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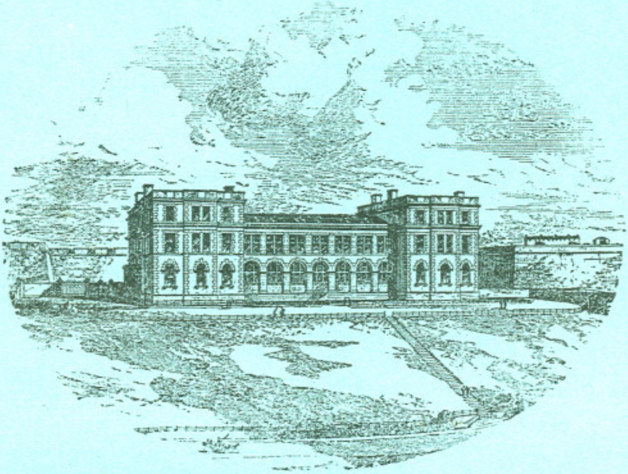

THE PLYMOUTH LABORATORY.

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Preliminary Notice on the Experimental Hybridization of Echinoids.

By

Cresswell Shearer, Walter De Morgan and **H. M. Fuchs.**

With 7 Text Figures.

INDEX.

I. Introduction and Previous Work.

THIS paper is a preliminary account of a series of experiments on the hybridization of Echinoids, first commenced by one of our number (De Morgan) at Plymouth in 1909, and which will form part of a larger work now approaching completion. This will comprise the hybridization of *Echinus acutus*, *E. esculentus*, and *E. miliaris*, together with an account of the cytology of the hybrid crosses and the control of paternal and maternal influence by chemical means.*

Within the last two decades a large amount of attention has been devoted to the problem of parental influence in Echinoderm hybrids. Most of this work has been done at Naples, where the majority of sea-urchins seem to possess a longer breeding period than those of the colder waters of our own coasts. In America within the last year or two considerable attention has been devoted to this subject. In the hands of different observers, however, this work has led to very conflicting results, and has proved most indecisive in settling the main points at issue.

In 1889 Boveri (3) was the first to investigate the hybrids between

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* The full paper will contain a large number of skeleton figures and drawings of Plutei and coloured plates of the hybrid urchins. The investigation of the cytology has been undertaken by L. Doncaster, and will form Part II of the paper.

different Echinoids, making use of *Sphaerechinus* eggs and *Echinus* sperms at Naples. He found that the hybrid larvæ were intermediate between those of the two parents. In 1894 Seeliger (16) made the same cross at Trieste, but found that many of his hybrids were of the purely paternal type; thus contradicting Boveri's result. In the following year Morgan (12) repeated this work, getting the same results as Seeliger. In 1895 Boveri (4) replied to Seeliger and Morgan substantiating his earlier results, but suggesting that at Trieste the Plutei had other characteristics from those at Naples. This does not apply, however, to Morgan's results obtained at Naples on the same material as that used by Boveri.

In the same year Vernon (19) commenced a new era in the work by a thorough investigation of the effects of environment on the larvæ. In 1898 he followed up his first work with the investigation of inheritance in various hybrid forms. He made crosses between *Sphaerechinus, Strongylocentrotus* and *Echinus.* His hybrid larvæ were mostly maternal, but some species seemed to have a greater capacity for transmitting their characteristics than others. In the same year Driesch (6) crossed *Strongylocentrotus, Sphaerechinus, Echinus,* and *Arbacia* and obtained hybrid larvæ of a purely maternal type.

In 1900 Vernon (21) hybridized *Strongylocentrotus* and *Sphaerechinus* at Naples and claimed to find that the parental influence in the resulting hybrids varied with the season of the year at which the experiments were made. In spring they resembled *Strongylocentrotus,* while in summer they were like *Sphaerechinus.* In the latter case, however, none of the larvæ were of the pure *Sphaerechinus* type. He suggested that this variation was due to a seasonal fluctuation in the relative ripeness of the sexual products.

In 1902 Steinbrück (17) studied the cross *Strongylocentrotus* ♂ × *Sphaerechinus* ♀ and came to the conclusion that, while there was a seasonal variation in dominance, it was not due to the relative ripeness of the eggs and sperm, but to changes in temperature; for, by raising the temperature of the water in which his larvæ were kept, in the spring he caused them to assume the summer form. In the same year Driesch (7) did some further work on the hybridization of *Strongylocentrotus, Sphaerechinus,* and *Echinus,* and substantiated his earlier results of maternal influence.

In 1906 Fischel (8) working at Villefranche, crossed *Arbacia, Echinus,* and *Strongylocentrotus* and contradicted Driesch, finding that the sperm had an important influence and that the hybrids showed undoubted paternal characters, although these were, he admitted, of a very minor degree as compared with the maternal.

In 1909 Hagedoorn (9), working in Loeb's Laboratory, Pacific Grove, Cal., crossed *Strongylocentrotus purpuratus* and *S. franciscanus* and found a purely motherly dominance in the shapes of the skeletal apical rods. In the following year Loeb, King, and Moore (10) repeated these experiments at the same place, but reached very different results. They came to the conclusion that each character was inherited separately, that is, quite apart from whether it is of maternal or paternal origin: that of a pair of allelomorphic characters one is invariably dominant over the other in the hybrid: that the characters of the Pluteus are inherited on strictly Mendelian lines. Thus, for instance, they found the club-shaped ends of the skeletal rods to be dominant over the arched form, the round, dome-shape of the larvæ to be dominant over the pyramidal, the rough spinous character of the skeletal rods dominant over the smooth, and so on through a number of characters. They made no attempt to rear their larvæ to metamorphosis and to follow these characters in the later stages, neither did they attempt to trace, if possible, the characters in the later generations, in the usual Mendelian manner.

Lastly, Tennent (18) working at the Tortugas Laboratory, off the coast of Florida, in the midst of the warm water of the Gulf Stream, crossed among other forms *Toxopneustes* and *Hipponöe*, always finding his hybrids to resemble *Hipponöe*. He then altered the concentration of the OH-ions in the seawater in which the cross was made, and found by this means that the dominance was changed to the *Toxopneustes* side. Here again, as in all the previous work, it is doubtful if characters sufficiently definite have been adopted as an index of parental influence. For instance, the skeletal support of the post-oral arm of *Toxopneustes* is a single rod, whereas in *Hipponöe* it is a lattice structure. If, in his hybrids, more than one rod appeared in the arm, Tennent considered it as an indication of *Hipponöe* influence. But as previous observers have noted, and we have repeatedly found in our own experiments, extra rods appear, under unfavourable conditions, even in forms which do not normally possess them.

From the above brief review of the subject, it is plain that the opinions expressed by the different investigators have been most conflicting, and that the conclusions they have drawn have been, in many instances, diametrically opposite to one another, although the work was frequently done with the same material. This is in great part due to the uncertain nature of the evidence on which these results have been based, evidence which has been drawn from the early development alone. No successful attempt has been made to rear the hybrids, in order to follow the nature of the parental influence in the later and less variable stages. The chief index of paternal or maternal influence has been the skeleton; but this, unfortunately, exhibits a large amount of irregular variation dependent on small metabolic changes, a variation in many cases quite independent of heredity.

The present work was commenced, therefore, with the object of discovering fixed specific characters, into which we felt certain no irregular variation entered. After considerable investigation we came to the conclusion that these could only be looked for in the later period of larval life. For in our experiments we have found that every culture jar had its own rate of development and showed minor variations with regard to the early larval characters, such as skeleton, pigmentation, and shape, so that it was plain that no definite results could be hoped for from the investigation of young stages alone.

Thanks to the methods elaborated by Dr. Allen (1) of rearing marine larvæ and of feeding them on pure cultures of diatoms from which bacteria and infusoria are as far as possible eliminated, and of keeping the larvæ in sterilized sea-water, it is now possible to rear the Plutei of Echinoids with great facility through metamorphosis to the young fully formed Sea-Urchin.* This suggested to us the idea of making a full investigation of the later stages of the pure and hybrid forms, and we hope to show that we have found in them immutable and distinct specific characters which give much more definite evidence than those hitherto used in this work.

We have chosen *Echinus acutus*, *E. esculentus*, and *E. miliaris* as the three commonest forms on our coasts, and also for the reason that we already possess in MacBride's work (1) a clear account of the main features of the normal development of these species. Of these three forms, *E. acutus* and *E. esculentus* at Plymouth are found in deeper water, while *E. miliaris* is a shore species. It is therefore highly probable that laboratory conditions are more favourable to the latter than to the two former; and this would seem to be borne out by the fact that *E. miliaris* develops, under laboratory conditions, much more quickly than the other two, and that in all crosses into which *E. miliaris* enters the rate of development is accelerated.

While there are marked specific differences between *E. miliaris*† (which has by some authors been placed in a separate genus, *Par-*

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* While previous investigators have unsuccessfully attempted to rear hybrid Plutei through metamorphosis, Doncaster (5), making use of the cross *Strongylocentrotus lividus* ♂ × *Echinus microtuberculatus* ♀, seems to have been the first to have accomplished this. One of the present authors (Shearer) had the opportunity in 1902 of examining these hybrids at Naples.

† It may be here noted that *E. miliaris*, on the Devonshire coast, exhibits several distinct varieties, which differ chiefly in size, in length of spines, and in pigmentation.

echinus) and *E. esculentus* or *E. acutus*, there is much less distinction between the two latter, which we suspect to be merely varieties of one and the same species, although according to Bell (2) the number and disposition of the plates differ considerably. We were quite unable, from a superficial examination, to tell to which species a large number of specimens, brought into the laboratory during the course of our experiments, belonged. In shape and character of their spines they were as much *E. esculentus* as *E. acutus*, and without a close examination of their plates it was quite impossible to identify them.

In the "Ingolf" Echinoidea I (14), Mortensen mentions some specimens, which "combine to a curious degree the character of both *E. esculentus* and *acutus*, var. *Flemingii*, so that it is quite impossible to decide with certainty to which of these species they belong, and the supposition of their being hybrids between the two species seems very obvious." In his recent paper (15) he gives a photograph of one of these supposed hybrids, with a description. We ourselves have long suspected that such hybridization must take place between *E. esculentus* and *E. acutus* at Plymouth, where they live side by side on the same beds, their breeding periods overlapping very considerably. If crossing between these two forms is such an easy matter in the laboratory, why should it not take place in a state of Nature? This is a question that has also been raised by Doncaster (5). We believe that considerable hybridization does take place, and the forms to which we have drawn attention above, and which agree in many respects with the one figured by Mortensen (15), seem to bear out this conclusion. In our full paper we shall give photographs and go into details of the plates of these intermediate forms.

Mortensen (15) also gives a figure of a form which he considers to be a cross between *E. esculentus* and *E. miliaris*. We have already mentioned in a footnote, page 124, that there are several varieties of *E. miliaris* at Plymouth, and one of these bears a considerable resemblance to Mortensen's supposed hybrid. This variety differs, however, in the character of its spines from the young hybrids of this cross which we have reared.

We have been unable to detect differences between the larvæ of *E. esculentus* and *E. acutus*, except that the skeletal apical rods of the latter are more robust and less arched than those of the former. For this reason, in the present paper, we have not considered in any detail the development of *E. acutus*, beyond pointing out that, when crossed with *E. miliaris*, it behaves similarly to *E. esculentus*. In our forthcoming paper we will go into these points in full.

We have tried to eliminate from our cultures, as far as possible, all abnormal or unhealthy Plutei. No drawings or observations have been made of any such larvæ, and all crosses showing an unusually large percentage of abnormalities have been thrown away and the experiments repeated. It is remarkable, however, how frequently abnormalities, especially with regard to the development of the arms and skeleton, are to be seen in Plutei taken from the Plankton.

Although we have had no difficulty in rearing Plutei in considerable numbers through metamorphosis, and some of our young hybrid urchins are now, after two years, one centimetre in diameter,* we have not succeeded so far in bringing them to a stage of sexual maturity. This is doubtless due to the fact that we have been unable to furnish them with the proper food. For it would seem, from what we have been able to discover regarding *E. miliaris,* that individuals of this species at least can become sexually mature within the first year of their existence, in a state of nature, and can attain the size of some six centimetres. As none of the *E. miliaris* hybrids in our cultures in the laboratory have shown any such rapid rate of growth, we feel that we have only partially succeeded in our feeding methods.

During the first few weeks after metamorphosis the young Echini thrive readily on the "red weed" (*Delesseria*), but after this they soon cease to grow, and evidently at this stage a further change of food is necessary. What exactly this change should be we have so far been unable to find out, and we have simply allowed them to remain in culture jars in the hope that they will find their proper food among the algae growing there.

It is the ultimate object of our work to bring the hybrid urchins to sexual maturity and, if possible, investigate the characters of the second generation. Our experiments of the last three years seem to point to the improbability of accomplishing this under laboratory conditions, and we are at present devising a method for confining our young hybrid urchins on the sea bottom in their natural habitat.

It is obvious, however, that all laboratory conditions differ in many essentials from those obtaining in nature. For this reason we have, this year, raised Plutei from the first in the sea, by confining them in jars in the chambers of a floating box, which is anchored some miles out, in the clearer water of Plymouth Sound. We have not noticed that our Plutei reared under these conditions grow more rapidly than those kept in the Laboratory, and this would seem to be due to the lack of food. It seems to be impossible to get any protected water where the Plutei might be confined, as rich in the Diatom

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* One hybrid, raised at Cambridge, is now 3 cm. in diameter. For figures of same, *vide Nature*, Vol. LXXXVII, p. 111, 1911.

flora as that of the rapidly changing tidal waters that sweep over the beds where the Echini find their natural habitat. Nevertheless a number of the Plutei reared under these conditions have metamorphosed.

II. MATERIAL.

The experiments were commenced in the Laboratory of the Marine Biological Association, Plymouth, in 1909, and continued through 1910 and 1911. Similar results were obtained from each series of experiments.

Echinus esculentus and *E. acutus* are obtainable at Plymouth in fairly large quantities by trawling in depths of about 25 fathoms.

Echinus miliaris is a shore species. It is not so numerous as the other species, and can only be collected at very low tides.

The percentage of ripe Echini in a haul is small. The quantity of material consumed was consequently large, and our thanks are due to Dr. Allen and the staff of the Laboratory for the trouble taken to provide us with a constant supply of material.

To ensure successful fertilization it is essential that the sperm and ova should be thoroughly mature. Partially ripe sperm and ova are capable of fertilizing, and of being fertilized, but the larvæ of such a union do not develop normally, and seldom reach a late stage, although they may remain alive for many days.

It might be assumed that the ideal condition would be to use Echini on the point of discharging their genital products, and such under normal conditions would be correct, but we have frequently noticed that under irritation or when in an unhealthy condition the animals discharge their sperm and ova. In a few instances we have observed them discharging in vast quantities when in a moribund condition, the urchins dying afterwards within a few hours. It is almost unnecessary to add that, when eggs or sperm, shed in this manner, are brought together, fertilization either fails to take place, or the resulting Plutei are abnormal and unhealthy.

If a ripe ovary is gently shaken in water, the ova will float away without any or but a small quantity of follicular tissue. Rough shaking should be avoided, as by it many partly ripe and immature eggs will be detached. The ovary from which the greatest number of eggs is obtained with the least agitation, and the least admixture of the tissue, will generally be found to give the best result. Under the microscope the nuclei of such eggs will not be visible.

It is more difficult to judge the ripeness of the spermatozoa, as they are motile some time before they are actually mature. The mature male gonad when torn exudes a milky fluid, which under the microscope appears to seethe with vibratile spermatozoa; but it is obviously difficult to compare the relative rapidity of motion in sperms from different individuals.

III. METHODS.

A portion of a ripe ovary was placed in a finger bowl containing either "outside water" or "Berkefeld water."[*]

As soon as the ova floated out, the piece of ovary and any pieces of tissue were removed and a small quantity of a culture of sperm added. Excess of sperm should be avoided, as it speedily fouls the water, which should be changed if it appears milky. If sperm and ova were ripe, the fertilization membrane was thrown out in a few minutes, and segmentation followed. In about twenty-four hours free-swimming blastulae appeared. These were removed to jars containing about 2000 cc. of Berkefeld or outside water. A couple of small pipettes-full of a culture of the diatom *Nitschzia closterium* were then added for food, and the blastulae left to develop.

All the usual precautions for sterilizing jars, pipettes, scissors, etc., were rigidly observed, and before being opened the Echini were immersed in tap water to destroy any sperm adhering to the test. In all experiments proper controls were kept, and in any case where these went wrong the entire batch of material was thrown away and the experiment repeated.

IV. DESCRIPTION OF EXTERNAL CHARACTERS OF NORMAL AND HYBRID LARVÆ.[†]

(*a*) EARLY DEVELOPMENT OF $\frac{\textit{E. ESCULENTUS} ♂}{\textit{E. ESCULENTUS} ♀}$

The four-armed pluteus stage is reached in about four to six days from date of fertilization. It then has a symmetrical body, well rounded at the posterior pole, with slender arms, longer than the depth of the body. It is slightly pigmented. A few days later (from seven to nine days) the third pair of arms (postero-dorsal) appears, and between the second and third weeks the anterior epaulettes. The

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[*] In using the term "outside water," we mean water brought into the Laboratory in 3–4 gallon glass carboys, collected outside the Plymouth Breakwater in the tidal water of the English Channel, and therefore three or four miles from land. These flasks are always allowed to stand for four or five days in the Laboratory before being used, thus ensuring the absence of live sperm. By "Berkefeld water" we mean ordinary Laboratory tank-water, which is of considerably lower alkalinity than "outside water," which has been treated with animal charcoal, aerated and filtered through a Berkefeld filter and then stored in sterilized flasks. See Allen and Nelson (1).

[†] The nomenclature used in this paper is that of Mortensen (13).

pluteus has still a rounded posterior pole, but the arms are longer in proportion to the depth of the body. About this time the invagination which will form the oral disc of the future *Echinus* appears. At the end of about a fortnight the fourth pair of arms is formed, and about this time the posterior pole begins to flatten until it assumes the appearance of Figure 1. In about three weeks the posterior epaulettes are well advanced, and the pedicellariae have appeared.

It may be here stated that the times given for the appearance of different organs are only very approximate. There is the widest difference in rate of development not only between the individuals in each jar, but between the larvæ of separate cultures reared from ova and sperm of the same Echini under apparently the same conditions.

EXTERNAL CHARACTERS OF LATE LARVA (FIG. 1).

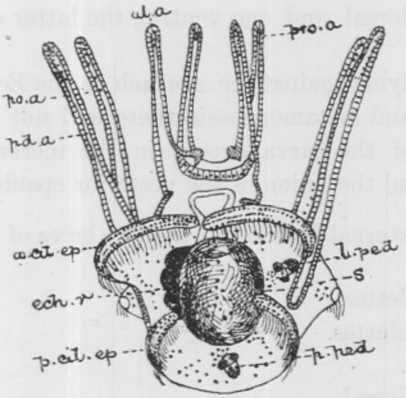

FIG. 1.—Larva of *E. esculentus* ♂ × *E. esculentus* ♀. Dorsal view. ×36. 22 days old. The specimen is tilted forward so that the posterior surface is shown. *a. cil. ep.* — Anterior ciliated epaulette. *al. a.* — Antero-lateral arm. *ech. r.*—Echinus rudiment. *l. ped.*—Lateral pedicellaria. *pro. a.*—Pre-oral arm. *p. cil. ep.*—Posterior ciliated epaulette. *p. ped.*—Posterior pedicellaria. *pd. a.*—Postero-dorsal arm. *po. a.*—Post-oral arm. *s.*—Stomach.

The body is rather deeper than wide, and the posterior pole somewhat flattened. It is studded with pigment spots of various shades from yellow to reddish brown, and of various shapes and sizes. In the specimen from which the drawing was made the body was regularly pigmented, while little, if any, pigment appears on the arms except at the extremities. Pigment, however, varies widely among individuals of the same culture in depth of colour and distribution, and this seems natural, since MacBride (11) has shown that the pigment is contained in wandering amoebocytes and is an excretory product in process of removal.

There are four pairs of long, slender arms, and along their edges run the ciliated bands. Around the anterior margin of the body are the "anterior ciliated epaulettes." These arise as four horizontally placed crescentic bands of cilia, constricted off from the main ciliated band, at the base of the post-oral and postero-dorsal arms. They gradually grow together, and before metamorphosis form a complete ring round the anterior pole of the body. In the figure they have not yet united dorsally. They are spotted with brown pigment, and carry powerful cilia, which in these late stages are the principal means of locomotion. At the posterior end are the "posterior ciliated epaulettes." These are pigmented and ciliated like the anterior epaulettes, and eventually will extend round the posterior pole of the larva.

There are three pedicellariae: one at the posterior pole, and two on the right side, one dorsal, and one ventral, the latter of which is not seen in the figure.

On the left hand, lying against the stomach, is the Echinus rudiment. Between this stage and metamorphosis there will not be much change in the appearance of the larva, except in the increased size of the Echinus rudiment and the union of the posterior epaulettes.

Thus the salient external features of the late larva of $\frac{\textit{E. esculentus} \ ♂}{\textit{E. esculentus} \ ♀}$ are :—

The anterior epaulettes.
The posterior epaulettes.
Three pedicellariae.
  One right side dorsal.
  One right side ventral.
  One at the posterior pole.

(*b*) EARLY DEVELOPMENT OF $\frac{\text{E. MILIARIS} \ ♂}{\text{E. ESCULENTUS} \ ♀}$

There was a good deal of variation among the four-armed plutei of this hybrid. The majority inclined to the pointed, clear, lightly pigmented *miliaris* type, but did not develop a preoral lobe. On the other hand, there were some that showed distinctly *esculentus* characters. In the course of development the posterior pole becomes more rounded, and, when the anterior epaulettes were formed, either the larvæ were distinctly *esculentus* in shape, or intermediate between *esculentus* and *miliaris.* The posterior epaulettes did not show much advance until the end of the fourth week, by which time the right dorsal pedicellaria was well established.

EXTERNAL CHARACTERS OF LATE LARVA (FIG. 2).

The body is not so wide as the pure *E. miliaris*, or so deep and flattened at the posterior pole as the pure *E. esculentus*. There is a good deal of brown pigment arranged in rather regular patches on the body, and also on the arms which are intermediate in form between the stumpiness of pure *miliaris*, and the length and slenderness of pure *esculentus*. The deeply pigmented anterior epaulettes have nearly surrounded the anterior margin of the body. The posterior epaulettes are not so far advanced. There are three pedicellariae, one at the posterior pole, and two on the right side, dorsal and ventral, the latter of which does not appear in the figure. On the left side is the young Echinus, well advanced, and with protruding tube-feet. The chief external features of this hybrid are:—

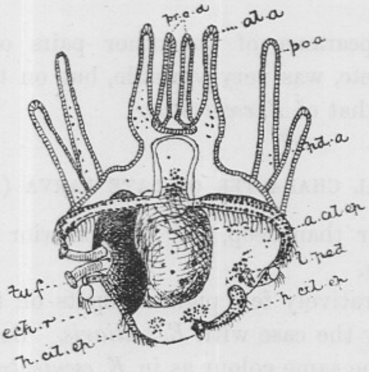

FIG. 2.—Larva of *E. miliaris* ♂ × *E. esculentus* ♀. Dorsal view. × 36. 36 days old. This larva is more advanced than that shown in Fig. 1, and the tube-feet of the the young Echinus are protruded. *tu.f.*—Tube-feet. The remainder of the lettering as in Fig. 1.

The anterior epaulettes.
The posterior epaulettes.
Three pedicellariae.
  One right side dorsal.
  One right side ventral.
  One at posterior pole.

(*c*) EARLY DEVELOPMENT OF $\frac{\textit{E. MILIARIS} ♂}{\textit{E. MILIARIS} ♀}$

The four-armed pluteus of *E. miliaris* is readily distinguished from that of *E. esculentus*. It is smaller, the posterior end is more pointed, and the arms are shorter as compared with the length of the body. It carries less pigment on the body than *esculentus*; and, in the early stages, there is often a very regular line of small pigment spots along the ciliated bands.

A marked preoral lobe overlies the mouth; and the larva generally presents a peculiar glassy, transparent appearance.

As the larva develops it retains its elongated form until about the time that the epaulettes appear. The posterior pole then gradually becomes more rounded, and finally assumes the shape depicted in Fig. 3.

The time of appearance of the other pairs of arms, epaulettes, Echinus rudiment, etc. was very variable, but on the average did not greatly differ from that of *E. esculentus.*

EXTERNAL CHARACTER OF LATE LARVA (FIG. 3).

The body is wider than deep, and the posterior pole more rounded than in *E. esculentus.*

There are comparatively few pigment spots on the body and arms, and this is generally the case with *E. miliaris.* Besides this pigment, which is of much the same colour as in *E. esculentus,* about the end of the third week, when the epaulettes are forming, a large mass of bright green pigment appears at the base of each. It always makes its first appearance at these points, but afterwards appears in spots on the arms, and just before metamorphosis is widely diffused. This green pigment is not found in *E. esculentus.* The arms are shorter and more stumpy than in those of *E. esculentus.*

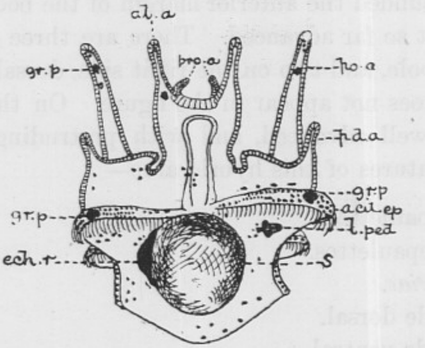

FIG. 3.—Larva of *E. miliaris* ♂ × *E. miliaris* ♀. Dorsal view. × 36. 20 days old. *gr.p.*—Green pigment. Other lettering as before.

There is only one set of epaulettes, the anterior, and they gradually close together and encircle the anterior pole of the body. They are dotted with yellowish brown pigment, generally of a lighter shade than in *E. esculentus*, and carry strong cilia.

There are two pedicellariae on the right side, one dorsal and one ventral; the latter not being shown in the figure. On the left of the stomach is the Echinus rudiment. Between this stage and metamorphosis there is not much change except in the size of the Echinus rudiment, and the amount of green pigment.

The chief external features are:—

Anterior epaulettes only.
Two pedicellariae.
  One right side dorsal.
  One right side ventral.
Masses of bright green pigment.

(*d*) EARLY DEVELOPMENT OF $\frac{\textit{E. ESCULENTUS} \, ♂}{\textit{E. MILIARIS} \, ♀}$

The four-armed plutei differ but little from the pure *E. miliaris* larvæ of the same stage, excepting that they carry rather more pigment. A preoral lobe is present, but is not marked as in pure *E. miliaris*. The typical elongated *E. miliaris* shape is retained until about the time when the epaulettes make their appearance, when the posterior pole gradually assumes a flatter curve as in the figure.

EXTERNAL CHARACTER OF LATE LARVA (FIG. 4).

The body is not so deep, and the arms are longer than those of the pure *E. miliaris*, but the general appearance is very similar. The whole body, and the ciliated bands, are spotted with pigment of various shades of reddish brown.

There is besides a large mass of green pigment at the bases of the epaulettes, and another mass of the same has appeared between their ends, and later on more will probably appear.

There is only one set of epaulettes, which ultimately encircle the anterior margin of the body. They are spotted with brown pigment and carry strong cilia.

There are two pedicellariae, one dorsal and one ventral, on the right side (the latter not shown in Fig. 4).

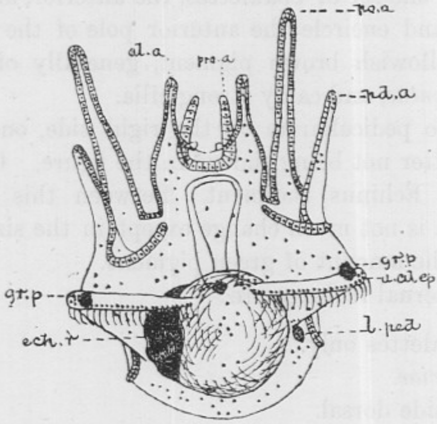

FIG. 4—Larva of *E. esculentus* ♂ × *E. miliaris* ♀. Dorsal view. ×36. Forty days old. Although it is twice the age of that shown in Fig. 3, there is little difference between them, except in size. Lettering as before.

This pluteus is twenty days older than the pure *E. miliaris* (Fig. 3), but beyond an increase in size there is little difference.

The chief external features of this hybrid are :—

The anterior ciliated epaulettes.

Two pedicellariae.

One right side dorsal.

One right side ventral.

Masses of green pigment.

(*e*) EXTERNAL CHARACTERS OF THE LATE LARVÆ $\frac{\textit{E. ACUTUS}\ ♂}{\textit{E. ACUTUS}\ ♀}$

Regarding *E. acutus* and *E. esculentus* as distinct species, it might be expected that their late larvæ would show some marked specific differences. Such differences we have, however, been so far unable to discover. The late larva of *E. acutus* resembles that of *E. esculentus* in number and position of pedicellariae, of ciliated epaulettes and in the absence of green pigment. Superficially the larva of *E. acutus* has a smaller body and slenderer arms, with darker and more abundant pigment than that of *E. esculentus*, but hybrids between these species afford no criteria by which parental influence can be discriminated.

Hybrids, however, between *E. acutus* ♂ and *E. miliaris* ♀ resemble those between *E. esculentus* ♂ and *E. miliaris* ♀, in the absence of the posterior pedicellaria and of the posterior ciliated epaulettes and the possession of green pigment masses, while in the reciprocal cross the characters common to *E. acutus* and *E. esculentus* make their appearance.

SUMMARY OF LATE LARVAL CHARAC

	Anterior epaulettes.	Posterior epaulettes.	Green pigment.	Right dorsal pedicellaria.	Right ventral pedicellaria.	Posterior pedicellaria.
E. esculentus ♂ *E. esculentus* ♀	+	+	○	+	+	+
E. miliaris ♂ *E. esculentus* ♀	+	+	○	+	+	+
E. miliaris ♂ *E. miliaris* ♀	+	○	+	+	+	○
E. esculentus ♂ *E. miliaris* ♀	+	○	+	+	+	○

Considering only these six external characters the experiments of 1910–11 show that :—

1. The egg of *Echinus esculentus* fertilized by its own sperm produces a late larva having anterior epaulettes, posterior epaulettes, no green pigment, one right side dorsal pedicellaria, one right side ventral pedicellaria, one posterior pedicellaria.

2. The egg of *Echinus esculentus* fertilized by sperm of *Echinus miliaris* produces a late larva having anterior epaulettes, posterior epaulettes, no green pigment, one right side dorsal pedicellaria, one right side ventral pedicellaria, one posterior pedicellaria.

These characters are the same as 1.

3. The egg of *Echinus miliaris* fertilized by its own sperm produces a late larva having anterior epaulettes, no posterior epaulettes, green pigment, one right side dorsal pedicellaria, one right side ventral pedicellaria, no posterior pedicellaria.

4. The egg of *Echinus miliaris* fertilized by sperm of *Echinus esculentus* produces a late larva having anterior epaulettes, no posterior

epaulettes, green pigment, one right side dorsal pedicellaria, one right side ventral pedicellaria, no posterior pedicellaria.

These characters are the same as 3.

V. CHARACTERS OF THE SKELETON.

While we have already stated that the skeleton is extremely variable, and is a doubtful index of parental influence, what evidence we have been able to derive from the study of a large number of larvæ seems distinctly to bear out some of the more recent contentions. While we reserve the publication of a large number of figures of the skeletal apical rods, we have inserted one typical example of each cross (Fig. 5). Here the evidence would seem to be distinctly in favour of the dominance of one character over another, as brought out by Loeb, King, and Moore (10). For, with reference to Fig. 5, it will be seen that the normal apical skeleton of *E. esculentus* is slender, arched, and somewhat spinous, that of *E. acutus* is more robust and bears a greater number of spinous processes, while that of *E. miliaris* is straight and club-shaped, bearing a few blunt knobs.

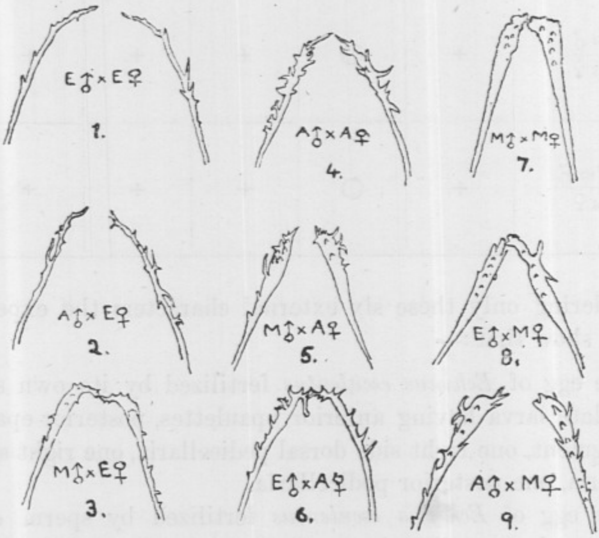

Fig. 5.—Skeletal apical rods of four-armed Plutei. 1. *E. esculentus* ♂ × *E. esculentus* ♀. 2. *E. acutus* ♂ × *E. esculentus* ♀. 3. *E. miliaris* ♂ × *E. esculentus* ♀. 4. *E. acutus* ♂ × *E. acutus* ♀. 5. *E. miliaris* ♂ × *E. acutus* ♀. 6. *E. esculentus* ♂ × *E. acutus* ♀. 7. *E. miliaris* ♂ × *E. miliaris* ♀. 8. *E. esculentus* ♂ × *E. miliaris* ♀. 9. *E. acutus* ♂ × *E. miliaris* ♀.

Loeb states that the spinous condition, as is exhibited for example by *E. esculentus* and *E. acutus* (Fig. 5), is dominant over the smooth, as seen in *E. miliaris*; and that the clubbed condition, like that of *E. miliaris*, is dominant over the arched form, as that of *E. esculentus*. This inheritance is stated to take place irrespective of whether the characters are paternal or maternal. This we find to be the case in our crosses, but we also find that the female has a stronger influence on the character of the hybrid skeleton than the male. As an illustration of the dominance of the spinous condition over the smooth, it will be seen in Fig. 5 that in the cross *E. acutus* ♂ × *E. miliaris* ♀ and its reciprocal the rough condition appears. Again, with regard to the clubbed and arched conditions, in the cross *E. esculentus* ♂ × *E. miliaris* ♀ and its reverse the clubbed form appears in the hybrids. It is also clear from the figure that maternal influence is stronger than the paternal.

VI. THE CHEMICAL CONTROL OF INHERITANCE.

In 1910 Tennent (18), working at Tortugas, made the cross *Toxopneustes* ♂ × *Hipponöe* ♀ and its reciprocal, producing hybrid larvæ which had, in both cases, the characteristics of *Hipponöe*. The skeleton was used as an index of parental influence, and we have already discussed the doubtful value of this evidence. He altered the concentration of the OH–ions in the water in which the fertilizations were made, by adding small definite quantities of Sodium hydrate and of Acetic and Hydrochloric Acid. By this means he claims to have altered the dominance, so that the hybrid skeleton now resembled that of *Toxopneustes*. While, from a close examination of his figures, it is clear that a considerable percentage of his Plutei showed skeletal abnormalities, his main result may or may not be considered as proved. If it is true, the conclusion is obviously a very important one, and one which should be tested with other material and at other places. For this reason, although the inheritance of the late larval characters of the hybrids at Plymouth is strictly maternal, and is not determined by any particular species, yet these characters are of such a definite nature, that we thought it to be a good opportunity for repeating this work.

