface (fig. 5), upon which it opens by a fine pore. In specimens that I have examined I have found from nine to fifteen clusters down the side of each arm. Each cluster having a separate opening, the genital pores form a row along the side of each arm, in position these rows lying one on each side of the interradial septa. The pores may not be made out directly in either the living or the preserved individuals, but their positions may be seen easily during the discharge of the reproductive elements. The ovaries in the fresh material are orange in color, the testes light yellow or almost white.

THE MUSCULAR SYSTEM.

Although the body-wall is thick and exceedingly stiff the starfish is very mobile. The aboral musculature is shown in fig. 4, where it will be seen that in each arm there are three principal muscle bands, each with numerous branches.

GENERAL.

Of the organs which are described and figured those which seem of greatest interest are the intestinal cæca. These, as above stated, were found in some instances to be greatly distended, stimulation causing their contraction. Upon allowing individuals, from which the aboral wall had been removed, to remain undisturbed in sea-water, the organs again became distended and later contracted of their own accord. In this behavior we have support to the idea of the analogy of the intestinal cæca of the starfish to the respiratory trees of the holothurian, an idea which has been based in the main upon the similarity of position of these organs.

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FIGURES.

Figures 1 to 6 were drawn with crayon on Ross's stipple paper to the full size of the object and have been reduced two-thirds in reproduction.

Abbreviations.

an., anus.
amp., ampulla.
card. st., cardiac portion of stomach.
i. p., interradial septum.
madr., madreporite.
mad. can., stone canal.
op. resp., opening of intestinal cæcum.
ov., ovary.
mes., mesentery.
pol. ves., Polian vesicle.
pyl. coec., pyloric cæcum.
rad., radial canal.
r. c., ring canal.
resp., intestinal cæcum.
retr., retractor muscle of stomach.
Tiedm., Tiedemann body.
ts., testis.

Fig. 1.

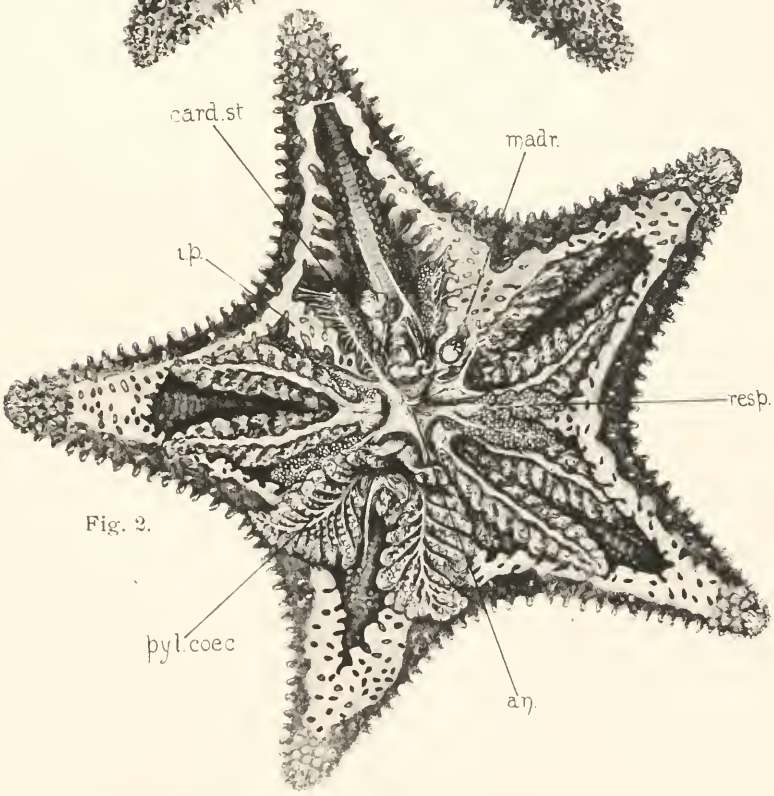

Fig. 2.

V. H. KEILLER DEL.

Pentaceros reticulatus.

1. Aboral Surface.
2. Dissection to show digestive system.

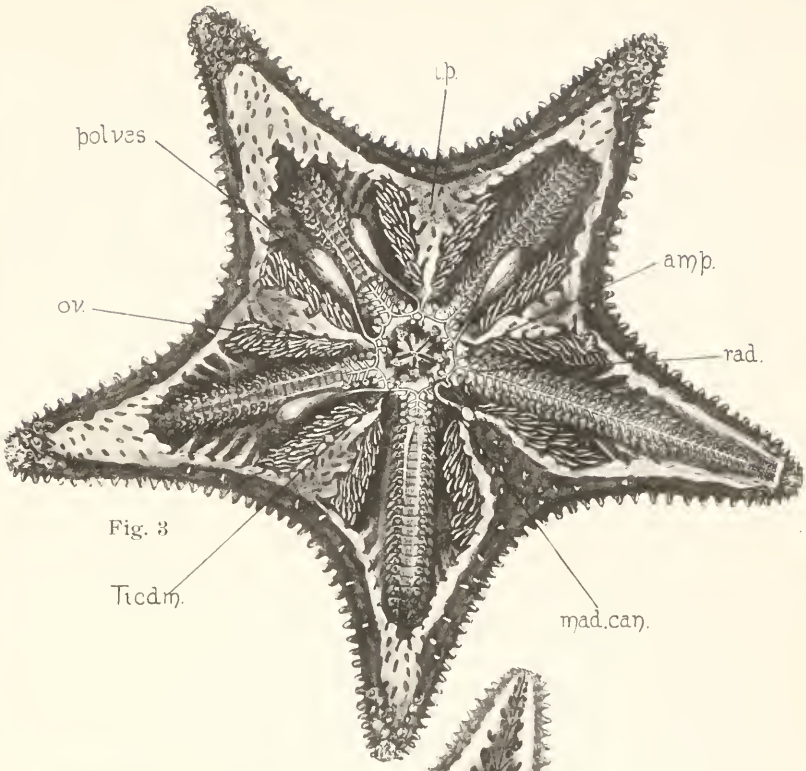

Fig. 3

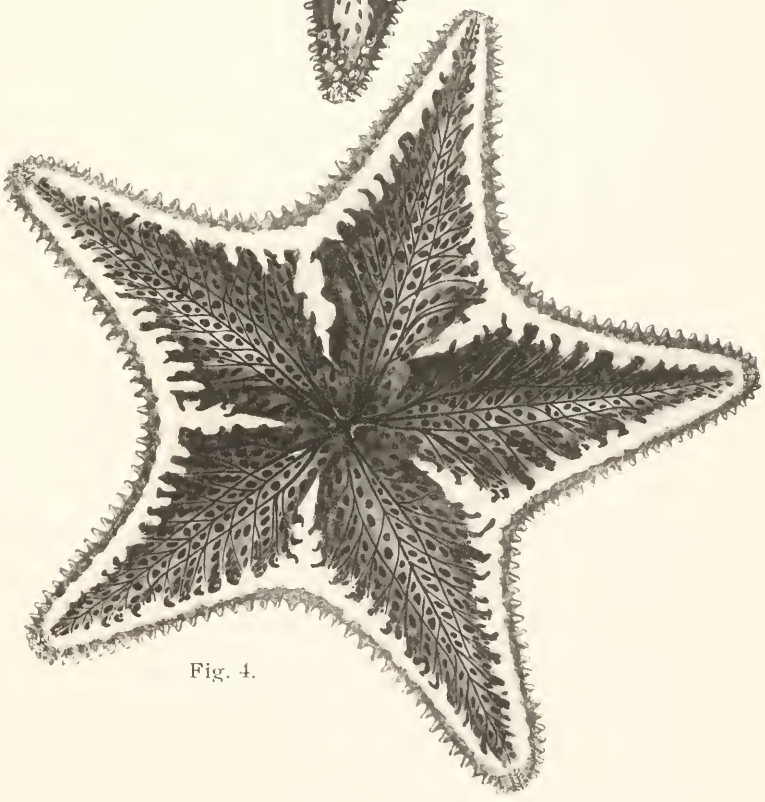

Fig. 4.

V. H. KEILLER DEL.

Pentaceros reticulatus.

3. Dissection to show water- vascular and reproductive systems.
4. Inner suface of aboral body-wall, showing aboral musculature.

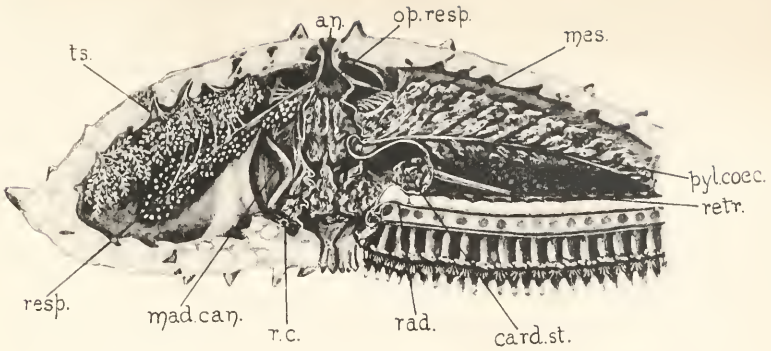

Fig. 5

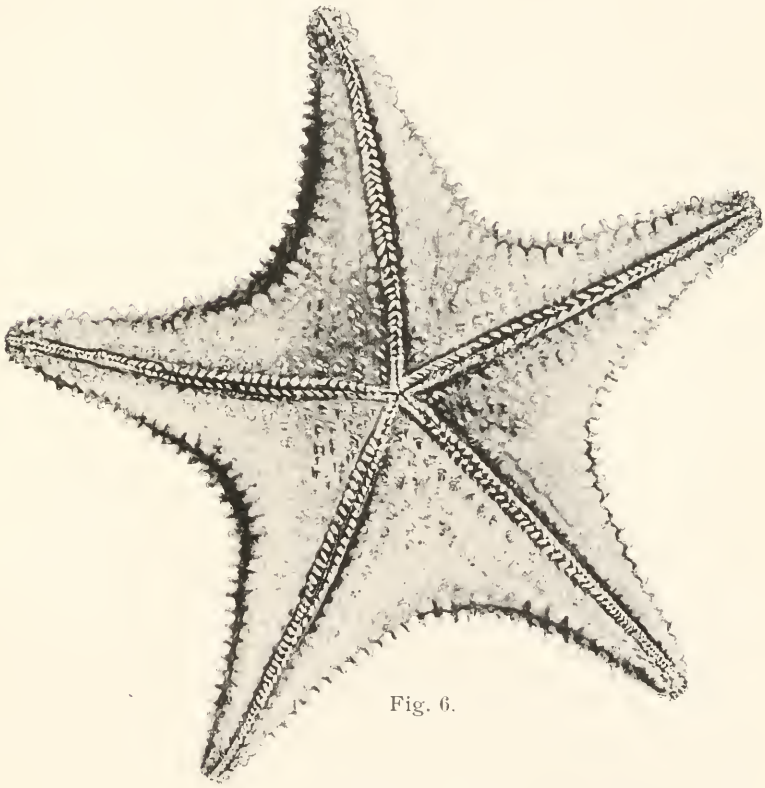

Fig. 6.

V. H. KEILLER DEL.

Pentaceros reticulatus.

5. Section of plane of madreporic interradius.
6. Oral Surface.

XII.

ECHINODERM HYBRIDIZATION.

By DAVID HILT TENNENT,
Bryn Mawr College.

6 plates, 7 text cuts.

ECHINODERM HYBRIDIZATION.

By David Hilt Tennent.

In 1907 I began work in the field of Echinoderm hybridization, primarily with the object of obtaining material for a study of the behavior of the nuclear material in cross-fertilized eggs. Some phases of the subject, which in the beginning occupied a secondary place in my mind, have compelled my attention up to this time. The results of these investigations, although incomplete in many ways, are here presented. The study of the nuclear activities will be completed as rapidly as circumstances permit, the mass of my material for this study now being relatively large.

Those who are at all familiar with this subject realize that a knowledge of facts which can be determined only by cytological studies is absolutely necessary before we can reach a definite conclusion regarding the phenomena which are described and discussed in this paper. My own results (Tennent 1908) and the later and more complete observations of Baltzer (1909 *a* and *b*) and of Herbst (1909) have shown that we may expect much from further investigations.

In 1907 at Beaufort I succeeded in making eight crosses,

1. *Arbacia punctulata* ♀ × *Mellita pentapora* ♂.
2. *Arbacia punctulata* ♀ × *Moira atropos* ♂.
3. *Mellita pentapora* ♀ × *Moira atropos* ♂.
4. *Moira atropos* ♀ × *Arbacia punctulata* ♂.
5. *Moira atropos* ♀ × *Mellita pentapora* ♂.
6. *Moira atropos* ♀ × *Toxopneustes* ♂.
7. *Toxopneustes* ♀ × *Mellita* ♂.
8. *Toxopneustes* ♀ × *Moira* ♂.

In 1908, having realized since the work of the previous summer the absolute necessity of a more complete knowledge of variation occurring in plutei raised under laboratory conditions, I made a study of the development of *Toxopneustes variegatus* from the fertilized egg through the metamorphosis of the pluteus into the adult. The account of that study of variation is now in press and will appear in the Journal of Experimental Zoölogy. During the same summer the study of the *Toxopneustes-Moira* cross was continued and some of the plutei derived from this cross were kept alive and in good condition for 45 days.

I wish to express my thanks to the Hon. George M. Bowers, U. S. Commissioner of Fisheries, for the privilege of working in the Beaufort Laboratory, at which this work was begun, and to Mr. Henry D. Aller, Director of the Laboratory, for many courtesies extended to me. I wish also to express my thanks to the Carnegie Institution of Washington and to Dr. A. G. Mayer, Director of the Marine Laboratory at Tortugas, for the splendid opportunity for continuing my investigations and for assistance in completing the plates which are used in illustrating this article. Most of the figures were drawn from the author's sketches by Mrs. Mary T. Walter or by Mr. K. Morita.

In 1909, at The Marine Laboratory of the Carnegie Institution at Tortugas, the scope of the investigation was widened greatly by the acquisition of new forms and the discovery that two of these forms crossed reciprocally with equal facility. This, I believe, has given me more favorable material than has been obtained by any other investigator. The crosses made during this summer are shown in the following list. The *Hipponoë-Toxopneustes* crosses are of especial interest.

1. *Hipponoë esculenta* ♀ (= *Tripneustes esculentus*) × *Cidaris sp.* ♂.
2. *Hipponoë esculenta* ♀ × *Ophiocoma riisei* ♂.
3. *Hipponoë esculenta* ♀ × *Pentaceros reticulatus* ♂.
4. *Hipponoë esculenta* ♀ × *Toxopneustes* ♂.
5. *Toxopneustes* ♀ × *Echinaster sp.* ♂.
6. *Toxopneustes* ♀ × *Hipponoë* ♂.
7. *Toxopneustes* ♀ × *Holothuria floridana* ♂.

In 1910, at Tortugas, experiments with *Hipponoë* and *Toxopneustes* were repeated and the observations of the previous summer were verified.

This paper embodies that part of my work on cross-fertilization in Echinoderms which pertains to the morphology of the embryos obtained, and to methods of controlling the dominance of maternal and paternal characters.

Work on Echinoderm hybridization has been in the main of four types:

(1) Morphological studies, usually simple descriptions of larvæ obtained from various crosses.