With this object the crosses *E. esculentus* ♂ × *E. miliaris* ♀ and *E. acutus* ♂ × *E. miliaris* ♀, and their reciprocals were made. The eggs were placed in sea-water, the OH–ion concentration of which had been altered by the addition of .25 to 1 cc. N/10 NaOH in the one case, and HCl or Acetic Acid, in the other, per 200 cc. of sea-water. As soon as the blastulae swam to the surface, they were transferred to "outside" or to "Berkefeld sea-water." At the same time, cultures of the same crosses were made, which were fertilized and raised from the commencement in sea-water of the normal OH–ion concentration.

A large number of larval skeletons were examined at about ten days old, drawings of which will appear in the forthcoming work, but in no case was the inheritance at all affected. At a late stage (30–40 days), again, a large number of Plutei were examined, but in no case could any alteration in the inheritance of the posterior ciliated epaulettes, the pedicellariae or the green pigment masses, be detected. Figs. 6 and 7 show typical 28-day Plutei of the cross *E. acutus* ♂ × *E.*

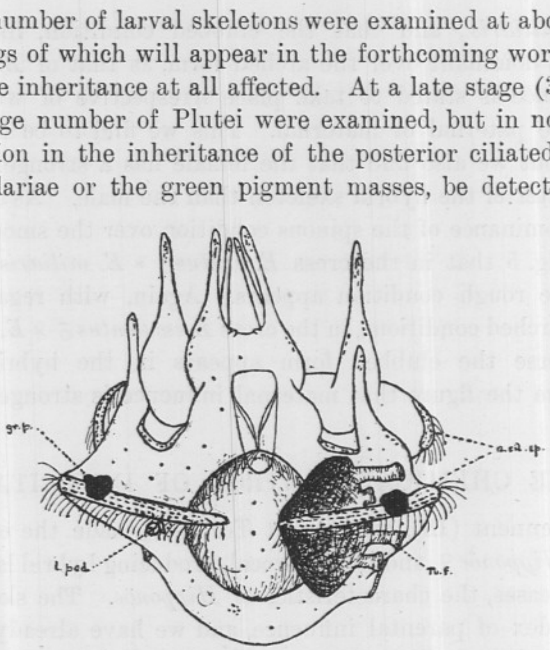

FIG. 6.—Larva of *E. acutus* ♂ × *E. miliaris* ♀. Dorsal view. 28 days old. Fertilized in water with raised OH-ion concentration. *a. cil. ep.*—Anterior ciliated epaulette. *gr. p.*—Green pigment. *l. ped.*—Lateral pedicellaria. *tu. f.*—Tube-foot.

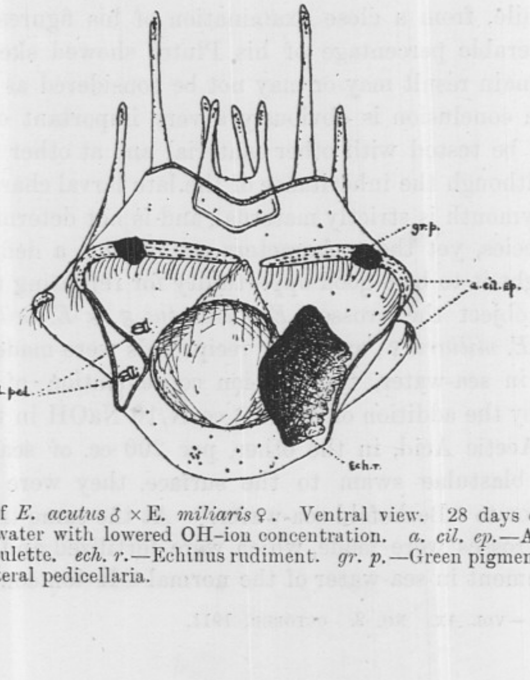

FIG. 7.—Larva of *E. acutus* ♂ × E. *miliaris* ♀. Ventral view. 28 days old. Fertilized in water with lowered OH-ion concentration. *a. cil. ep.*—Anterior ciliated epaulette. *ech. r.*—Echinus rudiment. *gr. p.*—Green pigment mass. *l. ped.* Lateral pedicellaria.

miliaris ♀ which had been fertilized in water treated respectively with alkali and acid. It will be seen that they differ in no essentials from the hybrids raised in normal sea-water. The inheritance of the maternal pigment masses, absence of posterior ciliated epaulettes and absence of posterior pedicellaria is unchanged.

VII. THE CHARACTERS OF THE YOUNG SEA-URCHIN.

In all cases the young fully formed Urchins, in superficial features, such as the character of the spines, pigmentation, etc., show a motherly influence, but so far we have not made a close examination of the number and structure of the test plates. The young Urchins always secrete a thick layer of mucus on the surface of the test, which effectually prevents any examination of the plates in the living state, and any such examination necessitates killing the hybrids. We wish to reserve for a future occasion our statements on this head.

There is this important feature about the tube feet of *E. miliaris*, to which attention has been drawn by MacBride (11). He has shown that the young *E. esculentus* and *E. acutus* walk by means of five tube feet, each forming the termination of one of the radial canals, and each provided with a sucker, in the centre of the disk of which is a sense organ consisting of elongated cells. In each radius there are in addition the rudiments of a pair of tube feet, which are mere buds in these species, but in *E. miliaris* these accessory tube feet are functional at metamorphosis. So we have this important distinction between the normal just metamorphosed *E. miliaris* and *E. esculentus* or *E. acutus*, that the former has five principal tube feet and ten secondary ones, while the two latter have only the five principal ones, the secondary ones being entirely rudimentary at this stage. This, therefore, offers a definite index of parental influence after metamorphosis. On this head also we wish to defer our statements, to incorporate them later with the evidence offered by the study of the structure and number of the hybrid test plates.

VIII. SUMMARY OF CONCLUSIONS.

1. As the result of extensive investigation of the early larval history of our various crosses, we have come to the conclusion that these are too variable to afford any definite evidence of parental influence, and especially is this true with regard to the skeleton, heretofore considered the chief index of inheritance.

2. What little evidence we have been able to derive from the study of a very large number of skeletons of both normal and hybrid crosses, seems to show that, while the paramount influence is always maternal,

there is considerable evidence for the contention put forward by Loeb, King, and Moore (10) that the minor skeletal characters are inherited independently of either parent. We are able to bear out their statement that with regard to the apical rods, the spinous condition of which is dominant over the smooth, and the clubbed condition over the arched, quite independently of their origin either from the paternal or the maternal side.

3. Regarding the early larval characters, therefore, as of too variable a nature, we have reared the normal and hybrid crosses to the young Urchin stage, in the hope of finding, in the late development, more definite characters for the solution of the question of inheritance. In the presence or absence of the posterior ciliated epaulettes, of the green pigment masses and of the posterior pedicellaria, we claim that we have found such definite characters, and we find them to be invariably inherited through the egg. A full summary of these facts has already been given in § IV, page 135.

4. We have shown that an alteration of the alkalinity of the water affects the inheritance neither of the skeletal characters nor of those of the late larva.

5. The young hybrid Urchins, some of which have already reached a considerable size and are now two years old, are, in superficial appearance, of the pure motherly type.

6. We suspect that a considerable amount of hybridization occurs under natural conditions between *E. esculentus* and *E. acutus.*

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The Action of some Denitrifying Bacteria in Tropical and Temperate Seas, and the Bacterial Precipitation of Calcium Carbonate in the Sea.

By

G. Harold Drew.

TABLE OF CONTENTS.

INTRODUCTION.

It is generally conceded that the plankton of tropical and sub-tropical seas is far less in quantity than that found in colder waters.

The zoö-plankton depends ultimately for its food on the phyto-plankton; hence any factor limiting the growth of the phyto-plankton, which is capable of functioning in tropical and not in temperate or Arctic waters, might offer an explanation of this phenomenon. It has been shown by various investigators that this factor is not temperature, light, or salinity, and it has been suggested that the explanation may lie in the relative deficiency in tropical seas of the nitrates or nitrogenous compounds which are so essential for all plant life. A matter of common observation in support of this view is the remarkable scarcity of algal growth in the shallow waters of tropical shores as compared with that in similar situations in temperate regions, and the fact that in the tropics, wherever sewage or other nitrogenous waste is poured into the sea, a free growth of algæ is found.

At present no reliable and accurate chemical method of estimating the combined nitrogen in sea-water exists, hence this theory cannot be directly put to the test. On the other hand, the existence of denitrifying bacteria in temperate waters has long been known, and it would seem a fair deduction that should this bacterial destruction of nitrates take place with greater intensity and completeness in tropical than temperate waters, an explanation of the relative scarcity of plankton in the former would be offered, and it was with the object of investigating this question that the present work was undertaken.

My thanks are due to the Marine Biological Department of the Carnegie Institute of Washington, U.S.A., for their kindness in accommodating me in their Laboratory at Loggerhead Key, Dry Tortugas, and at their temporary Laboratory in Port Royal, Jamaica, B.W.I., and also to the Marine Biological Association of the United Kingdom for giving me facilities for work in their Plymouth Laboratory.

METHODS.

At the Dry Tortugas Laboratory, the motor-yacht *Anton Dohrn* enabled me to obtain samples of water from the middle of the Gulf Stream, and a number of smaller motor-boats were always available for shorter journeys. At Port Royal, Jamaica, it was necessary to depend on a sailing-boat, but owing to the remarkable regularity with which a breeze springs up every morning, no difficulty was encountered from this cause. At Plymouth the s.s. *Oithona* enabled me to obtain samples of water from a point seventy miles west of Ushant.

The observations were made at Port Royal during May, at the Tortugas during June, and at Plymouth in August, 1911.

For purposes of comparison an endeavour was made in every case to obtain samples of water from localities where truly oceanic conditions prevailed, and hence samples were not obtained from the English Channel, where previous experience had shown that the bacterial flora was abundant and varied, owing to contamination from the land.

Surface samples were collected in sterilized wide-mouthed stoppered bottles holding about twelve ounces. Care was taken to avoid contamination from the sides of the boat by always collecting the samples from the bows when the boat was going ahead.

Deep samples were collected at the Tortugas in retort-shaped glass flasks of about 300 c.c. capacity, with narrow, recurved, long-drawn-out necks. These were sterilized, exhausted, and sealed: they were then lowered in an apparatus in which the extremity of the neck could be broken off at any desired depth by sending a messenger down the sounding wire, when the flasks became completely filled with water. After hauling up, a little water was shaken from the neck, and it was then sealed with the blow-pipe. By this method risk of contamination from more superficial layers of water as the apparatus is drawn up is avoided, since the changes in pressure and temperature as it ascends tend to cause a continuous outflow through the narrow neck until the surface is reached. The samples were taken back to the Laboratory, and cultures were made within three hours of collecting.

A somewhat similar apparatus was used for obtaining deep samples from the station seventy miles west of Ushant, but the glass bulbs were smaller and the tube leading from them was bent at right angles to itself. Considerable difficulty was caused by the breaking of the tube, owing to the force of the inrushing stream of water impinging on the wall where it was bent at right angles. After collecting the sample, the tubes were sealed with melted paraffin wax, and this method does not appear to have caused any bacterial contamination of the samples. Attempts to make Agar plate cultures in Petri dishes on board did not give satisfactory results, as, owing to the motion of the boat, the jelly set in irregular waves and lumps. Consequently the samples were kept in ice, and cultures were made from them at Plymouth twenty-four hours after collection. It is clear that if in the future attempts are made to make plate cultures on board a small boat, a very delicately swung table will be necessary, or else the roll tube culture method must be employed.

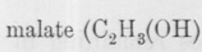
In Jamaica no apparatus for obtaining deep samples was available, so the primitive method of lowering a sterilized stoppered bottle with a string tied to the stopper was employed. At the required depth the stopper was pulled out until the bottle was nearly full, and then allowed to fall back in place. This method can only be used for very slight depths, owing to the pressure of the water at greater depths making it impossible to withdraw the stopper: a source of error also is introduced in that the inrushing water passes in close proximity to the stopper and its attachments, and may carry in bacteria which have adhered to them when passing through the surface layers.

The media most commonly employed for isolating the bacteria in plate cultures had the following composition:—

I. Peptone Agar.

Peptone	2·0 grammes.
Potassium nitrate (KNO_3) . . .	0·5 ,,
Sea-water	1000·0 c.c.
Agar Agar. . . .	12·0 grammes

II. Potassium Malate Agar.

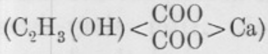
Potassium malate  $(C_2H_3(OH)<\genfrac{}{}{0pt}{}{COOK}{COOK})$	1·0 grammes.
Sodium phosphate ($Na_2HPO_4,12H_2O$)	0·25 ,,
Potassium nitrate (KNO_3)	0·5 ,,
Sea-water	1000·0 c.c.
Agar Agar	12·0 grammes.

The medium was only filtered through glass wool, so that a very slight floccular precipitate of Calcium phosphate was retained.

For other purposes a simple solution of Peptone in sea-water was employed (2 grammes to 1000 c.c.), and media were also used consisting of this Peptone solution with the addition of 0·5 per cent of various carbohydrates, such as Cane-sugar, Dextrose, Laevulose, Mannite, Lactose, etc., with sufficient Neutral Red solution to colour them.

In the case of Gelatin media, it was necessary to keep them artificially cooled to a temperature of between 20° and 25° C., as in the Tortugas the Laboratory temperature occasionally rose as high as 37° C., at which temperature Gelatin media will not remain solid.

When comparing various samples of water as to their power of causing denitrification in culture media, the following uniform method was employed:—

> 10 c.c. of each sample was added to 1000 c.c. of a modification of Gran's medium (see *Studien über Meeresbacterien*, by H. H. Gran, Bergens Museums Aarbog, No. 3, 1901). This culture fluid was sterilized in glass flasks and has the following composition:—
>
> | | |
> |---|---|
> | Potassium nitrate (KNO_3) | 0·5 grammes. |
> | Sodium phosphate (Na_2HPO_4, $12H_2O$) | 0·25 grammes. |
> | Calcium malate  $(C_2H_3(OH)<\genfrac{}{}{0pt}{}{COO}{COO}>Ca)$ | about 5·0 grammes. |
> | Sea-water | 1000·0 c.c. |
>
> Calcium malate is only slightly soluble in water (about $\frac{1}{20}$th per cent), and so can be added in excess.

The reduction of the nitrate to a nitrite was tested for by the addition of 5 c.c. of 10 per cent Sulphuric acid and 2 c.c. of a 1 per cent solution of Metaphenylene diamine hydrochloride to 25 c.c. of the culture. The production of a brown coloration (due to the formation of Bismark brown) is an indication of the presence of a nitrite, and is an extremely delicate reaction.

The formation of Ammonia was tested for by the addition of 5 c.c. of a 10 per cent solution of Potassium hydrate, and 5 c.c. of Nessler's reagent: the white precipitate formed on the addition of the Potassium hydrate does not appreciably interfere with the test, though it renders it somewhat less delicate.

The presence of nitrates or nitrites remaining in the culture fluid after the bacterial action was tested by the extremely delicate Brucine and Diphenylamine reactions.

THE INVESTIGATION OF SAMPLES OF WATER TAKEN OFF PORT ROYAL, JAMAICA.

In Jamaica, a measurement of the rate of denitrification in the modified Gran's medium inoculated with samples of sea-water was made, but the isolation of the bacteria on solid media was not attempted, owing to lack of apparatus.

Samples of sea-water were collected in sterilized stoppered bottles from the surface, and from depths of three and six fathoms, in positions about five miles from shore, where, from a consideration of the wind and tide, the water was probably under truly oceanic conditions, and unaffected by the neighbouring land.

The cultures were kept in a moderately dim light, and the room temperature ranged between 25° and 31·5° C. The average temperature during the growth of each culture was noted.

In a typical culture made from surface water, and for which the average temperature was 29° C., the first indication of the formation of a nitrite, as given by the Metaphenylene diamine reaction, appeared after twenty-seven hours: after thirty-eight hours the brown colour produced in this reaction was very intense, the culture became cloudy, and on testing with Nessler's reagent, slight Ammonia formation was apparent. After forty-eight hours the culture became very cloudy and a scum of bacterial growth developed: the nitrite and Ammonia reactions remained unaltered. After sixty-three hours the nitrite reaction was somewhat less marked, the Ammonia reaction was unaltered, and bubbles of gas began to appear. After seventy-two hours many bubbles of gas were being produced and the nitrite and Ammonia reactions were very slight. After eighty-six hours the bubbling had ceased, and no nitrite or Ammonia was present in the cultures. Testing the culture for nitrates by the Diphenylamine and Brucine methods, showed that no nitrates or nitrites were left in the solution. In the absence of a gas analysis apparatus the nature of the gas evolved could not be exactly determined, but considering that it was non-inflammable, did not turn lime-water milky, and that the nitrate originally present had been destroyed, it seems strongly probable that this gas was pure Nitrogen.

Thus at a temperature of 29° C., 0·5 grammes of Potassium nitrate were decomposed in eighty-six hours, and it was found that if a further 0·5 grammes of Potassium nitrate was added to the culture, it was in turn rapidly decomposed: this could be repeated indefinitely until the other constituents of the medium were used up, or the concentration of waste products became too high.

The rate of denitrification varied considerably with the temperature, and in cultures kept at a temperature between 10° and 12°C., no growth or denitrification occurred. Denitrification was more rapid in cultures from water taken from a depth of three or six fathoms than from the surface. It was also especially rapid with samples taken from the thick, muddy waters of a mangrove swamp, where organic matter was plentiful.

The bacteria present in the cultures were very minute, actively motile bacilli with rounded ends.

THE INVESTIGATION OF SAMPLES OF WATER TAKEN ROUND THE DRY TORTUGAS AND IN THE STRAITS OF FLORIDA.

Cultures made in the modified Gran's medium from various positions round the Tortugas gave results in close accord with those found in Jamaica, and the average rate of denitrification was the same; hence it appears probable that much the same bacterial conditions obtain in both places. Cultures were made on various solid media, and pure cultures of the bacteria were isolated. Petri dishes with porous earthenware covers were used and were found of great advantage, as by this means the formation of drops of water on the covers is prevented, and the water of condensation evaporates as soon as formed: in addition a free air supply is ensured. If evaporation is progressing too rapidly, the whole Petri dish can be covered with a bell-jar lined with damp filter-paper after the first day.

By plating samples of surface water from various positions as far as possible removed from the influence of the land, an average of fourteen colonies per 1 c.c. sea-water was obtained. These colonies appeared to be of two kinds, one much more plentiful than the other. Subcultures made from these colonies in Gran's medium showed that the bacteria forming the most common type of colony produced an active denitrification, while the others grew very slowly in this medium, and produced no denitrification. The chief characteristics of the denitrifying form are as follows:—

On the Potassium malate, or Peptone Agar media, colonies are visible as minute white specks after six to eight hours, when the room temperature averages 29·5° C. After about eighteen hours the colonies are well developed, they are white in colour, circular, but with finely irregular outline, and have a granular appearance. Superficial colonies are much elevated at first, but as growth proceeds, spread rapidly over the surface of the Agar. The deep colonies remain small, circular, and discrete. Growth is somewhat more rapid on the Peptone Agar than on the Potassium malate Agar, and the older colonies develop a brownish tinge in the centre when growing on the former medium.

On Gelatin Peptone (5 per cent Peptone, kept at between 20° and 25° C. to ensure the medium remaining solid) growth was very slow; in stab cultures growth proceeded slowly from the surface downwards, forming a funnel-shaped depression of liquefied gelatin. Acid formation occurs in Glucose, Mannite, and Cane-sugar, but not in Lactose media.

Growth is totally inhibited at a temperature of 10° C., but takes place slowly at 15° C.

Growth is much retarded by exposure to bright sunlight, but the bacteria are not killed by a ten hours' exposure.

The bacteria are facultative anaërobes, but growth under anaërobic conditions is very slow.

In Gran's medium growth and denitrification are rapid, but no growth occurs if the Potassium nitrate be omitted, or if the Calcium malate be replaced by Calcium carbonate. Growth in a pure solution of Peptone in sea-water is very slight, but becomes abundant if Potassium nitrate be added, when denitrification quickly ensues.

In its cultural reactions this bacterium is very similar to certain denitrifying forms found in the English Channel. The chief points of difference consist in its much greater denitrifying power, and the higher temperature necessary for its growth, by which it would be prevented from spreading into colder waters.

The characteristics of the scarcer, non-denitrifying, form of bacterium found on the Peptone Agar plates made from surface samples are as follows:—

Growth on the Potassium malate Agar medium is very slow and indefinite. On Peptone Agar growth is somewhat slower than in the case of the denitrifying form. On the surface, circular cream-coloured colonies are formed having a brownish centre, the edges are smooth and regular, and the colony remains discrete and does not tend to spread over the surface. The deep colonies are smaller and usually ovoid in shape, and of a somewhat darker colour than those on the surface. Growth does not occur on gelatin media.

Acid formation takes place in Glucose, but not in Cane-sugar, Lactose, or Mannite media.

Growth is much retarded by exposure to a strong light, and cultures on sloped Peptone Agar are completely killed by four hours' exposure to bright sunlight.

The bacterium is a strict aërobe.

Free growth takes place in Gran's medium, but develops much slower than in the case of the denitrifying form : no growth occurs if the Potassium nitrate be omitted entirely, but takes place freely if a mere trace in excess of that normally present in the sea-water be added, though no denitrification results. Attempts were made to discover whether this bacterium had any nitrifying or denitrifying action in various culture media, but uniformly negative results were obtained. Nitrites were neither oxidized to nitrates, nor reduced to Ammonia or free Nitrogen, and Ammonium salts were unaffected. No growth was obtained in any culture medium that did not contain at least a trace of nitrates, so it was not practicable to ascertain whether the bacterium had a nitrifying action without the necessary facilities for quantitative work.

On one occasion samples were obtained from various depths up to 90 fathoms at a point in the Gulf Stream, 25 miles south of the Dry Tortúgas.

The samples were plated in the Peptone Agar medium, and counted with the following average results :—

Depth fathoms.	Denitrifying forms.	Non-denitrifying forms.	Number of colonies developing from 1 c.c. of sample.
0	9	2	11
10	25	4	29
40	2	2	4
60	5	3	8
90	5	6	11

If any deductions can be made from one series of observations, it would seem probable that the non-denitrifying bacteria are a deep-water form, and this would be upheld by the ease with which they are killed by exposure to sunlight. On the other hand, the denitrifying bacteria would appear to be a surface form, reaching their maximum a little below the surface. This would be substantiated by the results obtained in Jamaica, by which it was shown that denitrification occurred much more rapidly in cultures made from samples collected at depths of 3 and 6 fathoms, and might possibly be explained by the fact that the growth of the bacteria is inhibited by strong light.

INVESTIGATION OF SAMPLES FROM A POINT 70 MILES WEST OF USHANT.

The samples were plated in the Peptone Agar medium after having been kept on ice for twenty-four hours, for the reason previously explained.

The colonies were well developed after forty-eight hours, and appeared to be all of one kind. A count gave the following average results:—

Depth in fathoms.	Number of colonies developing from 1 c.c. of sample.
0	7
10	9
20	6
30	5
50	6
70	30
80	20

The increase in the number of colonies at 70 and 80 fathoms is somewhat remarkable, but no conclusions in this respect can be drawn from one series of observations.

The appearance and cultural characteristics of the bacterium obtained from these samples were identical with those of the denitrifying form investigated at the Tortugas, with the exception that acid formation did not take place in media containing Cane-sugar.

1000 c.c. of the modified Gran's medium inoculated on board with 10 c.c. of a surface sample immediately after collection, and kept at an average temperature of 20° C., showed the first trace of nitrite formation after 70 hours. After 84 hours a very strong nitrite reaction was obtained, and a slight Ammonia reaction was given with Nessler's reagent. The process of denitrification, even after the lapse of weeks, did not extend beyond this, and no bubbles of gas were formed. Other experiments made with subcultures from Agar and Gelatin media gave similar results, so that it appears that this bacterium cannot entirely break down nitrates at a temperature of 20° C. The optimum temperature for denitrification produced by this bacterium appears to be about 20° C., as the process was less rapid at average temperatures of 17° C. and 25° C. At a temperature of 32° C. rapid growth took place, but no denitrification resulted.

It should be noted that these temperature observations were only made with subcultures from colonies on Peptone Agar and Peptone Gelatin media, and there is reason to believe that the power of denitrification becomes diminished after cultivation on such media. Further and more accurate temperature experiments are required, in which the culture medium is directly inoculated with freshly collected samples of water.

INVESTIGATION OF SAMPLES OF WATER FROM THE MARQUESAS KEYS, AND THE EXPERIMENTAL PRECIPITATION OF CALCIUM CARBONATE BY BACTERIAL AGENCY.

The Marquesas Keys constitute a coral atoll which forms part of the long chain of Keys separating the Gulf of Mexico from the Straits of Florida. Within the atoll the water is very shallow, and the bottom consists of a fine chalky mud many feet deep. Samples of the water from within the atoll were sent to me at Plymouth by post, and examined fourteen days after collection.

On plating on Peptone Agar, 800 colonies per 1 c.c. of the sample were obtained. These colonies appeared to be all of one species, and in appearance and all cultural characteristics were identical with the denitrifying form previously described as occurring around the Tortugas.

A suspension of these bacteria from a culture on Peptone Agar was made in sterile sea-water, and a similar suspension, containing roughly the same number of bacteria, was made from a third subculture on Peptone Agar of the bacteria obtained from the station seventy miles west of Ushant. 1 c.c. of each of these suspensions was then added to 1000 c.c. of the modified Gran's medium; some of these cultures were kept at an average temperature of 20°C. and others at 32°C., with the following results:—

At 20° C. cultures from Marquesas showed trace of nitrite after 45 hours.
„ „ „ gave strong nitrite reaction „ 53 „
„ „ 70 miles W. Ushant showed trace of nitrite . . . . . } „ 140 „
„ „ 70 miles west of Ushant showed strong nitrite reaction . . . } „ 162 „

In both cases a slight amount of Ammonia was recognizable by Nessler's reagent when the nitrite reaction was strong, but decomposition of the nitrite did not proceed further even after fourteen days.

At 32° C. cultures from the Marquesas showed trace of nitrite after 18 hours.
,, ,, ,, gave strong nitrite reaction ,, 22 ,,
,, ,, 70 miles west of Ushant never gave nitrite or Ammonia reactions.

The cultures from the Marquesas showed a slight amount of Ammonia formation, but the decomposition of the nitrite did not proceed further.

From these experiments it appears that the bacteria from subcultures from the Marquesas have a much greater denitrifying power than those from subcultures from a point seventy miles west of Ushant, and that as the bacteria from the Marquesas appear to be of the same species as those investigated at the Dry Tortugas, their power of causing complete denitrification in the modified Gran's medium has been lost by cultivation on Peptone Agar, or during the fourteen days journey from the Marquesas.

The presence of the thick layers of fine chalky mud within the Marquesas Keys, and elsewhere in many places near the Florida coast, led to a consideration of the possibility of its precipitation by bacterial agency.

Since these bacteria grow freely in Gran's medium, the Calcium salt of a simple organic acid is a sufficient source of organic food for them, and it seems probable that they would thrive in sea-water containing the products of decomposing vegetable matter, provided that the nitrate supply and conditions of light and temperature were suitable. Such conditions should be especially well fulfilled by the drainage into the sea of a well-wooded country with a calcareous subsoil, and the soluble organic Calcium salts carried to the sea in this way, would there be precipitated as Calcium carbonate by the action of the bacteria. In addition, the elimination of the acid radicle from the nitrate in the process of denitrification, by whatever stages it may occur, must leave the alkaline base free to destroy the normal equilibrium of the salts in sea-water, and by increasing the alkalinity, would also result in the precipitation of Calcium carbonate.

To test this theory, cultures were made in a medium having the following composition:—

Calcium succinate	2·5 grammes.
Potassium nitrate	0·5 ,,
Sea-water	1000·0 c.c.

Calcium succinate is soluble in these proportions, and the medium is quite clear. Free growth was manifested by the cloudiness of the medium forty-eight hours after inoculation, and nitrite formation was apparent. After ninety-six hours the medium appeared quite milky, and this milkiness was due to the presence of exceedingly fine particles of a substance which was soluble in dilute hydrochloric acid with evolution of gas, and was presumably Calcium carbonate. These particles were so minute that they remained in suspension in the liquid, and could only be satisfactorily separated from it by centrefugalizing. The addition to this culture of very fine particles of hydrated Calcium sulphate, or of larger particles of sand, resulted in the aggregation around them of the particles of Calcium carbonate, forming a concentrically laminated concretion around a central nucleus. These concretions were hard, and of almost crystalline appearance under the microscope, and were soluble in dilute Hydrochloric acid with evolution of bubbles of a gas which when the operation was performed on a microscopic slide, could be completely absorbed by running in a solution of Sodium hydrate under the cover slip. Once this process of concretion has been initiated, it appears to progress independently of the presence of particles which act as nuclei, and a large concretion may often be found having a number of smaller concretions around it, or continued into a chain of small spheres, the whole presenting somewhat the arrangement shown by freely budding yeast cells. The deposition of this form of Calcium carbonate also takes place on the sides of the flask, and more especially over any area where the glass is scratched or roughened.

It would seem a reasonable suggestion that similar bacterial agency may have played a part in the formation of oölitic limestones, which are composed of concretions of Calcium carbonate around central nuclei often consisting of particles of sand or shells. In the same way bacterial action may have been of great importance in the precipitation of the various chalk strata, and indeed in the formation of many rocks largely composed of Calcium carbonate.

If this view as to chalk and oölitic limestone formation is correct, it would seem probable that the precipitation of these rocks must have occurred in comparatively shallow seas, which received the drainage from a country in which extensive weathering of rocks containing Calcium salts was progressing, and where there was a considerable amount of vegetation from the decay of which the organic matter necessary for bacterial growth would be derived.

DISCUSSION OF RESULTS.

It seems that the rate of denitrification in equal volumes of the same culture medium, inoculated with equal volumes of samples of sea-water, must be a function of the number of bacteria in the sample, the temperature at which the cultures are grown, and the specific power of denitrification of the individual species of bacteria. Considering the rapid multiplication of bacteria when the food supply is plentiful, up to a maximum determined chiefly by the accumulation of the waste products of their own metabolism, it appears that the factor of the number of bacteria in the sample may be neglected within the limits of these experiments. For example, the number of bacteria in 1000 c.c. of Gran's medium at the end of twenty-four hours would probably be much the same, whether it were inoculated from a sample containing eight or sixteen bacteria per 1 c.c. Similarly, it was a matter of experience that the first trace of nitrite formation was observable at about the same time, whether 5 or 10 c.c. of a given sample had been used for inoculation.

Consequently it appears that, for purposes of comparison, and within the limits of the experiments described, if the temperature be the same for the cultures compared, the rate of denitrification is a measure of the specific denitrifying power of the particular species of bacteria.

Thus it has been shown that in cultures grown at temperatures approximating that of their normal environment, the denitrifying power of bacteria in seas in the American tropics is considerably greater than that of bacteria in the water to the north of the Bay of Biscay, and that they have the power under favourable conditions of completely breaking down nitrates, which those from the Bay of Biscay do not possess.

It has also been shown that these bacteria possess the power of precipitating Calcium carbonate from solutions of simple organic Calcium salts, and it is here suggested that some similar action has played an important part in geologic times in the precipitation of various rocks, wholly or in part composed of Calcium carbonate.

Investigation and comparisons of denitrifying power of samples of the coastal waters of the English Channel have been purposely avoided, as previous experience had shown that the bacterial conditions in this region are so very complicated. It should, however, be mentioned that in this coastal water denitrifying bacteria are present which are capable of entirely decomposing nitrates into free Nitrogen, e.g. the *Bacillus repens* (Gran), but that this complete denitrification is only possible at a temperature far higher than that of their normal environment, and that at a temperature of 15° C. even the formation of nitrite from the nitrate takes place with considerable slowness.

A point that has not yet been considered is the origin of the nitrate supply in the sea. Nitrates are absorbed by diatoms and the phytoplankton in general, and are presumably built up into complex nitrogenous compounds within the plant. If these compounds, on the death of the plant, are broken up and the Nitrogen again rendered available for use in the form of nitrates, a series of reactions must be gone through which may well be performed by bacterial agency, and this also applies to the waste nitrogenous products of animal metabolism. In addition, it has been shown that nitrates are actually destroyed by the denitrifying bacteria, which would thus tend to keep the nitrate concentration down to the level necessary for their own existence, and would come into competition for this essential with other forms of plant life. If the bacteria are successful in decomposing nitrates to the extent of entirely removing the Nitrogen from all chemical combination, as seems probable from the experiments in cultures, it follows that there must be some source of nitrates in order that the concentration in the sea may remain constant. The existence of nitrifying bacteria, which are capable of oxydizing the Nitrogen of the air to form nitrates, and are symbiotic with some of the Algæ found round the shores of the North Sea, has already been demonstrated, and it seems possible that forms having a similar nitrifying action remain to be discovered in the open sea.

As they at present stand, the observations noted in this paper can at most be considered as offering a slight indication of the part played by certain bacteria in the metabolism of the sea.

To obtain a real insight into the question, it would be necessary to make extensive bacterial and chemical investigations in tropical, temperate, and Arctic seas, and more especially to make a study of the bacterial flora at different depths at some spot where considerable depth, unaffected by currents, could be obtained. The probability that bacterial growth plays an important part in causing the precipitation of certain bottom deposits in coastal waters, and conceivably also in the deep sea, is a subject that appears well worthy of further investigation.

The Problem of Sex Determination in *Dinophilus gyrociliatus.*

By

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The group of primitive Annelids *Dinophilus* comprises some eight or nine species. They are remarkable for the fact that some show a well marked sexual dimorphism, in which the male is rudimentary, without any mouth or digestive tract, while in others the sexes are the same size and exhibit no signs of this dimorphism. The group as a class, therefore, is readily divisible into two subdivisions, in one of which all the species are sexually dimorphic, unpigmented, while in the other they are highly pigmented, a bright red, and are sexually monomorphic. The former may be called the Leucodinophilidae, while the latter may be called the Erythrodinophilidae. The known species, many of which are of doubtful specific value, may be arranged under these two subdivisions, as follows :—

Leucodinophilidae	1.	*Dinophilus*	*gyrociliatus*, Schmidt, 1857.
	2.	"	*Conklini*, Nelson, 1907.
	3.	"	*apatris*, Korschelt, 1882.
	4.	"	*metameroides*, Hallez, 1879.
	5.	"	*pygmaeus*, Verrill, 1892.
Erythrodinophilidae	6.	"	*vorticoides*, Schmidt, 1848.
	7.	"	*Gardineri*, Moore, 1899.
	8.	"	*tæniatus*, Harmer, 1889.
	9.	"	*gigas*, Weldon, 1886.

Of the Leucodinophilidae the first three species, *D. gyrociliatus*, *D. Conklini*, and *D. apatris*, are closely related, and are probably one and the same. The form on which the following work has been done is one of these three species, though exactly which of the three I have been unable to decide. I have placed it under the head of *D. gyrociliatus*, as this is the oldest of these names. Figures of the male and female drawn to scale, are shown in Fig. 1. It will be seen that the female is very much larger than the male, and that the sexual dimorphism is well marked. It was obtained some three years ago from some sandy material collected in Plymouth Sound. I introduced it subsequently into the tanks of the Plymouth Laboratory, where it has since established itself, and breeds.

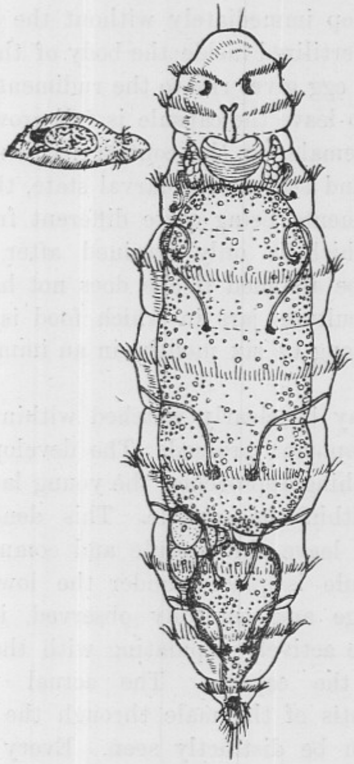

FIG. 1.—*Dinophilus gyrociliatus.* Rudimentary male and full-grown female. The female shows the broken nature of the ciliated bands in the head region and the solenocyte bearing nephridia.

Korschelt was the first to point out that a marked sexual dimorphism is present in *D. apatris,* where the male is small and rudimentary. He also observed that the female laid two kinds of eggs together in one capsule. Some of these were almost six times the size of the others, and were destined to give rise to the females, while the small ones gave rise to the rudimentary males. The large female eggs are laid in the proportion of three to one of the small male ones. Here is apparently a clear case in which we get sex determination in the ovary long before fertilization. Korschelt took it for granted that the eggs were only fertilized after being laid. The presence of this species of Dinophilus at Plymouth afforded me the opportunity of thoroughly investigating this question again, and of determining the manner of formation of these two kinds of eggs.

As Korschelt has determined, the two varieties of eggs are laid together, but they develop immediately without the presence of the male, and are therefore fertilized inside the body of the female. In a few days the small male egg gives rise to the rudimentary male, which at the time it is ready to leave the capsule is full-grown and sexually mature (Fig. 1). The female, on the contrary, when she leaves the capsule, is very small, and still in the larval state, the ciliation and arrangement of the segments being quite different from that in the full-grown condition, which is only attained after a considerable period, and may never be assumed if she does not happen to obtain proper food. Thus in culture jars in which food is not found, the females will remain as long as six months in an immature condition, and never grow up.

The young worms may be clearly watched within the capsule as segmentation and development proceed. The development is direct, and as the time for hatching approaches, the young larval females are seen to spin round within the capsule. This denotes that they are about to hatch and leave the capsule and commence their free existence. If the capsule is placed under the low power of the microscope at this stage and carefully observed, it will be seen that the little males are actively copulating with the small females at this time within the capsule. The actual transference of the sperm from the testis of the male through the penis into the body of the female can be distinctly seen. Every female as she passes out of the capsule is seen to carry a small mass of sperm, collected under the gut at the junction of stomach and intestine at the point where the ovary will subsequently appear. The rudimentary males seldom or never leave the capsule, dying shortly after the females escape. Examination of any immature free-swimming females, taken from culture dishes at random, always shows that they are all fertilized. If they are carefully fixed and sections cut from them at this stage, it will be seen that the germ cells have not been differentiated, and although a mass of sperm is collected at the point where the ova will subsequently appear, no trace of them can be detected. These only appear at a much later date, when the female has grown considerably in size. They are seen then as a few small refractive cells in the living state, and as small nuclei surrounded with hardly any cytoplasm in the stained condition, beneath the gut and amongst the mass of sperm. Shortly after they appear it is seen that each one is joined by a spermatozoon, the head of which has become embedded or attached to its nuclear wall, so that ultimately the nucleus of each primitive ovum is seen to be composed of one part derived from the spermatozoon, and the other part the female portion. These two elements of the nucleus never fuse, but retain their individuality throughout all the öogonial divisions. The double nucleus divides amitotically, each half separately. In the majority of the divisions the male and female portions of the nucleus divide equally, so that a similar quantity of nuclear material, both male and female, gets into each daughter cell. There are probably about forty to fifty öogonial divisions in all. In these the male and female portions of the nucleus divide and move apart simultaneously, the male portion usually dividing first. Now and again, however, the female half of the nucleus seems to divide before the male portion, so that the male portion gets left behind and is shut off entirely in one of the daughter cells. Therefore of the two resulting cells of this division, one has the whole of the male part of the original nucleus and its share of the female portion, while the other has only half the female and no male substance. This appears to be the sex determining factor; for of these two daughter cells, the one that has received the whole of the male element, plus the female element, becomes the female, while that which has received the female portion alone becomes the male. Both these kinds of eggs, once the sex determining division has taken place, grow rapidly. They seem to accomplish this through the power of absorbing and building up into themselves all the other immature egg cells with which they happen to come in contact, and in which the divisions of the two portions of the male and female substance has been equal. The outcome of this process is that the male egg is not fertilized, while the female egg is. It is, however, impossible to speak in the strict sense of the word of the male egg as unfertilized, as it has been directly under the influence of the sperm in all the early öogonial divisions previous to the sex determining one. For all the primitive germ cells are joined in the first place by a spermatozoon, irrespective of the fact that only some of these will give rise to ova later, and that the majority will be only nurse cells. It is only in the late stages, shortly before the female egg is laid, that the two portions of the nucleus, the male and female actually fuse beyond recognition. As the two kinds of eggs, male and female, are not found in a simple ratio, but in the proportion of three or two, to one male, it is probable that some other division takes place in the case of the female egg. I have been unable to decide this point so far, from my sections.

I have made a careful study of the maturation divisions, and as Korschelt has determined in *D. apatris*, there are two polar bodies given off by both kinds of eggs, the first polar body in turn dividing after it has been given off. There are twenty somatic chromosomes. In both eggs there is a regular reduction in the first maturation division, ten dumb-bell shaped chromosomes going out and ten remaining in the egg. In the female egg, however, this process is somewhat different from that in the male, as there seems to be something similar to a synapsis stage in the former which is missed out in the latter. In both eggs after the extrusion of the first polar body, the ten chromosomes remaining in the egg divide, bringing back the number to twenty again. In the second polar body, in the male egg, apparently twenty or eighteen chromosomes go out and the same number remain in the egg. The second maturation division in the female egg, I have been unable to obtain satisfactorily in sections so far, and it may prove that the second polar body in this egg is simply derived from division of the first, and that only one polar body is actually given off by the female egg. In the first and second segmentation divisions in both eggs there are apparently twenty chromosomes. It is, however, very difficult to make out their number in the male egg very accurately on account of their small size, there are at least eighteen or more, and probably twenty, as in the female egg.

The details of the maturation divisions I wish to reserve for my full paper on the subject, shortly appearing in the *Quarterly Journal of Microscopical Science.*

A Table showing certain Cultural Characteristics of some of the Commonest Bacteria found in the Laboratory Tanks at Plymouth.

By

G. Harold Drew.

THE following table is merely intended as a rough classification of eight of the most common species of bacteria found in the Laboratory Tanks. Only the forms which occur free in the water in the greatest numbers have been considered, and the moulds and higher bacteria which can be cultivated from scrapings from the walls of the tanks have not been included.

Until further and more detailed work has been done on the subject, it seemed better to designate the various species by numbers instead of names, since the majority have probably not previously been described.

The culture media employed were similar to those ordinarily used for bacteriological work, with the exception that fish broth (made from dogfish) was substituted for meat broth, and that sea-water was used in place of tap-water. All media were faintly alkaline to Neutral Red.

The medium designated as "Gran's medium," has the following composition:—

Sodium chloride	8·8 grammes.
Potassium nitrate	0·25 „
Sodium phosphate (Na_2HPO_4, 12 H_2O)	0·125 „
Calcium malate	in excess of its solubility.
Distilled water	250·0 c.c.

and is fully described by H. H. Gran in the *Bergens Museums Aarbog*, No. 3, 1901.

The sample of water, from which the bacteria classified in the table were isolated, was collected from just below the surface of one of the tanks in the upper Laboratory at 5.30 p.m. on December 6th, 1909. 0·1 c.c. of this sample plated on Fish-broth, Peptone Agar at 30° C., gave an average of 130 colonies of all kinds. Samples collected on other occasions show that the eight species described below may be considered as always present and abundant in the tank water.

TABLE SHOWING SOME OF THE DISTINGUISHING CHARACTERS OF THE COMMONEST BACTERIA IN THE AQUARIUM TANKS.

No	Morphological characters of bacteria grown in Fish broth, Peptone medium.	Appearance of superficial colonies on Fish broth, Peptone Agar at 30° C.	Stab cultures on Fish broth, Peptone Gelatin at 15° C.	Stab cultures on Fish broth, Litmus, Lactose, Peptone Agar at 30° C.	Stab cultures on Fish broth, Neutral Red, Glucose, Peptone Gelatin at 15° C.	Denitrification in Gran's medium at 15° C.	Further notes.
1	Actively motile rods with rounded ends, often appearing as diplobacilli. Length of rods about 1½ times diameter.	Circular brown colonies, becoming white at edge as growth proceeds. Slow growth. Margin smooth and regular.	Slight superficial growth; very faint growth along stab. No liquefaction.	Slight superficial growth; very faint growth along stab. No colour change.	Slight growth on surface and to within ½ inch below; very faint growth in deeper parts. No colour change.	No growth.	Growth in media containing Lactose or Glucose was slightly though distinctly more rapid than in other media.
2	Actively motile rods and diplo-bacilli. Extremely small.	Circular white colonies, uniform, with denser spot in centre. Margin smooth and regular. Rapid growth.	Free liquefaction on surface. Free growth in deeper parts, but no liquefaction.	Moderate woolly looking growth all along stab. No colour change.	Strong growth all along stab. Liquefaction on surface. Acid formation.	Moderate growth. Nitrite formation recognizable after five days.	
3	Small actively motile vibrio.	Circular cream-coloured colonies, much elevated. Brownish spot in centre. Highly refractive. Margin smooth and regular. Rapid growth.	Uniform growth along stab. No liquefaction.	Uniform growth along stab. No colour change.	Uniform growth along stab. No colour change. No liquefaction.	Moderate growth, but no nitrite formation.	Growth in media containing Lactose or Glucose was more rapid than in other media.
4	Long spirillum, actively motile.	Large white colonies with irregular outline, denser spot in centre. Colonies rapidly spread over surface.	Very slight growth all along stab. No liquefaction.	Very faint growth near surface. No colour change.	Very faint growth near surface. No colour change or liquefaction.	Moderate growth, but no nitrite formation.	Presence of Lactose or Glucose appears to retard growth.

5	Small actively motile vibrio.	Circular white colonies with very granular appearance. Margin finely irregular. Colonies much elevated above surface, and of slow growth.	Free growth. Funnel-shaped area of liquefaction rapidly spreading downwards.	Growth all along stab. No colour change. Woolly appearance.	Free growth. Funnel-shaped liquefaction. No colour change.	No growth.	
6	Minute actively motile rods.	Large white, cloudy-looking colonies, circular with finely irregular outline.	Growth on surface only. No liquefaction.	No growth.	No growth.	Rapid growth, and denitrification apparent in four days.	
7	Small spirillum, non-motile.	White, translucent, highly refractive colonies. Circular. Margin smooth and regular.	Free growth all along stab. No liquefaction at first, but after five days it begins to appear.	Free growth all along stab. No colour change.	Free growth all along stab. No colour change.	No growth.	
8	Small actively motile rods.	Circular white colonies of very rapid growth, showing denser spot in middle. Uniform smooth margin. In old cultures the whole medium becomes of a golden-brown colour.	Rapidly developed funnel-shaped area of liquefaction, soon resulting in the complete liquefaction of all the medium.	Free growth all along stab. Woolly appearance. No colour change beyond slight reduction in deeper parts.	Rapid liquefaction with acid formation.	Rapid growth, and denitrification within four days. Ammonia formation apparent at fifth day.	Further experiments with Litmus Gelatin Peptone media containing Glucose, Lactose, Cane Sugar, Starch, etc., gave results which point to the conclusion that this is the *Bacillus repens* described in detail by H. Gran.

Note on the Early Larvæ of *Nephthys* and *Glycera.*

By

H. M. Fuchs, B.A.

With 10 Text-figures.

THE larva of *Nephthys* was originally described by Claparède and Mecznikow (2) in 1868, from specimens obtained from the Plankton at Naples. Since then it has been figured by Fewkes (3), Häcker (5), and Gravely (4). The larva of *Glycera* is, as far as I am aware, hitherto undescribed.

The larvæ described below were reared in the laboratory at Plymouth, at the suggestion of Dr. Cresswell Shearer, from artificially fertilized eggs. Both *Nephthys Hombergi** and *Glycera convoluta* can be obtained from the sand in Jennycliff Bay at low water. *Nephthys* occurs more commonly than *Glycera*, but large specimens are not frequent. On the other hand, very young specimens are completely absent. It is at present altogether unknown what becomes of the young of these worms from the time when they sink down from the Plankton until they grow to about an inch in length. From June to August most of the larger individuals contain ripe sperm and ova, which are frequently discharged in captivity, within a day or two from the date of capture. It was found unnecessary, however, to wait until the generative products were shed naturally. The method of procedure was as follows: The worms were slit up with a pair of scissors and placed in "outside" sea-water in sterilized finger-bowls, the males being kept apart from the females. As soon as the sexual products emerged from the body cavity the worm was removed, several being treated in this manner to ensure the presence of ripe eggs. A few drops of liquid from the suspension of sperm was then added to the bowl of eggs and the latter stirred and allowed to settle. Wilson (9) has stated for *Nereis* and Treadwell (8) for *Podarke* that if the eggs are cut out of the body of the female, segmentation is irregular. This is not the case with *Nephthys* and *Glycera.* As soon as the larvæ swam to the surface they were pipetted off and placed in jars of filtered sea-water.

All attempts to feed the larvæ failed, and in consequence, although they could be kept alive for three weeks or a month, after about

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* It is just possible that a few small specimens of *N. caeca* were included with the *N. Hombergi.*

fourteen days no further development took place. I have to thank Dr. Allen for providing me with pure cultures of Diatoms and other Algæ, but I was unable to find any food which the larvæ would eat. Nothing is easier than the rearing of Serpulids in a laboratory, and especially is this the case with regard to *Pomatoceros*, which is a hardy shore form, found growing even at the mouths of drains. It breeds naturally and in great profusion in the tanks at Naples, the young worms settling down and forming their tubes without having any care bestowed on them. But very few of the free-living Polychaetes have been reared to a late stage. *Chaetopterus* is an example of one which has been raised (Allen and Nelson 1) and Lillie (6) was successful with *Arenicola*. I have found it very easy to rear the larvæ of *Nereis dumerilii*, which are derived from yolky eggs, as far as thirty segments or more. Quite otherwise, however, is it with those free-living Polychaetes which have a pelagic larva. It would thus seem that it is possible to rear from the egg such forms as the Serpulids, which have a typical Trochophore, and those which have yolky eggs, but no method has as yet been devised by which the majority of the errant forms can be fed and raised.

The egg of *Glycera* is discoidal, and it does not swell up in sea-water. It is granular, but comparatively transparent, with a lighter coloured nucleus. The egg of *Nephthys* is also flattened at the poles, but is opaque. The cleavage is equal.

Unlike the larva described by Claparède and Mecznikow (2), the larva of *Nephthys Hombergi* is granular and so opaque as to render the study of the internal organs in optical sections very difficult. Neither in shape nor in transparency can it be called a typical trochophore. An early stage is shown in Fig. 1. The upper hemisphere is more conical and of smaller diameter than the lower, and is surmounted by an apical patch of cilia. The mouth is situated mid-ventrally on the lower hemisphere, and strong cilia can be seen working within it. Brown

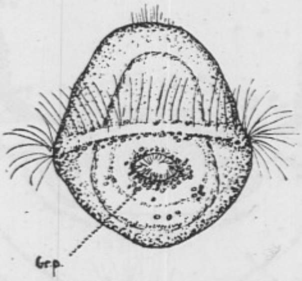

FIG. 1.—Larva of *Nephthys*. 2 days. From the ventral aspect. *br. p.*—Brown pigment.

pigment is scattered on the surface in small chromatophores, which ar concentrated below the trochal ring and round the mouth. Fig. shows a later stage, the general shape of the larva being the same a before. The alimentary canal is divided into stomach and intestine and there is an anal patch of cilia. Fig. 3 shows a trochophore of four

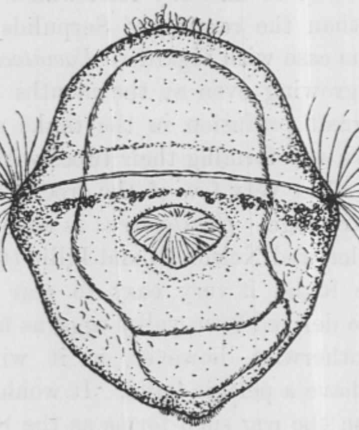

FIG. 2.—Larva of *Nephthys*. 6 days.
From the ventral aspect.

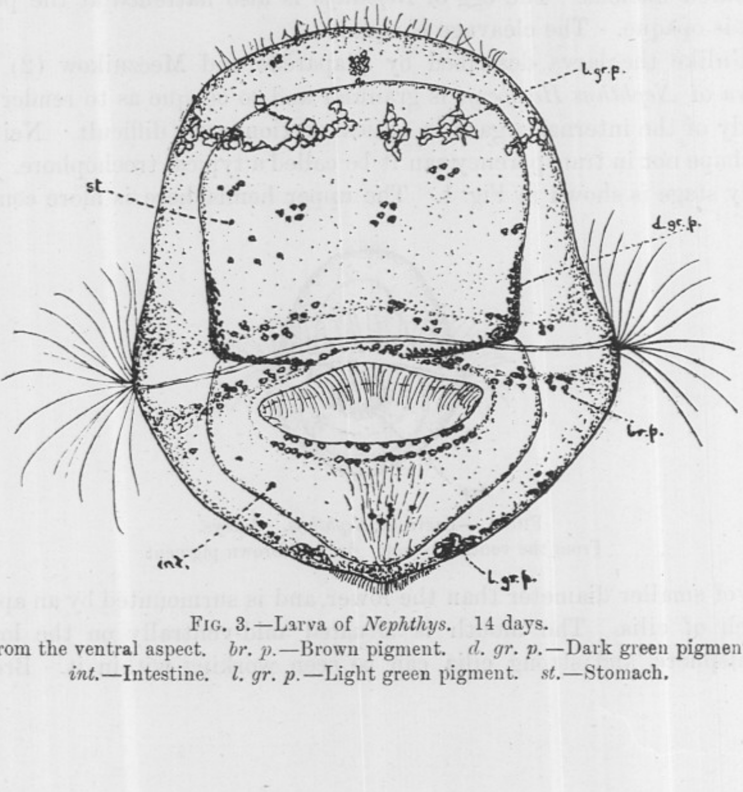

FIG. 3.—Larva of *Nephthys*. 14 days.
From the ventral aspect. *br. p.*—Brown pigment. *d. gr. p.*—Dark green pigment.
int.—Intestine. *l. gr. p.*—Light green pigment. *st.*—Stomach.

teen days old. The upper hemisphere is larger and blunter than the lower. There is a patch of cilia extending from the lower lip of the mouth towards the anus, which is terminal. The apical cilia are usually motionless. Light green pigment has appeared in an incomplete band round the upper hemisphere, and a pair of patches on either side of the anus. These latter are characteristic of the larva of *Nephthys* (3). The large stomach, which fills the greater part of the upper hemisphere, has a dark green pigment in its basal walls, the remainder of the gut being yellow in colour. The intestine is sac-like.

The larva described above differs very much from that figured by Claparède and Mecznikow (2). It resembles in some points that of Fewkes (3), but is of a different shape. Häcker (5) and Gravely (4) described the Metatrochophore and later stages only.

Swimming blastulæ of *Glycera convoluta* appear some ten hours after fertilization, and the trochal ring is formed in twenty-four hours. An early larva is shown in Fig. 4. It is spherical and granular with a ciliated trochal ring and an apical tuft of long cilia. Fig. 5 shows

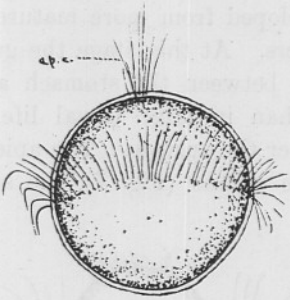

FIG. 4.—Larva of *Glycera*. 26 hours.
ap. c.—Apical cilia.

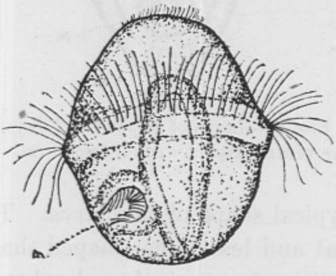

FIG. 5.—Larva of *Glycera*. 58 hours.
Left side view. *m.*—Mouth.

a somewhat later larva in lateral optical section. The larva is more elongated and the apical patch has become diffuse, without any long cilia. The buccal aperture is seen to be strongly ciliated. Fig. 6 is a trochophore of three days old. Little weight can, however, be attached to the ages of the larvæ, as in the same culture some larvæ, probably those which have developed from more mature eggs, will grow much more rapidly than others. At this stage the gut has thick walls, and there is a constriction between the stomach and intestine, which is much more evident than in later larval life. A very light green pigment is scattered over the surface. The apical patch seems to contain a pair of vibratile organs (Fig. 7), the significance of which is unknown.

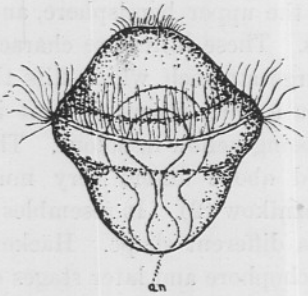

FIG. 6.—Larva of *Glycera*. 3 days.
From the dorsal aspect. *an.*—Anus.

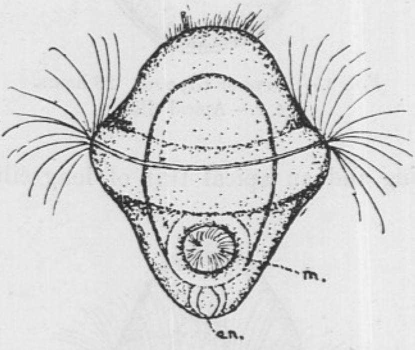

FIG. 7.—Larva of *Glycera*. 4 days.
From the ventral aspect. *an.*—Anus. *m.*—Mouth.

Fig. 8 shows the typical shape of this larva. The upper hemisphere is rather more conical and less dome-shaped than the lower. This is the reverse of the condition in a typical trochophore. Large and granular green pigment corpuscles are scattered over the surface. They are

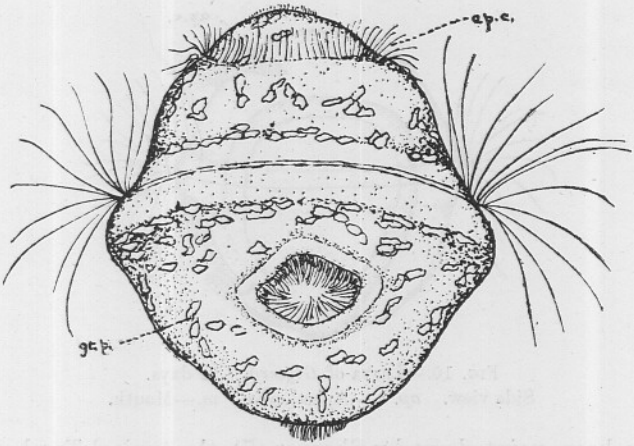

FIG. 8.—Larva of *Glycera*. 3 days.
From the ventral aspect. *ap. c.*—Apical cilia. *gr. p.*—Green pigment.

more numerous on the lower hemisphere, and especially below the trochal ring. The apical cilia have assumed the form of a ring round the upper hemisphere. This band is situated in a slight depression of the surface and is incomplete dorsally (Fig. 9). There is a patch

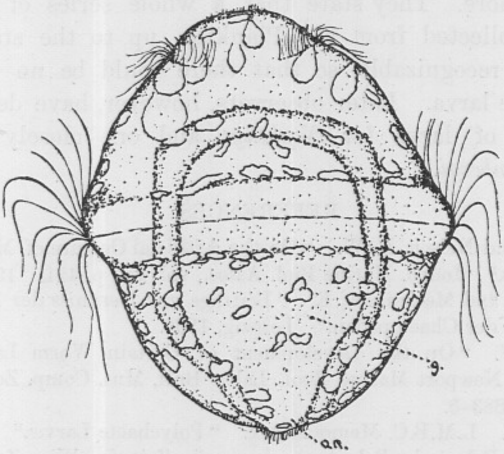

FIG. 9.—Larva of *Glycera*. 4 days.
From the dorsal aspect. *an.*—Anus. *g.*—gut.

of cilia extending from the lower lip to the anus (Fig. 10). The mouth is diamond shaped and is situated mid-ventrally on the lower hemisphere. The gut is large and sac-like, without any definite division into stomach and intestine.

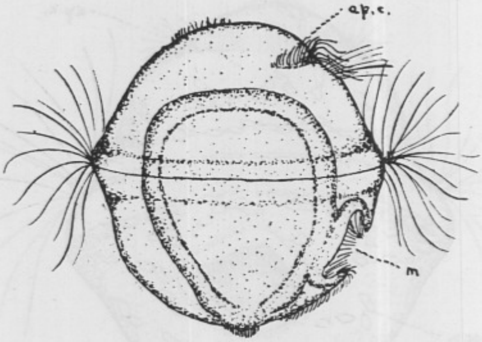

FIG. 10.—Larva of *Glycera*. 10 days. Side view. *ap. c.*—Apical cilia. *m.*—Mouth.

As has been pointed out by Shearer (7), the typical Trochophore is confined to a very limited number of Annelids, occurring, in fact, only in Serpulids and *Polygordius*. The larvæ of the remaining forms, which have a pelagic stage, mostly resemble the two described above. They differ from the true Trochophore in form, opacity, shape of the gut, in the absence of a head-kidney and in the small amount of blastocoele. The early *Nephthys* larva figured by Claparède and Mecznikow (2), however, seems to approach closely to the form of a true Trochophore. They state that a whole series of intermediate forms were collected from the Plankton, up to the stage when the bristles were recognizable, so that there could be no doubt of the identity of the larva. Later observers, however, have described quite another type of larva for *Nephthys*, and one closely approaching that of *N. Hombergi*.

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The Relation of the Heart-Beat to Electrolytes and its Bearing on Comparative Physiology.

By
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An objection often raised to experiments on tissues treated with artificial solutions and isolated from nervous control is that results obtained under such highly abnormal conditions can be of no real value. This criticism arises essentially from a misconception as to the aim of the experiments in question, but it is encountered so frequently that it may not be out of place to consider briefly why it is *necessary* for the physiologist who would learn something of the more intimate mechanism of the cell to work with tissues under artificial conditions.

Let us suppose that the action of some chemical substance on the heart-beat is the subject of investigation. It is found, we will suppose, that on injecting a solution of the substance into the circulation of an intact animal the beat of the heart is modified in some way—it may be in frequency or force, or in the rhythmic sequence of its chambers. If on repetition of this simple experiment, made on animals as far as possible under normal conditions, the same result is consistently obtained, the result may be of therapeutic interest. For its interpretation in terms of what is already established about the mechanism of the body, a searching physiological analysis is, however, required. The effect on the heart may be direct or it may be due to one of a variety of indirect actions of the substance.

Thus, for instance, it may be due to an action of the substance—

(1) on sensory nerve endings connected to the cardio-inhibitory or cardio-accelerator centres in the medulla;
(2) on the nerve cells in these centres;
(3) on cell stations in the course of the vagus or sympathetic nerves to the heart;
(4) on the respiratory movements, modification of which affects the condition of the blood in such a way as to influence the heart, either directly or through action on the nerve centres which can control it;

(5) through an alteration in the blood pressure (which may affect the heart by a local or a central point of attack) due to constriction or dilatation of arterioles, this being caused either by reflex, central or local action;

(6) on the heart muscle itself.

By an elaborate series of experiments, involving the section of nerves, the use of drugs such as atropine and curare, the enclosure of organs in plethysmographic apparatus; by taking records of blood pressure and by ventilating the lungs steadily with the aid of a mechanically-driven respiration pump, it would be possible to determine by which of the various possible means the effect of the injected substance on the heart was produced.

Suppose, now, that by such experiments the point of action of the substance has been traced to the heart muscle itself. (Be it noted that the elucidation of this point has involved the imposition of more and more artificial conditions.)

The experiment remains of comparatively little value to the physiologist, who is endeavouring, by the investigation of the effects of substances on the activity of the heart, to learn more about its actual mechanism. For the experiment can only in the vaguest sense be called quantitative. However carefully the dose of the drug is weighed out, the concentration of the substance in the blood reaching the heart cannot be known at all accurately, since the dilution depends not only on the rate at which the injection is made and on the rate of flow in the vein into which the injection is made, but also on the blood flow in all the other veins leading to the heart. Moreover, it is seldom justifiable to assume that the injected substance remains unchanged in the blood. Blood is a highly complex fluid: the carbonates, phosphates, and in particular the proteins present in it, enter into chemical or adsorptive relation with many injected substances. Of course, from a therapeutic standpoint this does not matter. If, for instance, the injection of a particular drug is followed by marked strengthening of the heart-beat, it matters little to the practitioner whether the substance actually presented to the heart as a result of his injection is the same chemical substance as that which left the hypodermic syringe, or a product of some complex reaction between that substance and the blood or tissue fluids. But when the object is not simply the production of the effect, but the explanation of how it is produced; when the response of living cells to changes in their chemical environment is being used to throw light on the mechanism and properties of the tissues themselves, it is clearly of the first importance that the change in the environment in any experiment should be known with the utmost precision.

Practically every experiment involves the imposition of some abnormal conditions. In a state of nature, conditions are varying continually; the fixation of any condition in itself constitutes an abnormality. After determining artificially one condition, it is fallacious to speak of the remaining conditions as being "normal." If they too are not experimentally controlled, they are unknown. The choice lies, therefore, between a type of experiment in which most of the conditions are unknown but where there has been but little operative interference, and another type where the conditions are simplified and controlled by artificial means. The former may yield information of much value to the physician, but it is to the latter that we must turn for advances in our knowledge of cell physiology.

If, now, we are concerned with the problem of how and why the heart gives rhythmic contractions, the first step will be to determine and to define the simplest conditions under which the heart will continue to beat. Removal of the heart from a freshly killed animal suffices to show that the essential mechanism is self-contained. The movements of the heart, though susceptible of control by the central nervous system, are yet able to continue when all nervous connection is severed, and indeed it is known that in the chick they begin in the heart muscle some time before any nervous connection is established. It might be suggested that the excised heart lying in a watch-glass and continuing to beat was under the simplest conditions imaginable. Very little consideration is needed to show that this is not the case. In the first place, its temperature is not being controlled; and secondly, the heart muscle is in contact with a layer of fluid of complex and changing composition. As so often in scientific work, an elaboration of apparatus is necessary in order to secure a simplification of experimental conditions. That the composition of the fluid in contact with the muscle may be known as closely as possible it is desirable that a large volume of the fluid should be available, and that the portion in contact with the tissue shall be frequently renewed. For this purpose it is not sufficient to immerse the heart, or portions of it, in a large vessel of the solution. Such a method, it is true, has often been adopted, but it is far from being satisfactory. The best method is to perfuse the heart with fluid, letting the solution enter the venous end of the heart at a small and constant pressure, and allowing it to escape at the aortae, which may be opened up to allow of a free flow. In this way, in the hearts of animals, where the coronary system has attained to no great development,* the heart muscle, and in particular that of the thin-walled auricles and sinus venosus, is brought into very intimate relation with a constantly renewed layer of fluid. The stream of fluid may be said to control the temperature and the chemical environment of the cells. The efficiency of the perfusion method is shown by the fact that with a suitable fluid the heart may be kept beating regularly and strongly for many hours, while slight changes in the composition of the perfused fluid with respect to an important ingredient are followed by evident change in the character of the beats within a very few seconds.

As might be expected, the blood or serum from the animal whose heart forms the subject of experiment forms a perfusion fluid effective in maintaining the activity of the heart. But the classical researches of Ringer revealed the all-important fact that these highly complex, protein-containing solutions could be satisfactorily replaced (in the case of the frog's heart) by certain solutions of simple inorganic salts. The presence of the inorganic salts of the serum in the fluid in contact with the cells of the heart muscle is of more immediate importance for the activity of these cells than is the presence of any organic food-stuffs. It is true that it has been shown that the addition to the salt solution of a small amount of glucose keeps the heart going for longer periods than are attained without it; and according to some the addition of proteins has a beneficial effect, though this is denied by others who have studied the point. The fact remains that with a solution of simple inorganic salts in distilled water containing some dissolved oxygen the hearts of various animals can be kept beating outside the body for very many hours.†

During this time, while the heart continues to contract regularly and with sufficient force to enable it to pump through fluid with such energy that if it were still connected to the arteries it could fulfil its normal function of propelling fluid through the vascular system, and while the frequency and form of the contractions remain essentially like those observed in the heart of the uninjured animal, we may say that the heart muscle is exercising its normal function, and although the conditions are frankly abnormal in many respects, yet they embrace those factors of environment which are *immediately* necessary for the normal behaviour of the cardiac muscle cells.

We must next consider what are the essential constituents of a salt solution which will answer this purpose.

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* In mammalian hearts it is necessary to perfuse the coronary system.

† In working with the hearts of Elasmobranchs, as Baglioni (Zentralbl. f. Physiol. 19, 385, 1905) showed, it is necessary to add urea to a neutral perfusion fluid.

The "Ringer's solution" most commonly employed in the physiological laboratory contains chiefly sodium chloride with small quantities of potassium and calcium chlorides and of sodium hydrogen carbonate. The actual concentrations of the salts employed differ according to the animal used; for the frog the total concentrations should be about ·125 Mol. If the concentration of sodium chloride be called 100 the relative concentrations of the other salts may be taken as 2·5 of potassium chloride, 2 of calcium chloride, and 1·5 of sodium hydrogen carbonate. This solution, simplified by the omission of the last-named ingredient, still maintains the heart-beat in a satisfactory manner. We shall return in a moment to the function of the sodium bicarbonate. Its presence is not essential. Removal of the potassium salt leads to an alteration in the character of the beats, the systolic phase becoming prolonged, but does not necessarily cause arrest or even weakening of the beats. With a solution consisting only of distilled water with sodium and calcium chlorides and some dissolved oxygen, if the concentration of the Ca is carefully adjusted, the frog's heart may be kept beating vigorously for prolonged periods.

Removal of the calcium from the perfusion fluid is immediately followed by great weakening of the contractions, which in the course of a few minutes become exceedingly feeble and before long cease altogether, the heart remaining in a relaxed state. When the calcium is replaced the beats start again at once and become as vigorous as at the start. In the frog, as Ringer showed, the calcium may effectively be replaced by strontium. It may also be replaced by barium, but only to a very limited extent, for in a short time barium causes arrest of the heart in a firmly contracted state.