(2) Physiological studies, usually investigations of an analytical nature based on observations of the effect of changes in environment and made in the attempt to account for the resemblance of the offspring to one or the other of the parents.

(3) Cytological studies, researches carried on with the aim of correlating microscopic characters of the hybrid material with those of either or both of the parents.

(4) Chemical studies, which have for their aim the solution of the problem of fertilization in particular and in which the Echinoderm egg is used incidentally because of its favorable nature.

It will be a matter of some surprise, even to those familiar with this work, to note the number and variety of the crosses which have been made. In the list on the following page I have included all that have come to my attention.

It is an interesting fact that in most of these cross-fertilizations it is the echinoid egg that has been fertilized by the sperm of other echinoids and of asteroids, ophiuroids, crinoids, holothuroids and mollusks, while reciprocal inter-class crosses have not been reported. This may or may not be a matter of significance. In my own experience I have found that the eggs of *Pentaceros reticulatus* are not readily fertilizable by the sperm of *Hipponoë*, either after being allowed to stand or after treatment with NaOH. In other experiments, in which I subjected the *Pentaceros* eggs to treatment with CO_2 for from 4 to 10 minutes before

fertilization, I obtained segmentation, but I am not prepared to say whether the segmentation was parthenogenetically produced or was the result of fertilization.

We have sufficient evidence, I believe, to warrant the belief that each egg requires a certain definite environment for fertilization by foreign sperm. That necessary environment has been determined only for the sea-urchin egg.

TABLE OF SUCCESSFUL ECHINODERM CROSS-FERTILIZATIONS.

Arbacia punctulata ♀
× *Mellita pentapora* ♂ (Tennent).
× *Moira atropos* ♂ (Tennent).

Arbacia pustulosa ♀
× *Dorocidaris* ♂ (Vernon).
× *Echinocardium* ♂ (Stassano).
× *Echinus* ♂ (Driesch, Stassano, Vernon).
× *Sphærechinus* ♂ (Driesch, Hertwig, Stassano).
× *Strongylocentrotus* ♂ (Driesch, Hertwig, Vernon).

Asteracanthion berylinus ♀
× *Asteracanthion pallidus* ♂ (Agassiz).

Asterias forbesii ♀
× *Arbacia pustulata* ♂ (Morgan).

Dorocidaris ♀
× *Strongylocentrotus* ♂ (Vernon).

Echinocardium cordatum ♀
× *Arbacia pustulosa* ♂ (Stassano, Vernon).
× *Echinus* ♂ (Vernon).
× *Sphærechinus* ♂ (Stassano, Vernon).
× *Strongylocentrotus* ♂ (Vernon).

Echinocardium mediterraneum ♀
× *Echinus* ♂ (Vernon).
× *Sphærechinus* ♂ (Vernon).
× *Strongylocentrotus* ♂ (Vernon).

Echinus acutus ♀
× *Arbacia* ♂ (Vernon).
× *Sphærechinus* ♂ (Vernon).

Echinus microtuberculatus ♀
× *Arbacia* ♂ (Driesch, Vernon).
× *Echinocardium* ♂ (Stassano, Vernon).
× *Echinus acutus* ♂ (Vernon).
× *Strongylocentrotus* ♂ (Driesch, Hertwig, Vernon).
× *Sphærechinus* ♂ (Driesch, Morgan, Vernon).

Hipponoë ♀ (= *Tripneustes*).
× *Cidaris* ♂ (Tennent).
× *Ophiocoma* ♂ (Tennent).
× *Pentaceros* ♂ (Tennent).
× *Toxopneustes* ♂ (Tennent).

Mellita ♀
× *Moira* ♂ (Tennent).

Moira ♀
× *Arbacia* ♂ (Tennent).
× *Mellita* ♂ (Tennent).
× *Toxopneustes* ♂ (Tennent).

Psammechinus miliaris ♀
× *Asterias rubens* ♂ (Giard).

Psammechinus (pulchellus) ♀
× *Spatangus* ♂ (Köhler).
× *Sphærechinus* ♂ (Köhler).
× *Strongylocentrotus* ♂ (Köhler).

Spatangus ♀
× *Strongylocentrotus* ♂ (Köhler).
× *Psammechinus* ♂ (Köhler).

Sphærechinus ♀
× *Echinus* ♂ (Driesch, Hertwig, Morgan, Vernon).
× *Psammechinus* ♂ (Stassano).
× *Strongylocentrotus* ♂ (Driesch, Hertwig, Marion, Morgan, Steinbrück, Vernon).
× *Antedon* ♂ (Godlewski).

Strongylocentrotus lividus ♀
× *Arbacia* ♂ (Driesch, Vernon).
× *Dorocidaris* ♂ (Köhler, Vernon).
× *Echinus* ♂ (Driesch, Vernon).
× *Psammechinus* ♂ (Köhler).
× *Spatangus* ♂ (Köhler).
× *Sphærechinus* ♂ (Hertwig, Köhler, Morgan, Vernon).

Strongylocentrotus purpuratus ♀
× *Asterias capitata* ♂ (Loeb).
× *Asterias ochracea* ♂ (Hagedoorn, Loeb).
× *Asterina* ♂ (Loeb).
× *Chlorostoma* ♂ (Loeb).
× *Mytilus* ♂ (Kupelwieser).
× *Pycnopodia* ♂ (Loeb).

Toxopneustes ♀
× *Echinaster* ♂ (Tennent).
× *Hipponoë* ♂ (Tennent).
× *Holothuria floridana* ♂ (Tennent).
× *Mellita* ♂ (Tennent).
× *Moira* ♂ (Tennent).

The failure to make a thorough attempt at the determination of the necessary conditions for fertilization by foreign sperm has been due, in my own case, to the fact that it seemed of advantage to make immediate use of the crosses that were readily made and to delay until some future time the investigation of methods necessary for making other crosses.

METHODS OF TREATING ECHINOID EGGS BEFORE FERTILIZATION.

The methods for increasing the number of fertilized eggs in hybrid cultures which have been most employed are: (1) Treatment with alkalis. (2) Treatment with fresh water. (3) Subjection to increased temperature. (4) Excess of sperm. (5) Allowing eggs to stand.

The method of Loeb has been most used, *i.e.*, treatment of the eggs with an alkali before fertilization. This method has been used with signal success by Herbst and Godlewski.

Herbst (1906, p. 183) obtained his best results by adding between one and two drops $\frac{n}{10}$ NaOH to 20 c.c. sea-water, and further pointed out that the optimum concentration is an individual affair.

Doncaster (1903) found that the percentage of fertilizations was increased in diluted sea-water. Herbst (1906) also tried the effect of fresh water, allowing the eggs to remain in fresh water 1 to 3 minutes. Some of the eggs were destroyed, but most remained unharmed by the treatment. As to the value of this method when compared with others mentioned, he does not feel prepared to decide.

Herbst's (1906*a*) experiments on the influence of warmth are of interest. In general it seemed that the optimum was about 24° C.

Born's method of excess of sperm is of somewhat doubtful value when used for Echinoderm crosses. With this method polyspermy is the usual result.

The method used by the Hertwigs (1885), of allowing the eggs to stand for some time before fertilization, has been used by several investigators. Vernon was at first inclined to regard it as a most useful method, but later came to look upon it as of somewhat doubtful value, especially when one desired older larvæ. Driesch also does not regard the method favorably. In my own work it is the method that I have employed almost exclusively and it has given me exceptionally good results. I have found that there is an individual or more properly a species optimum for the length of time that the eggs should stand. For example, with the eggs of *Arbacia*, *Hipponoë*, and *Toxopneustes*, with which most of my work has been done, three different periods of time were allowed to elapse before the eggs were fertilized.

The *Arbacia* eggs were fertilized with *Moira* sperm 7 hours after their removal from the ovary. The *Hipponoë* eggs were best fertilized with *Toxopneustes* sperm $2\frac{1}{2}$ hours after removal from the ovary. The *Toxopneustes* eggs were best fertilized with *Hipponoë* sperm 6 hours and with *Moira* sperm (at Beaufort) 5 hours after removal from the ovary.

I have tried other methods which will be mentioned in other parts of this paper, but never with the success that followed that of allowing the eggs to stand. The percentage of fertilization following this treatment in the *Arbacia*, *Hipponoë*, and *Toxopneustes* crosses was from 75 to 95 per cent.

In another paper (Tennent 1910*a*) I have described in detail the methods that were employed in my work at Beaufort. Similar care was exercised in the work at Tortugas in order to avoid results which might be due to careless laboratory manipulation. All dishes and instruments used were sterilized and especial care to avoid chance fertilization was taken.

AIM OF THE INVESTIGATION.

The aim of the research is the formulation of a statement of the conditions governing the resemblance of the offspring to the parent.

Herbst (1906 *a*, p. 173) has stated the purpose of his investigations simply when he asks the question, "Why do the offspring sometimes stand as a mean between the two parents; why do they sometimes incline more to one or more to the other; or again, why do they resemble one parent completely or almost completely while the characters of the other are repressed?"

We are acquainted to some extent with three types of heredity: (1) Blended heredity. (2) Particulate or Mosaic heredity. (3) Alternative heredity. In the vast amount of research that is being done to-day we lose sight of the fact that this is simply an artificial distinction and that the actual aim of studies of this kind is not the elucidation of one type in particular, but the determination of a "law" of heredity, a statement of the phenomena of inheritance that will be broad enough to include all types.

The study of Echinoderm crosses impresses the fact of the actual singleness of type upon one with peculiar force. In the embryos of one cross we may find all three "types" of heredity exhibited, and yet, so far as we can determine, these embryos have been subjected to the same environment.

How far-reaching this diversity of form may be is impossible to say, for we may make this statement only with respect to the larvæ. No one has yet been able to carry hybrid embryos through their metamorphosis to the adult condition, not to mention the absolutely necessary further step of obtaining individuals of a second generation. This will be a difficult, but probably not an impossible achievement, which will require for its completion the opportunity for working continuously at a marine laboratory for some years.

SURVEY OF OUR PRESENT KNOWLEDGE OF ECHINODERM CROSSES.

The primary stimulus to the investigation of Echinoderm crosses was given by Boveri (1889, 1895) in his effort to determine the localization of the force directing heredity, *i.e.*, to determine whether this force is resident in the nucleus or in the cytoplasm. The Hertwigs had previously seen the entrance of the sperm into enucleated egg fragments and had expressed the opinion that probably nothing would come from such fragments.

Boveri (1895) made the cross between *Echinus microtuberculatus* ♂ and *Sphærechinus granularis* ♀ and obtained an intermediate form (text

figs. 1, 2, 3, and 4). He also fertilized fragments of *Sphærechinus* eggs with *Echinus* sperm and obtained hybrids of three kinds: (1) Hybrids of the same size as the ordinary larvæ but intermediate in form. (2) Dwarf larvæ of mixed form. (3) Dwarf larvæ of pure *Echinus* type.

Boveri concluded that the dwarf larvæ of pure *Echinus* type had been derived from the fertilization of enucleated *Sphærechinus* egg fragments and that the experiment showed the lack of cytoplasmic influence.

Seeliger (1894) and Morgan (1895) criticized this conclusion, Seeliger showing that in the *Sphærechinus* ♀ × *Echinus* ♂ cross all of the larvæ were not of an intermediate but that some were of the paternal type,

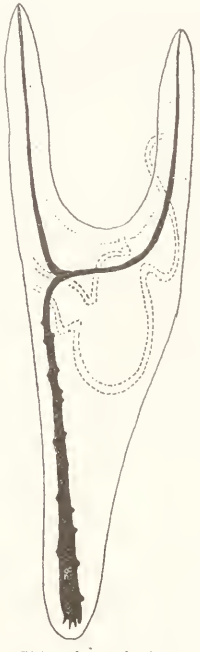

FIG. 1.—Side view of pluteus of *Echinus microtuberculatus* (Boveri).

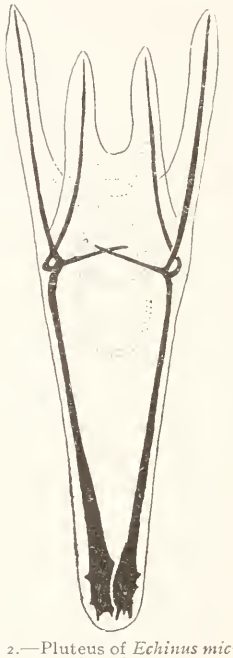

FIG. 2.—Pluteus of *Echinus microtuberculatus* (Boveri).

and that in Boveri's experiments dwarf larvæ of the *Echinus* type might have been derived from nucleated fragments. Morgan found that among the crossed larvæ a large percentage showed the pure *Echinus* form. Steinbrück (1902), in his study of *Sphærechinus* ♀ × *Strongylocentrotus* ♂ crosses, concluded that hybrids are not always of a mid-form, but show an extraordinary variability and exhibit a complete series between paternal and maternal form. In my own investigations, in certain circumstances, I have found a complete series and I have found that it is possible, by changing the environment, to increase or decrease the percentage of larvæ resembling one or the other parent.

To Boveri himself, while admitting the validity of the criticisms of his original proposition, must be given the credit of furnishing us with a rational interpretation of these variations, based on cytological

studies (Zellen-Studien 5 and 6), and it is on the basis of this interpretation that the more recent work of Herbst, Baltzer, and others is founded.

The beginning of a second phase of work in Echinoderm hybridization was marked by the appearance of Vernon's papers. As a preparation for his work on hybridization, Vernon (1895) made a careful study of the effect of environment on the development of Echinoderm larvæ, using *Strongylocentrotus lividus* as the subject of his research. As means of changing the environment he used (1) Differences in temperature, (2) Differences in concentration of sea-water, (3) Differences in light, (4) Chemical agents.

Strongylocentrotus eggs were placed in water of 8° or 25° C. for an hour, or even for a minute, at the time of impregnation. After 8 days the resulting plutei were 4.4 per cent smaller than those from eggs

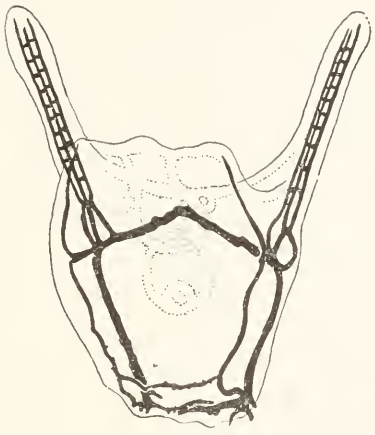

FIG. 3.—Pluteus of *Sphærechinus granularis* (Boveri).

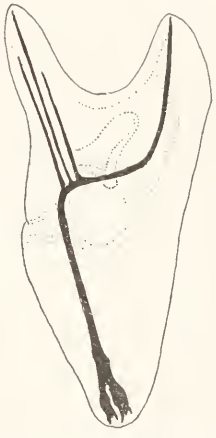

FIG. 4.—Hybrid pluteus *Echinus microtuberculatus* ♂ × *Sphærechinus granularis* ♀ (Boveri).

fertilized at 17° to 22° C. Larvæ allowed to develop in water 17° to 22° C. were 2 per cent or more larger than those allowed to develop at temperatures above or below these limits.