It should be noted that practically *any* soluble salt of calcium may be used in place of the chloride. The ionic theory is peculiarly acceptable to the physiologist in that it gives concrete expression to the fact so often encountered, as here, that all solutions of a particular metal, where on physico-chemical theory it should be in the same ionic condition, present in common certain definite physiological actions. We may say that the calcium ion is essential for the activity of the heart. There is reason to think that the calcium forms with some constituent of the heart muscle a readily dissociated compound, whose presence is a cardinal factor in the activity of heart muscle. The closely similar strontium ion can form a compound so similar to the calcium compound that it can fill the same niche in the architecture of the muscle, while the barium ion may best be pictured as forming a compound of similar composition but probably almost irreversible, perhaps through great insolubility. No metal other than these has been found to replace calcium.

It is of interest to find that these relations of the heart muscle to Ca, Sr, and Ba are essentially the same, not only in other vertebrates which have been studied, but also in the heart of the mollusc Pecten.

So far then the features of immediate importance in the perfusion fluid for the heart appear to be the presence of certain concentrations of sodium and of calcium ions, the presence of dissolved oxygen and the maintenance of the temperature within certain limits.

Keeping these conditions constant, let us consider the effects of varying the composition of the fluid in certain other respects.

Since we deal always in physiological work with aqueous solutions, and since water is to a slight extent electrolytically dissociated, our solutions always contain hydrogen ions and hydroxyl ions. In pure water these are of course present in equal numbers, the concentration of each, expressed in gram-ions per litre, being at room temperature about 10^{-7}.

It follows directly from the general principle expressed in the law of mass action that the addition to the water of any substance tending to increase the concentration of hydrogen ions will reduce the concentrations of hydroxyl ions. A similar shift in the equilibrium will be produced by the addition of any substance tending to remove hydroxyl ions. The ratio of the $H^{\cdot}$ to OH' is increased. An inverse change is produced by the addition of OH' or the removal of $H^{\cdot}$.

Knowing the dissociation constant of water, if the hydrogen ion concentration of any aqueous solution is given it is a matter of simple arithmetic to calculate the hydroxyl ion concentration and the ratio between the hydrogen and hydroxyl ion concentrations for that solution.

To define the position of this $H^{\cdot}$, OH' equilibrium for any solution it is sufficient therefore to state the hydrogen ion concentration of the solution. This is expressed most conveniently as a logarithm.

To avoid the constant repetition of the phrase "hydrogen ion concentration" we may adopt Sörensen's notation in which the symbol P^{+}_{H} is followed by the logarithm of the hydrogen ion concentration, the minus sign being understood. Thus describing a solution as being of P^{+}_{H} 7·07 means "the hydrogen ion concentration of the solution is equal to $10^{-7.07}$ gram ions per litre."

An aqueous solution whose P^{+}_{H} at 18° C. is 7·07 is neutral in reaction. If the P^{+}_{H} is numerically less than 7·07 the solution is acid, if greater it is alkaline. For instance, the P^{+}_{H} of centi-normal hydrochloric acid is 2, that of milli-normal sodium hydroxide 11, and so forth. Now the solution of pure salts of strong acids and strong bases in distilled water, neutral by intention, becomes in the course of preparation very faintly acid as it reaches equilibrium with the small concentration of CO_2 present in the air. The reaction of pure water or of a solution of salts of the type we have just mentioned, which do not themselves affect the reaction, is very easily shifted by minute additions of acid or alkali. Thus one drop (·05 c.c.) of normal hydrochloric acid would suffice to change the P^{+}_{H} of fifty litres of water from 7 to 6. But if the originally neutral solution contained a weak acid balanced against a salt of the same weak acid with a strong base, the addition of the same amount of acid as before would not appreciably disturb the P^{+}_{H}. Such a condition is realized, for example, in a saline solution containing a little sodium bicarbonate and carbonic acid. Such a solution may be prepared with the same P^{+}_{H} as the purest water: it will differ from water in that its P^{+}_{H} is much more stable—that is to say, it is displaced very much less by the addition of the same small traces of acid or of alkali. Substances having this effect on addition to a solution are spoken of by Sörensen as "buffers." Amphoteric electrolytes such as the amino-acids and their compounds the proteins tend also to stabilize the reaction of solutions in which they are present. The explanation of the action of all these substances lies in the fact that when they are present in a solution the hydrogen ion concentration is a function of more than one equilibrium. It should be noted in passing that in nature such "buffers" play an important rôle. For instance, the carbonates, phosphates, and proteins in the blood serve to limit the changes in P^{+}_{H} of that fluid resulting from the production of acid metabolites by the tissues, the secretion of acid or of alkaline fluids, and the removal of carbonic acid in the lungs, to such values that the limits of safety are never passed. If the blood could be deprived of these substances, it is probable that the acid produced in moderate exercise for example would so raise the $H^{\cdot}$ concentration as completely to disorganize nerve cells and heart, instead of altering it only to such an extent as to cause the blood to act as a chemical messenger or hormone, modifying the activities of nerve cells and heart in such a way as to restore the normal value of the P^{+}_{H} of the blood. For this is what happens in actual life.

The action of acids and alkalies on the heart was described by Gaskell nearly thirty years ago. Let us note in detail the effects of acid. Gaskell used lactic acid, but the description he gave of its action on the heart applies equally to the effect of other acids, such as hydrochloric or even carbonic. The action on the heart is due to what they have in common: an excess of the hydrogen ion. The essential features of the action on the heart are these: The acid solution diminishes the force of the systole, increases the diastole, and causes arrest of the heart in a state of complete relaxation. If now the acid solution is washed out with "neutral saline" (i.e. P^{+}_{H} about 6·5),* there is no recovery for some time, and then very feeble beats may start. But if a slightly alkaline solution is used the recovery is prompt, and the beats become as strong as at the start within a few seconds, or at most in a minute or two. Further experiment shows that the persistence of the after effect of the acid bears a definite relation to the P^{+}_{H} of the acid solution and to the duration of its application. With a minimal dose of acid applied for the shortest time necessary to cause arrest of the heart, recovery on perfusion with "neutral saline" may be fairly prompt and complete, but if the dose is rather greater or applied longer the effect may persist for an hour or more during subsequent perfusion with neutral saline. This is to be regarded as the typical effect of acid on the heart. If the solution is made too alkaline the heart is also stopped, but in this case in systole. In the case of the frog's heart the divergence from neutrality needed to produce the systolic *alkaline* arrest is much greater than that needed to produce the diastolic *acid* arrest. Roughly, the limits are about P^{+}_{H} 10 and P^{+}_{H} 5·5 respectively. It is not surprising then to find that "neutral saline" restores the heart from the alkaline arrest much more quickly than from the acid arrest, for this solution is much nearer the "acid" limit than the "alkaline" limit.

So far then we have these facts. *The normal activity of heart muscle (by which we mean its power of giving automatic rhythmic contractions) is possible only when the hydrogen ion concentration of the solution in contact with it lies within certain limits. If these limits are considerably exceeded on the acid side, not only is the heart stopped, but some alteration is impressed on the muscle, which is removed only very slowly, if at all, by a "neutral" solution, but instantly by an alkaline solution.*

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* The slight deviation from strict neutrality is due to CO_2 from the air. It is convenient to use such a solution and it will maintain the heart-beat for hours.

In working at the action of salts on the heart I was led, for reasons which need not be entered into here, to investigate the action of salts of the rare earth metals such as lanthanum, yttrium, neo-dymium—in all eleven were studied.

These salts in aqueous solution yield trivalent ions, $\mathrm{La}^{\cdot\cdot\cdot}$ $\mathrm{Nd}^{\cdot\cdot\cdot}$, etc. Unlike the salts of other metals yielding simple trivalent ions (such as aluminium, gold, iron, etc.), these salts of the rare earths do not render the solution acid in reaction—they do not undergo hydrolytic dissociation. I found that remarkably small concentrations of these salts in "neutral" solution sufficed to stop the heart. Thus the frog's heart is invariably stopped within a few minutes, generally within thirty seconds, by a solution containing ·00001 gram molecules per litre of either of these rare earths, and I have often seen it stopped by a concentration of one-millionth molecular. I find the same action on the heart of the rabbit and of the ray as on that of the frog.

Studied in detail on the frog's heart, *the effect of the simple trivalent ion parallels that of acid in every particular. The mode of arrest, the absence of recovery, or the slow and imperfect recovery on washing out with "neutral saline," and the immediate and complete recovery on the use of alkali, are all reproduced exactly.*[*]

How are these phenomena to be interpreted?

Can we discover any physico-chemical explanation for the close similarity in their action on the heart of these ions, the hydrogen ion and the ion of the trivalent rare earth, which are so different chemically, so widely different in their mobilities and their volumes?

Let us first review very briefly what other considerations have led physiologists to infer as to the nature of that subtle mechanism by which muscular tissues like that of the heart transform chemical into mechanical energy with such high efficiency.

The recent work of A. V. Hill on the time relations between heat

[*] Quantitative investigation shows that these simple trivalent ions are more powerful than the chemically equivalent concentration of hydrogen ions in their effect on the heart. And thus it happens that if the activities of solutions yielding trivalent ions which do undergo hydrolytic dissociation are compared with those which do not, the latter are found more powerful in their action on the heart. For instance, taking equimolecular solutions of aluminium, scandium, and lanthanum chlorides, the $\mathrm{Al}^{\cdot\cdot\cdot}$ liberated in the first solution combines to some extent with the OH' in the water, forming an almost undissociated hydroxide and liberating an equivalent of $\mathrm{H}^{\cdot}$ (3 $\mathrm{H}^{\cdot}$ for every $\mathrm{Al}^{\cdot\cdot\cdot}$). Thus the solution becomes acid. In the lanthanum solution there is no hydrolysis, for lanthanum hydroxide is strongly dissociated. Thus the solution remains "neutral," and contains its full complement of $\mathrm{La}^{\cdot\cdot\cdot}$. The case of scandium is intermediate: its solution is less acid than the Al solution. Now it is found that while all three solutions produce the same kind of effect on the heart, the lanthanum solution is the most and the aluminium solution the least powerful of the three.

production and shortening in muscle disproves the hypothesis that the shortening is an effect due to the action of local rise of temperature on some constituent of the muscle structure. The muscle is certainly not a heat engine.

The so-called osmotic theory of contraction—which supposes that as a result of the liberation of a high concentration of electrolytes within certain membranous compartments of a particular shape, an increase of hydrostatic pressure is set up within them causing them to bulge and shorten, thus producing the shortening of the whole muscle—meets with the objections that the osmotic pressure which would be necessary to give rise to mechanical effects of the magnitude encountered in muscular contraction would necessitate extraordinarily high concentrations of electrolytes, and such changes could scarcely be completed in the very brief interval which often suffices for the completion of a muscle twitch.

The sort of explanation which has most to recommend it is that which refers the act of shortening to a mechanism in which the alteration of surface tension at the junctions of different phases is the motive power. Anyone who has studied the behaviour of a globule of mercury, lying in a dish of dilute sulphuric acid to which a trace of potassium chromate has been added, when touched by a clean iron wire, will recognize in the sudden drawing together of the globule movements which recall more forcibly than any other artificial scheme the movements of living contractile tissue. Here, too, the evolution of a considerable amount of kinetic energy is the result of a very small amount of chemical change.

As to the mechanism by which surface tension alterations can be supposed to play a part in the muscle, there are two chief hypotheses. Both suppose that as a result of the process of excitation in the muscle there is a liberation of excess of some electrolyte in the tissue. According to one view, an ion of this electrolyte combines chemically with some protein constituent of the muscle to form a new ion-protein compound possessing different physical properties—the protein being so disposed in the muscle that an alteration in its surface tension will exert a pull on the whole structure and lead to shortening.

Another view is that the electrolyte is liberated in a system presenting surfaces possessed of differential permeability towards the ions and that thus the liberated electrolyte sets up differences of electrical potential across these surfaces resulting in mechanical strain and alteration in the contour of these surfaces. This last view, though it is at present of necessity somewhat vague, has on the whole more to recommend it than either of the others. The electrical phenomena which accompany the contraction as well as the excitation processes in muscle provide important evidence, and other facts might be adduced in favour of the idea that an essential part in the mechanism of the contractile process in muscle is played by surfaces or membranes possessed of differential permeability towards ions. It is familiar that such membranes form an integral part in the theories of *excitation* propounded by Nernst and elaborated by Lapicque and by Lucas and Hill.

The existence of membranes or surfaces in muscle is granted by the histologist to almost any desired extent. In the case of skeletal muscle indeed, it is scarcely an exaggeration to say that every histologist who has undertaken the investigation of its structure has described and attached his name to some new membrane, line or band.

It is clear then that we are not making any new or rash assumption in stating that *certain surfaces or membranes form an integral part of the muscle mechanism, and the ionic permeability of these membranes* is a factor of importance in that mechanism.*

The possession by a membrane of differential ionic permeability is no imaginary attribute: simple experiment shows it to be a very usual property. This fact is brought out most clearly by experiments on the influence of membranes on concentration cells.

Direct experiment shows also that the ionic permeability of membranes—and a great variety of them have been tested—is profoundly influenced by certain features of the solutions in contact with them. For a given membrane the factors likely to influence its ionic permeability are—

(1) The P^{+}_{H} of the solution with which it is treated.

(2) The presence in that solution of polyvalent ions. The simple trivalent positive ions are enormously more powerful than the divalent.

When a membrane such as a piece of peritoneal membrane, the lining of an egg-shell or a thin sheet of gelatine, is treated with a very small concentration of acid, or of one of the simple trivalent ions of which we have spoken, its ionic permeability is modified. Direct experiment shows that this altered condition is retained, it may be for minutes or hours, while the membrane is washed with a "neutral" solution free from the polyvalent ions—the persistence of the effect depends on the nature of the membrane, the concentration of the solution used to produce the

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* Here and in the following pages we employ the word membrane with the understanding that it need not necessarily mean anything more than the surface of separation between two phases in the system.

alteration and the length of time it was allowed to act—but if the membrane is treated with a faintly alkaline solution the effect of the acid or the trivalent ion is removed at once.

The parallel with the action of these same agencies on the heart is sufficiently striking. And the parallel does not end here.

Seeking to test further whether this potent action on the heart is indeed a general property of trivalent ions, I employed solutions of certain cobaltammines which yield complex trivalent ions in neutral solution. The compound used most frequently was luteo-cobalt chloride $[Co(NH_3)_6]Cl_3$, which yields the ion $[Co(NH_3)_6]^{\cdot\cdot\cdot}$. Through the generosity of Prof. Werner I was able to test also four other complex trivalent ions. All agreed in showing very much weaker action on the heart than the rare earth solutions. A concentration of the complex ion of the order of 1000 times as great as of the simple ion is needed to stop the perfused heart. A similar difference is shown in the action of these ions on the gelatine membrane. Even large concentrations have only a slight effect on the ionic permeability of a membrane of this type.

The influence of hydrogen ion concentration and of trivalent kations on the heart is paralleled in detail by the action of these ions on the ionic permeability of certain artificial membranes.

The ionic permeability of a membrane can be shown, both theoretically and experimentally, to depend—other things being equal—on the electric charge of the membrane.

Now it can be demonstrated by a variety of methods that most membranes in water, or in a solution like our "neutral saline," possess a negative charge. This charge is reversed with great ease by acid or by the rare earth solution. This can easily be shown, for instance, by the use of dyes, which are themselves charged colloids. A piece of gelatine soaked in "neutral saline" stains very feebly with eosin, strongly with methylene blue. But after treatment with acid or rare earth it takes up eosin with great avidity and is hardly tinged by methylene blue. Eosin consists of negatively charged particles, methylene blue of positively charged particles. If the membrane has a negative charge it can hold the latter, if a positive charge, the former. This method also reveals the fact that the complex trivalent ions are much less potent than the simple trivalent ions in altering the charge of the gelatine membrane.

In these membranes we are, of course, dealing with substances in the colloidal state. If instead of using extended sheets of material we turn our attention to colloidal solutions, where one phase of the colloidal system consists of discrete particles floating freely in the other, it is possible to study the action of electrolytes more simply still. For in such systems the alteration of electric charge on the particles is often followed by an alteration in their state of aggregation, which may be manifested as an alteration in the colour of the solution, or as is very usual by actual precipitation. For instance, when the particles are negatively charged, as in colloidal gold or in a solution of egg-white, the addition in suitable concentrations of electrolytes capable of conferring positive charges on the particles causes agglutination of these particles.

Testing a large number of colloidal solutions by this method, it becomes apparent that there are great differences in the relative efficiencies of different ions in affecting the charge of different surfaces. Thus considering the simple and the complex trivalent ions one finds that many colloidal solutions are quite as readily precipitated by them as by the simple trivalent ions (e.g. colloidal gold, arsenious sulphide, boiled diluted egg-white, and many more), while others which are readily precipitated by the simple trivalent ions are unaffected by even large concentrations of the complex ions (e.g. unboiled diluted egg-white, haemoglobin, etc.). Of a large number of colloidal solutions, those which are very sensitive to complex as well as to simple trivalent ions are found to belong to that class of colloids known as lyophobe or suspensoid; those sensitive to the simple but insensitive to the complex trivalent ions, to the class called lyophil or emulsoid.

Clearly, what has been stated would indicate that the membranes in the heart muscle are of an emulsoid colloidal material. This indeed confirms in one special instance a conclusion drawn from entirely different considerations by Martin Fischer as to the nature of the body proteins in general. The study of the action of these electrolytes on the heart will give more precise information than this. When the ratios of the activities of the simple and the complex ions on surfaces of various compositions have been worked out in detail, we shall be enabled to define precisely some physico-chemical constants for each surface. As has been remarked, the investigations of colloid chemistry have not as yet been carried far enough for this to be done, but the methods are available, and the research would present no insuperable difficulty. Just as the classification of liquids of two phases into suspensions, colloidal solutions and true solutions, is now recognized to be an arbitrary though convenient subdivision, there being no real boundaries between the classes; so it will probably be found among the colloidal solutions themselves. We shall learn in course of time to describe each colloidal solution in terms of certain essential factors which will include such terms as the viscosity of the phases, the amount of surface presented by them, and the electric charge they carry. At present this cannot be done in any quantitative sense, yet we may illustrate from material already available the nature of the biological interest which will accrue from knowledge of this kind.

In the first place, new light is thrown on the relations between the living heart muscle and its normal environment.

We have considered the importance of the P^{+}_{H} of the solution. Take now the case of magnesium. $\mathrm{Mg}^{\cdot\cdot}$ is the only simple divalent ion which can be expected to act on the heart simply quâ divalent ion. As we have shown, $\mathrm{Ca}^{\cdot\cdot}$, $\mathrm{Sr}^{\cdot\cdot}$, and $\mathrm{Ba}^{\cdot\cdot}$ enter probably into special chemical relation with some constituent of the tissue, while the other metals which give divalent ions (such as beryllium, zinc, cadmium, etc.) form solutions which are hydrolyzed.*

It is generally stated that an emulsoid colloid is very insensitive to salts. This is not true, at any rate in the case of some typical emulsoids, when simple trivalent ions are considered. Quite small concentrations of the rare earth solutions can produce large effects on colloidal materials which present all the other characters of emulsoids. Many experiments on a variety of colloidal solutions lead me to the conclusion that a fairer statement of the characteristic relations between emulsoid colloids and electrolytes would be this: that as compared with the suspensoids there is a very great difference in the relative concentrations of simple di- and tri-valent ions needed to produce the same effect. While for suspensoids the ratio of the activities of di- to tri-valent ions is taken ordinarily as of the order of 3 to 100, for emulsoids it is rather of the order of 1 to 10,000. It is certain that these ratios vary widely with different colloids, and that from a more precise study of these ratios than has at present been undertaken much more will be learnt about the colloids themselves, but the broad distinction appears to hold.

On the artificial scheme of membranes, then, magnesium produces effects tending in the same direction as those produced by the simple trivalent ions and by the hydrogen ion, but is enormously less potent than these.

Precisely the same is true of the action of $\mathrm{Mg}^{\cdot\cdot}$ on the heart.

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* Incidentally it should be noted that the effect of hydrolysis, as regards the activity of the solution on the heart and on colloids such as gelatine, is precisely opposite in the case of these solutions of divalent ions from that described for trivalent ions. For the H ions, though less powerful than the equivalent concentration of simple trivalent ions, are much more powerful than the equivalent concentration of divalent ions in these relations. Consequently a solution of beryllium, which is hydrolyzed, affects the heart, and colloid systems of the class sensitive to $\mathrm{H}^{\cdot}$, much more powerfully than the non-hydrolyzed solutions of magnesium.

In the ray heart, for instance, a concentration of ·000001 M to ·00001 M of $Ce^{\cdot\cdot\cdot}$ or $Nd^{\cdot\cdot\cdot}$ is sufficient to cause prompt arrest in diastole. The same effect can be produced by $Mg^{\cdot\cdot}$, but the concentration needed is about ·02 M. If our explanation of the action of magnesium on the heart is true, it should of course be found that the dose of Mg needed to arrest the heart depends greatly on the P^{+}_{H} of the solution in which it is applied. An increase in the numerical value of the P^{+}_{H} should tend to counterbalance the effect of magnesium. This is abundantly confirmed by experiment. For example: a ray heart was brought to rest by raising the concentration of $Mg^{\cdot\cdot}$ in the solution, perfusing it to ·025 M. The diastolic arrest occurred after two or three contractions. When the heart had remained completely at rest for a minute with this solution running through, without changing the concentration of $Mg^{\cdot\cdot}$ the P^{+}_{H} of the solution was raised from about 6·5 to 9. Within a few seconds the heart started beating, and continued to beat in this solution as strongly and regularly as in the original solution which contained no excess of $Mg^{\cdot\cdot}$.

Consideration of the relations of colloidal materials to simple and complex trivalent kations enables us to make certain inferences as to the nature of these colloidal substances from which we can predict their relations to certain other ions. Applying this principle to the heart, we arrive at conclusions as to the nature of the colloidal membranes in its structure from which we can predict the action of magnesium upon the heart-beat.

Finally, I wish to indicate certain directions in which experiments of this kind may assist in the development of a scientific comparative physiology.

Two instances which I have encountered in the course of work at the Plymouth Laboratory this summer may serve as illustrations.*

The facts already stated about the general similarity in the relations of the heart muscle of widely different species to Ca, Sr, and Ba encourage the belief that the general character of the mechanism is the same in these varieties of heart muscle. Can we find differences in the relations of the membranes to "charging" electrolytes? What kind of differences are to be looked for?

A study of the properties of colloids by a variety of methods leads to the following conclusions with respect to the part played by the

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* I am much indebted to Miss Dale, of Newnham College, for her skilful assistance in this work.

nature of the colloid itself in determining its relations to electrolytes.

(1) Different surfaces in the same solution may take on different electrical charges.

(2) Different surfaces possessing the same charge in some particular solution may require different concentrations of the same electrolyte to produce equal changes in their charges.

The physiological expression of a difference in the heart membranes of the kind indicated in the first of these conclusions would be of this type: that one kind of heart should behave in a neutral solution as another would behave in an acid or in an alkaline solution, other conditions being equal.

The heart of the mollusc Pecten maximus differs from all the vertebrate hearts studied in this way. On perfusing it with "neutral saline" it stops instantly in systole. It will beat, however, if we alter the P^{+}_{H} of the solution in the direction of slightly increased acidity; if the change is carried a little further, diastolic arrest—the characteristic acid effect—is then produced. Now the P^{+}_{H} of the solution which perfuses the heart of the living Pecten—the blood of the animal—is about 6·5 to 7, that is to say, very slightly on the *alkaline* side of our "neutral saline." How then can the heart beat in such a solution? The answer is simple. The blood of Pecten contains a large concentration of magnesium. The divalent $Mg^{\cdot\cdot}$ is present in sufficient concentration to bring the charge of the membranes to the value required for their proper differential permeability.

The assertion that the blood of Pecten is practically the same as sea-water overlooks one important fact about it. Though its osmotic pressure and concentrations of Na, Mg, K, Ca, etc. are closely the same as in sea-water, there is a distinct difference in the P^{+}_{H} of the two fluids. The P^{+}_{H} of sea-water varies from about 7·9 to 8·3, while that of the Pecten blood is rather below 7. The difference may be demonstrated by adding a drop of a solution of rosolic acid to a little sea-water and to a similar quantity of the colourless blood of Pecten. The colour given by the former is bright pink, that of the latter yellow or yellowish pink.

Thus we find that sea-water, despite its high concentration of magnesium, on perfusion instantly stops the Pecten heart in systole.

But if by the addition of a little dilute hydrochloric acid we change the P^{+}_{H} of the sea-water to the same value as that found for the blood of Pecten (about 1·5 cc. of ·1 M HCl in 100 cc. of sea-water), we have at once a solution which sustains the beat of the heart admirably. A solution of the same composition as this but without magnesium causes the heart to stop in systole. Further, as is predicted theoretically, it is possible to replace the Mg by a very small concentration of a simple trivalent ion. Thus the Pecten heart which has stopped beating in "neutral saline" can be made to beat again and be kept beating for a considerable time by the addition to the solution of a concentration of about ·00003 M of $Ce^{\cdot\cdot\cdot}$ or $Nd^{\cdot\cdot\cdot}$. Here we have one trivalent ion doing the work of some 40,000 divalent ions, and it is therefore not surprising that the solution with rare earth does not, as a rule, keep the heart going so regularly as the solution with magnesium. Higher concentrations of the simple trivalent ions (e.g. ·0001 M) stop the Pecten heart in diastole exactly in the same way as a too acid solution.

We may express the difference between the heart of Pecten and the hearts of the vertebrates which have been studied, by saying that the membranes have different iso-electric points. This kind of difference is most probably related to a difference in the chemical composition of the membranes; it being a well-established fact that different protein substances found in the animal kingdom do show differences of this kind, according to the predominance of the "acidic" or "basic" amino-acids included in their composition.

It is by no means a new suggestion that the differences between different species are at root differences in the chemical composition of their tissues; but as far as I am aware it has not before been pointed out by what mechanism such differences can affect the physiological behaviour of the living heart.

The second conclusion formulated above as to the relations between different surfaces and the same electrolytes finds biological application in the differentiation of more closely allied species.

The hearts of the elasmobranchs Raia and Scyllium are kept beating satisfactorily for many hours by perfusion with the same "neutral" solution containing sodium, potassium, calcium, and magnesium chlorides and urea, the solution being thoroughly aerated and of P^{+}_{H} about 6·5. The concentration of Mg in the solution generally used was ·005 M. Removal of the Mg from the solution only causes the beats to get rather faster. Increase of the concentration of magnesium causes slowing of the heart-beats, and if carried far enough, stoppage. Qualitatively these phenomena are alike in the hearts of the ray and of the dogfish. But studied quantitatively the ray heart is found to be much more sensitive to Mg than is the dogfish heart. Thus taking the mean of a number of observations on ray* hearts, the concentration needed to give a 50 per cent reduction in the rate of beat (calling the rate in the solution with ·005 M Mg = 100) was only ·009 M. For Scyllium it was ·05 M. To stop the ray heart the concentration of $Mg^{\cdot\cdot}$ needed was about ·02 M, while to stop the heart of Scyllium a concentration usually greater than ·1 M was required.

A corresponding difference is found in the sensitiveness of these hearts to the simple trivalent ions. Thus ·00001 M $Ce^{\cdot\cdot\cdot}$ readily stops the heart of Raia, while a concentration approaching ten times this magnitude is needed to produce the same effect on the heart of Scyllium.

In these respects the heart of the angel fish, *Rhina squatina*, was found to resemble that of the dogfish rather than that of the ray. The curve representing its behaviour in the presence of various concentrations of Mg lies between that plotted for Raia and that for Scyllium, but much nearer the latter than the former. This is interesting, as in the ordinary morphological classification Rhina is placed between the Scylliidae and the Raiidae. It is said that Rhina resembles rays rather than dogfish in its general habits, yet it is possible that the character indicated by these experiments represents something deeper seated than the details of the responses given by the central nervous system.

It is at any rate conceivable that by the extension of work along these lines we may learn to express genetic affinity in physico-chemical terms.

One further point. It will be recollected that the behaviour of a membrane depends not only on its original composition and on the nature of the solution immediately surrounding it, but also to some extent on its previous history. I have found that the blood plasma of Scyllium and of Rhina is of P_{H}^{+} numerically slightly higher than the plasma of *Raia* (*clavata* or *blanda*). It is possible that the difference in sensitiveness of the hearts of these animals to the

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[*] Two species, *R. clavata* and *R. blanda*, were used in these experiments. No difference could be detected in the behaviour of the hearts of these two species towards electrolytes. Using rays of very different sizes no constant differences were found between the large and the small specimens.

agencies we have named is to be explained in part by the fact that their membranes have been bathed for years before the experiment in solutions of slightly different P^{+}_{H}.

There are striking differences in the relations of the hearts of different species of animals to hydrogen ion concentration and to polyvalent ions: these correspond exactly to differences found in the effects of these ions on the ionic permeability of different colloidal materials. A mechanism is thus suggested which explains how differences in the chemical constitution of a tissue component may cause differences in the physiological behaviour of the living tissue.

I venture to hope that these fragmentary remarks will make it apparent that a field of research lies open here, containing treasure not alone for the physiologist who desires to elucidate the mechanisms by which vital processes are wrought in the individual, but also for the zoologist, who in looking for clues of genetic affinities has learnt the limitations of pure morphology and the truth of the axiom of physiology that by studying what a cell can do we shall best learn what it is.

For the furtherance of the science of Comparative Physiology the co-operation of morphologists and physiologists with physical, organic, analytical, and colloid chemists is necessary. This country is not lacking in any of these. The study of the science can be carried on best where the greatest variety of animals, living and in good condition, can be provided. This condition is most perfectly fulfilled at a marine station.

At Plymouth the fauna is rich, and the facilities provided in the Laboratory for keeping animals in condition for physiological experiment are admirable. It is earnestly to be wished that more workers in this country would interest themselves in a branch of zoology and of physiology pregnant with interest for both sciences, and that their efforts might secure for the Plymouth Laboratory permanent accommodation and equipment for physiological work, on a scale approaching that provided by marine stations in other countries.

In conclusion, I would express my warmest thanks to the Director and Staff of the Laboratory of the Marine Biological Association at Plymouth for much valuable assistance and advice.*

September, 1911.

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Types of Crustacean Blood Coagulation.

By

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HAVING been engaged at intervals during the last few years in studying from a physiological point of view the coagulation of crustacean blood —Tait (08), (10, A and B), (11)—and finding more variation in this regard in different crustacea than has hitherto been recognized, it suggested itself to me to inquire if the observed differences are correlated with any special physiological peculiarities, and, further, if they depend in marked degree on phylogenetic relationship. It is not a simple matter to settle either of these questions, and I make no pretence to have done so. At the same time, I have thought it worth while to put my observations on record, in the hope that the matter may thus sooner arrive at a satisfactory settlement.

The literature of the subject and details regarding the technique of examination of the blood and other particulars I hope shortly to publish elsewhere. Suffice it here to state that at least three distinct modes of blood coagulation may be recognized in crustacea :—

A. Simple agglutination of the blood corpuscles without any subsequent jellying of the blood plasma. (This is probably the most primitive and essential device both in invertebrates and vertebrates for procuring arrest of hæmorrhage from a wound.)

B. Agglutination of the blood corpuscles with subsequent general jellying of the plasma.

C. Jellying of the plasma in two successive stages, the preliminary cell-agglutination being relatively insignificant. The first plasma coagulation consists of localized (primitively globular) clots, which occur around or in immediate relation to special blood corpuscles, originally discovered by Hardy (92), and by him named "explosive corpuscles." At a later stage a second jellying process occurs, which this time involves the whole of the remaining plasma.

Although it is not my intention to discuss in detail the basis on which crustacean coagulation is separated into these three types, it would be a mistake to convey the impression that a hard and fast line of separation can be drawn between them. The classification is arrived at by examining the blood always under the same conditions—that is to say, *entirely removed from the vessels and placed either in a dish or on a glass slide.* These are, in fact, the conditions under which observations on coagulation are generally made. The classification here given represents a more extended knowledge than that hitherto in vogue—originally due to Heim (92)—which recognizes two groups, viz. A and B above.

COAGULATION C.

It will be convenient to begin with coagulation C, which is associated with the presence of explosive cells, and which from its complexity must be regarded as a somewhat specialized form of coagulation.

This form seems to be specially frequent in Isopoda. I have found it in *Conilera cylindracea*, in *Idotea baltica* and *emarginata*, in *Ligia oceanica*, in *Oniscus*, and in one or more species of *Porcellio*, i.e. in individual members of three sub-orders out of six.* On the other hand, I have failed to find it in *Gnathia maxillaris*, in *Dynamene rubra*, in *Sphaeroma serratum*,† and in *Jaera marina*, though possibly owing to the fact that the amount of blood obtained from these relatively small specimens was too meagre to allow of a satisfactory examination.

Among Amphipoda the occurrence of coagulation C seems to be much less frequent. It is not present in *Gammarus marinus*, in *Gammarus pulex*, in *Orchestia littorea*, nor in certain specimens of *Caprella* examined by me. On the other hand, I have recorded its presence in *Gammarus locusta*—Tait (10, B). This was not, however, under the conditions above specified—that is to say, in blood wholly removed from the animal and examined separately on glass, nor have I as yet observed it under these conditions.

As regards the Mysidacea coagulation C is absent in at least one species of *Mysis* (the only member of this order examined by me).

Crustacean blood coagulation has been chiefly studied in the

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* The zoological classification referred to in the present paper is that given by Calman in Part VII of Lankester's *Treatise on Zoology.*

† In the course of this work I found that *Sphaeroma serratum* (with black chromatophores) undergoes colour change in response to its background, like that undergone by *Idotea*—V. Bauer (05)—and by *Ligia*—Tait (10, C)—whereas *Oniscus* and *Conilera*, the latter possessed of orange and lemon-yellow chromatophores, do not.

Decapoda, and it is of especial interest to know if coagulation C occurs among them. Of this order I have examined *Pandalus montagui* and *P. brevirostris*, *Hippolyte varians* and *H. viridis*, *Palaemon serratus*, *Crangon vulgaris*, *Palinurus vulgaris*, *Homarus vulgaris*, *Astacus fluviatilis*, *Galathea squamifera* and *G. strigosa*, *Porcellana longicornis* and *P. platycheles*, *Eupagurus bernhardus* and *E. prideauxii*, *Ebalia tuberosa*, *Corystes cassivelaunus*, *Carcinus maenas*, *Portunus puber*, *P. marmoreus*, *P. arcuatus* and *P. depurator*, *Atelecyclus septemdentatus*, *Cancer pagurus*, *Xantho hydrophilus* and *X. incisus*, *Inachus dorynchus*, *Macropodia rostratus*, *Hyas coarctatus* and *Maia squinado;* and have found coagulation C only in two members of the list, viz. in Palinurus and in Astacus. It is thus an uncommon form of coagulation in the Decapoda, while it is doubtful if it occurs at all in the Brachyura.

The examples hitherto mentioned exhaust the crustacean forms in which I have looked for the presence of coagulation C. I shall now say a word or two regarding this form of coagulation in its physiological aspect.

The clotting associated with the presence of explosive cells is a sufficiently striking one. Thus the blood of Palinurus forms the stiffest jelly of any crustacean blood I have examined; Ligia blood also forms a specially firm coagulum; while the onset of coagulation is in all cases rapid. In the solidity of the jelly formed, coagulation C represents the most advanced form of coagulation to be met with in crustacea.

Assuming that the object of plasma coagulation is to provide an additional mechanism for stopping a wound (cell-agglutination being, as above indicated, the primary and most essential mechanism for this purpose) it would follow that, *ceteris paribus,* a hæmorrhage in an animal possessing explosive cells would cease sooner than a hæmorrhage in other crustacea. This being so, one would look in isopods, in Palinurus and in Astacus for some special cause necessitating the existence of an extraordinary mechanism for arrest of hæmorrhage.

One naturally thinks of the process of autotomy in this connection. The reflex surrender of damaged limbs is generally conceded to be a method of preventing undue loss of blood. Where the reflex is present in least degree one might expect on the whole to find the highest degree of coagulability of the blood, and *vice versa.*

Now isopods do not show autotomy. In Astacus the reflex is said to be present—Huxley (80)—though in my own experience and in that of others, e.g. Fredericq (83), it is not readily demonstrable even in specimens presumably fit and healthy. In isopods and in Astacus, therefore, the association is what one would *a priori* expect. In

Palinurus, however, whose blood forms such a stiff coagulum, the power of autotomy is present in very marked degree. Thus the form of coagulation associated with the presence of explosive cells does not imply absence or defective power of autotomy.

If, again, we direct our attention to the crustacea that possess incoagulable, or rather non-coagulating, blood-plasma (type A), we find the evidence equally contradictory. Thus in Maia and in Cancer, both of them forms with non-coagulating plasma, power of autotomy is marked. In the spider-crab, *Inachus dorynchus*, whose type of blood coagulation likewise falls under group A, the presence of autotomy is unusually difficult to demonstrate. It seems that no constant relationship exists between power of autotomy and any special form of blood coagulation.

The assumption with which we originally started, viz. that the property of coagulability in blood plasma exists or has been evolved for the sole purpose of arresting hæmorrhage, may however be unwarranted. All the microscopical observations made on the arrest of hæmorrhage from a vessel, whether in invertebrates or in vertebrates, including mammals, go to show that the opening is plugged chiefly by adhesion and agglutination of cells at the cut surface. The blood of a hæmophilic person forms a perfectly firm clot: in spite of the presence of the clot, however, blood continues to ooze for hours and even days from a wound. Again, we meet with coagulability in physiological fluids other than blood. Milk clots in the stomach: yet no one has suggested that this coagulation has a merely mechanical function. Considerations such as these warn us against drawing premature conclusions in regard to the purpose for which coagulability exists in the blood plasma. In circulating blood there are multitudinous chemical processes constantly going on, the nature of which is entirely hidden from us. Who can say that coagulability is not primarily concerned in some of these hidden processes?

As a matter of fact, when we compare the actual time taken for natural arrest of hæmorrhage from the terminal segment of one of the limbs of Maia and of Palinurus respectively, we find that, while the hæmorrhage is, to begin with, equally profuse in both cases, the Maia wound is closed as soon as the Palinurus wound. Maia blood is characterized by the absence of all plasma coagulation. Palinurus blood is highly coagulable. Until we have further knowledge as to the *raison d'être* of coagulability in blood plasma, attempts to correlate by *a priori* methods, different types of blood coagulation with special physiological conditions can be but shots in the dark.

I shall conclude this physiological discussion by referring shortly to two other conditions with which at one time I imagined coagulation C might possibly be correlated. Having discovered coagulation C in isopods long before I found it in any decapod, I looked for other physiological peculiarities characteristic of isopods as opposed to decapods. An obvious one is the peculiar method of moulting observable in isopods. A moulting isopod throws off first the covering of the abdomen along with that of the posterior three thoracic segments. At a later date the covering of the head and anterior four thoracic segments is got rid of.* In decapods the moulting process does not occur in two stages. Once again, a fact to which attention has not been called, so far as I know, isopods do not turn red on boiling as so many decapods do. The discovery, however, of coagulation C in decapods, which moult in a different fashion and turn red on boiling, disposed of any possibility of establishing a correlation with these two phenomena.

COAGULATIONS A AND B.

Before discussing coagulation C in its zoological bearing, it may be well to make some statements regarding the distribution of the other two forms of coagulation, viz. types A and B. To determine the existence of one or other of these two types a greater quantity of blood is necessary than when one looks merely for the presence of coagulation C. Partly for this reason my data are somewhat meagre, and refer only to fairly large crustacea of the order Decapoda. The results agree to some extent with those of Heim (92).

Coagulation A is present in *Cancer pagurus, Maia squinado, Inachus dorynchus, Macropodia rostratus,* and *Hyas coarctatus.*

Coagulation B is present in *Carcinus maenas, Palaemon serratus, Portunus puber*, and *Homarus vulgaris.*

The last four animals are arranged in order according to the extent and firmness of the plasma jelly that forms in their blood after withdrawal. In *Carcinus maenas* the bulk of the plasma remains indefinitely fluid; in Palaemon the jelly, which is soft, involves almost all the plasma; in Portunus there is a complete and fairly firm jelly; while the plasma of Homarus clots with exceptional firmness.

I should like to make two comments on these results. In the first place, there is a complete series of gradation of plasma coagulation to be observed in group B. The amount of plasma jelly formed in the blood of *Carcinus maenas* is so slight that we might almost

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* I do not know that anyone has called attention to the fact that the separation between the two cast-off portions of the integument occurs just at the anterior limit of the heart, as determined by the researches of Delage (81).

include the animal under group A. This indicates a difficulty in drawing a sharp line of distinction between group A and group B. In the second place, the coagulation observable in Homarus blood (at the other end of the series) approaches, in regard to its mere firmness apart from the mechanism involved in its production, most nearly to the coagulation seen in the blood of Palinurus or of Ligia.

ZOOLOGICAL SIGNIFICANCE.

From what has been said it will be apparent that the various categories into which crustacea fall according to the form of blood coagulation observed in them do not coincide in any striking fashion with the subdivisions into which they are grouped by zoologists. Within the order Decapoda alone we meet with all three types of coagulation. When, within this order, we consider the animals characterized by one given type of coagulation, we find that they are not necessarily close allies from a morphological point of view. Thus Palaemon, Homarus, and Portunus, similar as regards blood coagulation, represent extremes of decapod structure; from a blood coagulation point of view, again, we should group Astacus with Palinurus rather than with Homarus, an arrangement that would appeal to no morphologist; and so on.

On the other hand, there are indications that one and the same type of blood coagulation may sometimes keep constant in the members of a given zoological group. The prevalence in so many isopods of coagulation C, a type rare in decapods, is one example. Another is the apparent universality of coagulation A among the Maiadae. Then, again, among the decapods coagulation C is confined to the macrurous Reptantia.

The question, so far as it concerns the zoologist, now comes to be: Do the facts above related afford any justification for utilizing the physiological method of inquiry in the task of deciding upon the inter-relationships of crustacea? It seems that while a valid case for the actual applicability of this method has not been established, a case has at least been established for the desirability of further research along this line. As a further justification for this standpoint, I would quote the following sentence from Calman (09):—

> "The classification of the Decapoda is a very difficult problem, and none of the schemes hitherto proposed can be regarded as entirely satisfactory. The traditional classification of the group into the long-tailed Macrura and short-tailed Brachyura was established by Latreille

in 1806; but the difficulty of defining these groups is shown by the varying limits which have been assigned to the intermediate group of Anomura established by Milne-Edwards in 1834. Boas, in 1880, was the first to make a radical departure from this system. He pointed out that the Brachyura and Anomura were only single branches of the Decapod stock, and by no means equal in systematic value to the Macrura, which included several other branches not more closely connected with each other. In other words, just as in the classification of the Malacostraca as a whole, so within the Order Decapoda, the retention of the primitive 'caridoid facies' does not necessarily imply close affinity between the groups exhibiting it."

Again, referring to the Amphipoda, which were ranked by Leach along with the Isopoda in his group Edriophthalmata, he says (p. 239): "It seems very likely that their affinity to the Isopoda is not so close as has been supposed."

These statements are quite in accordance with the grouping that one would adopt from considerations of blood coagulation.

PHYSIOLOGICAL EVOLUTION.

Lastly, and this concerns principally the physiologist, we have in Crustacea an exceptionally appropriate assemblage of types in which to study the evolution of blood coagulation. In the blood or body-fluid of all invertebrates apart from arthropods, the only form of "blood coagulation" that occurs is an agglutination of the corpuscles—Cuénot (91), see also Geddes (80); there is no jellying of the plasma. In arthropods alone among invertebrates we meet with a true jellying or solidification of "fibrinogen" normally present in solution in the plasma. Now, in the class Crustacea, and even within the order Decapoda, we find some animals whose blood does not jelly, others in which the jellying process is present but insignificant, and others again in which it is very conspicuous. In this group of animals, therefore, we have a readily available series of types showing every gradation of evolution from complete absence of jelly (e.g. the spider-crabs) to the occurrence of very firm jelly-coagulation (e.g. the lobster and the rock-lobster).

Further, in the same class of animals we find that the jellying process, when present in what is possibly its most developed state, is associated with the presence of corpuscles having special physiological attributes. Assuming that the functional peculiarities of these cells represent a high degree of selective adaptation, we have to inquire by what steps the specialization has been brought about. The physiological features in question cannot be supposed to have sprung suddenly into existence in a few special animals. The explosive property and the jelly-producing property must be present in various stages of development in the blood-cells of different Crustacea. Crustacean blood-cells offer excellent material for the study of functional evolution.

Generally speaking, such studies have been much neglected in the past. Keith Lucas in two notable communications (09, A, B) has recently sketched the lines on which such investigation should proceed, and indicated the general bearing of the results that may be expected thus to accrue. I hope shortly to adduce some facts related to the coagulation of crustacean blood, which further illustrate this question of functional evolution.

A considerable portion of the work recorded in this communication was done during the occupancy of a table (granted by the British Association and by London University) in the Marine Laboratory, Plymouth. The expenses of that portion of the research done in Edinburgh were defrayed by the Carnegie Trust. I have to record my thanks to Dr. Allen, the Director of the Plymouth Laboratory, and his assistant, Mr. Orton, for help in the determination of species.

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The Amphipoda collected by the "Huxley" from the North Side of the Bay of Biscay in August, 1906.

By

E. W. Sexton.

WITH PLATE III.

THE Amphipoda dealt with in this paper were collected in August, 1906, by Dr. Allen, to whom I am indebted for the opportunity of examining them.

The collection contained thirty-five species (belonging to twenty families), of which one only, *Tryphosites alleni*, is new to science. Five others, *Stenothoe richardi*, *Syrrhoites walkeri*, *Syrrhoe affinis*, *Eusirus biscayensis*, and *Rhachotropis rostrata*, are recorded for the first time since their original discovery. The geographical range of five species has been considerably extended, viz. *Syrrhoe affinis*, *Tmetonyx similis*, *Sympleustes glaber*, *Epimeria parasitica*, and *Laetmatophilus tuberculatus*; the four last forms have not been hitherto recorded with certainty south of Norway.

Several of the genera have been discussed separately in four papers which I have published elsewhere [**34–37**].

The classification followed is that of Stebbing in his classical monograph on the Amphipoda in *Das Tierreich*. Previous records of the capture of any species in the Bay of Biscay are given below the account of the species.

The measurements in all cases, unless otherwise stated, are taken from the tip of the rostrum to the tip of the telson.

TRIBE GAMMARIDEA.

FAM. LYSIANASSIDAE.

GEN. Trischizostoma, Boeck.

Trischizostoma nicæense (Costa).

STATION XII. One specimen, an ovigerous female, measuring 23 mm. For synonymy and description, see Sexton (**34**).

Recorded once before from the Bay of Biscay by Chevreux as *Guerinella nicœensis* (**15**).

GEN. **Ichnopus**, A. Costa.

Ichnopus spinicornis, Boeck.
(Stebbing, *Das. Tierr.*, p. 52.)

STATION X. Six specimens, all females, the largest measuring 13 mm., the others small, averaging 5·5–6 mm. in length.

This species has been recorded before from the Bay of Biscay, trawled twice by the *Hirondelle* (Chevreux (**14**), p. 15), and once by the *Britannia* (Walker, (**46**), p. 159).

GEN. **Tmetonyx**, Stebbing.

Tmetonyx similis, G. O. Sars.

STATION II. One specimen, an ovigerous female, measuring 18 mm.

This specimen is a very large full-grown female, which has apparently developed the secondary sexual characters of the male. Tattersall has recorded a similar instance in his recent paper on Schizopoda (*Fisheries, Ireland, Sci. Invest.*, 1910, II [1911], p. 16), in which a full-grown ovigerous female had assumed the rostrum characteristic of the adult male.

The *Huxley* specimen agrees in every particular with Sars's description and figures (**30**), p. 93, pl. 33, with the exception of the antennae. Sars's description of the flagella is as follows: In the superior antenna "flagellum more than twice the length of the peduncle, and composed of about twenty articulations, the first of which is rather large, about equal in length to the four succeeding ones combined; accessory appendage scarcely attaining one-third of the length of the flagellum and six articulate. Inferior antennae but little longer than the superior, flagellum composed of about twenty-four articulations." In the specimen examined the first joint of the *primary flagellum* equalled in length the six following joints taken together; 19-jointed; *accessory flagellum* 7-jointed. The inferior antenna was twice the length of the superior; flagellum composed of 42 joints. Both pairs of antennae were calceoliferous, one calceolus on each joint, from the 5th–16th in the superior; and in the inferior from the 3rd–24th, then on alternate joints to the 30th. The calceoli do not form a continuous row, but alternate in the same way as those of the *Tryphosa antennipotens* figured by Stebbing in his *Challenger* Report, pl. 6.

This is the first record of the species from the Bay of Biscay.

Gen. **Tryphosites**, G. O. Sars.

Tryphosites alleni, Sexton.

Station XII. Two specimens, ovigerous females, measuring 10 mm. in length.

For description and figures see (**37**).

Fam. **METOPIDAE.**

Gen. **Proboloides, Della Valle.**

Proboloides gregarius (G. O. Sars).

(Stebbing (**41**), p. 189.)

Station XIII. One specimen, an ovigerous female, 5 mm. long.

First gnathopod. The palmar margin is sharply serrated, as well as the inner edge of the finger. The finger carries a small decurrent tooth subapically, two setules inset in the notch and four others at intervals along the margin. The 4th and 5th joints are furnished posteriorly with strong flat dentate bristles in addition to the feathered setae, three on the 4th joint and two on the 5th.

Recorded once before from the Bay of Biscay by Walker (**46**), p. 159.

Fam. **CRESSIDAE.**

Gen. **Cressa,** Boeck.

Cressa dubia (Bate).

(Stebbing (**41**), p. 191.)

Station VII. Depth, $\frac{\cdot}{444}$ fathoms ; one specimen, a male, 2 mm. long.

The previous records from the Bay of Biscay (Chevreux (**13**), p. 478, and (**10**) p. 121) are from quite shallow water, 6 m.

Fam. **STENOTHOIDAE.**

Gen. **Stenothoe,** Dana.

Stenothoe richardi, Chevreux.

(Stebbing (**41**), p. 194.)

Station XIII. One specimen, a male, measuring 4·5 mm. in length; trawled in 412 fathoms.

The only other specimen known, also a male, 5 mm. long, was taken by the *Princesse Alice* (Chevreux (**12**), pp. 427, 432–5), in August, 1894, in this same region, to the south of *Huxley* Station. The method of capture was the same, the trawl; the depth recorded 1262–748 mètres.

FAM. **ACANTHONOTOZOMATIDAE.**

GEN. **Iphimedia,** Rathke.

Iphimedia obesa, Rathke.

(Stebbing (**41**), p. 214.)

STATION V. One specimen, nearly 12 mm. in length, several young remaining in the pouch.

Boeck (**4**), p. 246, erroneously describes the maxilliped-palp as 4-jointed, the 4th joint rudimentary, although in the figure (Pl. XVIII, fig. 11 h.) it is represented as 3-jointed. Sars gives the number of joints as three, "dactylus wholly wanting" (**30**), p. 377. The *Huxley* specimen agrees with Sars's figure, except that the apex of the 3rd joint is truncate, not rounded. The large sensory spines on this joint are each composed of a stout feathered shaft and a small endpiece, which is inserted in the cleft at the apex of the shaft.

Recorded by Chevreux (**13**), p. 479, and (**14**) p. 63; and by Walker (**46**), p. 159. Chevreux's records are from much shallower water, 10–50 mètres.

FAM. **TRONIDAE.**

GEN. **Syrrhoites,** G. O. Sars.

Syrrhoites walkeri, Bonnier.

(Stebbing (**41**), p. 281.)

STATION XII. One specimen, a large female, 9 mm. in length.

This is the first record of the species since its discovery by the *Caudan* Expedition in the Gulf of Gascony, 1895.

The *Huxley* specimen is larger and older than the female described by Bonnier (**6**), pp. 647–50, measuring 9 mm., as compared with the "un peu moins de 7 mm." of Bonnier's largest specimen. The greater age is shown by the greater number of joints in the flagella of the antennae, Bonnier's female having nine in the superior flagellum and seven in the inferior, while the *Huxley* specimen has eleven and nine respectively. The incubatory lamellae are fully developed, no eggs remaining in the pouch.

GEN. **Syrrhoe,** Goës.

Syrrhoe affinis, Chevreux.

1908. *S. affinis,* Chevreux (**16**), pp. 7–9, fig. 4.)

STATION IX. Two specimens, measuring 9·5 mm., ovigerous females, one with eggs nearly hatched.

The following notes may be found useful in supplementing the short account given by Chevreux:—

Body. Peraeon-segment 7, and pleon-segments 1–4 dorsally serrate as in *S. papyracea* Stebbing, to which it bears a strong resemblance. The number of the serrations differs in the different segments, and occasionally the number on one side of a segment differs from the number on the other side; each serration has a long fine setule inset. In peraeon-segment 7 the serrations number twenty on the right side of the prominent central tooth and eighteen on the left; they extend across the segment almost to the posterior angles, each of which is produced into a small tooth with a setule inset above. The 1st pleon-segment has twenty-five serrations, the middle one much the longest; postero-lateral angles acutely produced, as in the 2nd pleon-segment also. This latter has twenty-one serrations, the middle one again the longest; five sensory spines inset along the inferior margin of the epimera, which are much dilated anteriorly as well as posteriorly. There are eighteen dorsal serrations on the 3rd pleon-segment, the two middle ones, of unequal length, much longer than the others; the epimera are considerably dilated inferiorly, the hind margin with seventeen upturned serrations, each with a setule inset. The 4th pleon-segment has twenty-five of the serrations; while the 5th carries two or three similar serrations on either side, just above the insertion of the uropods.

Head. Strongly vaulted in front, equalling the first four peraeon-segments taken together, in length; rostrum depressed, reaching beyond the middle of the 1st joint of the antenna, sharp-edged, apex very acute; lateral corners not much produced, truncate.

Sideplate 1. Front margin lightly curved forward; front angle subacute, hind angle rounded; posterior margin produced proximally as in Sideplate 2; inferior margin dilated. Inferior margin of Sideplate 2 narrowed and rounded. In Sideplate 3 the truncate portion of the hind margin has a crenulate appearance owing to the insertion of four or five setules. Sideplate 5 not much produced anteriorly, expanded and rounded posteriorly. In Sideplate 6 the anterior portion is hardly developed at all; the posterior portion is greatly produced downwards with its inferior margin truncate, one serration at the posterior angle. Sideplate 7 also with one tooth posteriorly, as noted by Chevreux.

Antennae. Superior antenna. 1st joint of the peduncle thick, more than twice as broad as the succeeding joints, with a strongly upcurved apical tooth; 2nd joint nearly as long as the 1st; 3rd not quite three-quarters the length of the 2nd, all carrying ciliated hairs and setae. *Primary flagellum* 15-jointed, with a long sensory filament on alternate joints; 1st joint long, nearly equalling the 3rd joint of the peduncle in length; 2nd joint very short; 3rd joint half as long again as the 2nd; apical joint tipped with long setae and one ciliated hair. *Accessory flagellum* as long as the 1st and 2nd joints of the primary taken together.

Oral parts much as in *S. papyracea* Stebbing.

Upper Lip (Pl. III, fig. 1) partly carinate, distal margin truncate, corners rounded and edged with microscopic spinules.

Lower Lip, outer lobes large and rounded, covered with fine hairs, with two double-tipped sensory spines on the inner margin of each, near the apex, similar to those described by Stebbing in *S. papyracea; inner lobes* inflated, covered with fine hairs; *mandibular processes* widely divergent, and narrowed distally.

Mandibles (Pl. III, figs. 2 and 3). Right mandible, *cutting-plate* margin irregularly crenate, produced below into two large teeth, with one small one between them; *accessory plate* large, dentate, the three sharp teeth above separated from the two large rounded ones below by a transverse row of three minute sharp denticles, the uppermost tooth with a denticle at its base. In the figure the plates are laid back to show their structure, which, owing to their great curvature, it is impossible to see in the natural position. In the left mandible, the *cutting-plate* margin is crenate, produced below into two large rounded teeth; *accessory plate* with five rounded teeth, the lowest the largest. There are six spines in the right *spine-row,* two large ones covered with minute tubercles, and four slender flexible ones, feathered on both sides; eight in the left spine-row, three large and five slender; the 1st spine is falciform in both rows. *Molar* prominent, strongly denticulate, with a feathered seta inset posteriorly, the teeth on the posterior edge of the crown longer and sharper than those on the anterior portion, front edge sinuous.

Maxilla 1 very like *S. papyracea.* *Outer plate* truncate with eleven spines in two rows, seven long spines in one row, and four shorter in the other. In the row of long spines, the two innermost are finely plumose, the next three denticulate with from twelve to sixteen denticles plumose on the outer side near the tip, the two outermost spines with five and three larger denticles respectively. In the other row, the two inner spines are simple, the 3rd carries one large and two small denticles, and the 4th spine is very stout, curved, and simple. The *inner plate* is fringed with ten plumose setae, the apical two much shorter than the others. The *palp* is considerably longer than the outer plate, with both margins microscopically serrate; 1st joint short, with three setules on the outer margin; 2nd joint nearly three times as long as the 1st, apex truncate with four or five long spines inset, and about ten or eleven stiff setae set in pairs along the upper part of the inner margin; spines and setae very finely serrate.

Maxilla 2. *Inner plate* broader than the *outer;* the arrangement of the setae as in *S. papyracea.*

Maxillipeds (figs. 4 and 5). *Inner plate* reaching to the distal end of the 1st joint of the *palp;* upper margin truncate, furnished with four stout feathered spines, two curious bent spines directed inwards, and one feathered seta. The larger of these spines (fig. 4) is situated midway on the margin, the smaller one at the inner angle. Just below the smaller spine, the outer surface of the plate is deeply hollowed, and on the lower edge of this little hollow or groove stands a strong coupling spine (fig. 5). The upper half of the inner margin carries five long stout spines, set one behind the other, feathered on both sides for half their length like the spines on the upper margin. *Outer plates* reaching almost to the distal end of the 2nd joint of the palp, with a row of twenty-one strong flat spines, extending along the inner margin to the outer side of the rounded apex. These spines, downturned and apically dentate, increase gradually in length to the eighteenth, the remaining and outermost three are setiform. On the outer surface five or six pairs of setae are inset submarginally, and nearly the whole of the surface is covered with a furry armature of microscopic spinules. The 2nd joint of the *palp* is expanded on the inner side and fringed with long setae, with the fur-like spinose armature distally. The 3rd joint is much expanded on both sides, the finger thus appearing to be set in the middle of the distal margin; the whole joint is fringed with long setae, and carries a graduated row of cleft-tipped spines on the distal outer angle. The nail is more than half the length of the finger, with one setule above and two below.

Gnathopod 1 (Pl. III, figs. 6 and 7), 2nd joint long, lightly curved, carrying six to eight extremely long and delicate plumose setae posteriorly. These setae are sparsely plumose, the feathering being very long and fine; similar setae are found on the basal joints of Gnathopod 2, and peraeopods 1 and 2. The 3rd joint has one of these setae at the distal angle. 5th joint with the anterior margin fully twice as long as the anterior margin of the 6th; posteriorly it is a little expanded, covered with the fur-like spinose armature, and furnished with several densely crowded rows of spines on the margin; one row directed forward of small coarsely dentate spines, one row of small spines, another row of medium-sized, and a row of long cleft-tipped ones similar to the one figured (fig. 6). There appears to be another row beyond this of the medium-sized, and in addition three long fine, thread-like setae are found near the distal end, the longest being nearest to the hand and much exceeding it in length. The hand widens a little distally, hind margin pectinate; palm slightly oblique, fringed with long setae, and carrying at the palmar angle, besides the very large palmar spine, a group of five long spines, the apical flagella of which are of great length. The finger is a little longer than the palm, and crosses the palmar spine. For the construction of finger and spine, see fig. 7.

Gnathopod 2, 5th joint very slender, considerably more than twice as long as the 6th, furnished on the distal posterior margin with dense rows of setae. The setae appear to be arranged in groups set closely together along the margin, each group containing four graduated setae. The hand widens distally; it resembles that of Gnathopod 1 in the construction of the finger and palmar spine and in the arrangement of the long setae on the palm. The branchial vesicles are large, as long as the basal joint; incubatory lamellae large and wide, exceeding the basal joint in length.

Peraeopods 1 and 2, practically subequal in length; branchial vesicles large, incubatory lamellae long and narrow. 2nd joint very long and slender, with long plumose setae on both sides; 4th, 5th, and 6th joints subequal, fringed posteriorly with long fine setae; 6th joint with a stout spine and a dense fringe of these setae distally, almost concealing the finger, and exceeding it in length. The finger, as in all the peraeopods, has a long nail, both finger and nail pectinate along the outer curve; with two setules inset close to the nail, and one long plumose seta proximally on the outer margin. In peraeopod 2 the 5th and 6th joints have the anterior margin strongly pectinate.

Peraeopod 3, 2nd joint almost circular, one setiform spine and five short stout ones on the lower part of the anterior margin, nine deep serrations posteriorly; 4th, 5th, and 6th joints increase rapidly in length and decrease in thickness, all furnished with long sensory spines some of which are remarkable for the extraordinary length of their apical filaments. Another variety of spine peculiar to this peraeopod is to be found on the posterior margins of the 5th and 6th joints, eight on the 5th and seven on the 6th, each inset with a small long-filamented spine; the shaft is laminar, and the apex of the spine is produced to nearly the length of the apical filament (Plate III, fig. 8). The branchial vesicle and incubatory lamella are small, the hairs on the latter very long.

Peraeopod 4, 2nd joint large, rounded, more produced posteriorly than in Peraeopod 3; two setiform and six short stout spines anteriorly, ten serrations posteriorly. 5th joint half as long again as the 4th; 6th much longer than the 5th; finger not quite one-third the length of the 6th. The spines on the 4th joint are the longest, those of the 6th the shortest and most numerous; the apical filaments of all the spines are very long, but do not reach the remarkable length of those of the 3rd peraeopod.

Peraeopod 5, as Chevreux describes it; 2nd joint longer than broad, six setiform and six short spines anteriorly, thirteen serrations posteriorly. The setae as in Peraeopod 4.

Pleopods. The rami in all three pleopods are about twice as long as the peduncles; inner ramus with seventeen joints, outer ramus with eighteen; five cleft spines on the inner ramus. The coupling-spines are set in a little hollow with two long ciliated hairs; apices recurved; the upper spine has two large recurved teeth on one side, five small ones on the other; lower spine with three large and six small.

Uropod 1. Peduncle considerably longer than the outer ramus; it carries a row of seven long spines on the inner margin, six on the outer margin, the distal portion of which is produced in a long curved process reaching more than half-way down over the outer ramus. Outer ramus with three short spines on the margin, one large strong spine and two small inset at the apex. The inner rami are both broken but are evidently longer and broader than the outer. *Uropod* 2, outer ramus twice as long as the peduncle, inner ramus nearly four times as long. The peduncle carries two long stout spines at the outer angle. Outer ramus narrow, outer margin with three long sensory spines, inner margin with ten or eleven short stout simple spines; one broad stout spine and two small ones at the apex. Inner ramus broad and laminar; apex acutely produced: inner margin edged with a thick row of about thirty short stout simple spines; outer margin with sixteen long sensory spines inset at regular intervals. *Uropod* 3, badly mutilated in both specimens. Inner ramus two and a-half times longer than the peduncle; it appears to have small spines on the outer margin, and plumose setae proximally on the inner margin; both rami broad and laminar.

Telson. In the *Huxley* specimens the cleft is longer than Chevreux figures it. A pair of long ciliated hairs are inset on either side of the cleft near the margin, and several small flat spines are scattered over the surface proximally; the apices each with a setule in the fork.

Distribution. Taken by the *Princesse Alice*, 10 July, 1901, 33° 59′ 30″ N.; 8° 12′ 45″ W.; trawl; 851 mètres; 2 females, 7 mm. long. Chevreux (**16**).

Taken by the *Huxley*, 25 August, 1906; 48° 7′ N.; 8° 13′ W.; Agassiz trawl; 240 fathoms; bottom deposit, fine sand.

FAM. **CALLIOPIIDAE.**

GEN. **Apherusa,** A. O. Walker.

Apherusa bispinosa (Sp. Bate).

(Stebbing (**41**), p. 305.)

STATION IV. 109 fms.; 44 specimens; 30 of these were ovigerous females, 6·5-3·75 mm. in length.

STATION X. 146 fms.; 3 specimens, 1 male and 2 females, the larger of which measured 7 mm.

These captures are interesting as being the first authenticated records of the occurrence of this species in the open sea far from land. The depth at Station X., 146 fathoms, is the greatest hitherto recorded. *A. bispinosa* is generally regarded as a purely littoral or sublittoral form, and is usually found living among the algae close inshore. Sars, however, mentions (**30**), p. 440, "another form or variety living in somewhat greater depths," agreeing in all essential details with the littoral form, but distinguished from it by the larger size, the comparatively larger and less pigmented eyes, and the lighter hue of the body. The *Huxley* specimens are of this latter type. They are more slender and more spinose than the shore form.

The 3rd joint of the palp of the *mandible* in full-grown specimens is subequal to the 2nd in length, not shorter, as given by Sars for the shore form.

The *antennae* are filiform and longer than in the shore animal; the joints of the flagella very attenuated. The flagellum of the superior antenna is furnished with two sensory filaments on each of the first four joints, and two on alternate joints to the 14th in the female and to the 20th in the large males. The inferior antennae in the female are a little longer than the body; half as long again in the male. Unfortunately all were broken; one female of 5 mm. length had the inferior antenna 5 mm. long, fifty-one joints in the flagellum; and a male 7 mm. long had sixty-three joints still remaining, the broken antenna measuring 7·5 mm.

The proportions of the joints of the *gnathopods* are as given by Sars, but the hand and finger differ (Pl. III, fig. 9). The hand is broader, as in *A. clevei* Sars, with the palm oblique and subequal to the hind margin in length; the palmar margin is microscopically serrulate, with the two specialized bristles characteristic of the family, inset on either side of the finger. The finger is much longer in proportion than in the littoral form with four serrations on the inner margin in Gnathopod 1, three in Gnathopod 2.

The postero-lateral corner in *pleon-segment* 1 is produced to a small acute recurved point, as mentioned by Sp. Bate (**3**), p. 250. In pleon-segment 3 the hind margin above the postero-lateral corner is divided into eight serrations in the large full-grown specimens, seven in the small but sexually mature animals, each serration with a setule inset; the upper tooth of the bidentate projection is very acutely produced.

All the margins of the *uropods* are microscopically pectinate, with the exception of the inner margin of the inner ramus of *Uropod* 3. The distal half of the margin of the *telson* is also pectinate; apex distinctly tridentate with two setules inset; two pairs of mobile sensory plumose hairs.

Previous records: By Chevreux as *Halirages bispinosus* (**9**), p. 304; as *A. bispinosa* (**14**), p. 70; and by Walker (**46**), pp. 158–9.

FAM. **PLEUSTIDAE.**

GEN. **Sympleustes**, Stebbing.

Three species of this genus were taken, two from deep water 412 fathoms; and one hitherto regarded as an Arctic or sub-Arctic form *S. glaber* from a much less depth, 75 fathoms.

Sympleustes latipes (M. Sars).

(Stebbing (**41**), p. 317.)

STATION XIII. Three specimens, immature, measuring respectively 7·5, 4·5, and 2·5 mm.

Taken once before in the Bay of Biscay by the *Caudan* Expedition (**6**), p. 645, in 1410 metres.

Sympleustes grandimanus (Chevreux).

STATION XIII. Five specimens, females, 3–7·5 mm. in length.

For synonymy and discussion of this species, see Sexton (**35**), pp. 857–64.

Sympleustes glaber (Boeck).

(Stebbing (**41**), p. 318.)

STATION II. One specimen, a small female with ova, 4·5 mm. in length.

This species has not been recorded before south of the Kattegat. The *accessory flagellum* of the superior antenna, as in other species of the genus, is quite rudimentary, 1-jointed tipped with two or three setae (cf. Sexton (**35**), pp. 853 and 859).

FAM. **PARAMPHITHOIDAE.**

GEN. **Epimeria**, A. Costa.

Epimeria parasitica, M. Sars.

(Stebbing (**41**), p. 321.)

STATION IX. Twenty specimens.
„ XII. Twenty-three specimens.

This species is here recorded for the first time with certainty out of Norway. It is there found living in a semi-parasitic state on the skin of *Holothuria tremula* (**31**), p. 131, and (**30**) p. 367; the *Huxley* specimens on the other hand were taken free swimming in considerable numbers, at two stations. They are larger than the Norwegian specimens, quite half of them measuring 13 mm. in length as compared with Sars's statement: "length of adult female scarcely exceeding 9 mm." They appear to live at greater depths than *E. cornigera.* The incubatory lamellae of the females were well developed, but no eggs were found in the pouches.

Epimeria cornigeria (Fabricius).

(Stebbing (**41**), p. 323.)

STATION II. Two specimens, 17·5 and 18 mm. respectively.
„ V. Sixty-three specimens, average length 20 mm; the largest measured 23·5 mm.; 11 were half-grown, and 5 small. No eggs remaining in the pouches.
„ IX. Two large specimens, mutilated.
„ XI. Three specimens, largest 17 mm.
„ XII. One specimen, 22 mm. in length.

Several previous records by Chevreux (**14**), p. 62, but only one or two specimens taken at a time.

FAM. **ATYLIDAE.**

GEN. **Nototropis**, A. Costa.

Nototropis vedlomensis (Bate and Westwood).

(Stebbing (**41**), p. 331.)

STATION IV. One specimen, a female, 8 mm. in length.

The method of capture was by swab and townet attached to the dredge working at the bottom, depth 109 fathoms. Chevreux's records are from shallow water; under the name of *Atylus vedlomensis* as fairly common on sandy bottoms in the Bay of Croisic, 4–10 m. (**9**), p. 304; and as *Paratylus vedlomensis* at Concarneau, 15–19 m. (**13**), p. 480.

FAM. **EUSIRIDAE.**

GEN. **Eusirus**, Kröyer.

Eusirus longipes, Boeck.

(Stebbing (**41**), p. 341.)

STATION IV. Three specimens, males, the largest 8 mm.

Previous records: Chevreux (**14**), pp. 65, 171, 172; and Walker (**46**), p. 160.

Eusirus biscayensis, Bonnier.