The normal breeding season of *Strongylocentrotus* is from December to March. Larvæ from fertilizations made in August were 20 per cent smaller than those obtained in April, May, or October. June and July larvæ were intermediate in size—this due to immaturity.

The addition of 50 c.c. distilled water to a liter of sea-water gave larvæ 15.6 per cent larger than larvæ grown under normal conditions; 25 c.c. distilled water to a liter of sea-water, 9.5 per cent larger; 150 c.c. distilled water to a liter of sea-water, 4.3 per cent smaller. Larvæ developed in more concentrated sea-water were unchanged; larvæ grown under normal conditions from impregnations made in concentrated sea-water were 1.6 per cent larger. Larvæ grown in semi-darkness were 2.5 per cent larger; in darkness 1.3 per cent smaller; in blue light (copper sulphate), 4.5 per cent smaller; in violet blue light (Lyons blue),

7.4 per cent smaller; green light, 4.8 per cent smaller; red light, 6.9 per cent smaller; yellow light, 8.9 per cent smaller.

The body length was uninfluenced by the number of larvæ in a given volume of sea-water, provided this number be kept below 30,000 per liter. It was also uninfluenced by the amount of sperm added on impregnation. Larvæ grown in water containing an additional amount of carbonic acid were slightly larger than normal.

The larvæ were not much influenced by partial de-aëration or by oxygenation of the water in which they were developing.

The aboral and oral arms reached their maximum length after 8 days' development, after which they underwent absorption. The body length increased regularly up to the sixteenth day. The arms of larvæ impregnated at 8° were 8 per cent shorter than normal; at 25° about 2.5 per cent shorter. In larvæ developed above 22° the aboral and oral arms were respectively 10.8 per cent and 8.5 per cent longer than those developed at 18° to 20°. The body length of larvæ developed in diluted water was increased on an average by 9.1 per cent, while the arm lengths were increased by 7.7 per cent and 10.5 per cent. The absolute arm lengths were not affected. Arm lengths in darkness, semi-darkness, green and violet lights were 10 per cent or more shorter than those grown under normal conditions.

The variability of larvæ with respect to body lengths declined after the fifth day. The variability reached a maximum at 18° to 20°, the temperature most favorable to development.

In this work Vernon notes the common occurrence of multiple rods in the anal arms, this variation sometimes reaching 35 per cent.

Vernon (1898) determined the specificity of reaction to temperature in *Strongylocentrotus*, *Sphærechinus*, and *Echinus:* The *Strongylocentrotus* pluteus body was largest at 23.7°; the *Sphærechinus* pluteus body at 15.9°; the *Echinus* pluteus body at 20.4°.

Steinbrück's (1902) study of *Strongylocentrotus* showed that the occurrence of multiple rods in the anal arms is a common variation. In a study of *Toxopneustes* (Tennent 1910) I have shown in the purely bred larvæ a type or line variation and the tendency of the eggs of a given female to vary as a whole in some direction. Hagedoorn (1909) has shown the common occurrence of similar variations in the skeleton of *Strongylocentrotus purpuratus.*

Herbert's (1906) research on purely bred larvæ seems to have escaped the attention of most investigators. He subjected embryos *Echinus Sphærechinus* and *Strongylocentrotus* to temperature changes.

For *Echinus* he notes with increased temperature: (1) A striking increase in the number of multiple rods. (2) The average body length at 24° to 25.75° is less than at lower temperature. (3) The average arm length is longer than at lower temperatures. (4) In frequent cases the beginning of lattice formation.

For *Sphærechinus* he notes with increased temperature: (1) An increase of multiple rods. The number of rods may be increased one or two and in infrequent cases from three to six; but the number of rods which reach entirely to the end of the arm is raised only about one.

(2) The average body length at 13° to 14° is greater than at 24° to 26°. (3) The rods are longer than in the cold temperatures. (4) That the number of cross connections in the anal rods increases.

For *Strongylocentrotus* he notes with increased temperature: (1) Plutei at 26° show an increase of multiple rods. (2) The average body length at 24° to 25° is somewhat greater than at 13° to 14°. (3) In infrequent cases the beginning of lattice formation.

SUMMARY OF THE RESEARCHES ON PURE FORMS.

Summary of Vernon's observations.

(1) Multiple anal arm rods sometimes appear in *Strongylocentrotus*.
(2) The optimum temperature for fertilization, 17° to 22° C.
(3) The optimum temperature for growth, 17° to 22° C.
(4) The optimum temperature for arm growth, 17° to 22° C.
(5) The optimum light for growth is ordinary diffuse daylight.
(6) There is a species temperature optimum for *Strongylocentrotus*, *Sphærechinus*, and *Echinus*.

Summary of Herbst's observations.

(1) Increased temperature gives an increase in multiple rods.
(2) There is a species temperature optimum for body size.
(3) In *Strongylocentrotus* and *Echinus*, in a small percentage of cases, lattice formation is caused by increased temperature.

The outcome of these researches has been to show that some of the varieties of skeletal structure found in hybrids may occur as normal variations in purely bred larvæ, and further that such variation may be induced by a change in the external conditions, notably by changes in temperature.

After considering the cross-fertilization results I shall refer again to these observations on purely bred forms, when their importance will be evident.

OBSERVATION ON CROSSES.

Vernon (1898, 1900) working with *Arbacia*, *Dorocidaris*, *Echinocardium cordatum*, *Echinocardium mediterraneum*, *Echinus acutus*, *Echinus microtuberculatus*, *Sphærechinus*, and *Strongylocentrotus*, out of 64 possible direct and cross-fertilizations tried 49. Of these, 29 gave plutei of 8 days' growth, 9 gave segmenting ova, blastulæ, or gastrulæ, and 11 gave no sign of cross-fertilization whatever.

It is in a measure unfortunate that the fine observations of Vernon's earlier paper should be based on body proportions alone. This situation is to some extent relieved by the supplementary and the new observations on the skeletal structures described in the later paper.

Perhaps the most important of Vernon's results was the determination of a seasonal variation in the character of the plutei obtained from the crosses. In general, with *Echinus-Arbacia*, *Echinus-Strongylocentrotus*, *Sphærechinus-Echinus*, and *Sphærechinus-Strongylocentrotus*, *i.e.*, with the most successful crosses, the summer larvæ were of the maternal type, while the autumn and winter larvæ were of the paternal

type. Vernon concluded that this variation was due to the relative maturity of eggs and sperm.

Besides this major conclusion, two minor conclusions are of importance:

(1) Respecting reciprocal crosses; 9 out of 13 possible reciprocal crosses were attempted, from which, plutei were obtained from both crosses in 7 instances. Vernon concluded that "The capacity for reciprocal crossing seems, therefore, to be the rule rather than the exception."

(2) Respecting the Hertwigs' conclusion with regard to cross-fertilization and the staleness of the eggs, he confirmed the observations as to fertilization itself, but decided that there was a less tendency for such stale eggs to develop to plutei than for eggs fertilized in a fresh condition.

Herbst (1906, 1907) had little faith in the idea of a greater or lesser ripeness of reproductive elements and sought for another controlling factor. "Das ist aber für unsern Zweck ein erfreuliches Resultat, denn jetzt ist die Möglichkeit vorhanden, dass wir an Stelle des dunklen innern Faktors der verschiedenen Reife einen scharf präzisierbaren äusseren in die Hand bekommen können, von dem die Ausgestaltung der Bastarde abhängig ist," expresses his attitude towards the question. He suggested, as possible means of control, change in temperature, change in salt-content, or change in concentration of OH ions, the last of which might be connected with the presence of larger or smaller quantities of algæ or connected with the reserve-stuff content of the eggs, which would be dependent on variation in the interaction of sea-urchin and environment.

Herbst selected the skeleton as the character for study. In his investigations with *Sphærechinus* ♀ × *Echinus* ♂ and *Sphærechinus* ♀ × *Strongylocentrotus* ♂ he considered in detail the effect of changes in temperature on the character of the skeletal rods of the anal arms and on the number of "roots" of anal arm supports, defining these roots as any outgrowth from the horizontal part of the oral bar, anal crossbar, or body-skeleton directed into the anal arm.

For the purpose of comparing Herbst's results with my own I include parts of four of Herbst's tables, Nos. III, VI, X, and XII (1906, pages 192, 203, 225, 230).

Herbst's Table III $\frac{\text{Str. } ♂}{\text{Sph. } ♀}$	Temp. 11° to 19° C.	Temp. 24° to 27° C.
Number of plutei with lattice structure	19	37
Number of arms with lattice structure	24	53
Number of arms with multiple bars	28	5

Herbst's Table VI, $\frac{\text{Str. } ♂}{\text{Sph. } ♀}$	Temp. 11° to 19° C.	Temp. 24° to 27½° C.
Anal arm rods with 1 root	35	10
Anal arm rods without lattice str.	24	5
Anal arm rods with 2 roots	54	54
Anal arm rods with 3 roots	10	27
Anal arm rods with 4 roots	0.6	9

Herbst's Table X, $\frac{\text{Ech. } ♂}{\text{Sph. } ♀}$	Temp. 11° to 19° C.	Temp. 23° to 29° C.
Number of plutei with lattice structure	12	37
Number of arms with lattice structure	19	54
Number of arms with multiple bars	28	15

Herbst's Table XII, $\frac{\text{Ech.}\,♂}{\text{Sph.}\,♀}$	Temp. 11° to 19° C.	Temp. 23° to 29° C.
Anal arm rods with 1 root	35	6
Anal arm rods without lattice structure	25	3
Anal arm rods with 2 roots	54	30
Anal arm rods with 3 roots	9	38
Anal arm rods with 4 roots	1	11
Anal arm rods with 5 roots	0	7
Anal arm rods with 6 to 7 roots	0	2

It will be seen at once that in temperatures of 23° to 29° C. there is a greater number of plutei and a greater number of arms with lattice structure than in temperatures below 20° C. Similarly the influence of higher temperatures in increasing the number of roots will be noted.

We have already seen the influence of temperature on pure forms. We may now interpret the pure form and cross-fertilization results.

1. *Sphærechinus* ♀ × *Strongylocentrotus* ♂ and *Sphærechinus* ♀ × *Echinus* ♂, through abundance of beginnings of lattice formation and greater number of crossbars, show in the warmth, on an average, more resemblance to the mother than to the father.

2. We can not say that warmth causes this, since in pure *Sphærechinus* larvæ also the number of crossbars is raised in the warmth.

3. Similarly for the anal arm roots, *Sphærechinus* ♀ × *Strongylocentrotus* ♂ warmth plutei are more like the mother than are the cold plutei. We can not make a similar assertion for the *Sphærechinus* ♀ × *Echinus* ♂ plutei.

4. In both combinations it is impossible for us to speak with respect to the number of arm-bar roots, of a proportionally strong resemblance to the mother.

5. Plutei of the *Sphærechinus* ♀ × *Strongylocentrotus* ♂ have body proportions like the mother, but we can not speak of a stronger appearance of maternal body proportions in the higher than in the lower temperatures. With the *Sphærechinus* ♀ × *Echinus* ♂ combination we can not assert even the first.

6. The average body length of the *Sphærechinus* ♀ × *Strongylocentrotus* ♂ hybrids is swung more toward the maternal side in the warmer than in the colder temperatures.

7. We have recognized a second example of such oscillation in the combination *Sphærechinus* ♀ × *Echinus* ♂, where the maternal characters, with respect to body length, become prominent in the cold.

8. In the pairing of sea-urchins with one like character, there may appear in the descendants a weakening of this character.

Herbst's conclusions regarding the influence of temperature at different times are of importance:

1. In order to obtain an increase in fenestrated rods it is immaterial whether we expose the unfertilized eggs, or the blastulæ without mesenchyme, to the higher temperature.

2. It is not sufficient for increasing the number of larvæ with lattice structure, if we expose the germs only temporarily to the warmer temperature and then carry them back. The higher temperature must be

applied throughout the gastrula stage if the number of rods with latticed structure is to be increased.

3. The number of anal arm supports with more than one root is already determined by the gastrula stage, before the triradiate spicules have given rise to rods. In the cultures transferred to warmth at the gastrula stage, there is no such increase in number of arm supports with three or four roots as in warm cultures in which the larvæ remain to the completed pluteus.

Doncaster (1903) concluded that the different hybrids owe their peculiarities to the temperature of the water in which they developed. Herbst's conclusion (1906) is that Vernon's seasonal variation is partly dependent on temperature. Besides this, another unknown factor has played a rôle, this factor varying not only during the time of year but also varying in the two years. Herbst feels himself forced upon the idea of a variation of hybrid form which is certainly not connected with the direct influence of temperature in the formation of the larvæ.

THE CONTROL OF DOMINANCE.

Herbst (1906 *b*) found a method of controlling the appearance of maternal characters in the combination of parthenogenesis and fertilization. By subjecting the eggs of *Sphærechinus* to treatment which would cause their parthenogenetic development and fertilizing them with *Strongylocentrotus* sperm before the nucleus had fairly begun its processes of division, Herbst obtained a displacement of heredity to the maternal side. The method of treatment was much the same as that which I used in a study of the star-fish egg (1906), a method which has also been used by Fischel and by Loeb (1907). The effect of the treatment is shown in the following table; the displacement toward the maternal side (*Sphærechinus*) being well marked.

(Herbst, (1906 *b*), Table I, page 482. Anal arm rods, *Sphærechinus* ♀ × *Strongylocentrotus* ♂. Number of plutei studied in each case 50.)

Treatment of eggs before fertilization.	Condition of nucleus at time of fertilization.	Number of plutei with lattice structure.	Anal arm rods with lattice structure.	Partially *Sphærechinus* rods.	Perfect *Sphærechinus* rods.	Typical *Sphærechinus* plutei.
1. Untreated	Unchanged.	29	38	0	0	0
2. Eight minutes in sea-water + acetic acid.	Indistinct, with halo.	45	82	9	9	0
3. Five minutes in sea-water + acetic acid.	Some indistinct. Some distinct. Nuclei larger than normal.	48	90	9	33	8

Herbst suggested that this displacement might be caused by—

(1) The growth of the maternal nuclear substance.
(2) An alteration in the condition of the cytoplasm.
(3) Or both factors together may influence the displacement.

Pursuing the subject further (1907), he determined the critical stage of displacement. A striking shift takes place when the nucleus at the

moment of fertilization has become enlarged, although it need not have reached its maximum size. The most favorable moment for the displacement of the course of heredity coincides with the stage of parthenogenetic development in which the egg nucleus has reached its greatest volume, so that (in part of the eggs) even a giving up of nuclear sap to the cytoplasm may have taken place. If the eggs are fertilized at this moment, resemblance to the mother follows. We may term this the high point for displacement.

Boveri, in his Zellen-Studien 5 and 6, has given us a nomenclature for certain phenomena, which will be found useful:

(1) The pronucleus in the egg = a hemikaryon.
(2) The egg nucleus = a thelykaryon.
(3) The sperm nucleus = an arrhenokaryon.

Thus, all nuclei which arise from isolated egg or sperm nuclei are hemikaryons. The first cleavage nucleus and its descendants are amphikaryons. Through reduction in oögenesis and spermatogenesis hemikaryons arise from the amphikaryons. A normal embryo arising from the fertilized egg is amphikaryotic; one from a fertilized egg without an egg nucleus, *i.e.*, from an enucleated egg, is arrhenokaryotic; one arising from an artificially parthenogenetic egg is thelykaryotic. The two latter are in the same sense hemikaryotic. So we may speak of amphikaryosis, hemikaryosis, etc. If the chromosomes of the first cleavage nucleus have doubled without nuclear division we have a diplokaryon and a diplokaryotic organism. Organisms which have in one region normal nuclei, in another abnormal, containing only the derivatives of an egg nucleus or of a sperm nucleus, are respectively partially-thelykaryotic or partially-arrhenokaryotic.

In Zellen-Studien 6 the nomenclature is slightly changed:

1. Instead of hemikaryon we have monokaryon.
2. A dikaryon or amphikaryon, as before.
3. A trikaryon = one egg nucleus + two sperm nuclei.
4. A diplokaryon = tetrakaryon contains four times the elements of the monokaryon.

Boveri had shown (Zellen-Studien 5) that the surface of the nuclei of the somatic cells of the sea-urchin is directly proportional to the number of chromosomes in the developing egg. Marcus (1906) had shown the relation between temperature and nuclear size. With this knowledge, Herbst proceeded to ascertain the fate of the sperm nuclei in hybrids with altered heredity. He reasoned that if one compared nuclei of parthenogenetic larvæ with those of normal larvæ and with those of larvæ having had the double treatment, the earlier nuclei of the former, having half as many chromosomes, should have half as great a nuclear surface as those of normally fertilized eggs, and therefore the same should be true when compared with the nuclei of doubly treated eggs. The matter, however, was not so simple. The study of the nuclei

of the ciliated bands and the body parts of 28 parthenogenetic larvæ gave no half nuclei. The nuclei were either of normal size or were above normal size. This leads to the conclusion that there must have been many cases of monaster formation.

Notwithstanding this complication, the comparison of the nuclear size of purely parthenogenetic larvæ with those of hybrids with displaced heredity proved of value. Herbst succeeded in fitting the larvæ that he obtained to the types suggested by Boveri and in demonstrating the actual participation of the sperm nucleus in the activities of fertilization and of segmentation.

Herbst first made a study of the nuclear size of parthenogenetic larvæ. He found:

1. Diplothelykaryotic larvæ. This was the most numerous form. They may have arisen from a single monaster formation of a Monokaryon (hemikaryon).

2. Tetrathelykaryotic larvæ. These may have arisen by two monaster divisions; they have very large nuclei.

3. Larvæ with nuclei intermediate in size between (1) and (2). These may have arisen,

(*a*) From eggs of over-normal nuclei. These nuclei may have arisen from one monaster division; they would be diplothelykaryotic.

(*b*) From eggs of under-normal nuclei. These nuclei may have arisen from two monaster divisions. They would be tetrathelykaryotic.

(*c*) From eggs of normal nuclei. These nuclei may have arisen from two monaster divisions, but in this monaster formation some of the chromosomes must have remained undivided.

Proof of the copulation of egg and sperm nuclei was drawn from the occurrence of partially-thelykaryotic larvæ. In such larvæ (see Herbst 1907, figs. 3 and 4) the skeleton of the anal arms is of the *Sphærechinus* type on one side and of the hybrid type on the other. This condition may be due to the fact that the chromatic matter of the maternal and paternal nuclei separated in the first division. A slight difference between the two sides may be due to the influence of cytoplasm given off by the male nucleus. Some indication of paternal influence may be seen even in the maternal half. On the hybrid side of the pluteus, large nuclei are found; on the maternal side small nuclei.

A comparison of other larvæ of the cultures was made with these partially-thelykaryotic larvæ, *i.e.*, a comparison of nuclei was made. Herbst reasoned that if he found larvæ whose nuclei corresponded in size with those of the hybrid side of the partially-thelykaryotic larvæ, he would be at liberty to conclude that in these cases a copulation of the two sex nuclei had taken place. If he found, on the contrary, small nuclei, he must conclude that in these the copulation had not taken place and that he had hemikaryotic plutei.

(1) Hybrids with large nuclei; maternally directed heredity (Herbst's figs. 6 and 7): These larvæ were of a pronounced maternal type. Their nuclei are large, like those of the hybrid half of partially-thelykaryotic larvæ.

(2) Hybrids with small nuclei; from cultures with maternally directed heredity: Out of 79 larvæ, there were 11 with small nuclei. A comparison with other nuclei shows that these are typical half nuclei. We may distinguish two types of plutei of this class:

(*a*) Plutei of the maternal type with small nuclei: A pluteus of this type is shown in Herbst's (1907) fig. 10. Here only the female nuclei have taken part in the development. The probable explanation of this condition is that it is a case of partial fertilization and subsequent separation of the two nuclei. In this event it must be acknowledged that the sperm has been of some influence before its elimination.

(*b*) Plutei of the paternal type with small nuclei: A pluteus of this type is shown in Herbst's (1907) fig. 14. This is a typical arrhenokaryotic pluteus, but shows evidences of its hybrid origin. The nuclei must be designated as half nuclei. When regarded in the light of Boveri's (1889, 1895) suggestion, to which we have already referred, it will be seen that we have here larvæ with only paternal nuclear material which shows hybrid characters. These larvæ are more properly partially-arrhenokaryotic plutei. They have arisen from multiple fertilization.

Displacement of heredity toward the maternal side occurs in many ways. It is not essential that the processes of fertilization be modified from the beginning. In some cases it is a delay of fertilization, *i.e.*, of copulation of egg and sperm nucleus, that is responsible for the displacement. This delay may have various consequences:

(1) It may lead to a failure of the sex nuclei to unite and in some way to an elimination of the sperm. Thus half nuclei may originate and give us wholly or nearly pure *Sphærechinus* plutei in the cultures with maternally directed heredity.

(2) It may lead to copulation after the egg nucleus has completed the first steps of division. According as this is a dyaster or a monaster, we may have two subdivisions;

(*a*) The sperm nucleus may copulate with one of the two cleavage nuclei. From such eggs partially-thelykaryotic larvæ may arise. These larvæ are found infrequently.

(*b*) The sperm nucleus may copulate with the egg nucleus after the latter has reached a double size through monaster formation. Monasters are common, so this case is not infrequent. It is to be expected when a halo is present around the nucleus at the moment of fertilization. There are probably other possibilities in methods of displacement. Herbst believes that the cause of displacement lies in an altered ratio of egg nuclear and sperm nuclear size. If the egg nuclear substance be in excess, then there will be a preponderance of maternal characters, while if the sperm nuclear substance be in excess there will be a preponderance of paternal characters in the descendants.

Because of the great importance of this (1907) paper I give a brief summary of Herbst's conclusions.

(1) The critical stage for displacement occurs when the egg nucleus at the moment of fertilization has shown a distinct increase in size.

(2) There is a difference in intensity of displacement corresponding with the stage at which fertilization takes place; the maximum displacement coinciding with the greatest expansion of the egg nucleus before its solution. After the passing of the maximum size there is a gradual falling of displacement, but not to zero.

(3) Larvæ with maternally directed heredity, as a rule, have larger nuclei than the ordinary hybrids of the same culture.

(4) The hybrids with nuclei larger than the normal may have arisen by copulation of a diplothelykaryon and an arrhenokaryon.

(5) Partially-thelykaryotic larvæ may arise when the sperm nucleus copulates with one of the daughter nuclei succeeding dyaster formation.

(6) Hybrids of the maternal type with small nuclei may have arisen because of the elimination of male nuclear material.

(7) Hybrids of the paternal type with small nuclei are probably arrhenokaryotic, the maternal nuclear material having been eliminated.

(8) Hybrids may be partially arrhenokaryotic in character.

(9) Displacement to the maternal side may be caused in various ways.

(10) The course of heredity may depend on relative proportion of female nuclear mass.

NEW INVESTIGATIONS.

There is, as is well known, a characteristic period of time between fertilization and the appearance of the first cleavage furrow. I bring these statistics together in this place for the sake of comparison. Fertilizations were made at temperatures varying between 26° and 31° C. *Arbacia*, 40 to 47 minutes; *Hipponoë*, 60 to 75 minutes; *Mellita*, 30 to 60 minutes, usually 35 minutes; *Moira*, 35 minutes; *Toxopneustes*, 37 to 45 minutes. These variations are not altogether correlated with differences in temperature.

DESCRIPTION OF NORMAL PLUTEI.

One is much impressed by the more rapid earlier development of the forms considered in this paper than of the forms described by European investigators. The following outline of the normal development of one series of *Toxopneustes* eggs may be of interest.

(Fertilization made at 12^h40^m p. m. Temperature of water, 28° C.)

First cleavage	1^h20^m	meridional	2 cells.
Second cleavage	1^h50^m	meridional	4 cells.
Third cleavage	2^h15^m	horizontal	8 cells.
Fourth cleavage	2^h30^m	horizontal (micromeres)	12 cells.
Fifth cleavage	2^h30^m	meridional of upper 4	16 cells.
Sixth cleavage	2^h50^m	meridional of lower 4	20 cells.
Seventh cleavage	2^h50^m	meridional of upper 8	28 cells.
Eighth cleavage	3^h00^m	horizontal (mic. to form second set)	32 cells.
Ninth cleavage	3^h15^m	horizontal (div. of lower 8)	40 cells.
Tenth cleavage	3^h35^m	meridional (up. 16 to 32)	56 cells.
Eleventh cleavage	3^h35^m	meridional (next 8 to 16)	64 cells.
Twelfth cleavage	3^h45^m	meridional (first mic. to 8)	68 cells.
Thirteenth cleavage	3^h50^m	meridional (lower 8 to 16)	76 cells.

Fourteenth cleavage.....	4^h00^m	meridional (second 16 of eleventh to 32) 92 cells.
Fifteenth cleavage.....	4^h30^m	meridional (upper 32 of tenth to 64) 124 cells.
	5^h45^m	Blastulæ rotating within membrane.
	6^h00^m	Membrane thrown off. Blastulæ swim.
	6^h30^m	Most swimming at surface.
	8^h25^m	Mesenchyme formation beginning, 4 cells in blastocoel.
	9^h25^m	Beginning gastrulation.
	3^h00^m	a.m. Pigment spots and skeleton beginning.
	12^h00^m	noon. Many plutei with anal arms.

The explanation of this more rapid development may be found in the differences in temperature between the localities in which the work has been done.

In hybridization work it is desirable to cross forms whose plutei possess striking structural differences. The most advantageous Echinoid crosses upon which any detailed work has been done by the European investigators are the *Sphærechinus*×*Strongylocentrotus* and the *Sphærechinus* × *Echinus* combinations. These are advantageous because the *Echinus* and *Strongylocentrotus* anal arm skeletons consist of a single, slender rod, while the *Sphærechinus* anal arm skeleton is of the fenestrated type, being made up of three rods united with one another by crossbars. The same advantage is afforded by American crosses in which one of the forms used is *Toxopneustes*. The plutei of *Toxopneustes* have a single rod as the support in the anal arms. The plutei of *Arbacia*, *Hipponoë*, *Mellita*, and *Moira* have anal arm skeletons of the latticed type.

In my observations, then, *Toxopneustes* will correspond to *Echinus* (text figs. 1 and 2) and *Strongylocentrotus* (text fig. 7), while *Arbacia*, *Hipponoë*, *Mellita*, and *Moira* will correspond to *Sphærechinus* (text figs. 3 and 6).

ARBACIA.

The normal *Arbacia* pluteus (plate 1, fig. 8) is distinctly pyramidal in form. The skeleton (plate 1, fig. 9) is rather heavily formed, the body skeleton terminating in an irregular club-like enlargement. The supports of the anal arms are of the ladder-like type. I have not succeeded in keeping *Arbacia* plutei alive in laboratory cultures for more than ten days. The older plutei and young adults which have just completed their metamorphosis are readily obtained in surface towings.

HIPPONOE.

In the *Hipponoë* pluteus (plate 1, figs. 3 and 4) the posterior end of the body is truncated. The anal arm rods are fenestrated. The oral arm rods are continued posteriorly as the dorsal body skeleton and unite with the ventral body skeleton to form a basket. All of the rods are heavy in character. These plutei live well in laboratory cultures.

MELLITA.

The *Mellita* pluteus (plate 1, figs. 10, 11, and 12) is rounded posteriorly. The oral arm rods are continued posteriorly as the dorsal body skeleton and unite with the ventral body skeleton to form a complicated basket. The anal arm rods are fenestrated.

MOIRA.

The *Moira* pluteus (plate 1, figs. 5, 6, and 7) in the older stages is characterized by the possession of a posterior unpaired spine whose method of origin is shown in plate 1, fig. 5. The anal arm rods are fenestrated.

TOXOPNEUSTES.

The *Toxopneustes* pluteus (plate 1, figs. 1 and 2) is more slender in form than the others considered. It differs from them further in that the skeletal rods of the anal arms are single, slender rods whose surface is roughened by the presence of thorn-like projections. The dorsal and ventral body skeletons are not connected posteriorly. A detailed consideration of these plutei of various ages may be found in my paper on variation in *Toxopneustes* plutei (Tennent 1910).

THE CROSSES.

ARBACIA:

Two crosses with the *Arbacia* egg were made, one with *Mellita* and one with *Moira*. A fertilization membrane was formed. In both instances cleavage took place about 40 minutes after fertilization, the time of beginning of cleavage thus not being changed by the use of foreign sperm. The *Arbacia* egg is small and deeply pigmented, and is not adapted for study of the nuclear activities in the living conditions. I have shown elsewhere (1908) that the preserved egg is especially favorable for the study of chromosomes in cross-fertilized eggs.

ARBACIA ♀ × MELLITA ♂:

The greater number of plutei obtained from the *Arbacia* ♀ × *Mellita* ♂ cross (plate 2, fig. 22) could be referred neither to the maternal nor to the paternal type. In most cases the skeleton of the anal arms consisted of from two to four unconnected or irregularly connected rods. No plutei of a pronounced paternal type were found. The posterior basket was undeveloped. About 2 per cent were of the maternal type shown in plate 2, fig. 22, although here the hybrid characters of the larvæ are at once evident. The plutei are not intermediate in type, but form a series. The body is in general of the *Arbacia* type.

ARBACIA ♀ × MOIRA ♂:

The *Arbacia* ♀ × *Moira* ♂ cross was readily made and was very successful for segmentation, but was of little use in the older stages. The eggs were allowed to stand for 7 hours and were then fertilized with active *Moira* sperm. The hybrids were irregularly intermediate in character. No trace of a posterior unpaired spine could be seen. The hybrids inclined somewhat to the maternal form, yet showed distinctly their hybrid origin.

TOXOPNEUSTES:

It will be remembered that the *Toxopneustes* plutei are distinctive in form, in that they alone have single rods in the anal arms. The plutei of all other forms worked with have fenestrated rods. Any cross with

Toxopneustes is therefore of especial value. The favorable nature of the *Toxopneustes* egg for the observations of nuclear phenomena during the living stage has been mentioned by other investigators. It has been possible to see, in this egg, the fusion of the pronuclei and to convince myself that the processes of fertilization were being completed.

TOXOPNEUSTES ♀ × HIPPONOË ♂:

This and the reciprocal cross *Hipponoë* ♀ × *Toxopneustes* ♂ are the ones that have made the experimental portion of my work possible. The *Toxopneustes* eggs were fertilized 6 hours after their removal from the ovary, a series of fertilizations showing that the highest percentage of fertilizations was obtained if the eggs were allowed to remain in sea-water for this length of time. A fertilization membrane was formed. Cleavage began 40 minutes after fertilization, this being the period characteristic of the egg.