STATION XII. Seven specimens; 6 females, 12–13·5 mm. in length, and 1 male barely 13 mm. long, the first hitherto recorded.

This is the first record of the species since its discovery by the *Caudan* Expedition. The original description was taken from a mutilated specimen. I have, therefore, redescribed and figured certain of the anatomical details (**35**), pp. 865–9.

GEN. **Rhachotropis**, S. I. Smith.

Rhachotropis helleri (Boeck).

(Stebbing (**41**), p. 351; and Sexton (**35**), pp. 869–76.)

STATION XII. Twenty specimens; 1 male, 8·75 mm., and 19 females, 10–12·5 in length.

This species has been discussed and the fully developed animal figured in the above-mentioned paper.

Rhachotropis rostrata, Bonnier.

(Stebbing (**41**), p. 353; and Sexton (**35**), p. 869.

STATION XII. Four specimens, males, 9–10 mm. in length.

This is the first record of the species since its discovery by the *Caudan* Expedition.

FAM. **GAMMARIDAE.**

GEN. **Maera**, Leach.

Maera tenuimana (Bate).

(Stebbing (**41**), p. 436.)

STATION V. One specimen, a male, 9 mm. long.

Previous records: by Chevreux as *M. Batei* Norman and *M. multidentata* Bate (**9**), p. 307; and as *M. Batei* Norman (**14**), p. 83; and by Walker (**46**), p. 160.

FAM. **PHOTIDAE.**

GEN. **Leptocheirus**, Zaddach.

Leptocheirus pectinatus, Norman.

= **Leptocheirus dellavallei**, Stebbing.

(Sexton (**36**), pp. 576–585, Pl. XIX.)

STATION II. One specimen, an ovigerous female, measuring 4·5 mm.

For the discussion of this species and proofs of the identity of the two forms *pectinatus* and *dellavallei*, see the paper referred to above.

FAM. **JASSIDAE.**

GEN. **Jassa**, Leach.

[**Jassa falcata** (Montagu).

No specimens of this species were taken during the cruise, but it is necessary to include a note relating to it in order to explain my reasons for using the specific name *falcata*, in the discussion of *J. pusilla*, instead of *pulchella* as established by Stebbing (**41**), p. 654. The species is a difficult one owing to the different forms assumed by the male during development, this causing great confusion not only in the species itself, many of the stages having been described as distinct species, but also leading to further confusion with other species, *pusilla* in particular.

There would seem to be at least two well-marked forms of *falcata*. During immaturity it is absolutely impossible to distinguish between them, but as they grow they differentiate into either—a form with the flagella of the antennae swollen, some of the joints coalesced, the hand also swollen, and thumb broad; or a form with the antennae slender, joints distinct, hand and thumb slender. In both forms there appear to be *two* distinct kinds of adult males, besides the several markedly different stages during growth. The females are exactly alike, except that some have antennae like the first form, and others like the second. Whether these will prove to be seasonal variations, or whether they are really two distinct species, I have not as yet sufficient evidence to speak with certainty. Experiments in rearing them, commenced in 1909 in the Laboratory here, have only been successful to a certain point; some of the stages have moulted but the series is still far from complete.

For figures of the first form see Nebeski (**23**), and for the second Sars (**30**), pl. 212; for the females Spence Bate's *variegatus* (**3**), p. 439, belongs to the first, *pelagicus* (**3**), p. 447, to the second form.

Through Dr. Calman's kindness I was able last year to examine in the British Museum the type specimens of Montagu's *falcatus*, Leach's *pulchella*, and Spence Bate's *variegatus*.

The type specimen of the species was taken by Montagu more than a hundred years ago at Torcross on the Devonshire coast. It is marked 603 a, and is referred to in the old manuscript register under that number as having been taken at Torcross. In the "List of the Specimens of Crustacea in the collection of the British Museum," 1847, by Adam White, it is entered (p. 89) as "*Cerapus falcatus* a. Devon (Torcross). From the collection of Col. Montagu." The specimen bears a sufficient resemblance to Montagu's drawing (**22**) t. 5, f. 2, to suggest that it was the actual one from which the drawing was made. It measures 8 mm. from the tip of the rostrum to the tip of the telson, and is of the type referred to above as the second form. The flagellum of the superior antenna has eight or nine joints, the inferior five. The second sideplate is of the form characteristic of the species, the anterior margin only half the length of the posterior margin of the preceding sideplate. The finger of the second gnathopod has a rather prominent process developed on the inner margin; this process I have found of frequent occurrence in the larger males. The small spines which are found behind the thumb process on the hind margin of the hand are represented in Montagu's figure as another process. They are naturally much more prominent in the dried specimen than in spirit specimens, owing to the shrinkage.

There are seven specimens marked "*Podocerus pulchellus*, Devon," in the old manuscript register, and numbered 296 a—g, which appear to be Leach's types. These are exactly the same form as Montagu's. Two have lost both gnathopoda and antennae; of the others, one is a young male with the thumb half developed, and the remaining four are adult males, thumb well developed, process on inner margin of the finger small. The antennae of all were broken, excepting two superior antennae, which had about seven joints each.

The tube marked *Podocerus variegatus* in Spence Bate's collection, as Mr. Walker pointed out (**44**), p. 472, contains more than one species, several of the specimens belonging to *J. pusilla*. Among the *falcata* were two young males and two or three full-grown ovigerous females of the type referred to above as the "first form," and figured by Bate as *P. variegatus* (**3**), p. 439.]

Jassa pusilla, G. O. Sars.

(Stebbing (**41**), pp. 655 and 739.)

Station VII. One specimen, a large ovigerous female, 5·5 mm. long.
„ XIII. Three specimens, 2 males, 5 and 5·5 mm. respectively, and 1 female, 4·75 mm.

The separation of this species from *J. falcata* (Mont.) is rendered difficult not only by their great similarity, but by the enormous range of variation found in *falcata*, which at a first glance suggests the possibility of *pusilla* being only a young stage of that species. Walker was inclined to consider them identical; he says (**45**), p. 314: "If it be admitted that Amphipoda may become sexually mature before they have attained their final moult, I think these species [*pusillus* and *Herdmani*] can hardly be maintained"; and again (**44**), p. 473: "I am disposed to consider *P. Herdmani* and *P. pusillus* (Sars) as examples of arrested development and mere varieties of *P. falcatus*."

Sars and Stebbing, on the other hand, consider them distinct species, but, as several of the characters given by them for distinguishing the one from the other are those subject to developmental modifications, I have thought well to discuss the different points in detail.

The two most useful and constant distinguishing features will be found in the second gnathopod, viz. the second sideplate and the hand. The inferior margin of this sideplate in *pusilla* forms a continuous line with the margins of the 1st and 3rd sideplates, its anterior margin being as long as the posterior margin of the 1st. In *falcata*, on the contrary, the anterior margin is much shorter than the posterior margin of the preceding sideplate, only half the length in full-grown specimens, giving a curious and characteristic appearance to the animal, as if the head and 1st peraeon-segment were divided from the rest of the body (the coloration adding to the effect, the head of the 1st segment and sideplates being invariably darkly pigmented, and the 2nd segment and sideplates light with a few patches of pigment). This character is found even in the young in the incubatory pouch, though with them the anterior portion of the sideplate is rounded, whereas in the full-grown animal it is angular (see figs. 10 and 11 for comparison).

With regard to the second distinguishing feature, the hand, the adult male of *pusilla* has the apex of the thumb bifid; in the adult male of *falcata* it is entire; and an examination of many thousands of specimens of this latter species shows that in it the characteristic bifid form of *pusilla* is never met with.

General aspect. The body is more compressed in *pusilla*, the sideplates longer in proportion, and the peraeopods longer and more slender than in *falcata*. The cuticle is thinner, and the coloration also differs, as Sars noted when first describing the species (**29**), p. 112. In *falcata* the pigment is in dark definite bands or patches composed of stellate markings or of dots thickly crowded together, retaining its colour even after years in alcohol. The distribution of colour can be plainly seen in Montagu's specimen even now after a century. In *pusilla*, on the other hand, the pigment shows only as diffused indefinite transverse bands along the posterior margins of the peraeon-segments, and occasionally on the pleon-segments as well; spirit specimens retain very little of the colour.

Size. The difference in size is given by Sars (**29**), p. 112, as a specific character, but, as Walker pointed out, it cannot be used as such, for though *pusilla* is always small, never exceeding 5–6 mm., and *falcata* attains a length of 10–12 mm., yet specimens of the latter, both male and female, have been found sexually mature at 4 mm.

Sideplates (Pl. III, figs. 10 and 11). The *proportions* of the sideplates are generally given as specific characters, but they cannot be relied on as such, varying as they do with the age of the animal. Stebbing gives the *falcata* (**41**), p. 654, "3rd and 4th in ♂ considerably deeper than 2nd and 5th"; and for *pusilla* ♂ "5th nearly as deep as 4th." The sideplates in this latter species are always wider and much deeper in proportion to the body than in *falcata;* Sideplate 2 is rounded anteriorly, considerably expanded inferiorly, and twice as wide as deep (fig. 11); the 3rd and 4th the deepest, as wide as deep; the 5th almost as long as the 4th. In *falcata* the relative proportions alter with each stage of development, the only constant feature being the short anterior margin of Sideplate 2. In the young in the marsupium 2·25 mm. long, the inferior margins of Sideplates 1–5 are on the same level; in specimens 5–6 mm. in length, the 3rd, 4th, and 5th are on the same level, the 1st and 2nd shorter; at 7 mm. length, the 5th is slightly shorter than the 4th; while in large specimens 9 mm. and upwards, the 1st, 2nd, and 5th are very noticeably shorter than the 3rd and 4th.

Antennae. Here again the proportions of the joints of the peduncles, and the number of joints in the flagella vary with the growth of the animal, and cannot be employed in specific distinction. The antennae in *pusilla* are much more slender, and the primary and accessory flagella much longer in proportion than in *falcata*. The joints of the primary flagellum are long and cylindrical, and the accessory flagellum is longer in proportion to the 1st joint of the primary than in the other species, equalling half the length of the 1st joint in the adult animal. The last joint of the peduncle of the inferior antenna is fringed with long setae. Of the *Huxley* specimens the two males each have five joints in the superior and five in the inferior flagella, the accessory flagellum 2-jointed, 1st joint long, narrow, and cylindrical, and the apical joint almost rudimentary. The smaller of the two females has five joints in both superior and inferior flagella. Two of the antennae of the larger animal are broken, those remaining being the superior on the left side with seven joints, and the inferior on the right side with six. Sars (**30**), p. 596, gives five joints for the superior and four for the inferior.

I have been able to compare the *Huxley* specimens with some taken by Mr. Crawshay near the Eddystone. In these, the increase in the number of the joints with growth is plainly shown. One young male, 3·75 mm. long, with the thumb just commencing to appear on the hand of Gnath. 2, had four joints in the superior and four in the inferior flagellum; a larger specimen, 4·25 mm., with the thumb further developed, had five in the superior and four in the inferior; two large fully adult males, 5·5 mm., with the bifid apex to the thumb, also had five in the superior and four in the inferior. Of the females, the smallest ovigerous one, 3 mm., had four in the superior, three in the inferior; other young ovigerous females, 4 mm., had four joints in both superior and inferior. In the larger ones, unfortunately, the antennae were more or less broken; one had five joints in both inferior flagella; the largest, 6·5 mm. long, had six joints on the right and five on the left superior, five joints on the left inferior; another had six joints on the right superior, four in the right and three in the left inferior.

There are two forms of antennae in both sexes in *falcata*. In the one form the whole of the inferior antenna is swollen, and all the proximal joints of the flagellum coalesced, so that only three joints can be traced, the long, swollen, coalesced 1st joint, a small stout joint, and a rudimentary terminal one; the accessory flagellum is 1-jointed and swollen. Dense fascicles of plumose setae are developed on the long joint of the flagellum and on the distal portion of the last joint of the peduncle. In the other form the inferior antenna is more slender; the joints of the flagellum are distinct five or six in number, the number frequently different on one side from the other; no plumose setae are developed, but the curved sensory spines are stronger and more numerous; the accessory flagellum is 2-jointed and cylindrical. This form is certainly near *pusilla*, but can be easily distinguished from it; it is much more heavily built; the last joint of the peduncle lacks the fringe of long setae, being only sparsely setose; the joints of the flagellum are short and thick; and the sensory spines of the flagellum also show a marked difference, being short and thick, and curved like hooks, while in *pusilla* they are long and slender, and hardly curved at all. The fascicles of plumose setae cannot be used as a distinguishing character, seeing that it is only in certain stages of development that they occur in *falcata.*

Gnathopod 2. All the specimens taken by the *Huxley* are fully adult, the two males showing the characteristic development of the thumb. There are two forms of the hand in *falcata,* correlated with the two forms of antenna. In the first form the hand is broad and swollen, thumb broad and truncate at the apex. In the second form the hand long and slender, with fascicles of plumose setae along the palmar margin, and the thumb is long and narrow, tapering to a subacute point. But in all the stages of development in *falcata* the apical margin of the thumb is entire, with not the slightest tendency to the bifid apex of the adult *pusilla.* The young male of *pusilla* resembles the young male of the second form of *falcata* in the development of the thumb, cf. figures given by Sars (**30**), pl. 212, p. 2, ♂ ÷, and pl. 213, 1, p. 2, ♂ ÷.

Norman (**27**), p. 93, considers the *Podocerus variegatus* of Bate and Westwood (not Leach) to be the female of *pusilla,* but an examination of Bate's specimens in the British Museum has shown it to be the female of the first form of *falcata;* it has the broad, heavily-built, inferior antenna, the short stout joints in the flagellum, and the second sideplate and hand characteristic of this species.

The finger in *falcata* develops with age an angular projection on the inner margin, very noticeable in the full-grown male. The finger in *pusilla* shows no sign of it.

The *Peraeopods* afford another character for distinguishing the species. In *pusilla* they are much longer in proportion to the animal's size, and more slender, the 6th and 7th joints more elongate; 7th joint lightly curved, not falciform as in *falcata;* 6th joint lacking the stout sensory spines carried by the latter species.

Distribution. J. pusilla appears to be a deep-water form, there being no authentic record of its occurrence in a less depth than 20 fathoms. *J. falcata,* on the other hand, is a littoral or sub-littoral form, building its nests in the algae and hydroids on buoys, dock-piles and rocks near the shore.

J. pusilla has been recorded by :—

Sars (**29**), p. 112, as *Podocerus minutus,* and (**30**), p. 597, as *Podocerus pusillus,* from the south and west coasts of Norway, and as far north as Hammerfest, " clinging to hydroidae growing in depths varying from 20–100 fathoms."

Robertson (**28**), p. 27, records an amphipod as *P. minutus* taken amongst the algae on the timbers of Millport Pier, Cumbrae. I have not been able to trace his specimen, but I consider this record is open to doubt, and that in all probability he mistook a young stage of *falcata* for Sars's species.

Scott (**32**a), as *Podocerus pusillus*, from the Firth of Forth.

Walker (**45**), p. 314, as *P. pusillus*, off Port Erin, no depth stated.

Norman (**27**), p. 93, records under *Bruzeliella pusilla*, "two females taken at Falmouth in 1884," but as he identified these with the *Podocerus variegatus* of Bate and Westwood, they are therefore, as I have shown above, females of the "first form" of *falcata*.

Chevreux (**9**), p. 315, as *P. minutus* on *Maia*, dredged south-west of Belle Isle in 80–100 m.

To these records must be added:—

Thirteen specimens taken by Mr. Crawshay, in June, 1906, 20 m. south-west of the Eddystone, in 42 fathoms; 5 males, 8 ovigerous females; from sponge coating *Inachus dorsettensis;* and numerous specimens taken by Mr. Barnard, March–April, 1911, near the Eddystone, also from sponge coating *Inachus.*

The *Huxley* specimens were from very deep water; one specimen, a female, from $\frac{\cdot}{444}$ fathoms; and three specimens, 1 male and 2 females, from 412 fathoms, all full-grown animals.

FAM. **COROPHIIDAE.**

GEN. **Erichthonius**, Milne-Edwards.

Erichthonius brasiliensis (Dana).

(Stebbing (**41**), pp. 671 and 740.)

STATION XII. 246 fathoms; 1 specimen, an ovigerous female, 6 mm.

„ XIII. 412 fathoms; 2 specimens, females, 1 measuring 5 mm. in length, with eggs; the larger one 6 mm., with six young ones still remaining in the marsupium; length of young, 1·25 mm.

The greatest depth hitherto recorded for this species is given by Chevreux as 130 metres.

Previous records: by Chevreux (**14**), p. 108; and (**9**) pp. 289, 301, and 316, found on *Maia squinado*, trawled in 60–80 metres, as well as on algae growing on the bottom.

GEN. **Unciola**, Say.

Unciola planipes, Norman.

(Stebbing (**41**), p. 679.)

STATION IV. One specimen, an ovigerous female, 6·5 mm. long; taken in a tow-net attached to the dredge working at the bottom.

Recorded by Chevreux (**14**), p. 110, from 50–180 m.

GEN. **Siphonoecetes**, Kröyer.

Siphonoecetes colletti, Boeck.

(Stebbing (**41**), p. 683.)

STATION V. Three specimens, 2 males and 1 female of 6 mm. length.
„ IX. One specimen, a male, 5·5 mm.
„ XII. Four specimens, all males, 7·5–8 mm.

Previous records: by Bonnier (**5**), p. 347, as *S. typicus* Kröyer; and by Chevreux (**9**), p. 317, as *S. typicus*, and (**14**) p. 108 as *S. colletti*. The greatest depth recorded by Chevreux is 180 metres; by the *Huxley* at Station XII, 246 fathoms.

FAM. **PODOCERIDAE.**

GEN. **Laetmatophilus**, Bruzelius.

Laetmatophilus tuberculatus, Bruz.

1859. *Laetmatophilus tuberculatus* Bruzelius (**8**), p. 11, Taf. 1, fig. 1.
1862. *Cyrtophium tuberculatum* Spence Bate (**2**), p. 275, pl. 46, fig. 9.
1868. *Cyrtophium armatum* Norman (**25**), p. 285.
1876. *Laetmatophilus tuberculatus* Boeck (**4**), p. 663.
„ „ *spinosissimus* „ „ p. 665.
1894. „ *tuberculatus* Sars (**30**), p. 630, pl. 226.
„ „ *armatus* „ „ p. 632, pl. 227, fig. 1.
1895. „ „ Norman (**25**a), p. 493.
1906. „ *tuberculatus* Stebbing (**41**), p. 696.
„ „ *armatus* „ „ p. 697.

STATION XIII. One specimen, a male, 4·5 mm. long.

This species was established by Bruzelius in 1859. In 1868 Norman described a specimen from the Shetland Isles under the name of *Cyrtophium armatum*. He noted its resemblance to Bruzelius's species, but considered it sufficiently distinguished from it by the following characters: "much more strongly tuberculated; and the gnathopods of different structure, the first smaller, the second larger, the hand broader and the basos spined." Sars in 1894, although he described the two species as distinct because of the peculiar armature of the body in *armatus*, was inclined to consider this latter form as merely a deep-sea variety of *tuberculatus*, giving the range of distribution as 20–50 fathoms for *tuberculatus* and as 50–300 fathoms for *armatus*.

The specimen taken by the *Huxley* was a large male, 4·5 mm. in length, with the spinose armature even more accentuated than in the figure given by Sars for *armatus*. Through the kindness of Canon

Norman I have been able to compare it with a Norwegian specimen of *tuberculatus*, also a large male, 4 mm. in length, and as a result of this examination, I think there can be no question but that the two species are identical, *armatus* being the fully-developed animal.

As I have shown before (**35**), p. 849, the Amphipoda undergo considerable modification, even after reaching sexual maturity, the characters most noticeably affected being: the *antennae*, the number of the joints in the flagella increasing and the proportions of the peduncle joints altering with growth; the *second gnathopods*, the hand of which increases in size and alters in shape to a far greater degree than the hand of the first gnathopod; and the chitinous *cuticle* of the body, spinose processes developing, and growing longer, more acute, and more numerous with age. An example of the development of the processes of the cuticle, bearing on the present case, will be found in the paper above referred to (p. 870), where the stages of growth in one species were traced; the cuticle in the very young animal was perfectly smooth, the spinose processes commencing as slight swellings, and developing at maturity into rounded upstanding tubercles, only assuming their characteristic shape with the further development of the animal.

It will be seen that the distinguishing points given by Norman to differentiate his species from Bruzelius's are precisely those which would be influenced by age and growth. With regard to the first point—the stronger armature of the body—I found on examining the specimen of *tuberculatus* that the tubercles are of exactly the same number and arranged in exactly the same manner as the spine-processes of *armatus*, those on the last peraeon-segment and the first two pleon-segments being larger than the others, as Norman noted in *armatus*. The 1st segment has two, one behind the other; the 2nd segment three, one median in front of the transverse furrow, followed by two side by side; the remaining peraeon-segments and two first pleon-segments each have a swelling in front of the furrow, with two tubercles side by side behind. This agrees with Bruzelius's description;* he states (p. 11) that the 2nd, 3rd, 4th, and 5th segments carry three tubercles each, one in front of the other two; but, except on the 2nd segment, as described above, the anterior tubercle is not distinctly defined. Boeck's account is the same. Sars, however,

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* "Tvärs öfver dess ryggsida går en intryckning, och på ryggen bär det två små knölar. Det andra, tredje, fjerde och femte segmentet hafva, liksom det första, en tvärs öfver ryggen gående intryckning, och tre små knölar, den ena framför de två andra. . . . Det sjette och sjunde segmentet, som äro hopvuxna med hvarandra, hafva på ryggen två par knölar. Deras epimerer äro åtskilda. De tre första abdominal-segmenterna äro korta, och af dessa bära de två första två knölar på ryggen." Bruz. (p. 11).

appears to have had younger specimens to deal with than either Bruzelius or Boeck. His description (p. 630) is as follows: "1st segment with two succeeding dorsal tubercles; 2nd segment with a slight tubercle in front of the sulcus; the succeeding segments scarcely tubercular, but having the dorsal contour somewhat rugged."

There is little to add to the full descriptions of the species, already referred to. In both specimens the 6th and 7th peraeon-segments are coalesced; the finger of Gnathopod 1 is bifid at the apex and furnished on both margins with small setae; the finger of Gnathopod 2 has the marginal setae as in Gnath. 1, and two specialized bristles at the articulation, one simple and one plumose; the pleopods have no cleft spines; the coupling-spines are of the same construction as those figured by Stebbing for *L. purus* (**39**), pl. 132, six in the older animal (*armatus*), and rami 9-jointed, five coupling-spines in *tuberculatus* and rami 8-jointed.

The species has only been recorded hitherto from the coast of Norway and the Shetland Islands.

TRIBE CYAMIDEA. STEBBING (42), p. 464.

FAM. CAPRELLIDAE.

GEN. Pseudoprotella, Mayer.

Pseudoprotella phasma (Montagu).

(For synonymy and distribution, see Mayer (**19**), p. 29; (**20**), p. 19; and (**21**), p. 37.)

STATION XIII. Five specimens; 1 female measuring 11 mm., and 4 males, 10–15 mm. in length.

This species has been frequently taken on the oceanic coast of France, but the depth at which the *Huxley* specimens were trawled, 412 fathoms, appears to be the greatest yet recorded for its occurrence.

GEN. Pariambus, Stebbing (39), p. 1268.

Pariambus typicus (Kröyer).

(See Mayer, *Podalirius typicus* (**19**), p. 75; (**20**), p. 92; and (**21**), p. 63.)

STATION V. One specimen, a female with ova, measuring 3 mm., depth 109 fathoms.

Recorded from the Bay of Biscay by Chevreux, found on *Maia*, *Asterias*, etc.

TRIBE PHRONIMIDEA, STEBBING (**42**), p. 473.

FAM. **VIBILIIDAE.**

GEN. **Vibilia,** Milne Edwards.

Vibilia armata, Bovallius.

STATION VIII. Surface: 1 specimen, a female, measuring 10 mm.

Recorded before from the Bay of Biscay by Stebbing (**40**), p. 31.

FAM. **HYPERIIDAE.**

GEN. **Hyperia Latreille.**

Hyperia galba (Montagu).

STATION X. Seven specimens. The largest, a female carrying eggs, measured 23 mm.; of the others 2 were ovigerous females, 16 mm.; 1 a large male, 14 mm.; the three remaining, 1 male and 2 females, were immature, the smallest 5·5 mm. in length.

GEN. **Euthemisto,** Bovallius.

Euthemisto compressa (Goës).

All the specimens were acutely carinate dorsally, but in many (cf. also the specimen described by Stebbing (**40**), p. 38) the projecting dorsal teeth were not developed.

STATION IV. Ten specimens; 7 ovigerous females, the largest 5·5 mm. long, 1 young male, and 2 adult males, 5 mm.

STATION X. 42 specimens; 36 females and 6 males; the largest measured 7 mm.

I agree with Tattersall (**43**), p. 36, in considering *E. gracilipes* Norman as a young stage of *E. compressa*. Both forms have been recorded from the Western Stations by the Int. Council Investigations.

Euthemisto bispinosa (Boeck).

STATION X. Two specimens; ovigerous females, the larger 8 mm. in length; the dorsal spinose processes not much developed in either.

With this record it will be interesting to compare a haul made on May 16, 1909, by the *Huxley*, not far from Station X., in 47° 47′ N., 7° 44′ W., when the tow-nets were choked with an immense swarm of large specimens of this species. In this haul the females outnumbered the males by three to one. They were much larger on the average, the largest females in the sample examined by me measuring 31 mm. from rostrum to tip of uropods, while the largest males were only 18–19 mm. There were only a very few small ones, and of these the smallest was 9 mm. long. The two specimens from Station X agree in all structural details with these large ones, the only difference being the degree of development of the dorsal processes on the two last peraeon-segments and the two first pleon-segments, which in the larger specimens are greatly produced. The variation in size is not confined to this species. Stebbing has noted a similar case in *Parathemisto oblivia* (**40**), p. 37; and it was one of Norman's principal reasons for separating *E. gracilipes* from *E. compressa* (**27**), p. 54.

The pouches of all the females (May, 1909) were greatly distended with either young just hatched or with eggs nearly hatched. Owing to the extrusion of most of the contents of the pouches it was impossible to estimate the number carried by any one female. It must, however, be considerable; one female, 20 mm. long, had 71 young, each measuring 1·5 mm., still remaining in the pouch. In view of Sars's remarks as to the peculiar armature of the 6th joint of the 3rd peraeopod forming a constant specific character in the very young specimen equally with the adult, it may be worth while to note here that in the larval *E. bispinosa* all five peraeopods are practically subequal in length, and the 3rd is not distinguishable from the others. The proportions of the joints are quite different also, e.g. the finger of the 3rd peraeopod is nearly as long as the preceding joint.

FAM. **LYCAEIDAE.**

GEN. **Brachyscelus,** Bate.

Brachyscelus crusculum, Sp. Bate.

STATION X.	One specimen, an ovigerous female, 8·5 mm. long.
,, XII.	One specimen, a large ovigerous female, 17·5 mm.

For the discussion as to the identity of *Thamyris mediterranea* Claus with *Brachyscelus crusculum* Sp. Bate, see Norman (**26**), p. 134; Tattersall (**43**), p. 26; and Stebbing (**40**), p. 41. I have not seen any males of this species, but an examination of the two female specimens mentioned above would seem to support Norman's view that *T. mediterranea* is only a young stage of *B. crusculum*. In the small female the 3rd uropods reach to nearly the level of the telson, as in Senna's figure 10 (**33**), p. 8, while in the large female the telson extends considerably beyond them, as in Chevreux's figure 8 (**11**), p. 74.

LIST OF SPECIES, AND THE STATIONS AT WHICH THEY OCCURRED.

STATION No.	II	IV	V	VII	VIII	IX	X	XI	XII	XIII
Latitude, N.	48° 24′	47° 48′	47° 48′	47° 36′	47° 30′	48° 7′	48° 7′	48° 10′	48° 7½′	48° 7½′
Longitude, W.	6° 28′	7° 25′	7° 46′	7° 31′	7° 31′	8° 13′	8° 13′	8° 11′	8° 13′	8° 13′
Depth in fathoms	75	109	109	444	Surface.	240	146	146	246	
Bottom deposit		Coarse sand and broken shells.	Coarse sand and shells.			Fine sand.	Fine sand.	Fine sand.	Fine sand.	Sand, mud, and hard ground.
Trischizostoma nicæense	...	...	...	...	...	...	...	...	1. f.	...
Ichnopus spinicornis	...	...	...	...	...	...	6. f.	...	...	...
Tmetonyx similis	1. f.	...	...	...	...	...	...	...	...	...
Tryphosites alleni n. sp.	...	...	...	...	...	...	...	...	2. f.	...
Proboloides gregarius	...	...	...	...	...	...	...	...	...	1. f.
Cressa dubia	...	...	...	1. m.	...	...	...	...	...	...
Stenothoe richardi	..	...	...	...	...	...	...	...	...	1. m.
Iphimedia obesa	...	...	1. f.	...	...	...	...	...	...	...
Syrrhoites walkeri	...	...	...	...	...	...	...	...	1. f.	...
Syrrhoe affinis	..	...	...	...	...	2. f.	...	...	...	...
Apherusa bispinosa	...	44 m. and f.	...	...	...	...	3. 1 m.	...	...	...
Sympleustes latipes	...	...	...	...	...	...	...	...	...	3.
Sympleustes grandimanus	...	..	...	...	...	...	...	...	...	5. f.
Sympleustes glaber	1. f.	...	...	...	...	...	...	...	...	...
Epimeria parasitica	...	...	...	...	...	20	...	...	23.	...
Epimeria cornigera	2.	...	63	...	...	2	...	3	1.	...
Nototropis vedlomensis	...	1. f.	...	...	...	...	...	...	...	...
Eusirus longipes	...	3 m.	...	...	...	...	...	...	...	...
Eusirus biscayensis	...	...	...	...	...	...	...	...	7. 1 m.	...
Rhachotropis helleri	..	...	...	...	...	...	...	...	20. 1 m.	...
Rhachotropis rostrata	...	...	...	...	...	...	...	...	4. m.	...
Maera tenuimana	...	...	1 m.	...	...	...	...	...	...	...
Leptocheirus pectinatus	1. f.	...	...	...	...	...	...	...	...	...
Jassa pusilla	...	...	...	1. f.	...	...	...	...	...	3. 2 m.
Erichthonius brasiliensis	...	...	...	...	...	...	...	...	1. f.	2. f.
Unciola planipes	...	1. f.	...	...	...	...	...	...	...	...
Siphonoecetes colletti	...	...	3. 2 m.	...	...	1. m.	...	...	4. m.	...
Laetmatophilus tuberculatus	...	...	...	...	...	...	...	...	...	1. m.
Pseudoprotella phasma	...	...	...	...	...	...	...	...	...	5. 4 m.
Pariambus typicus	...	...	1. f.	...	...	...	...	...	...	...
Vibilia armata	...	...	...	...	1. f.	...	...	...	...	...
Hyperia galba	...	...	...	...	...	...	7. 2 m.	...	...	...
Euthemisto compressa	...	10. 3m.	...	...	...	...	42. 6 m.	...	...	...
Euthemisto bispinosa	...	...	...	...	...	...	2. f.	...	...	...
Brachyscelus crusculum	...	...	...	...	...	...	1. f.	...	1. f.	...

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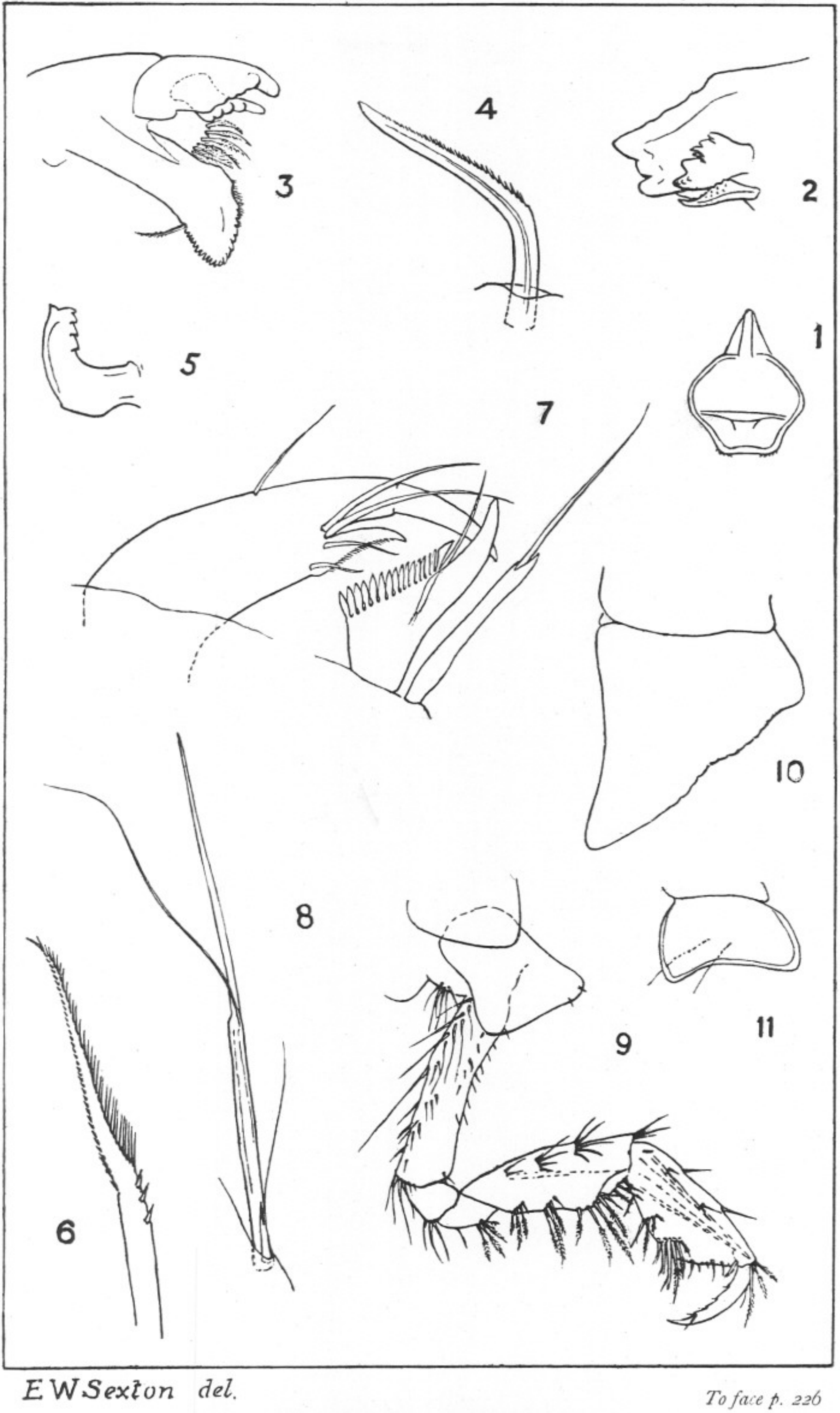
Journ. Mar. Biol. Assoc. Vol IX *Plate III*



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To face p. 226

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EXPLANATION OF PLATE III.