The character of the hybrids resulting from this cross is shown in plate 2, figs. 27, 29 to 31, plate 3, figs. 32 to 37, and plate 5, figs. 75 to 76. With the exception of the individual shown in plate 3, fig. 32, a pronounced *Hipponoë* dominance is shown. This dominance is expressed in the great number of fenestrated anal arm rods, in the number of multiple rods, and in the general form of the body skeleton. In the fertilizations made in 1910 the *Hipponoë* dominance was further expressed in the occurrence of the basket-like structure at the posterior end of the body. The best idea of the amount of inclination to the paternal type is shown in table I.

TABLE I.—*Summary of results of cross-fertilization in ordinary sea-water.*

[Number of plutei studied, 50. Temperature of water, 28.5° C.]

Year.	Cross.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneustes* plutei.	Perfect *Hipponoë* plutei.	Basket.
1909	*Hip.* ♂ / *Tox.* ♀	33	60	39	14	1	0	5	10
1910	*Hip.* ♂ / *Tox.* ♀	24	32	60	0	8	0	0	25

EXPLANATIONS OF HEADINGS OF THE COLUMNS.

"Plutei with lattice structure" are plutei which have parallel rods connected by crossbars in one or both anal arms.

"Anal arm rods with lattice structure" designates the total number of anal arms of the plutei considered in the preceding columns, which have a skeleton composed of parallel rods connected by crossbars.

"Arms more than one rod" indicates an anal arm skeleton of more than one straight rod.

"Perfect *Hipponoë* rods" are anal arm rods which are as perfect as those found in purely bred *Hipponoë* plutei.

"Perfect *Toxopneustes* rods" indicates a single straight rod with thorn-like protuberances.

"Perfect *Toxopneustes* plutei" and "Perfect *Hipponoë* plutei" indicate respectively plutei of the normal *Toxopneustes* and *Hipponoë* type.

"Basket" indicates the basket-like structures present in the posterior part of the body of purely bred *Hipponoë* plutei.

In this examination, in 66 per cent of the plutei studied, arms with latticed structure were found; no perfect *Toxopneustes* plutei, and 5 perfect *Hipponoë* plutei were seen.

In the study of the same cross made in 1910 there were 50 plutei examined, 48 per cent of which had the latticed structure and 50 per cent basket structure.

TOXOPNEUSTES ♀ × MELLITA ♂:

The *Toxopneustes* ♀ × *Mellita* ♂ cross was made after the eggs had stood in sea-water for 5 hours. A fertilization membrane was formed. The fertilization-cleavage period was characteristic of the egg. Most of the larvæ obtained were of the mixed type. In general the hybrids were of the maternal type in body form, but without exception had multiple rods in the anal arms. A few larvæ, such as those shown in plate 2, fig. 21, resemble the plutei of the pure maternal form, although the hybrid origin was very evident. The body skeleton was of the *Mellita* type, but without the posterior basket, and a very abnormal fenestration of the anal arm skeleton was evident. Some of the *Toxopneustes* ♀ × *Mellita* ♂ hybrids were kept alive for upwards of 10 days.

TOXOPNEUSTES ♀ × MOIRA ♂:

This cross was readily made after the *Toxopneustes* eggs had stood in water for 5 hours, experiments showing that the best results were

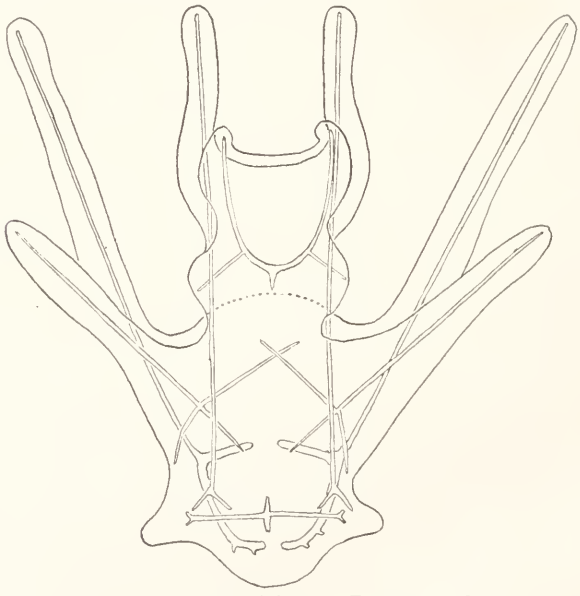

FIG. 5.—Pluteus *Moira* ♂ × *Toxopneustes* ♀.

obtained after the eggs were allowed to stand for this length of time. A fertilization membrane was formed. Cleavage took place in about 40 minutes after fertilization; the fertilization-cleavage period being that characteristic of the egg.

The greater number of hybrids of this cross were of the intermediate maternal type (plate 1, figs. 13 to 17; plate 2, figs. 18 to 20) having multiple rods in the anal arms. All of the plutei obtained in 1907 were of this character. From two crosses made in 1908, a small percentage, about 1 per cent, of hybrids of a purely maternal form were obtained (plate 2, figs. 18 to 20 and text fig. 5). These showed no trace of their hybrid origin, their perfect form suggesting that they were pure *Toxopneustes* larvæ which had arisen from chance fertilizations. This possibility, I believe, is excluded by the extraordinary care that was taken in making the fertilizations and by the care that was exercised in avoiding contamination while the larvæ were being reared. Herbst and Vernon also obtained similar larvæ of a striking maternal form.

The pluteus shown in plate 2, fig. 20, resembles in all respects a purely bred pluteus of the same age, the epaulets, Echinoderm rudiment, and the first pedicellariæ being well developed. I was not able to obtain adults from any of these plutei, although during the same season I carried laboratory fertilized *Toxopneustes* embryos through their metamorphosis. At the end of 45 days in one instance with the crosses I had 9 and in another 6 plutei in good condition. Upon trying to find them on the succeeding day nothing could be made out, although a careful search of the diatom mud in the bottom of the culture dish was made.

TOXOPNEUSTES ♀ × ECHINASTER ♂:

The result of this cross was not especially noteworthy. No fertilizations were obtained after the usual method of allowing the *Toxopneustes* eggs to stand 5 hours before treatment with sperm. In a second attempt the eggs were exposed to the action of CO_2 sea-water for from $1\frac{1}{2}$ to 10 minutes, and later fertilized after the method that I described five years ago (Tennent 1906). The most successful lot was that treated with CO_2 for 4 minutes. Inasmuch as segmentation did not begin until 2 hours after fertilization, I believe that the fertilization was ineffective and that the segmentation was parthenogenetic. A third attempt at this cross, when I again used the CO_2 treatment, exposing the eggs to the action of CO_2 sea-water for 4 minutes and fertilizing with *Echinaster* sperm 10 minutes after the egg had been transferred to sea-water, resulted in the occurrence of segmentation 55 minutes after fertilization. Cleavage was irregular and the "embryos" obtained were formless, ciliated clumps of cells.

TOXOPNEUSTES ♀ × HOLOTHURIA FLORIDANA ♂:

(1) The *Toxopneustes* eggs were allowed to stand for 2 hours and were then fertilized with *Holothuria* sperm. A fertilization membrane was formed at once. A small percentage of segmentation was obtained.

(2) *Toxopneustes* eggs were treated with $MgCl_2$ (1 c.c. to 100 c.c. sea-water) for 3 minutes. Segmentation began 45 minutes after fertilization with *Holothuria* sperm.

(3) Toxopneustes eggs treated with CO_2 for 4 minutes and fertilized with *Holothuria* sperm 50 minutes after transference to sea-water.

Good fertilization membranes were formed. A high percentage of cleavage was obtained, but the cleavage was irregular.

(4) *Toxopneustes* eggs treated with $\frac{n}{10}$ NaOH (2 c.c. to 100 c.c. sea-water) for 3 minutes and then fertilized with *Holothuria* sperm. Most of the eggs were destroyed by the entrance of the sperm.

(5) *Toxopneustes* eggs treated with $\frac{5}{8}$m $CaCl_2$ (2 c.c. to 100 c.c. sea-water) for 5 minutes and fertilized with *Holothuria* sperm 45 minutes

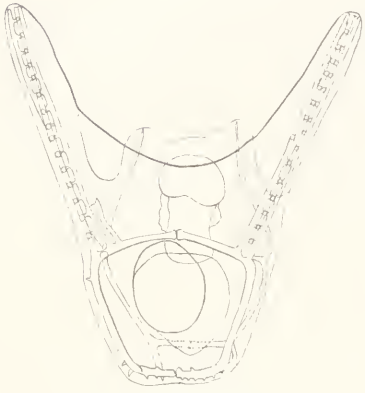

FIG. 6.— Parthenogenetic *Sphærechinus* pluteus (Herbst).

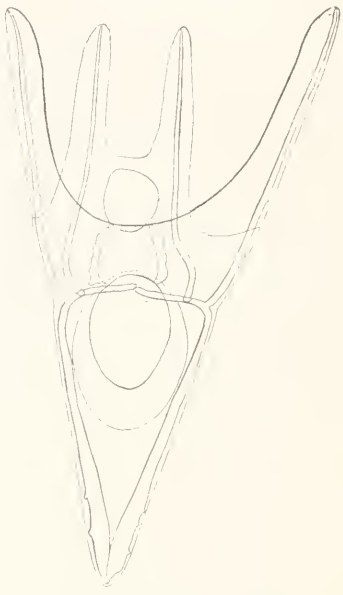

FIG. 7.—*Strongylocentrotus* pluteus (Herbst).

after transference to sea-water. A fertilization membrane was formed at once. Most of the eggs then burst. A few gave good segmentation.

In the eggs treated with $\frac{5}{8}$m $CaCl_2$ and with $\frac{n}{10}$ NaOH the sperm upon entering, in most cases, tears the egg to pieces. A deep notch or pathway made by the sperm may be seen and then the egg suddenly disintegrates. In this connection it is interesting to note that the *Holothuria floridana* egg has a very thick membrane and a well-defined micropyle.

MELLITA:

The *Mellita* ♀ × *Moira* ♂ cross was easily made. About 10 per cent of the eggs were fertilized after being well washed in sea-water. Most of the plutei were of the intermediate maternal type with multiple rods in the anal arm. A few were of a well-marked maternal form (plate 2, fig. 23), yet showing evidences of the hybrid origin. The body was rounded posteriorly, but the skeletal basket was absent. There was no trace of the posterior unpaired spine, the *Moira* character which it was hoped would appear.

MOIRA :

MOIRA ♀ × TOXOPNEUSTES ♂:

This cross was easily made by fertilizing the eggs immediately after they were removed from the ovary. For information regarding the method of obtaining the eggs I am indebted to Dr. Bartgis McGlone, who has also furnished me with two drawings of the *Moira* pluteus, which I have used in this paper. The *Moira* egg is beautifully transparent and well suited for study in the living condition. A fertilization membrane was formed. The copulation of the pronuclei could be observed. Segmentation began 35 minutes after fertilization, as in the straight fertilization. The crosses were of the intermediate type (plate 2, figs. 24, 25, 26). No trace of the maternal posterior, unpaired spine appeared, although the larvæ were kept alive for 7 days.

HIPPONOË (TRIPNEUSTES):

The *Hipponoë* egg, like the *Arbacia* egg, is not well adapted for study in the living state. The egg is small and dark in color. In comparison with the *Toxopneustes* egg it measures in micrometer eye-piece units, Ocular 2 Objective D, *Hipponoë* 19 × 19; *Toxopneustes* 26 × 25.

HIPPONOË ♀ × TOXOPNEUSTES ♂:

The *Hipponoë* ♀ × *Toxopneustes* ♂ cross was the most successful cross made with the *Hipponoë* egg. A series of experiments showed that the largest percentage of fertilizations was obtained when the eggs were fertilized 2½ hours after their removal from the ovary. During the work of 1909 the cleavage began 75 minutes after fertilization, as in the normally fertilized egg. In the experiments of 1910, although the fertilization-cleavage period in the normally fertilized eggs remained the same, cleavage in the cross-fertilized eggs began in 55 minutes, a reduction of 20 minutes. No fertilization membrane was ever obtained, the only membrane formed being the "Verbindungs-membran." In the plutei of this cross (plate 2, fig. 28; plate 3, figs. 38 to 43), there is a pronounced *Hipponoë* dominance as expressed in the skeleton of the anal arm. In the experiments of 1910 the third rod of the skeleton was never developed. The plutei live well in laboratory cultures.

TABLE II.—*Summary of results of cross-fertilization in ordinary sea-water.*

[Number of plutei studied, 50. Temperature of water, 28.5° C.]

Cross.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneusles* plutei.	Perfect *Hipponoë* plutei.
Tox. ♂ / *Hip.* ♀	37	58	40	30	2	0	12

It will be seen from the table that 74 per cent of the plutei have anal arms with rods having latticed structure. The normal sea-water fertilizations of 1910 were in accord with those of 1909. A count of 50 plutei made in 1910 gave results approximately the same as those of 1909.

HIPPONOË ♀ × CIDARIS ♂:

Hipponoë eggs were fertilized with *Cidaris* sperm 3 hours after their removal from the ovary. No fertilization membrane was formed. The fertilization-cleavage period was characteristic of the egg; segmentation very irregular; larvæ all of abnormal form.

HIPPONOË ♀ × OPHIOCOMA ♂:

Hipponoë eggs were fertilized with *Ophiocoma* sperm 3 hours after their removal from the ovary. No fertilization membrane was formed. The fertilization-cleavage period was characteristic of the egg. All larvæ abnormal.

HIPPONOË ♀ × PENTACEROS ♂:

Hipponoë eggs were fertilized with *Pentaceros* sperm 3 hours after their removal from the ovary. No fertilization membrane was formed. The fertilization-cleavage period was characteristic of the egg; the segmentation and larvæ irregular.

THE EXPERIMENTAL CONTROL OF DOMINANCE.

One of the most interesting and important problems connected with the results of Echinoderm hybridization is the determination of the factors influencing the appearance of maternal or of paternal characters in the hybrid embryo.

We have seen that Vernon (1898, 1900) showed a seasonal variation and tried to show that the characters of the hybrid pluteus are dependent on the relative ripeness of the sperm used in the crosses. Doncaster (1904) concluded, as a result of his experiments, that the temperature of the water in which the embryos developed is the determining factor. Herbst (1906*a*, 1906*b*, 1907), in an extended and able series of papers, expressed his conviction that while temperature was a contributing factor it was not the only one, and showed that the dominance might be swung toward the maternal side by a combination of artificial parthenogenesis and fertilization; the actual cause of this displacement lying in the preponderance of maternal nuclear material arising from the application of this method.

My own investigations have shed further light upon this unknown factor. My experimental evidence shows that while the theories of the investigators mentioned are correct, they do not present the whole truth. My material has been especially fortunate in that I have obtained a dominance of one species over another in both crosses. That is, when the fertilization was made in normal sea-water *Hipponoë* characters were dominant. By changing the conditions in which fertilizations were made I have been able to change this dominance and to show that the actual factor determining the dominance is directly concerned with differences of season and of temperature, but that these two factors are simply contributory.

In my experiments, *the factor determining the dominance is the variation in alkalinity of the sea-water in which the embryos develop.* This variation is probably dependent on season and temperature.

I know of no analyses of sea-water which show a variation, toward or away from neutrality, correlated with change of season and of temperature. We have evidence that sea-water in different localities shows a variation in reaction during the same season. It is merely a matter of speculation, then, when I suggest that the artificial conditions that I produced, which enabled me to control dominance at will, correspond to natural conditions at different seasons of the year.

Algæ, growing in the sea-water, are credited by Loeb (1906) with causing it to become alkaline. It would seem in general that with the higher temperatures of the summer months there would be an increased solution of phosphates and of carbonates to which the alkaline reaction may be due. Whatever the explanation may be, the results of my experiments show a definite effect of a change in environment on the character of the embryos, an effect due to the decrease in the concentration of OH ions, brought about by adding an acid to the sea-water.

The two crosses made which have served as the basis for this experimental work are *Hipponoë* ♀ × *Toxopneustes* ♂ and the reciprocal cross *Toxopneustes* ♀ × *Hipponoë* ♂. Both of these crosses gave plutei with *Hipponoë* characters. It will be recalled that the pure plutei differ from one another in that the *Hipponoë* plutei have anal arm skeletons of the fenestrated type and that a basket-like structure is present at the posterior end of the body, while the *Toxopneustes* plutei have simple rods as skeletons of the anal arms and no basket-like structure is present in the body.

In my presentation of the evidence of *Hipponoë* dominance, I shall confine myself closely to the evidence afforded by a comparative study of the skeletal characters of the purely bred and of the hybrid plutei. From a prolonged study of pure *Toxopneustes* plutei (1910), I am convinced that characters such as the form of the larvæ, in nearly similar forms, the number, pigment-content, and arrangement of the chromatophores, and comparative measurements of parts of the body, are of too variable a nature to afford safe criteria upon which to base conclusions of importance.

I shall regard the presence of more than one rod in the anal arm and the presence of a basket-like structure in the posterior part of the body as an indication of *Hipponoë* influence.

It may be urged that the intraspecific variation of the skeleton is so great that I have no right to use any part of the skeleton as the basis for the conclusions that I am making. We have seen that both Vernon (1898) and Steinbrück (1902) observed the occurrence of multiple rods in the anal arms of *Strongylocentrotus* plutei as a common variation. In my own investigations on *Toxopneustes* (in which cultures from some hundreds of individuals and measurements involving the careful examination of several thousand plutei were made) such a variation was found in the embryo from the eggs of but two individuals. In one case it was found in 1 per cent and in the other in 3 per cent of the plutei examined. In other crosses it occurred as occasional variation. The pure cultures made in 1909 and 1910 as controls for the hybridization experiment showed an occasional pluteus with this variation, but the

number was never great enough to exclude the advisability of considering the common appearance of more than one rod, a *Hipponoë* character, as an indication of *Hipponoë* influence.

THE EFFECT OF THE INCREASE IN THE ALKALINITY OF THE SEA-WATER.

With an increase of alkalinity of the sea-water, brought about by the addition of $\frac{n}{10}$ NaOH, the plutei obtained were of the *Hipponoë* type, the increased alkalinity causing little modification. A slight increase of *Hipponoë* influence is apparent.

A series of experiments for determining the effect of varying degrees of increase in alkalinity was carried out. I give one table (Table III) showing the effect of the addition of 20 drops $\frac{n}{10}$ NaOH (1 drop $= \frac{1}{18}$ c.c.) to 400 c.c. of ordinary sea-water. The eggs from which the plutei were obtained were placed, immediately after being removed from the ovary and washed, in the sea-water whose alkalinity had been raised. The *Hipponoë* eggs were fertilized 2½ hours later, in a similar solution, with *Toxopneustes* sperm. The *Toxopneustes* eggs were allowed to remain in a like solution for 6 hours and fertilized with *Hipponoë* sperm.

After fertilization the eggs were changed to more of the same solution, in which they were kept until it was possible to pour the swimming blastulæ into ordinary sea-water, which was changed from time to time during the days through which the investigation was in progress.

TABLE III.—*Summary of results of cross-fertilization in sea-water of increased alkalinity.*

[Number of plutei studied, 50. Temperature of water, 29° C.]

Year.	Cross.	Plutei with lattice structures.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneustes* plutei.	Perfect *Hipponoë* plutei.
1909	*Hip.* ♂ *Tox.* ♀	39	57[1]	40	15	2	0	3
1909	*Tox.* ♂ *Hip.* ♀	40	62	38	31	0	0	10

Figures of the skeletons of the *Toxopneustes* ♀ × *Hipponoë* ♂ cross which had been subjected to the NaOH treatment are shown (plates 2 and 3, figs. 29 to 37; plate 6, fig. 94); of the *Hipponoë* ♀ × *Toxopneustes* ♂ cross (38–43). The experiments of 1910 showed no change as the result of the increase in alkalinity. This is due, I believe, to the greater alkalinity of the sea-water over that of the previous summer.

THE EFFECT OF A DECREASE IN THE ALKALINITY OF THE SEA-WATER.

With a decreased alkalinity brought about by the addition of $\frac{n}{10}$ acetic or $\frac{n}{10}$ hydrochloric acid, the plutei obtained showed the effect of an influence tending to swing them toward the *Toxopneustes* type. The plutei whose skeletons are shown in the figures were taken at random, no attempt being made to select plutei which would support any theory. (See *Toxopneustes* ♀ × *Hipponoë* ♂, figs. 44–85, and *Hipponoë* ♀ × *Toxopneustes* ♂, figs. 86–93.)

Table IV gives a summary of results obtained with the acetic acid.

TABLE IV.—*Summary of results of cross-fertilization in sea-water of decreased alkalinity, acetic acid.*

[Number of plutei studied in each fertilization, 50. Temperature, 29° C.]

Year.	Cross.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hippo-noë* rods.	Perfect *Toxop-neustes* rods.	Perfect *Toxop-neustes* plutei.	Perfect *Hippo-noë* plutei.	Basket.
1909	*Hip.* ♂ / *Tox.* ♀	7	7	62	1	31	7	0	–
(1) 1910	*Hip.* ♂ / *Tox.* ♀	4	5	93	0	2	0	0	7
(2) 1910	*Hip.* ♂ / *Tox.* ♀	14	16	81	0	3	0	0	10
(3) 1910	*Hip.* ♂ / *Tox.* ♀	13	15	69	0	16	3	0	12
(4) 1910	*Hip.* ♂ / *Tox.* ♀	3	5	51	0	44	6	0	2
(5) 1910	*Hip.* ♂ / *Tox.* ♀	4	6	49	1	45	5	0	1

Further data :

1909. Eggs fertilized in 400 c.c. sea-water + 20 drops $\frac{n}{10}$ acetic acid.
(1) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ acetic acid. 3 days.
(2) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ acetic acid. 4 days.
(3) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ acetic acid. 5 days.
(4) 1910. Eggs fertilized in 500 c.c. sea-water + 2 c.c. $\frac{n}{10}$ acetic acid. 2 days.
(5) 1910. Eggs fertilized in 500 c.c. sea-water + 2 c.c. $\frac{n}{10}$ acetic acid. 2 days.

The table shows clearly that the effect of the addition of the acetic acid had been to swing the dominance toward *Toxopneustes* side. That this result is not due to the specific action of the acetic acid is indicated by the results of fertilization in sea-water whose alkalinity has been decreased by the addition of hydrochloric acid.

TABLE V.—*Summary of results of cross-fertilization in sea-water of decreased alkalinity.*

[Hydrochloric acid. Number of plutei studied in each fertilization, 50. Temperature, 29°–29.5° C.]

Year.	Cross.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneustes* plutei.	Perfect *Hipponoë* plutei.	Basket.
1909	*Hip.* ♂ *Tox.* ♀	5	8	52	4	40	10	0	–
(1) 1910	*Hip.* ♂ *Tox.* ♀	20	24	71	0	5	0	0	9
(2) 1910	*Hip.* ♂ *Tox.* ♀	17	17	79	0	4	0	0	13
(3) 1910	*Hip.* ♂ *Tox.* ♀	13	16	66	0	18	3	0	8
(4) 1910	*Hip.* ♂ *Tox.* ♀	6	9	51	0	40	4	0	2
(5) 1910	*Hip.* ♂ *Tox.* ♀	5	7	49	0	44	2	0	1

Further data:

1909. Eggs fertilized in 400 c.c. sea-water + 10 drops $\frac{n}{10}$ HCl.
(1) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ HCl. 3 days.
(2) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ HCl. 4 days.
(3) 1910. Eggs fertilized in 500 c.c. sea-water + 15 drops $\frac{n}{10}$ HCl. 5 days.
(4) 1910. Eggs fertilized in 500 c.c. sea-water + 30 drops $\frac{n}{10}$ HCl. 2 days.
(5) 1910. Eggs fertilized in 500 c.c. sea-water + 30 drops $\frac{n}{10}$ HCl. 2 days.

Table VI shows that the effect of the addition of the acid has been to swing the dominance in the *Hipponoë* ♀ × *Toxopneustes* ♂ cross to the *Toxopneustes* side:

TABLE VI.—*Summary of results of cross-fertilization in sea-water of decreased alkalinity.*

[20 drops $\frac{n}{10}$ acetic acid to 400 c.c. sea-water. Number of plutei studied, 50. Temperature, 29° C.]

Year.	Cross.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneustes* plutei.	Perfect *Hipponoë* plutei.
1909	*Tox.* ♂ *Hip.* ♀	12	23	68	4	9	3	1

To facilitate comparison I combine all the statistics together in Table VII.

TABLE VII.—*Comparison of results of cross-fertilization.*

Year.	Cross.	Medium.	Plutei with lattice structure.	Anal arm rods with lattice structure.	Arms more than one rod.	Perfect *Hipponoë* rods.	Perfect *Toxopneustes* rods.	Perfect *Toxopneustes* plutei.	Perfect *Hipponoë* plutei.	Basket.
1909	*Hip.* ♂ / *Tox.* ♀	sea-water	33	60	39	14	1	0	5	
1909	*Tox.* ♂ / *Hip.* ♀	sea-water	37	58	40	30	2	0	12	
1909	*Hip.* ♂ / *Tox.* ♀	sea-water+ NaOH	39	57	40	15	2	0	3	
1909	*Tox.* ♂ / *Hip.* ♀	sea-water+ NaOH	40	62	38	31	0	0	10	
1909	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	7	7	62	1	31	7	0	
(1) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	4	5	93	0	2	0	0	7
(2) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	14	16	81	0	3	0	0	10
(3) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	13	15	69	0	16	3	0	12
(4) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	3	5	51	0	44	6	0	2
(5) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ acetic	4	6	49	1	45	5	0	1
1909	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	5	8	52	4	40	10	0	–
(1) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	20	24	71	0	5	0	0	9
(2) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	17	17	79	0	4	0	0	13
(3) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	13	16	66	0	18	3	0	8
(4) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	6	9	51	0	40	4	0	2
(5) 1910	*Hip.* ♂ / *Tox.* ♀	sea-water+ HCl	5	7	49	0	44	2	0	1
1909	*Tox.* ♂ / *Hip.* ♀	sea-water+ acetic	12	23	68	4	9	3	1	

The first results of 1910 did not tend to confirm those of 1909. As I have already stated, another indication of *Hipponoë* dominance made its appearance, this being the basket. This character seemed especially difficult to control. The use of a greater amount of acid brought the results of the previous summer. One interesting thing in the work of 1910 was that the *Toxopneustes* ♀ × *Hipponoë* ♂ hybrids lived better in sea-water of reduced alkalinity than in the normal sea-water.

SUMMARY OF THE CONTROL OF DOMINANCE.

The outcome of the investigation may be summed up in the form of equations.

I. Ordinary Sea-Water.

$$\frac{\textit{Hipponoë}\ \♂}{\textit{Toxopneustes}\ ♀} = \text{Dominant } \textit{Hipponoë}.$$

$$\frac{\textit{Toxopneustes}\ \♂}{\textit{Hipponoë}\ ♀} = \text{Dominant } \textit{Hipponoë}.$$

II. Sea-Water + NaOH.

$$\frac{\textit{Hipponoë}\ \♂}{\textit{Toxopneustes}\ ♀} = \text{Dominant } \textit{Hipponoë}.$$

$$\frac{\textit{Toxopneustes}\ \♂}{\textit{Hipponoë}\ ♀} = \text{Dominant } \textit{Hipponoë}.$$

III. Sea-Water + Acetic or Hydrochloric Acid.

$$\frac{\textit{Hipponoë}\ \♂}{\textit{Toxopneustes}\ ♀} = \text{Dominant } \textit{Toxopneustes}.$$

$$\frac{\textit{Toxopneustes}\ \♂}{\textit{Hipponoë}\ ♀} = \text{Dominant } \textit{Toxopneustes}.$$

DISCUSSION OF RESULTS.

The task of bringing together and of harmonizing the results of various investigators is extremely difficult. Their results are diverse. In many cases the same forms worked with in the same localities, at the same time of year, but in different years, have given at one time one result, at another time another result. Yet the work has been done by investigators whose ability is unquestionable.

I may be criticized for having given so full a résumé of the papers of Herbst and of Vernon. I have done this for the purpose of giving an exact account of our present knowledge of Echinoderm hybridization, which I hope may be useful.

I have already stated that I regard the material with which I have worked as especially fortunate, more fortunate than that which has been obtained by any other investigator. This is for two reasons,

(1) Reciprocal crosses were readily made and the plutei from both were well formed.

(2) A clear preponderance of *Hipponoë* characters was evident, no matter which way the cross was made.

In the résumé of the literature of the subject that I have given and in my own work one thing stands out clearly: This is the "optimum."

Herbst determined an optimum concentration of OH ions in the sea-water of the Gulf of Naples; an optimum OH ion concentration in his cross-fertilization researches; an optimum temperature for development. Vernon determined optimum temperature, light, season, concentration of sea-water, etc. My own studies have shown an optimum time for fertilization, an optimum concentration of OH ions, etc.

Everything points to an optimum environment and a necessary environment for certain occurrences. This is only what we expect. One aim of scientific research is to determine the conditions in which certain phenomena will be exhibited. Our evidence has shown the

delicacy of the balance between an exhibition of *Hipponoë* characters and the appearance of *Toxopneustes* characters. We are not surprised that this should be so, now that we have seen it. It is what we might expect in forms which are so closely similar.

In comparing the change of dominance which I obtained by change in alkalinity of the sea-water with that which Herbst obtained by changing temperature, it is interesting to notice that the normal temperature of the sea-water in the tropical and subtropical regions in which my work has been done is higher than those of Herbst's experimental conditions.

In this comparison (see tables on pages 128 and 147) it will be seen that the changes caused by increased temperature and the changes caused by decreased alkalinity are approximately the same. I have reduced the amount of *Hipponoë* dominance in about the same amount that Herbst has decreased the amount of *Strongylocentrotus* influence. In the cross *Sphærechinus* ♀ × *Strongylocentrotus* ♂, Herbst has held the embryo to the egg type.

The difference between Herbst's observations and my own is more apparent than real. By one change in environment I have obtained embryos of a *Hipponoë* type; by another change of environment, I have obtained embryos of the *Toxopneustes* type. Herbst by combining artificial parthenogenesis and fertilization held the embryo to the egg type.