Fig.	1.	Upper Lip	*Syrrhoe affinis* Chevreux	x 42
,,	2.	Cutting plate, accessory plate, and spine. Right mandible	*Syrrhoe affinis* Chevreux	x145
,,	3.	Left mandible	,, ,, ,,	x 75
,,	4.	Curved spine, inner plate, Maxillipeds	,, ,, ,,	x435
,,	5.	Coupling-spine ,, ,, ,,	,, ,, ,,	x435
,,	6.	Bristle from joint 5, gnathopod 1	,, ,, ,,	x380
,,	7.	Finger and palmar spine, upper surface, gnathopod 2	*Syrrhoe affinis* Chevreux	x380
,,	8.	Sensory spines from distal end of joint 6, peraeopod 3	*Syrrhoe affinis* Chevreux	x265
,,	9.	Gnathopod 1, ovigerous female	*Apherusa bispinosa* (Bate)	x 75
,,	10.	Sideplate 2, adult male, 11 mm. long	*Jassa falcata* (Montagu)	x 42
,,	11.	Sideplate 2, adult male, 5·5 mm. long	*Jassa pusilla* (Sars)	x 42

On Some Colour Variations and Adaptations in *Actiniae*.

By

Chas. L. Walton.

THE significance of the colouration of various *Actiniaria* has been of much interest to me for some time, and particularly the extreme variability displayed by certain of the most abundant species. Descriptions of colouration and coloured plates help but little toward elucidating these problems, the examination of numbers of the animals living in their natural environment being absolutely essential before any conclusions can be drawn.

Taken as a whole, the colouration of *Actinians* seems to come under the following heads:—

I. Warning.
II. Aggressive.
III. Protective.
IV. Colours with some special physiological significance.

Examination has shown that these leading features are not necessarily confined to a species, or even to an individual, which may combine them in varying degrees, though one is usually partially or wholly dominant, this being chiefly governed by the environment. In other words, I believe that the colouration of a species or individual may be adaptable to more than one end; that local circumstances and environment govern this, and that those species which become most effectually adapted to these environmental circumstances will be everywhere found to be the most abundant.

Naturally this adaptation is not confined to colouration, but is observable in many other details, some of which will be mentioned, but the colouration is the main factor to which I wish to draw attention.

I have assembled a considerable mass of data, from which I shall here detail a selection of what I deem the most suggestive and instructive.

Actinia equina, Linn. This is quite the most abundant British species, and is extremely variable in colour; shades of red, brown, green, etc., alone or variously combined, are all abundant; several of the varieties are so well marked as to have been considered distinct species at various times.

While working at Aberystwyth, in Wales, I was struck, while collecting, by the fact that light had a great deal to do with the distribution of the variously coloured forms, those from exposed positions being dark red, etc., while those from under stones, or seaweed, or from caves, were mostly of light shades, or green. This has also been recorded for another member of the genus, *Actinia tenebrosa*, Farquhar, from New Zealand. In describing this species the author says: "This is the southern representative of the European species *A. equina*," etc. "This species is a good example of the effects of light on the colours of animals. Full-grown individuals, in situations well exposed to the rays of the sun, have the column greenish, or brownish black, and the disk and tentacles dusky crimson, while those on the under side of overhanging stones are reddish brown or crimson, the depth of colour varying according to the amount of light that reaches them. Specimens on the vertical sides of rocks (their favourite habitat) often have a patch of reddish brown on the side turned away from the light. I found a specimen under a large stone which had evidently never been in the light: the whole animal was yellowish white with a slightly greenish tinge."

No remarks are made as to any variation in the size, number, or colour of the acrorhagi, or "marginal spherules."

Investigation of Aberystwyth specimens of *A. equina* showed that a correlation exists between environment and these organs, which are as a rule of a bright blue. M. Haime (quoted by Gosse) gives the following table of number of spherules:—

18 if 5th cycle of tentacles not developed.
24 if 5 or 5½ cycles are developed.
48 if 6 cycles are developed.

My observations on some hundreds of specimens prove this to be correct if an average be taken—in effect there are considerable differences. Those individuals which are of dark shades and occur in exposed positions, tend to possess more acrorhagi than those which live in sheltered situations and which are of paler shades. In these last also the acrorhagi are fewer, smaller, and of a pale blue, whilst in the darker specimens the acrorhagi are larger, of a much deeper blue, and often irregular (bilobed, etc.) in form. The colour of the acrorhagi (lighter and darker) is of course due to the same circumstances that cause the colour differences in the rest of the individual; but the variation in size is not necessarily due to this cause, and in all probability results from the different environment. It seems probable that these organs are defensive (possibly offensive), and they certainly contain many nematocysts, and their colour may be interpreted as "warning." These organs are not plainly visible during complete expansion, and not at all during retraction; but when the anemone is startled (if the blue basal line be touched, etc.) the tentacles are partially withdrawn and the acrorhagi are then exposed and show very plainly. A good deal has been written respecting these organs, and they have been referred to as eyes, special sense organs, etc.

Messrs. G. Y. and A. F. Dixon in treating of this species, besides mentioning irregularities of form and colour, say: "Each spherule contains a prolongation of the general body cavity, and can consequently be dilated at will," and they relate that a specimen dilated greatly, and brought the spherules into contact with the glass of the aquarium. When the organs contracted and withdrew, portions remained forming conspicuous blue spots. Examination of these showed numerous spindle-shaped cells, as described by Hollard (Ann. de Sci. Nat. Zool., 3 ser., vol. xv., p. 272), and they conclude that the anemone had evidently engaged in an attempt to sting the glass front.

I have never been able to demonstrate the discharge of nematocysts from these organs, but have on more than one occasion observed great distention of the acrorhagi, so that the increased weight caused the anemone to bend over on one side, and so bring the projecting organs into contact with objects which happened to be in close proximity.

The pale specimens with few acrorhagi are frequently as large as those darker ones with many, so that the increase must be either due to more light, or, owing to inhabiting more exposed positions, there is greater need of protection by increased batteries of nematocysts.

Sagartia miniata (Gosse) is under most conditions to be classed amongst species whose colouration is of a "warning" nature, and is amply provided with acontia which are very freely emitted. As a rule they form very conspicuous objects on the sides of rocky pools. I have examined hundreds of specimens from the North Sea, which though showing many minor variations were all of the same type even when from a depth of 40 to 47 fathoms, where the bottom was black mud (see *Actiniae* of the s.s. *Huxley*, 1907); but Mr. L. R. Crawshay showed me living specimens from the Bay of Biscay deep water in which the colouration was dull, the scarlet being absent, probably owing to lack of light. This variety entirely lacks the bright colours of the typical form. I recently found a specimen which harmonised with its surroundings in a remarkable manner. It was affixed to the bottom of a rock pool at East Pentire, Newquay, Cornwall. This pool was full of a growth of dark *Algae*, with which colonies of red species showed as thin, irregular, red streaks and lines. The *S. miniata* was very large, and its outline most irregular; the scarlet-cored outer row of tentacles so exactly resembled the red *Algae*, and the rest of the animal the dark *Algae*, as to render it most difficult of detection. I watched for some time and observed an amphipod deliberately swim into the scarlet tentacles, doubtless deceived by their resemblance to the *Algae*.

Cereus pedunculatus (*Sagartia bellis*). I had long considered that many of the numerous varieties were to be interpreted as aggressive, but a careful study of pools at Newquay, etc., brought out additional and interesting cases of adaptations.

In pools similar to that described above, a variety of a dusky umber hue was abundant, with frequently streaks and shades of red upon the inner tentacles and disk. This type predominated in those pools where the *Algae* were dark, with red species intermingled. When fully expanded the anemones resembled the dark weeds; when alarmed and partially contracted the red showed up, and the animals were still in harmony with their surroundings; if further irritated, the edges of the salver-shaped margin were folded over and the anemones then formed bluish purple patches against a dark background: possibly a warning colouration. A second variant found in pools only a few yards from those just mentioned was lighter in colour, more variegated, and had the tentacles merely tinged with red, or not at all. This type was found in pools where "corallines" were the main growth, and when these specimens closed, their columns were of a pink shade (seldom purple), and thus in accordance with the environment instead of in contrast, as in the last examples.

On muddy shores the summit is dark, as I have observed at Plymouth, etc. These examples could be backed by many more from varying localities. In all, the complex colouring of the disk and tentacles approximates to the colour scheme of the pool, or portion of pool in which the individual has its habitat. I quote from my notes made on the rocks at Polzeath, near Padstow, Cornwall: "*C. pedunculatus* is fairly abundant on this (the Pentire) side of the bay, and I have examined a considerable number. The rocks hereabouts are reddish or greenish, and the pools are often coated with pink *Algae*. In the first I examined a dull red specimen was expanded, and just the shade of numerous tufts of the brown seaweeds growing around it; when closed the margin was of a pink hue, exactly that of the rock around it. Near by were others growing amidst pink corallines, the tips of which were dead and white; these were of a dull pink, freckled all over with white, and thus so resembled the corallines as to quite deceive me at first.

"Many were expanded at the mouth of cracks and crevices, into which they retired when touched; and most of these had the summit covered with fragments of shell, etc., attached to the suckers of the upper portion of the column, so that when contracted there remained no sign of the anemone. I ascertained that in these the colour of the under side of the waved margin was not in agreement with the environment.

"Throughout numerous pools in ever-changing conditions, this species in each case varied to suit the colour scheme, importing shades of red or yellow, or both, in threads and streaks, and so on through innumerable variations."

I have frequently had to resort to feeling before I could be sure whether what I saw was a tuft of some seaweed or a specimen of *C. pedunculatus*.

That this resemblance is of protective value is highly probable, but from numerous observations, both under natural conditions and in aquaria, of small *Crustacea* mistaking the anemones for *Algae* and so being caught and devoured, I consider it to be also, if not even predominantly, aggressive.

Specimens living under stones are usually of small size, and when so situated that they can receive even a modicum of light are in colour merely pale editions of the prevailing local varieties; but when, as is frequently the case, specimens are obtained from beneath several layers of stones and weeds and thus have lived in darkness, the colours are usually light shades of chrome-yellow, together with crimson and scarlet, generally in lines and streaks, the columns as a rule colourless. A certain proportion of these shades frequently forms some portion of the mixed colouration of the surface forms, and the curious predominance in cases where adaptive and selective conditions are in abeyance may point to a form originally so coloured.

Gephyra dohrnii, von Koch, I consider to be a true instance of protective resemblance. At the Marine Biological Laboratory, Plymouth, I recently examined several specimens living upon *Eunicella cavolini*, von Koch. An adult exactly agreed with the general tone of the *Eunicella*, but a smaller and younger one did not accord so well, being paler, and when expanded showed a number of irregular opaque white streaks upon the disk and tentacles. In the adults there were merely a few specks in the area of the mouth. This may also point to an ancestry not resembling the *Eunicella* in colour, or at any rate striped in the usual *Sagartian* style? That *Eunicella* is in a great measure protected from fish attacks finds support in the colouration of specimens of *Tritonea plebeia* and *Ovula patula* that dwell thereon.

A. C. Haddon, in his account of *Gephyra* from the Irish coast, figures three-colour variants, all of which occurred upon *Tubularia*. Fig 3, pl. xxxi., shows a "cherry-coloured" variety, which must have been in close accord with the "polyps" of the *Tubularia;* the others are much lighter and more resemble the forms from *Eunicella*. Andres figures a yellow *Gorgonid*, and the anemone whitish tinged with yellow, but his description gives "Colonna carnicina."

Sagartia undata (*S. troglodytes*). Although fairly well acquainted with several varieties, I regret that since I became interested in this subject I have not met with it in any abundance, and hence cannot say much regarding this most variable species. Gosse says (p. 92): "In the shallow pools that floor the largest caves at St. Catherine's, Tenby, the varieties *scolapacina* and *aurora* spread their pretty blossom faces at the bottom of the clear water. And yet it is not easy to discover them even when scores are thus exposed, for the mottled colouring of the disk and tentacles is so like that of the sand and mud of the pools that even a practised eye may overlook them without the closest searching." Others with orange disk, or tentacles, are evidently warningly coloured. Gosse (p. 91) gives "variety *hesperus*. Wholly pure white, gradually acquiring colour in a confinement of some months" (Lundy, W. Brodrick in litt.).

Tealia coriacea (*crassicornis*). Mr. F. Slade, of Horniman's Museum, in a letter to my friend Dr. Fleure, noted that specimens of *Tealia coriacea* (*crassicornis*) sent from Aberystwyth were better coloured and altogether better animals than those from the chalk of Sussex, and this I have since seen for myself. Gosse (p. 211) says of deep water specimens, "all colour lost in a semi-pellucid dusky grey . . . and specimens usually very large." I have examined large numbers of this deep-water form. H. N. Moseley describes from the Severn estuary, near Aust and New Passages, large numbers of *Actinia*, and *Tealia* (near Weston-super-Mare, very common), the colours of all dull, especially *Actinia*; which were dirty white or pale olive, and the *Tealia* transparent green. Near Aust were found a few *Tealia* vivid red, nearly as bright as marine specimens. "They were attached to a rocky channel, and when the tide fell a constant stream of water came from a large pool above in which the mud settled and clear water flowed off."

Anemonia sulcata is a decidedly puzzling species. Showing considerable variation, there are two varieties which are predominant:

the green with purple-tipped tentacles, and the brown and grey. Both these are most abundant, frequently inhabiting the same pool. The species is provided with very powerful sting cells (I have myself been well "nettled" when handling large specimens) and is evidently sufficiently protected from fish attacks, or at any rate to a considerable extent. Both varieties when under water present a decided resemblance to masses of *Algae*, and doubtless obtain much food in the form of deluded *Crustacea*, and I am inclined to consider the colouration as partially aggressive, with secondary warning colours in the purple tips of the tentacles, and the occasional scarlet area on the lower portion of those organs. It is to be noted that these characters usually only accompany the green variety named *smaragdina* by Gosse. The scarlet area on the tentacles is mentioned by Gosse (p. 162) as occurring at Herm, and I have seen it in varying degrees on several occasions. I will give one instance from my notes. Near low-water level at Polzeath (N. Cornwall) I came across two enormous individuals attached side by side. The columns were rich purple-brown, the tentacles some four or five inches in length, a most vivid green, and purple-tipped, while the bases of the tentacles resembled flame-coloured silk. I touched them with my finger, knowing that irritation usually produces an increase of brilliance in this species; the tentacles at once bent inward, thus exposing the lustrous, glowing areas to plainer view. F. G. A. Stuckey in his *Review of New Zealand Actiniara* says of *Anemonia olivacea*, Hutton: "This species is found in rock pools, often inhabiting quite small pot holes on the upper part of the rocks. Its colour is strongly protective." I have examined numbers on the west coast of the N. Island, and consider that this also is a case of "aggressive" colouration, as it greatly resembles tufts of green *Algae*.

I had frequently noted in many *Sagartids* that in some specimens the acontia would be emitted on the slightest provocation, while in others they were only protruded after severe irritation. I now regard this as largely due to varying environmental conditions. Those individuals which have been exposed to constant friction only emit the acontia after considerable irritation, whilst those which have been living in quiet and undisturbed surroundings emit the acontia with freedom and at the least touch. My reference to constant friction applies to individuals which, living within tide marks, are exposed to constant boil of surf, causing abrasion by shingle, etc., or constantly brushed by *Algae*.

This control of acontia is closely connected with and analogous to the control of nematocysts, etc., referred to in a former paper ("Notes on the Habits of some Sea Anemones," by H. J. Fleure and C. L. Walton), and is the result of differentiating reactions and a persistent inhibitory stimulus due to the constant repetition of such causes as induced the same, and which alone probably prevent its lapsing. We have already shown that such impressions are evanescent, and it would appear that the whole tissues of these animals are in what may perhaps be termed a more or less plastic state, and hence (at least in many forms) varying environmental conditions readily affect them, and adaptations rapidly result. Hence the abundance of very variable species.

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Notes on various British Anthozoa.

By
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I. **Sagartia ornata** (Holdsworth).

THIS species is described and figured by Gosse in his *Actinologia Britannica*, quoting its original discoverer Holdsworth, who obtained a number of specimens at the entrance of Dartmouth harbour from among the roots of *Laminaria*. In Section III of the Appendix, Gosse (p. 355) adds: "I have taken this at Torquay. It has also been found at Mizen Head, and sent me from Banff. The markings are true to the description, and leave no doubt of its distinctness as a species."

I recently (July, 1910) obtained specimens at Aberystwyth, attached to a fixed stone at the bottom of a deep pool near low-water mark on the reefs below the University. Dr. Fleure recognised them as having occurred some ten years previously in crevices on the reefs below the Castle; that colony, however, disappeared, this being the first subsequent rediscovery. The colony consisted of an adult, on either side of which was situated a young individual, and from the irregular form and development of these I consider them to have recently originated by fission from the base of the large specimen.

The adult, although agreeing in the main with the plate and descriptions of Holdsworth and Gosse, showed some variation, chiefly in lack of distinctness and definition of the colouration, which had a somewhat blurred appearance.

Size when expanded: height of column, 5 mm.; diameter, 10 mm. Outline of base irregular. Column—smooth, with suckers on the upper portion. Faintly grooved longitudinally by the insertion of the mesenteries. Disk: convex during expansion, smooth. Mouth of fair size, raised, tumid, the throat somewhat ribbed.

Tentacles fairly numerous (about eighty-six), rather stout, tapering to the tip, held flexed outwards during expansion. Acontia fairly freely emitted.

Colours: Column, flesh colour, the suckers pale. Disk, semi-transparent umber, with a light yellowish area around the mouth; the yellow rays irregular; a cycle of twelve white irregularly shaped (not cordate) spots on the disk between the yellow rays. Gonidial radii white. Throat pink.

Tentacles, light umber, with three white or yellowish rings; the area between the second and third rings rich dark umber Slight dark lateral longitudinal streaks near the tentacle bases.

II. **Bunodes thallia**, Gosse.

I have been so fortunate as to discover a considerable number of this rare species, which varies in an interesting manner. It was originally described by Gosse, who obtained a colony at Lydstep, and he mentions four specimens subsequently discovered near Ilfracombe. G. Y. and A. F. Dixon have described a variety with white tentacles from the Ore Stone, near Torquay, and I have since taken both varieties in the neighbourhood of Padstow and Port Isaac, and a few small examples near Newquay. A few years ago I searched the original Lydstep locality in the hope of rediscovering this species, but without success, nor did I find it anywhere in that district. Gosse (p. 196) says: "About a dozen individuals of different sizes were associated in the dark angles and pools of a little insular rock exposed at spring-tide, that lies just off the cove called the Drock, near Lidstep. They were not troglodyte in habit, but adherent to the open rock, and therefore easily detached. The species is social; clustering together in groups, mutually pressing each other's sides." The Newquay specimens were small, typical in form and colouration, and were adhering to the under surfaces of stones at St. Columb Porth. Polzeath, between Padstow and Port Isaac, is the locality where I have found most specimens. A number of small specimens occurred attached to the lower side of stones which were more or less fixed in pools on the reefs. The greater number, many of considerable size, were found in situations very similar to those mentioned by Gosse; shallow pools, or low detached rocks surrounded by fine sand, which also lined the floor of all the pools and covered the anemones with the exception of the tentacles. So exactly did the grey and olive frecklings of these organs resemble the sand and byssal threads of the *Mytilus* colonies which abounded there, that only prolonged scrutiny and even feeling with the hands enabled me to ascertain the numbers present. The summit of the column was almost invariably covered with fragments of shell and sand attached by the suckers on prominent warts; and even when exposed the general hue of the column greatly resembled the olive-coloured algæ growing in the pools. All these specimens were normally coloured, the chief variation being the warts. In those individuals which lived affixed beneath stones the warts were frequently by no means strongly developed, and the whole column lighter in colour (in one instance dirty white). Those living exposed in the shallow pools possessed very conspicuous warts, especially toward the summit, where they were crowded, prominent, or even somewhat clavate.

An isolated colony of about a dozen specimens was discovered in Port Quin Bay, nearer Port Isaac. They were of the variety described by G. Y. and A. F. Dixon, from Torquay. Living in a long crack between two bare, rounded rocks in a pool about half-way up the reef, their opaque white tentacles (which are also slightly longer and more tapering in this variety) gave them the appearance of a colony of some species of *Sagartia*. The pattern of the disk showed some variation, but in most cases the raying was obscure, dull grey and umber predominating. The rays were more pronounced in the younger examples.

III. The Actinian Fauna of Salcombe.

Allen and Todd (3) enumerated eight species as found in the estuary. During a recent visit I examined the reefs on either side of the harbour mouth, with the result that six further species were discovered. This is partly owing to the fact that I collected rather further seaward than Allen and Todd, although several occurred within the area they examined.

The following are the additional species:—

1. *Sagartia miniata*. Rocks between South Sands and Splat Cove, a few specimens.
2. *S. nivea*. Reefs near Mill Bay, a few. Molt Point, one specimen.
3. *S. sphyrodeta*. Between South Sands and Splat Cove, a few.
4. *S. pallida*. Splat Cove, two on the under side of a stone in a pool on the reef. Normal size.
5. *Bunodes verrucosa*. Molt Point, a few. Mill Bay, not uncommon.
6. *Corynactis viridis*. Very abundant in sheltered positions on the reefs between Mill Bay and the Blackstone.

IV. Actiniae collected between Bolt Tail and the River Avon, South Devon.

The following observations were made during January and February, 1910. The species obtained were all littoral. The examination of the coast was as thorough as the weather would permit, but many days were lost through rough seas, etc. The Actinian fauna of the S. Devon coast is now fairly well known. A recent short search on reefs near Sidmouth (December 25th, 1909) produced only a solitary specimen of *A. equina;* E. J. Allen and R. A. Todd record that species and also *Anemonia sulcata* from the Orcombe Rocks at the mouth of the Exe. Many records from Teignmouth, Torquay and district, and Dartmouth are given by Gosse and others. Allen and Todd enumerate eight species from Salcombe; and the rich fauna of the Plymouth area is well known. My only excuse, therefore, for these notes is that they help to link up the Plymouth and Salcombe records as regards this group.

The following species were obtained: 1, *Actinia equina;* 2, *Anemonia sulcata* (*Anthea cereus*)*;* 3, *Cereus pedunculatus* (*S. bellis*)*;* 4, *Sagartia miniata;* 5, *Sagartia rosea;* 6, *Sagartia venusta;* 7, *Sagartia nivea;* 8, *Sagartia sphyrodeta;* 9, *Phellia murocincta;* 10, *Bunodes verrucosa* (*B. gemmacea*)*;* 11, *Tealia coriacea* (*T. crassicornis*).

The rocks in the area explored consist mainly of slates, grits, and conglomerate; there is some sand in the bays, and a large area of it at the River Avon. With the exception of Hope Cove, the whole region is very storm-swept.

The chief peculiarity of the district is that, with the exception of *A. equina*, *A. sulcata*, and *S. sphyrodeta* (occasionally in very sheltered angles and hollows), all the species have their habitat under fixed stones. It is practically useless to look for them under any stone which is easily moved, and usually it is a matter of careful observation to decide the most likely spots, to then remove some stone less firmly fixed than the rest, loosen and remove others, until finally the lower layers are reached; there, fixed and sheltered from stormy seas and moving stones, will be found such anemones as inhabit this coast. I ascribe this habit of life to the attrition of much coarse grit and shingle, which is to be found in almost every pool. A result of this enforced hidden existence is that the individuals are smaller than usual, and the pigmentation weaker. Particularly is this the case in the various species of *Sagartia*, tending in many instances to the obliteration or disappearance of typical markings, rendering a clear separation of the various species and varieties a matter of some difficulty.

Actinia equina. This species is neither very abundant nor large in the area examined, with the exception of the remarkable variety *fragacea.* This, though never common, occurs in most places and always of large size, and I can bear out all that Gosse states regarding this variety (see p. 177). The handsome colouration, large size, absence of the blue basal line, and in this area different distribution—(it is usually solitary and extends downward into the *Laminarian* zone)—renders it most conspicuous.

Anemonia sulcata is the only really abundant species on many reefs; colouration and size normal.

Sagartia miniata. One normally coloured, and one specimen of the variety *brunnea* (Gosse, p. 43)—both from beneath stones. Hope Cove.

Sagartia rosea. Ten specimens were obtained from a reef-pool between Thurlestone Sand and Hope Cove. In the pool, which was sheltered by a large rock, stood many slabs of slaty rock, on edge, and wedged together. By loosening one, all were in time examined, and the anemones discovered attached to the lower sides and edges of the stones. They were small and easily detached. The column elongate, when expanded almost pellucid white, in some slightly tinged with pink, the mesenteries showing very plainly; grooved, studded with numerous minute scattered whitish suckers to which adhered fragments of sand, etc., and in a few a brown mucous coat was present when first obtained.

Disk, semi-transparent white; mouth, rose-red; throat and stomodaeum, orange-red, showing through the integuments during expansion. Tentacles, rose, with a darker core when contracted, forty-eight to seventy in number.

These specimens manifested an intense dislike for light, and always crept under stones; at night the column was greatly lengthened, and then presented a most graceful pillar-like appearance, the rose-red throat and stomodaeum being very striking. They were all singularly insensitive, and it was a long time before I could procure the expulsion of acontia; finally one was extruded from the mouth.

Sagartia nivea. A few specimens from under stones, mostly of the variety *obscurata* of Gosse. From Hope Cove I obtained an abnormally developed example. Diameter 10 mm. expanded. The abnormality consisted of some sixty of the tentacles, comprising three-quarters of the circumference, remaining short and obtuse, and of the olivaceous hue of the summit of the column. The inner cycle about 2 mm. in length, the outer more like papillae than tentacles. The remaining thirty were normal in size and colouration, white, slender, and about 5 mm. long. The anemone had the appearance of never being properly expanded.

S. venusta. Normal in colour, but small.

S. sphyrodeta. Both varieties are present in the district, *candida* fairly abundant; *xanthopis* at Hope Cove under stones, among *Laminaria.*

Phellia murocincta. Two specimens, Warren Point, attached to the under side of stones, top of the Laminarian zone.

I can now extend the range of this species to four localities: Torquay (Gosse), Thurlestone, Zennor and Polzeath (these two in Cornwall). This would seem to indicate that the species is not so very rare, but when contracted within its covering it so resembles the many excrescences or fragments of debris so abundant under all stones in tide pools that probably it is often overlooked. A note on the Zennor specimens has already appeared in the Journal (September, 1907). I will only add that the Thurlestone examples agreed with them. The investing "coat" was easily detached, and did not adhere about the base; after its removal the anemones became restless and roamed until they obtained sufficient fragments to form a new covering, when they again became stationary. They only expanded at night, and when contracted and the covering was removed resembled, as Gosse observes, "a young *Sagartia viduata*." Tentacles 36. The Polzeath specimen was very small and only obtained by chance. Having observed several specimens of *Mytilus barbatus* mingled with *M. edulis* in a small cave, I detached them and put them in a collecting bottle full of water; on arriving home I observed the anemone fully expanded upon one of the *M. barbatus,* mingled with *Sycon coronata*, etc.

V. **Hoplangia Durotrix,** Gosse.

Originally mistaken by Gosse for *Phyllangia americana,* and subsequently described in *Actinologia Britannica,* p. 338, from specimens dredged in Weymouth Bay in 1858, when a colony of "from 50 to 100 specimens of this little coral, clustered in groups," was obtained, some of which came into his hands. The animal was not described, being too decomposed. Gosse states, however, that the discoverer spoke of it as resembling *Caryophyllia,* and "told me that he remarked numerous tentacles, but did not notice whether they were knobbed."

During a recent visit to Plymouth I examined a colony of more than thirty specimens, chipped off Wembury reef by W. Searle, 2nd September, 1909. The corals were of varying sizes, as Gosse says, "clustered in groups," on the stone. They agreed with the original Weymouth specimens. Diameter of largest unbroken individual, 5 mm.; height varied up to 10 mm.; outline varied, some being oval, others nearly circular. The colony had been killed partially expanded, and showed a considerable number of thick, obtuse tentacles, which do not appear to be knobbed. Searle tells me that the animals were colourless, or at any rate, he did not notice any colours like those of *Caryophyllia.*

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Note on Abnormal Pigmentation of a Whiting infected by *Trematode larvæ.*

By

Prof. F. W. Gamble, F.R.S., and G. H. Drew, B.A., Beit Memorial Fellow.

In one of the tanks at the Plymouth Laboratory containing pipe-fish and sticklebacks, a whiting was found recently which exhibited black specks scattered over its pigmented areas and on the conjunctiva. The spots were fairly evenly distributed and averaged ·5 to ·1 mm. in diameter. Around each black point there was a clear unpigmented area.

Preparations showed that this abnormal colouration was due to a Trematode. Each black spot contained a cyst within which the parasite lay. The influence of the parasite has drawn towards the cyst all the neighbouring chromatophores, thus explaining the dense accumulation of pigment in each spot and the area of pallor surrounding it.

The Trematode is a species of *Holostomum*, probably *H. cuticola*, v. Nordmann; for that author described a similar infection in German Cyprinoids as long ago as 1832. The points of interest are the presence of this Trematode in British waters, the reaction of the chromatophores, and the migration of pigment-cells into the conjunctiva.

January 18, 1911.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY.

The Relation between Light and Pigment-formation in *Crenilabrus* and *Hippolyte*. By Prof. F. W. Gamble, F.R.S. (*Quart. Jour. Micros. Science*, Vol. LV, Pt. 3, 1910.)

THE work on which this paper is based was carried out at Plymouth in 1908 and 1909. The two species on which the main results were investigated were *Crenilabrus melops* and *Hippolyte varians*. Backgrounds of weed and of painted glass were employed in order to test the influence of reflected light. Light-filters transmitting red and green respectively were used to determine the result of subjecting *Hippolyte* to diffused monochromatic light.

The following are the chief results:—

(1) *Crenilabrus*. The effect of light reflected from backgrounds of weeds and diluted with white light is different from the effect of light transmitted through the same weeds and not largely diluted with white light. In the former case the young animals generally assumed the tint of their surroundings; in the latter the dominant colouring was that complementary to the brightest part of the transmitted spectrum.

(2) *Hippolyte*. Any female of this variably coloured prawn throws larvæ which are constant in coloration with the exception of the broods of certain green parents. In other cases the colour (red) of the Zoeae varies in intensity at the time of hatching with the amount of red pigment in the female.

Green females throw one of three kinds of larvæ: (1) all red; (2) colourless; or (3) a mixture of red and colourless in the proportion of 3 to 1.

The red larval pigment develops in the absence of light. The origin of the yellow pigment has not been timed, but post-larval specimens (4–5 mm. long) if kept in darkness develop into brown specimens.

In pure green light similar (nearly colourless) specimens develop into crimson specimens. The red and yellow pigments disappear.

In pure red light the yellow pigment develops, and this together with a variable amount of diffuse blue colouring give a green tint, or a yellowish one. The colour of the food has apparently some influence upon the result.

On a red background and on a green background in white light *Hippolyte* become orange and green respectively.

The production of sympathetic colouring in the shallower coastal waters is explained as a background effect. The production of crimson *Hippolyte* (and possibly of reddish varieties of other marine animals) in deeper water is explained as due to diffused green light.

There is no evidence that the pigments of the algal food are the sources of the pigments in *Hippolyte varians*.

F. W. G.

The Reproduction and Early Development of *Laminaria digitata* and *Laminaria saccharina*. By G. H. Drew. *Ann. Bot.*, Vol. XXIV, No. XCIII., January, 1910, p. 177.

Laminaria digitata and *L. saccharina* are among the commonest seaweeds found on the coasts of the British Isles; they form the greater part of the "kelp" which is collected in some places for commercial purposes.

In the autumn and winter dark brown patches appear near the extremities of the broad flattened fronds, and in these the reproductive cells are formed. When ripe, small pieces of these reproductive areas were placed in a special culture solution (Allen and Nelson's modification of Miquel's solution), and it was found that an immense number of minute, free swimming cells were liberated. These cells each have two flagella, by means of which they are enabled to swim actively, and they show a tendency to move towards any source of light. Though all are exactly similar in appearance, yet these cells must be regarded as the sexual cells of the plant. After a time they fuse in pairs, lose their flagella, and settle to the bottom of the culture fluid. A series of changes then takes place resulting in the formation of a chain of cells possessing colouring matter, and apparently capable of absorbing nutrition, and of growth. This chain of cells can be considered as a separate plant, which though of an extremely rudimentary type, yet has an independent existence. Eventually any cell of the chain may give rise directly to a young *Laminaria* plant.

Thus in the life cycle of *Laminaria*, an alternation of generations occurs: the *Laminaria* plant represents the sexual phase, and the microscopic chain of cells above mentioned represents the asexual phase.

G. H. D.

Some Notes on Parasitic and Other Diseases of Fish. By G. H. Drew.

Parasitology, Vol. II, No. 3, September, 1909, p. 193; second series, Vol. III, No. 1, April, 1910, p. 54.

AN examination of a number of diseased fish was undertaken primarily with the object of finding cancerous growths. Many of the specimens were obtained by the s.s. *Huxley* in the North Sea.

Four cases of cancer were found resembling in appearance and structure malignant cancerous growths in man. Two of these originated in the pancreas of two specimens of plaice, and two in the livers of rainbow trout. There seems no reason to suppose that these growths are not in every way comparable to true carcinoma as found in man. One case of a superficial growth in a dog-fish was found, closely resembling the growths known as *endotheliomata*, which are generally included in the term "cancer." A number of cases of "benign" tumours were found (*fibromata*, *myxo-fibromata*, etc.), chiefly in plaice and salmon, and these also showed the closest resemblance to similar tumours in human beings. It thus seems probable that, whatever may be the cause of cancer and other tumours in man, the same cause may be operative in the production of similar growths in fish.

Several cases of disease due to sporozoon parasites were investigated, and two new species of sporozoa were described.

A number of cases of an affection of the swim-bladders of trout, resulting in the death of a large number of fish, were investigated. It was found that nematode worms made their way from the intestine into the swim-bladder by burrowing through the tissues, and carried bacteria with them, which set up an acute septic inflammation.

An outbreak of a contagious disease among fish in the Hertfordshire Colne was also investigated, and it was recognized as a recrudescence of the "salmon disease," in this case attacking fish of many different species. The specific bacteria were isolated, and also the white filamentous fungus, which grows freely on the lesions caused by the bacteria, was identified.

G. H. D.

Some Points in the Physiology of Lamellibranch Blood Corpuscles. By G. H. Drew. *Quar. Jour. Micro. Sci.*, Vol. LIV, Part 4, February, 1910, p. 605.

The Origin and Formation of Fibrous Tissue as a Reaction to Injury in *Pecten maximus.* By G. H. Drew and W. De Morgan. *Quar. Jour. Micro. Sci.*, Vol. LV, Part 3, September, 1910, p. 595.

THESE investigations were carried out as a necessary preliminary to further work of an experimental nature on the mode of origin of tumours. Most of the work was done on the common cockle and scallop.

The different sorts of corpuscles present in the blood were described, and it was shown that they were capable of ingesting, and so destroying, bacteria; thus protecting the animal from bacterial attacks. It was also shown that if the animal were wounded, so that any blood escaped, the corpuscles adhered to the injured surfaces and then sent out long, slender processes which would join up with similar processes from corpuscles on the other side of the wound. A network is thus formed in which other corpscules become entangled and so block up the opening of the wound. Finally, the protoplasmic strands forming the network contract, and so draw together the injured surfaces, which soon heal up.