As to the explanation of this phenomenon, I believe that we have it in the optimum which was produced by the change in environment. In work with the pure forms Herbst, Vernon, and I have shown that uniformly the best results are obtained in certain definite conditions. I have shown that a higher degree of alkalinity is more favorable to *Hipponoë*, a lesser degree to *Toxopneustes*. If the fertilization be made in the environment most favorable to the sperm, and therefore possibly most favorable to the growth of its nuclear material, resemblance to the male parent has followed in both cases.

That the results are not uniform depends upon individual differences in the germ-cells, although in general the germ-cells in a given parent produce embryos of a similar character. This idea of individual differences is simply the idea of chemical variation in the germ-cells which has arisen during their growth, a variation due to food, functional reactions, etc.

I have done nothing which will explain the "cause" of these phenomena. I have simply shown that, in the material with which I worked, definite phenomena are exhibited in certain conditions. I believe that Herbst (1907) and I worked with essentially the same factors, although we applied them in a somewhat different manner—in his case the method of treatment being sufficient to cause parthenogenesis, in my work the method of application not being sufficient.

We have by no means exhausted all of the influential factors. Herbst found a partial control in temperature. From my investigations I believe that another partial control lies in the concentration of OH ions in the sea-water in which development is taking place. Given an optimum concentration, we may expect certain occurrences.

The "unknown factor" is a complex of conditions made up of factors, each one of which represents the optimum environment, an

environment in which the same thing may always be expected to occur. It is by a change from the optimum of one form to that of another that we may expect to bring about change in structure.

I stated in the beginning that the aim of this investigation is a formulation of a statement of the conditions governing the resemblance of the offspring to the parent. That aim has been at least partially accomplished by the research.

We have definite evidence that temperature and OH ion concentration of the sea-water are factors whose variation determines in part this resemblance to one or the other parent. In other words, the structure of the sea-urchin pluteus from cross-fertilized eggs may be influenced by the external environment to which the developing germ is subjected during its growth.

In a preliminary paper (1910*a*) I criticized Herbst's idea of nuclear control. Our evidence, I believe, all points to a physical-chemical control. This control is exerted on the germ-cell from its beginning. This control is the complex of factors forming the environment of the germ-cell during its growth in the body of the parent and (in forms in which fertilization and development take place outside of the body) the environment in which growth takes place. For the agent through which this control is exerted, much evidence shows that we may look to the nucleus.

A further generalization from my observations would be premature. We do not know how permanent the changes in structure may be. That knowledge can be gained only by a study of later generations raised from cross-fertilized eggs.

SUMMARY.

(1) The *Toxopneustes* ♀ × *Hipponoë* ♂ and the reciprocal cross *Hipponoë* ♀ × *Toxopneustes* ♂ were easily made after allowing the eggs to stand in sea-water for some hours before fertilization.

(2) In the embryos of both crosses made in ordinary sea-water, which was alkaline, the *Hipponoë* influence showed a tendency to predominate.

(3) In the embryos of both crosses made in sea-water of increased alkalinity, there was evidence of an increase of *Hipponoë* influence.

(4) In the embryos of both crosses made in sea-water of decreased alkalinity, a tendency toward *Toxopneustes* dominance was evident.

(5) The results thus show *Hipponoë* dominance in sea-water of a higher OH ion concentration and *Toxopneustes* dominance in sea-water of a lower OH ion concentration.

(6) I suggest that these variations in the alkalinity of the sea-water, which I have brought about artificially, may correspond to normal seasonal changes.

(7) If this be true, the winter (paternal) embryos and the summer (maternal) plutei of the combination *Sphærechinus* × *Strongylocentrotus* of other investigators had their origin in such normal seasonal changes of OH ion concentration.

(8) The results of this and of other investigations show species tendencies toward different grades of temperature and of alkalinity.

(9) The explanation of the preponderance of one character over another in Echinoderm hybrids seems to lie in the reaction of the species toward a complex of factors.

MODESTO, CALIFORNIA, *July*, 1910.

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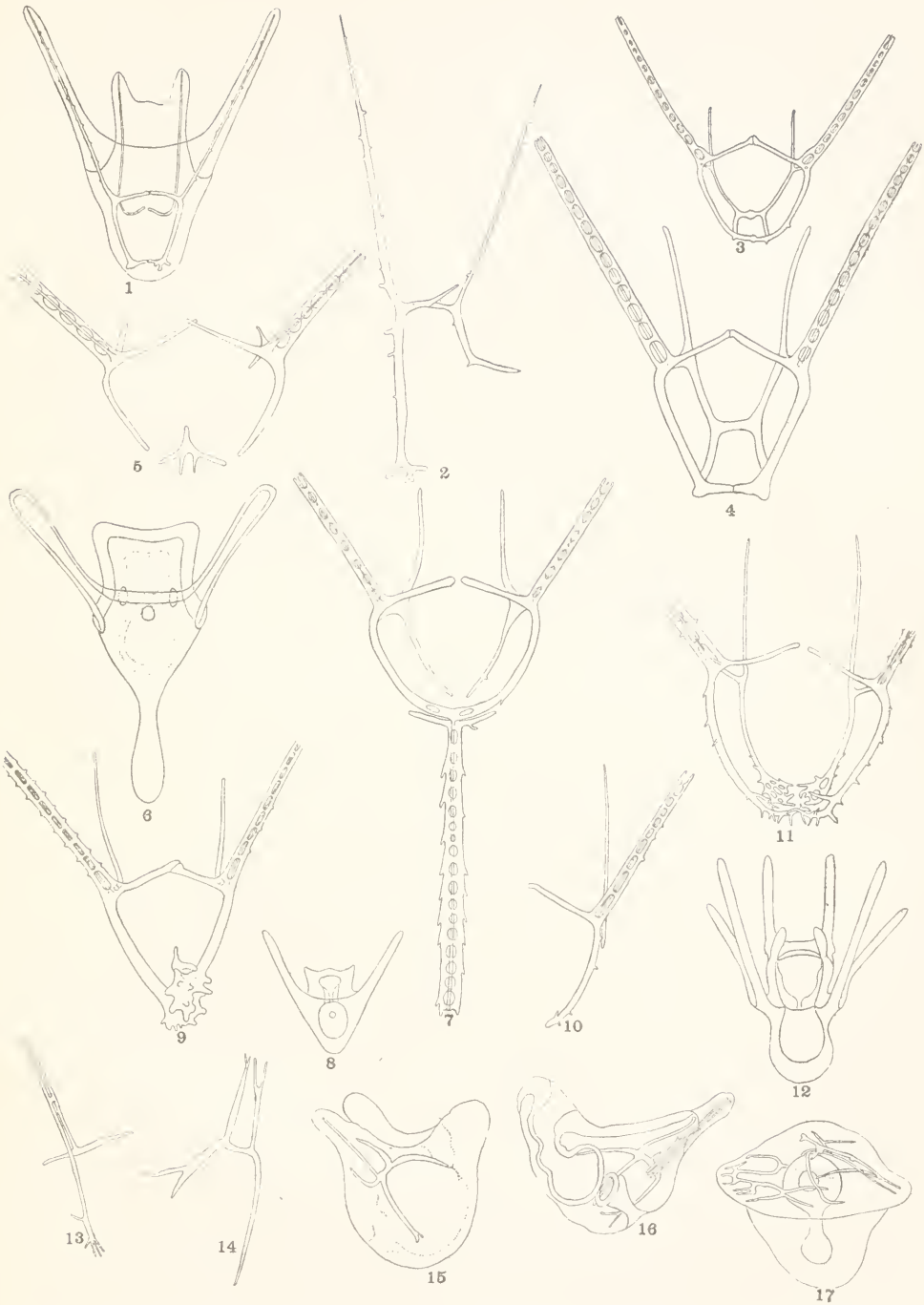
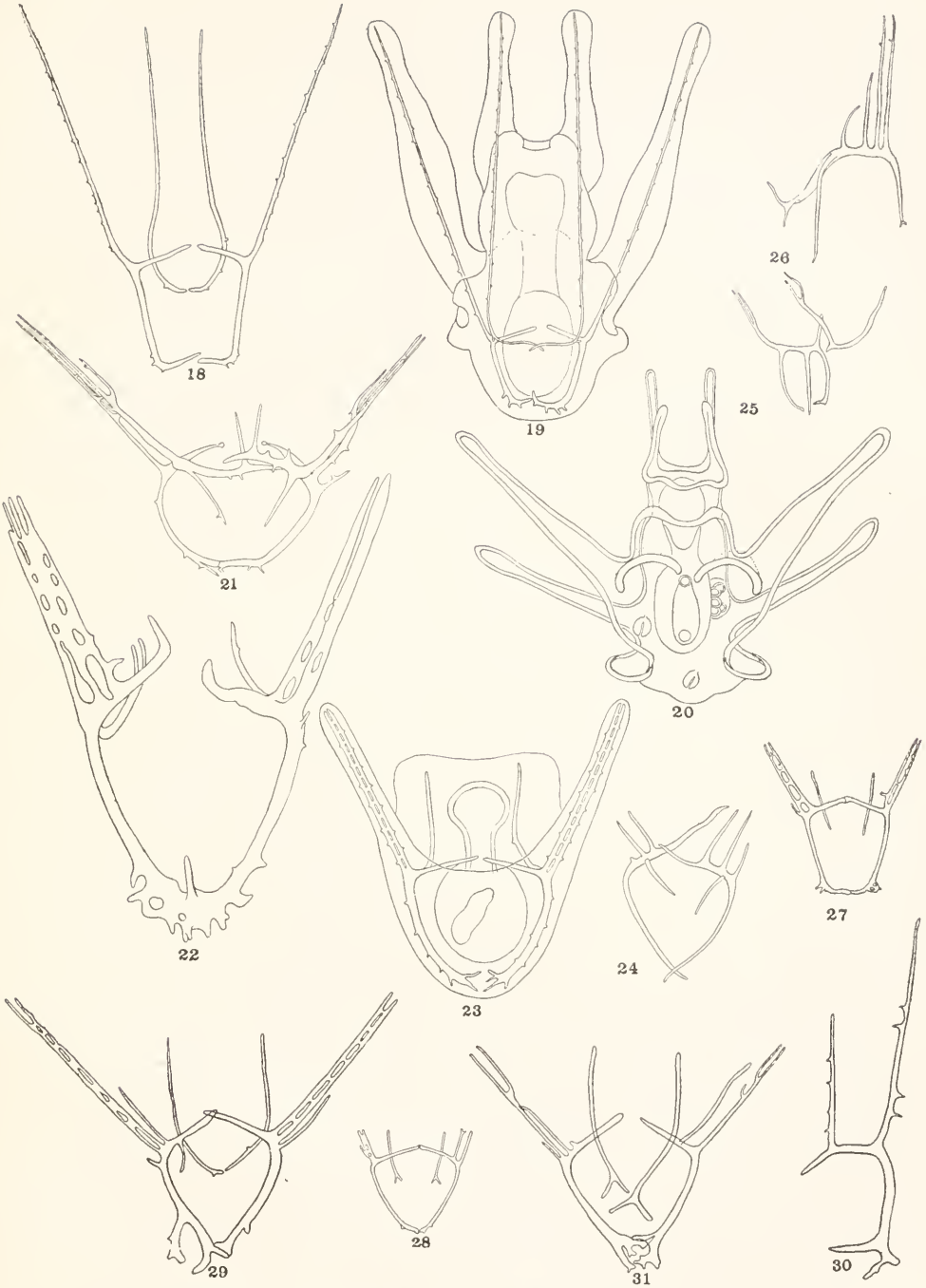
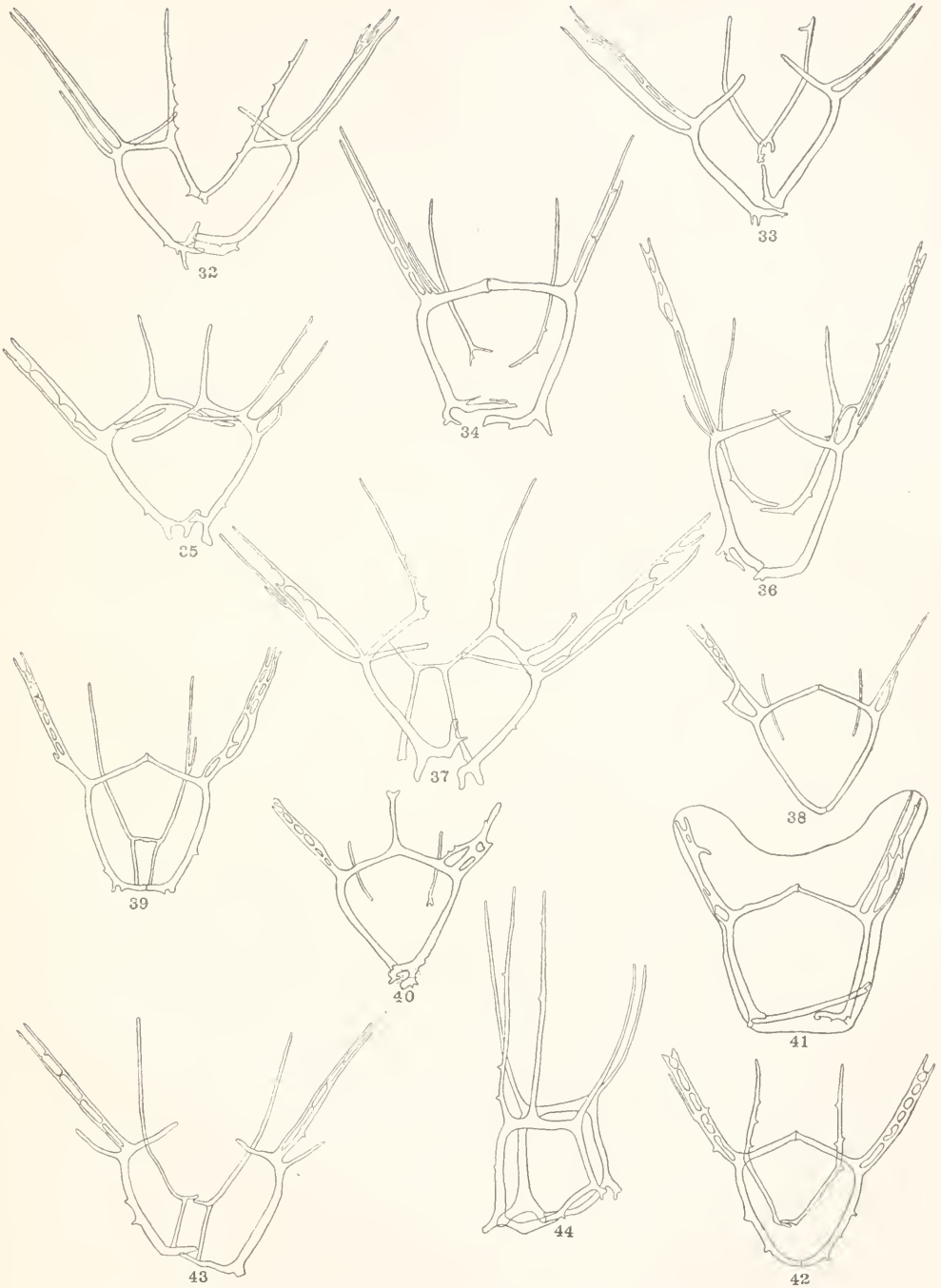


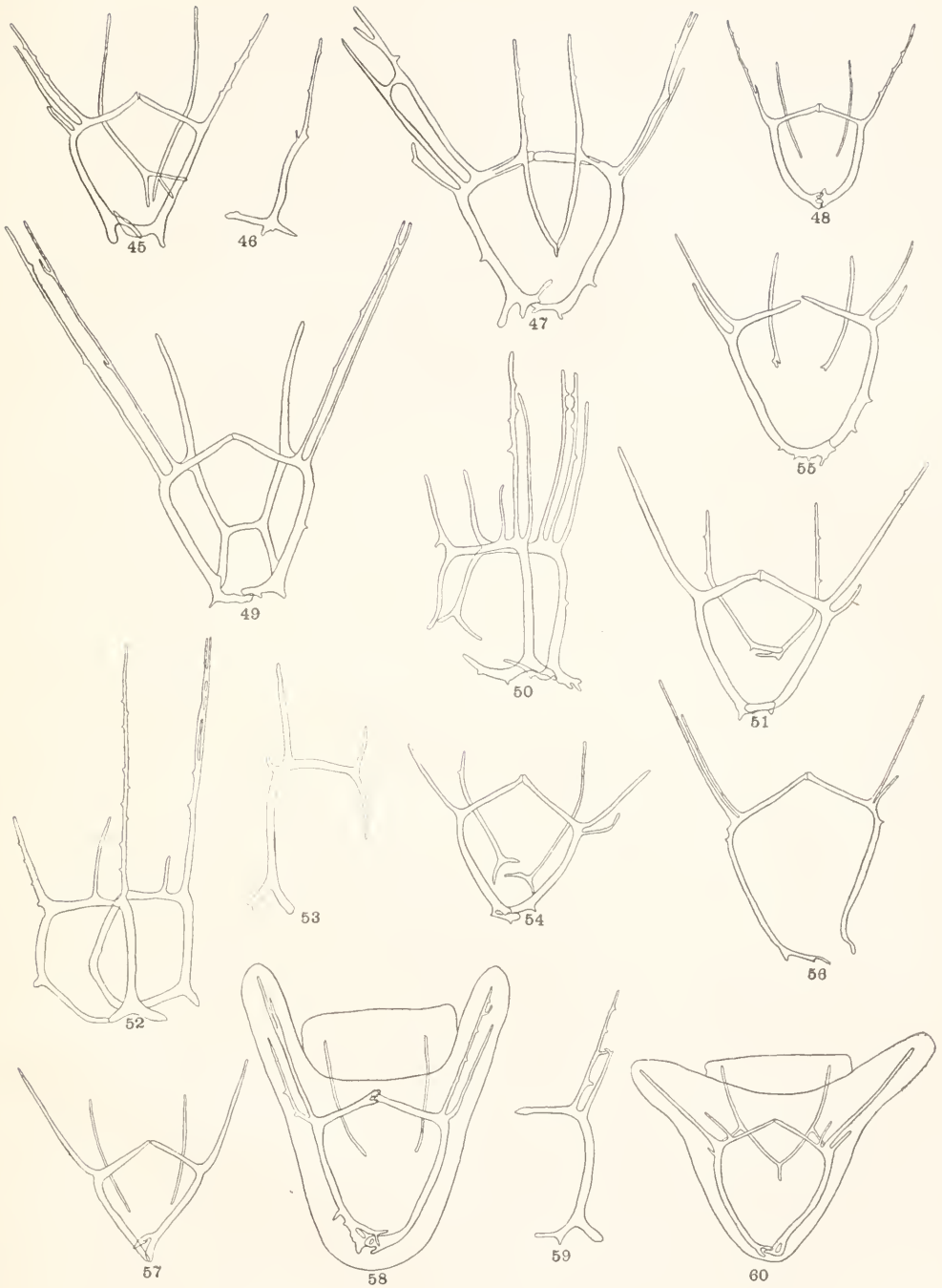
1. *Toxopneustes* skeleton and pluteus. 24 hours.
2. *Toxopneustes*, half skeleton. Side view. 24 hours.
3. *Hipponoë* skeleton. 24 hours.
4. *Hipponoë* skeleton. 4 days.
5. *Moira* skeleton. 15½ hours. (McGlone.)
6. Outline *Moira* pluteus. 24 hours. (McGlone.)
7. *Moira* skeleton. 29 hours.
8. Outline *Arbacia* pluteus. 40 hours.
9. *Arbacia* skeleton. 40 hours. Ends of arms not shown.
10. *Mellita*, half skeleton. 23½ hours.
11. *Mellita* skeleton. 48 hours. Ends of arms not shown.
12. Outline *Mellita* pluteus. 84 hours.
13–14. *Toxopneustes* ♀ × *Moira* ♂. Half skeleton. 42 hours.
15. *Toxopneustes* ♀ × *Moira* ♂. Side view. 48 hours.
16. *Toxopneustes* ♀ × *Moira* ♂. Whole skeleton. 48 hours.
17. *Toxopneustes* ♀ × *Moira* ♂. 62 hours.



18. *Toxopneustes* ♀ × *Moira* ♂. Half-skeleton. 60 hours.
19. *Toxopneustes* ♀ × *Moira* ♂. Pluteus and skeleton. 6 days.
20. *Toxopneustes* ♀ × *Moira* ♂. Pluteus. Free hand. 15 days.
21. *Toxopneustes* ♀ × *Mellita* ♂. Skeleton. 48 hours.
22. *Arbacia* ♀ × *Mellita* ♂. 48 hours.
23. *Mellita* ♀ × *Moiro* ♂. 36 hours.
24. *Moira* ♀ × *Toxopneustes* ♂. 36 hours.
25–26. *Moira* ♀ × *Toxopneustes* ♂. 36 hours.
27. *Toxopneustes* ♀ × *Hipponoë* ♂. 48 hours.
28. *Hipponoë* ♀ × *Toxopneustes* ♂. 48 hours.
29–31. *Toxopneustes* ♀ × *Hipponoë* ♂. NaOH. Fourth day.



32–37. *Toxopneustes* ♀ × *Hipponoë* ♂. NaOH. Fourth day. | 42–43. *Hipponoë* ♀ × *Toxopneustes* ♂. NaOH.
38–41. *Hipponoë* ♀ × *Toxopneustes* ♂. NaOH. Fourth day. | 44. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Fifth day.



45–46. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Third day.

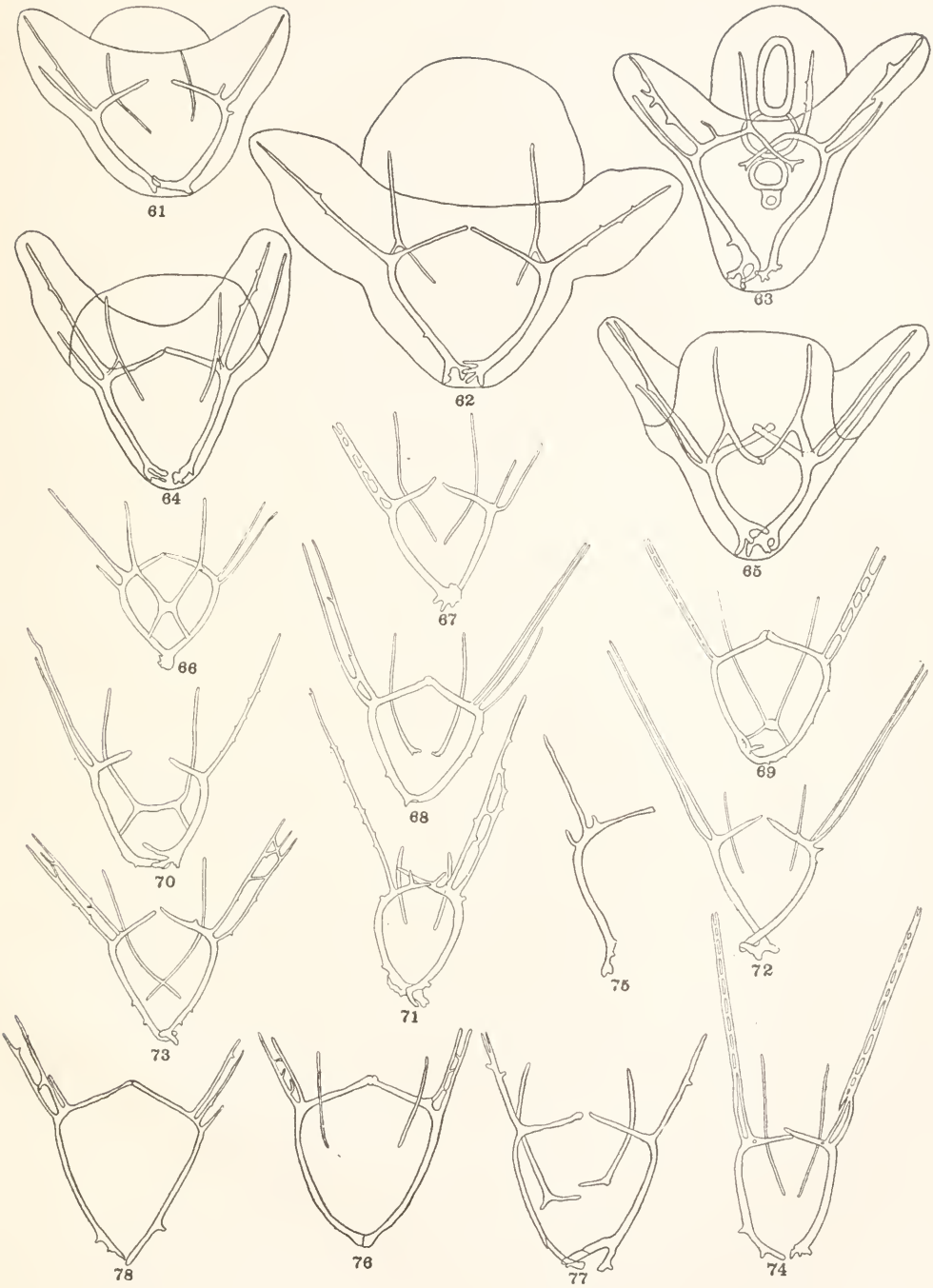
47. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Fifth day.

48. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Third day.

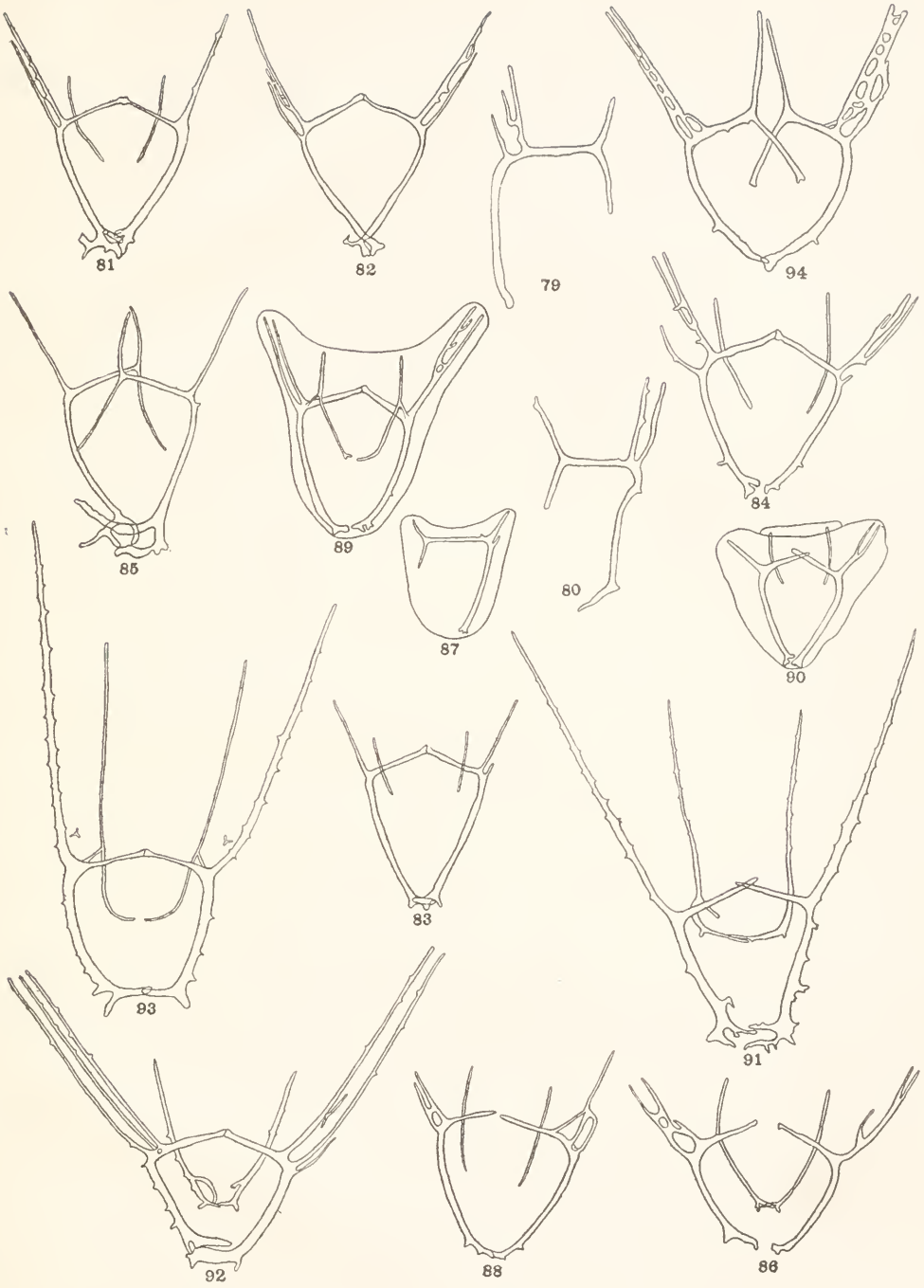
49–53. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Fifth day.

54–56. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid. Third day.

57–60. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid.



61–65. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid.
66–69. *Toxopneustes* ♀ × *Hipponoë* ♂. 1910. Ordinary sea water. 3 days.
70–72. *Toxopneustes* ♀ × *Hipponoë* ♂. HCl. 4 days.
73. *Toxopneustes* ♀ × *Hipponoë* ♂. 1910. Acetic acid. 4 days.
74. *Toxopneustes* ♀ × *Hipponoë* ♂. 1910. Acetic acid. 5 days.
75–76 *Toxopneustes* ♀ × *Hipponoë* ♂. Normal sea water.
77. *Toxopneustes* ♀ × *Hipponoë* ♂. Acetic acid.
78. *Toxopneustes* ♀ × *Hipponoë* ♂. HCl.



79. *Toxopneustes* ♀ × *Hipponoë* ♂. 1910 Acetic acid.
80–85. *Toxopneustes* ♀ × *Hipponoë* ♂. 1910. HCl.
86. *Hipponoë* ♀ × *Toxopneustes* ♂. NaOH.
87–90. *Hipponoë* ♀ × *Toxopneustes* ♂. Acetic acid.
91–93. *Hipponoë* ♀ × *Toxopneustes* ♂. Acetic acid.
94. *Toxopneustes* ♀ × *Hipponoë* ♂. NaOH.