The formation of fibrous tissue as a reaction to injury, and the consequent healing of wounds by "scar-tissue" formation, was also studied in detail. It was shown that any injurious foreign body which was implanted into the tissues of the animal was rapidly surrounded by an agglutinated layer of blood corpuscles, and that these were soon replaced by a dense mass of fibrous tissue. The foreign body thus becomes completely surrounded by a protective fibrous capsule, which, by shutting it off from the neighbouring tissues, tends to prevent its injurious action from spreading. It is perhaps an interesting point in the study of Evolution, to note that the process of "scar-tissue" formation, by which such animals as the scallops are enabled to recover from injuries, differs only in detail from the similar process which occurs in the highest types.

G. H. D.

Marine Biological Association of the United Kingdom.

Report of the Council, 1910-11.

The Council and Officers.

The usual four quarterly meetings of the Council have been held, at which the average attendance has been thirteen. The thanks of the Council are due to the Royal Society and to the Royal Astronomical Society for the use of their rooms for these meetings. A Committee of the Council has visited and inspected the Plymouth Laboratory.

The Plymouth Laboratory.

No considerable repairs or renewals of the engines, machinery, or equipment of the Laboratory have been found necessary during the year. The small room in the front on the ground floor of the east wing of the building has been fitted up as a Chemical Laboratory. Although the room is small it is a useful addition to the facilities of the Laboratory. The larger room at the back of the same wing has been specially fitted for work in experimental embryology. During the busy seasons of the year the demands for space for the different departments of our work are becoming very difficult to meet, and it was again necessary to hire a room at the Yacht Club below the Laboratory for the Easter Vacation Course.

The Boats.

Except for the addition of a small punt for harbour work the boats used are the same as last year. The steamer *Oithona* was laid up for the winter months, but is now again in commission, and is in sound working order.

The Staff.

Mr. F. J. Bridgman, Associate of the Royal College of Science, formerly demonstrator at the Imperial College of Science and Technology, South Kensington, has been appointed a Naturalist on the staff for the study of the biology of fishes, and Mr. J. H. Orton, B.Sc., of the same college, an Assistant-Naturalist for the study of invertebrates. The remaining members of the staff, Dr. E. J. Allen and Mr. D. J. Matthews, continue to occupy their former positions.

Occupation of Tables.

The following Naturalists have occupied tables at the Plymouth Laboratory during the year:—

K. H. Barnard, B.A., Cambridge (Amphipoda).
L. R. Crawshay, M.A., Plymouth (Fauna at Mid-Channel).
W. De Morgan, Plymouth (Hybridization of Echinus).
G. H. Drew, B.A., Plymouth (Tissue Transplantation in Pecten).
F. Martin Duncan, London (Photography of Marine Animals).
J. S. Dunkerly, B.Sc., London (Parasitic Protozoa).
E. S. Goodrich, F.R.S., Oxford (Embryology of Patella).
S. A. Arendsen Hein, Utrecht (Eyes of Fishes).
Herbert Henry, M.D., Sheffield (Parasites of Blood of Fishes).
Prof. Iwaji Ikeda, Japan (Parasitic Protozoa).
J. W. Jenkinson, D.Sc., Oxford (Regeneration in Hydrozoa).
W. O. R. King, M.A., Cambridge (Regeneration in Hydrozoa).
Miss M. V. Lebour, B.Sc., Leeds (Trematoda).
Keith Lucas, M.A., Cambridge (Physiology of Ciona).
J. F. Murphy, Cork (Fishes).
G. E. Nicholls, B.Sc., London (Nervous System of Fishes).
R. C. Pearson, B.A., Cambridge (General Zoology).
R. W. H. Rowe, B.Sc., London (Sponges).
J. T. Saunders, B.A., Cambridge (Polychaeta).
C. Shearer, M.A., Cambridge (Dinophilus).
Geoffrey Smith, M.A., Oxford (Blood of Carcinus).
T. H. Taylor, M.A., Leeds (Nematocysts of Coelenterata).
Miss Gerarda Wijnhoff, Utrecht (Nemertina).

In addition to the above the Easter Vacation Course in Marine Biology was attended by seventeen students. This course was conducted again this year by Prof. Walter Garstang, of the University of Leeds, who gave the first course of the kind held at the Laboratory in 1895.

Dr. Cresswell Shearer, M.A., brought a class of six students from Cambridge for a practical course in Experimental Embryology.

A class of four students from the Imperial College of Science, South Kensington, attended at the Laboratory in July for a course of practical work in Marine Biology in continuation of the course conducted at the College in London by Dr. E. J. Allen in the previous spring.

General Work at the Plymouth Laboratory.

Work on the cultivation of plankton organisms and on the rearing of marine larvæ, which has been in progress for some years, has been continued and advanced. Several interesting forms of diatoms and algæ not previously obtained in persistent cultures have been isolated. Mr. W. De Morgan, who has worked in co-operation with Dr. C. Shearer, has reared a large number of hybrid larvæ obtained by intercrossing three species of *Echinus* in as many different ways as possible. It is hoped that these experiments will throw light upon some theoretical questions of considerable importance.

Mr. G. H. Drew, who was last year appointed a Beit Memorial Fellow, has carried out a number of successful experiments on the transplantation of tissues in invertebrate animals, which have an important bearing upon the cancer problem. He has also made a special study of certain diseases which occur in fishes.

Mr. Orton has been making a general study of the distribution of the invertebrate fauna of Plymouth, and has paid particular attention to the Echinodermata and Crustacea.

Fishery Investigations.

Owing to the transference of the North Sea Investigations and the staff connected therewith to the Board of Agriculture and Fisheries, and to the sale of the steamer *Huxley* which thus became necessary, it has not been possible during the past year to devote nearly so much attention as formerly to purely economic fishery problems. In future it is proposed to confine the economic work of the Association to special scientific problems of a fundamental character, which bear directly upon fishery investigations. At the same time it must be pointed out that the Plymouth Laboratory will still afford precisely such training as is required by men who may afterwards be employed in scientific investigation in the service of the Government, and that the general scientific work of the Association, though it may have no immediate economic value, is of such a character as to form an important part of the necessary foundation upon which the applied science of fisheries must in future be built.

Mr. Bridgman has commenced an investigation of the age and growth-rate of Plaice in the western part of the English Channel, in continuation of similar researches which were carried on by Dr. Wallace in connection with the North Sea Investigations. A considerable amount of material has already been collected, and this will, it is hoped, be largely added to as the year advances. Mr. Hefford's report on the embryonic and early larval stages of fishes obtained at Plymouth has been published in the Journal of the Association.

A further Blue Book has been published by His Majesty's Stationery Office dealing with the work done by the Association in connection with the International Investigations. This contains the Third Report on the subject prepared by the Association. The results contained in the separate memoirs were summarised in the Report of the Council for last year. A further Blue Book, which will contain the Fourth Report on the International Investigations, is now in the press. The thanks of the Association are due to the Board of Agriculture and Fisheries for allowing these reports to be completed by the members of the staff after the direction of their work had been transferred to the Board.

Published Memoirs.

The following papers, either wholly or in part the outcome of work done at the Laboratory, have been published elsewhere than in the official publications of the Association:—

Allen, E. J., and Nelson, E. W.—*On the Artificial Culture of Marine Plankton Organisms.* Quart. Journ. Micr. Sci., vol. 55, 1910, pp. 361–431.

Drew, G. H., and De Morgan, W.—*The Origin and Formation of Fibrous Tissue produced as a Reaction to Injury in Pecten maximus, as a Type of the Lamellibranchiata.* Quart. Journ. Micr. Sci., vol. 55, 1910, pp. 595–610.

Drew, G. H.—*Experimental Metaphasia. 1. The Formation of Columnar Ciliated Epithelium from Fibroblasts in Pecten.* Journal of Experimental Zoology, vol. 10, 1911, pp. 349–379.

Eliot, C.—*British Nudibranchiate Mollusca. By the late Joshua Alder and the late Albany Hancock.* Supplement. Ray Society.

Shearer, C.—*On the Anatomy of Histriobdella Homari.* Quart. Journ. Micr. Sci., vol. 55, 1910, pp. 287–360.

Gamble, F. W.—*The Relation between Light and Pigment-formation in Crenilabrus and Hippolyte.* Quart. Journ. Micr. Sci., vol. 55, 1910, pp. 541–584.

Hodgson, T. V.—*The Pycnogonida of Devonshire.* Trans. Devonshire Assocn. XLII, 1910, pp. 425–439.

The Library.

The thanks of the Association are due for the following books and current numbers of periodicals presented to the Library during the past year:—

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American Museum of Natural History. Annual Report.
American Microscopical Society. Transactions.
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Board of Agriculture and Fisheries. Report on the Research Work of the Board in relation to the Plaice Fisheries of the North Sea.
—— Report of Proceedings at a Meeting of Representatives of Authorities under the Sea Fisheries Regulation Act, to consider a means to extend the scope and increase the effectiveness of the Annual Meetings.
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—— Dept. of Marine Biology. Annual Report of the Director.
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Falmouth Observatory. Meteorological and Magnetic Reports.
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—— On the Origin of certain types of Crinoid Stems. By A. H. Clark.
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—— A new European Crinoid. By A. H. Clark.
—— A new Fresh-water Amphipod from Virginia, with some notes on its biology. By G. C. Embody.
United States National Museum. Proisocrinus, a new Genus of Recent Crinoids. By A. H. Clark.
—— A new Ophiuran from the West Indies. By H. L. Clark.
—— Description of a new Isopod of the Genus Notasellus from the East Coast of Patagonia. By H. Richardson.
—— On some Ostracoda, mostly new, in the Collection of the United States National Museum. By R. W. Sharpe.
—— New Arenaceous Foraminifera from the Philippines. By J. A. Cushman.
—— Notes on the Marine Copepoda and Cladocera of Woods Hole and Adjacent Regions, including a Synopsis of the Genera of the Harpacticoida. By R. W. Sharpe.
—— Fresh-water Sponges in the Collection of the United States National Museum. Part IV. Note on the Fresh-water Sponge, *Ephydatia Japonica*, and its allies. By N. Annandale.
—— Description of a new parasitic Isopod from the Hawaiian Islands. By H. Richardson.
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Loeb, J. Ueber die Befruchtung von Seeigeleiern durch Seesternsamen. II. Mittheilung.

—— Maturation, Natural Death and the Prolongation of the Life of unfertilized Starfish Eggs (*Asterias Forbesii*) and their significance for the theory of Fertilization.

—— Studies on the Physiological Effects of the Valency and possibly the Electrical Charges of Ions. I. The Toxic and Antitoxic Effects of Ions as a Function of their Valency and possibly their Electrical Charge.

—— Experiments on Artificial Parthenogenesis in Annelids (Chaetopterus) and the nature of the process of Fertilization.

—— Ueber die Bedeutung der Ca- und K-Ionen für die Herzthatigkeit.

—— Ueber den Einfluss der Werthigkeit und möglicher Weise der elektrischen Ladung von Ionen auf ihre antitoxische Wirkung.

—— Über die angebliche gegenseitige Beeinflussung der Furchungszellen und die Entstehung der Blastula.

—— Ueber die physiologische Wirkung von Alkalien und Säuren in starker Verdünnung.

—— Einige Bemerkungen über den Begriff, die Geschichte und Literatur der allgemeinen Physiologie.

—— Zur Theorie der physiologischen Licht- und Schwerkraft-wirkungen.

—— Zur Theorie des Galvanotropismus III. Ueber die polare Erregung der Hautdrüsen von Amblystoma durch den constanten Strom.

—— Zur Physiologie und Psychologie der Actinien.

—— Beiträge zur Entwickelungsmechanik der aus einem Ei entstehenden Doppelbildungen.

Loeb, J., and Gerry, W. E. Zur Theorie des Galvanotropismus. II. Mitt. Versuche an Wirbelthieren.

Loeb, J., and Gies, W. J. Weitere Untersuchungen über die entgiftenden Ionenwirkungen und die Rolle der Werthigkeit der Kationen bei diesen Vorgängen.

Loeb, J., and Maxwell, S. S. Further Proof of the Identity of Heliotropism in Animals and Plants.

McIntosh, W. C. Note on Irish Annelids in the Museum of Science and Art, Dublin.

—— Notes from the Gatty Marine Laboratory.

Man, J. G. de. Beiträge zur Kenntnis der in dem weissen Schleimfluss der Eichen lebenden Anguilluliden, nebst Untersuchungen über den Bau des Essigälchens und der Gattung *Anguillula* Ehrb.

—— Über eine neue Art der Gattung Arete, Stimps.

Marceau, F. Sur les fibres musculairs dites doublement striées obliquement.

—— Note sur la structure du cœur chez les Céphalopodes.

—— Recherches sur la physiologie et en particulier sur les lois de la production de travail mécanique par les muscles adducteurs des Acéphales.

—— Recherches sur la structure des muscles du manteau des Céphalopodes en rapport avec leur mode de contraction.

—— Note complémentaire sur la structure du manteau des Céphalopodes en rapport avec leur mode de contraction.

—— Recherches sur le mouvement de bascule des valves de certains Acéphales pendant leur ouverture et leur fermeture et ses conséquences morphogéniques.

Masterman, A. T. On a possible case of Mimicry in the Common Sole.
—— Report on the Later Stages of the Pleuronectidae.
—— Second Report on the Later Stages of the Pleuronectidae (for 1909).
Mayer, A. G. The Converse Relation between Ciliary and Neuro-Muscular Movements.
—— Alpheus Hyatt, 1838–1902.
—— Alexander Agassiz, 1835–1910.
Mortensen, Th. *Tjalfiella tristoma*, n. g., n. sp. A sessile Ctenophore from Greenland.
—— Echinological Notes.
Needham, J. G. Practical Nomenclature.
Nicoll, W. Remarks on the Bionomics of Helminths.
—— On *Gasterostomum tergestinum* Stossich.
—— On the Entozoa of Fishes from the Firth of Clyde.
Nicoll, W., and Small, W. Notes on Larval Trematodes.
Norman, W. W. Do the Reactions of the Lower Animals against injury indicate pain sensations? With Additional Note by J. Loeb.
Perrier, E., and Anthony, R. Organisation d'une étude générale du Plankton de la Baie de la Hougue.
Piéron, H. De l'influence réciproque des phénomènes respiratoires et du comportement chez certaines Actinies.
—— Des réactions de l'*Actinia equina* à la désoxygénation progressive du milieu.
Police, G. La Pesca nel Golfo di Napoli. I. La Pesca con le nasse.
Popta, C. M. L. Étude sur la vessie aérienne des Poissons. Sa fonction.
Pütter, A. Der Stoffwechsel der Aktinien.
—— Die Ernährung der Wassertiere durch gelöste organische Verbindungen.
Rathbun, M. J. Decapod Crustaceans collected in Dutch East India and elsewhere by Mr. Thomas Barbour in 1906–1907.
—— The Stalk-eyed Crustacea of Peru and the adjacent coast.
—— The Danish Expedition to Siam, 1899–1900. V. Brachyura.
Redeke, H. C. Temperatuur en Zoutgehalte van het Zeewater bij drie Nederlandsche Lichtschepen en in het Marsdiep, 1906–1908.
—— Über den Sprott und die Sprottfischerei in Holland.
Regan, C. Tate. A Synopsis of the Sharks of the Family Cestraciontidae.
—— A Synopsis of the Sharks of the Family Scyliorhinidae.
—— A Synopsis of the Sharks of the Family Squalidae.
—— A Revision of the Sharks of the Family *Orectobobidae.*
—— Report on the Marine Fishes collected by Mr. J. Stanley Gardiner in the Indian Ocean.
—— A Preliminary Revision of the Irish Char.
—— A Revision of the British and Irish Fishes of the Genus Coregonus.
—— A Revision of the Fishes of the Genus Elops.
—— The Char (*Salvelinus*) of Great Britain.
—— The Species of Three-spined Sticklebacks.
—— Descriptions of new Marine Fishes from Australia and the Pacific.
—— On Colour-changes in Fishes.
—— A Collection of Fishes made by Dr. C. W. Andrews, F.R.S., at Christmas Island.
—— The Anatomy and Classification of the Teleostean Fishes of the Order Zeomorphi.

Regan, C. Tate. The Origin and Evolution of the Teleostean Fishes of the Order Heterosomata.
—— On the Caudal Fin of the Clupeidae, and on the Teleostean Urostyle.
—— The Origin of the Chimaeroid Fishes.
—— Notes on the Classification of the Teleostean Fishes.
—— A Synopsis of the Marsipobranchs of the Order Hyperoartii.
—— On the Systematic Position of *Macristium chavesi*.
Richters, F. Nordische Tardigraden.
—— Zwei neue Echiniscus-Arten.
—— Marine Tardigraden.
—— *Cladonema radiatum* bei Helgoland.
—— Islandische Tardigraden.
—— Bunonema.
—— Wiederbelebungsversuche mit Tardigraden.
—— Antarktische Tardigraden.
—— Vorläufiger Bericht über die antarktische Moosfauna.
—— Rotatoria, Tardigrada und andere Moosbewohner.
—— Beitrag zur Kenntnis der Moosfauna Australiens und der Inseln des Pazifischen Ozeans.
Richters, F. Tardigraden unter 77° S. Br.
—— Tardigraden-Studien.
—— Meer-Bärtierchen.
—— Tardigraden aus den Karpathen.
Schaeffer, A. A. Selection of Food in *Stentor caerulens* (Ehr.).
Scharff, R. F. The Irish Whale Fishery.
Shaw, H. O. N. *Cypraea* and *Trivia*.
—— Anatomical Differences between *Cypraea* and *Trivia*.
—— Notes on the References to Certain Groups, etc., used in the Classification of Mollusca.
Schaxel, J. Das Zusammenwirken der Zellbestandteile bei der Eireifung, Furchung und ersten Organbildung der Echinodermen.
Shearer, C. On the Development and Structure of the Trochophore of *Hydroides uncinatus* (Eupomatus).
Stephenson, J. On some Littoral Oligochaeta of the Clyde.
Sherborn, C. D., and Shaw, H. O. N. Sowerby's "Conchological Illustrations" and Gray's "Descriptive Catalogue of Shells."
Shipley, A. E. Grouse Disease.
—— William Henry Dallinger, 1842–1909.
—— "Foreword."
—— Report upon two small Collections of Pentastomids with the Description of a New Species of "Porocephalus."
Sterzinger, I. Über die Spirorbis-Arten der Nordlichen Adria.
Stuckey, F. G. A., and Walton, C. L. Notes on a Collection of Sea-anemones.
Sumner, F. B. An Experimental Study of Somatic Modifications and their reappearance in the offspring.
—— An Intensive Study of the Fauna and Flora of a restricted area of sea bottom.
Sun, A. Über einen Parasiten aus der Körperhöhle von *Ptychodera minuta*.
Sund, O., and Koefoed, E. Underskelser over Brislingen i Norske farvand vaesentlig paa Grundlag av "Michael Sars's" Togt 1908, med Tabellen over foreksomst af Egg og Yngel fra "Michael Sars's" Togt 1908.

Thomson, J. S. The Alcyonaria of the Cape of Good Hope and Natal. Alcyonacea.

Tracy, H. C. The Fishes of Rhode Island. V. The Flat Fishes. VI. A Description of two young specimens of Squeteague (*Cynoscion regalis*), with Notes on the Rate of their Growth.

—— Annotated List of Fishes known to inhabit the waters of Rhode Island.

Treadwell, A. L. *Haplosyllis cephalata* as an Ectoparasite.

—— Polychaetous Annelids from the Dry Tortugas, Florida.

Trybom, F. Svenska rödspättemarkningar 1909, med tillägg från äldre märkningar.

Vayssière, A. Sur un nouveau genre de la famille des Tritoniadés.

—— Sur les Tectibranches du Golfe de Marseille.

—— Sur le dimorphisme sexuel des Nautiles.

—— Sur un nouveau *Temnocephala*, parasite de l'*Astacoides madagascariensis*.

—— Étude anatomique sur le *Coléophysis* (*Utriculus*) *truncatula*, Brug.

—— Considerations sur les differences qui existent entre la faune des Opistobranches des côtes océaniques de la France et celles de nos côtes mediterranéennes.

—— Recherches zoologiques et anatomiques sur les Mollusques Opistobranches du Golfe de Marseille. III. Nudibranches.

—— Sur les Opisthobranches recueillis en 1883 par l'expédition du Talisman.

—— Etude Zoologique de l'*Archidoris stellifera* H. von Ihering.

—— Observations zoologiques et anatomiques sur l'*Ammonicera* nouveau genre de Gastéropode Prosobranche.

—— Notice sur les Travaux scientifiques.

Vejdovský, F. Neue Untersuchungen über die Reifung und Befruchtung.

Viguier, C. Nouvelles études sur le Plankton de la Baie d'Alger.

Vles, F. Les notions de Martin Lister (1694) sur la locomotion des Gasté-ropodes.

—— Sur la valeur des stries musculaires au point de vue spectrographique.

—— Sur les bruits émis par des *Helix* pendant leur progression.

—— Monographie sommaire de la Mye (*Mya arenaria* Linné 1767).

—— Sur un micromètre oculaire à vernier intérieur.

Walker, A. O. Notes on Amphipoda.

—— Marine Amphipods from Peru.

Whitehouse, R. H. Some Remarks on the Teleostean Caudal Fin.

—— The Caudal Fin of the Teleostomi.

Wijnhoff, G. Die Gattung Cephalothrix und ihre Bedeutung für die Systematik der Nemertinen.

—— Verslag aan Zijne Excellentie den Minister van Binnenlandsche Zaken, aangaande onderzoeking verricht door Gerarda Wijnhoff, phil. nat. doct. van 3| Mei—13 Juni en 6 Juli—14 September 1910 in het Zoologisch Laboratorium van de Marine Biological Association te Plymouth.

Willey, A. Convergence in Evolution.

Winiwarter, H. von. Nachtrag zu meiner Arbeit über Oogenese der Säugetiere.

—— Das interstitielle Gewebe der Menschlichen Ovarien.

Winiwarter, H. von, and Sainmont, G. Erfahrungen über die Flemmingsche Dreifärbung.

Woodruff, L. L. Two Thousand Generations of Paramecium.

Woodruff, L. L., and Bunzel, H. H. The Relative Toxicity of various Salts and Acids towards Paramecium.

Donations and Receipts.

The receipts for the year include the grants from His Majesty's Treasury (£1000) and the Worshipful Company of Fishmongers (£400, of which £100 had been paid in advance), Special Donations (£169), Annual Subscriptions (£156), Rent of Tables in the Laboratory (£75), Sale of Specimens (£505), Admission to Tank Room (£105)

The following is a list of the Special Donations:—

	£	*s.*	*d.*
G. P. Bidder, Esq. . . .	100	0	0
Professor G. C. Bourne, F.R.S. . .	3	19	0
Cambridge University—Zoological Dept.	25	0	0
F. Martin Duncan, Esq. . . .	1	1	0
G. H. Fox, Esq.		10	6
London University . . .	25	0	0
University College, London . .	5	0	0
Per the Director	8	6	8
	£168	17	2

Vice-Presidents, Officers, and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1911–12:—

President.

Sir E. Ray Lankester, K.C.B., LL.D., F.R.S.

Vice-Presidents.

The Duke of Abercorn, K.G., C.B.
The Duke of Bedford, K.G.
The Earl of St. Germans.
The Earl of Ducie, F.R.S.
The Earl of Stradbroke, C.V.O., C.B.
Lord Avebury, F.R.S.
Lord Walsingham, F.R.S.
The Right Hon. A. J. Balfour, M.P., F.R.S.
The Right Hon. Joseph Chamberlain, M.P.
The Right Hon. Austen Chamberlain, M.P.
W. W. Astor, Esq., M.P.
G. A. Boulenger, Esq., F.R.S.
A. C. L. Günther, Esq., F.R.S.
A. R. Steel-Maitland, Esq., M.P.
Sir John Murray, K.C.B., F.R.S.
Rev. Canon Norman, D.C.L., F.R.S.
Edwin Waterhouse, Esq.

Members of Council.

G. L. Alward, Esq.
W. T. Calman, Esq., D.Sc.
Prof. A. Dendy, D.Sc., F.R.S.
Sir Charles Eliot, K.C.M.G.
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S. F. Harmer, Esq., Sc.D., F.R.S.
Commander M. W. Campbell Hepworth, C.B., R.N.R.
Prof. J. P. Hill, D.Sc.
E. W. L. Holt, Esq.
Prof. E. W. MacBride, F.R.S.
P. Chalmers Mitchell, Esq., D.Sc., F.R.S.
Edgar Schuster, Esq., D.Sc.
Geoffrey W. Smith, Esq.
Prof. D'Arcy W. Thompson, C.B.

Chairman of Council.

A. E. Shipley, Esq., D.Sc., F.R.S.

Hon. Treasurer.

J. A. Travers, Esq., Tortington, Arundel.

Hon. Secretary.

E. J. Allen, Esq., D.Sc., The Laboratory, Citadel Hill, Plymouth.

The following Governors are also members of the Council:—

G. P. Bidder, Esq., M.A.
THE PRIME WARDEN OF THE FISHMONGERS' COMPY.
Sir Richard Martin, Bart. (Fishmongers' Company).
W. L. Birch, Esq. (Fishmongers' Company).
Prof. G. C. Bourne, D.Sc., F.R.S. (Oxford University).
A. E. Shipley, Esq., D.Sc., F.R.S. (Cambridge University).
Prof. W. A. Herdman, D.Sc., F.R.S. (British Association).

Dr. *Statement of Receipts and Payments for*

	£	s.	d.	£	s.	d.
To Balance from last year, viz. :—						
Cash at Bank	1,033	12	2			
Cash in hand	9	17	8			
	1,043	9	10			
Less Bank Loan	300	0	0	743	9	10
,, Current Income :—						
H.M. Treasury	1,000	0	0			
The Worshipful Company of Fishmongers : Balance of Grant	300	0	0			
Annual Subscriptions	156	3	0			
Composition Fee	15	15	0			
Rent of Tables	75	9	3	1,547	7	3
,, Extraordinary Receipts :—						
Donations, per Report				168	17	2
				£2,459	14	3

Examined and found correct.

(Signed) N. E. Waterhouse, F.C.A. W. T. Calman.

Wyndham Birch. L. W. Byrne.

27th June, 1911.

the Year ending 31*st May*, 1911. Cr.

		£ s. *d.*	£ s. *d.*
By Current Expenditure:—			
Salaries and Wages—			
Director		200 0 0	
Assistant Director		26 5 0	
Hydrographer		143 15 0	
Naturalist		54 13 0	
Assistant Naturalists		108 6 8	
Salaries and Wages, and Compensation paid		674 17 10	
		1,207 17 6	
Less Compensation recovered from Employers' Liability Assurance Corporation		45 1 3	1,162 16 3
Travelling Expenses			40 4 3
Library		137 11 11	
Less Duplicates sold		3 18 2	133 13 9
Lecture Expenses			10 3 0
Journal, Vol. IX, Part I		44 17 0	
Less Sales		17 15 11	27 1 1
Buildings and Public Tank Room—			
Gas, Water, and Coal		116 0 4	
Stocking Tanks and Feeding		34 9 1	
Maintenance and Renewals		193 2 6	
Rent, Rates, Taxes, and Insurance		55 18 10	
		399 10 9	
Less Admission to Tank Room		105 2 6	294 8 3
Laboratory, Boats, and Sundry Expenses—			
Glass, Apparatus, and Chemicals	199 11 1		
Less Sales	111 4 0	88 7 1	
Purchase of Specimens		56 10 10	
Maintenance and Renewals of Boats, Nets, Gear, etc.	368 11 3		
Less Sales, including equipment for Captain Scott's Antarctic Expedition	229 7 5	139 3 10	
Insurance of s.s. *Oithona*		18 17 7	
Coal and Water for Steamer		76 15 9	
Stationery, Office Expenses, Printing, etc.		171 13 8	
		551 8 9	
Less Sale of Specimens	505 4 11		
Expenses of Salmon Experiment undertaken on behalf of the Duke of Bedford, refunded	29 16 11	535 1 10	16 6 11
By Bank Interest			1 18 2
By the Trustees of the "Ray Lankester Fund"— By Deed dated 11th May, 1911, the Trusts regulating this Fund were declared, and the above sum has been invested in the names of Trustees nominated by Mr. G. P. Bidder. (See note to accounts for the year ending 31st May, 1910.)			750 7 6
By Balance:—			
Cash at Bank		316 0 4	
Cash in hand		6 14 9	
		322 15 1	
Less Bank Loan		300 0 0	22 15 1
This Balance is apportioned as follows:—			
Repairs and Renewals	200 0 0		
Less General Account overdrawn	177 4 11		
	£22 15 1		
			£2,459 14 3

Marine Biological Association of the United Kingdom.

LIST
OF
Governors, Founders, and Members.

1st OCTOBER, 1911.

* Member of Council. † Vice-President. ‡ President.

Ann. signifies that the Member is liable to an Annual Subscription of One Guinea.

C. signifies that he has paid a Composition Fee of Fifteen Guineas in lieu of Annual Subscription.

I.—Governors.

The British Association for the Advancement of Science, *Burlington House, W.*	£500
The University of Oxford	£500
The University of Cambridge	£500
The Worshipful Company of Clothworkers, 41, *Mincing Lane, E.C.*	£500
The Worshipful Company of Fishmongers, *London Bridge, E.C.*	£9705
Bayly, Robert (the late)	£1000
Bayly, John (the late)	£600
Thomasson, J. P. (the late)	£970
G. P. Bidder, Esq., *Cavendish Corner, Cambridge*	£1400

II.—Founders.

1884	The Corporation of the City of London	£210
1884	The Worshipful Company of Mercers, *Mercers' Hall, Cheapside*	£341 5*s.*
1884	The Worshipful Company of Goldsmiths, *Goldsmiths' Hall, E.C.*	£100
1884	The Royal Microscopical Society, 20, *Hanover Square, W.*	£100
1884	The Royal Society, *Burlington House, Piccadilly, W.*	£350
1884	The Zoological Society, *Regent's Park, London, N.W.*	£100
1884	Bulteel, Thos. (the late)	£100
1884	Burdett-Coutts, W. L. A. Bartlett, 1, *Stratton Street, Piccadilly, W.*	£100
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1884	Daubeny, Captain Giles A., *The Vicarage, Tottington, Bury, Lancs.*	£100
1884	Eddy, J. Ray, *The Grange, Carleton, Skipton*	£100
1884	Gassiott, John P. (the late)	£100

‡1884	Lankester, Sir E. Ray, K.C.B., F.R.S., 29, *Thurloe Place, South Kensington, S.W.*	£100
1884	The Rt. Hon. Lord Masham (the late)	£100
1884	Moseley, Prof. H. N., F.R.S. (the late)	£100
†1884	The Rt. Hon. Lord Avebury, F.R.S., *High Elms, Bromley, Kent*	£100
1884	Poulton, Prof. Edward B., M.A., F.R.S., *Wykeham House, Oxford*	£100
1884	Romanes, G. J., LL.D., F.R.S. (the late)	£100
1884	Worthington, James (the late)	£100
1885	Derby, the late Earl of	£100
1887	Weldon, Prof. W. F. R., F.R.S. (the late)	£100
1888	Bury, Henry, M.A., *Mayfield House, Farnham, Surrey*	£100
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1889	The Worshipful Company of Grocers, *Poultry, E.C.*	£120
1889	Thompson, Sir Henry, Bart. (the late)	£110
1889	Revelstoke, The late Lord	£100
1890	Riches, T. H., B.A., *Kitwells, Shenley, Herts*	£230
1902	Gurney, Robert, *Ingham Old Hall, Stalham, Norfolk*	£105
1909	Harding, Colonel W., *The Hall, Madingley, Cambridge*	£100
†1910	Murray, Sir John, K.C.B., F.R.S., *Challenger Lodge, Wardie, Edinburgh*	£100

III.—Members.

1897	Adams, W. R., 16, *Milestone Road, Cintra Park, Upper Norwood, London*	Ann.
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1884	Alger, W. H., 8, *The Esplanade, Plymouth*	C.
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1884	Bayly, Miss, *Seven Trees, Plymouth*	£50
1884	Bayly, Miss Anna, *Seven Trees, Plymouth*	£50
1884	Beaumont, W. I., B.A., *The Laboratory, Plymouth*	Ann.
1885	Beck, Conrad, 68, *Cornhill, E.C.*	C.
1884	Beddington, Alfred H., 8, *Cornwall Terrace, Regent's Park, N.W.*	C.
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1903	Bidder, H. F., 10, *Queen's Gate Gardens, London, S.W.*	Ann.
1910	Bidder, Mrs. M. G., *Cavendish Corner, Cambridge*	Ann.
1910	Bloomer, H. H., 35, *Paradise Street, Birmingham*	Ann.
1910	Borley, J. O., M.A., 43, *Parliament Street, London, S.W.*	Ann.
*1884	Bourne, Prof. Gilbert C., M.A., F.R.S., *Savile House, Mansfield Road, Oxford*	Ann.

1910 Bowkett, Sidney, *Claygate, Surrey* .... Ann.
1898 Bowles, Col. Henry, *Forty Hall, Enfield* .... Ann.
1910 Bradford, J. Rose, M.D., D.Sc., F.R.S., 8, *Manchester Square, London, W.* Ann.
1910 Bridgman, F. J., *The Laboratory, Plymouth* .... Ann.
1902 Brighton Public Library (Henry D. Roberts, Chief Librarian) .... Ann.
1886 Brooksbank, Mrs. M., *Leigh Place, Godstone, Surrey* .... *C.*
1884 Brown, Arthur W. W., 62, *Carlisle Mansions, Carlisle Place, London, S.W.* *C.*
1910 Brown, F. J., 10, *Belmont Road, Ilfracombe* .... Ann.
1893 Browne, Edward T., B.A., *Anglefield, Berkhamsted* .... Ann.
1892 Browne, Mrs. E. T., *Anglefield, Berkhamsted* .... Ann.
1897 Byrne, L. W., B.A., 7, *New Square, Lincoln's Inn, London, W.C.* .... Ann.

*1908 Calman, Dr. W. T., *British Museum (Natural History), Cromwell Road, S.W.* .... Ann.
†1884 Chamberlain, Rt. Hon. J., M.P., 40, *Prince's Gardens, S.W.* .... Ann.
1911 Chilton, Prof. C., *Canterbury College, Christchurch, New Zealand* .... Ann.
1884 Christy, Thomas Howard, 199, *Bramhall Lane, Stockport* .... *C.*
1911 Clark, Dr. J., *Flowerbank, Kilmarnock, N.B.* .... Ann.
1910 Clarke, G. B. R. Kitson, *Meanwoodside, Leeds* .... Ann.
1887 Clarke, Rt. Hon. Sir E., K.C., 5, *Essex Court, Temple, E.C.* £25
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1886 Coates and Co., *Southside Street, Plymouth* .... *C.*
1910 Cole, Thomas Skelton, *Westbury, Endcliffe Crescent, Sheffield* .... Ann.
1885 Collier Bros., *Old Town Street, Plymouth* .... *C.*
1900 Cooper, W. F., B.A., *Ashlyns Hall, Berkhamsted* .... Ann.
1909 Crawshay, L. R., M.A., *The Laboratory, Plymouth* .... Ann.

1910 Darbishire, A. D., M.A., *The Zoological Department, The University, Edinburgh* .... Ann.
1885 Darwin, Francis, F.R.S., 13, *Madingley Road, Cambridge* .... *C.*
1885 Darwin, W. E., *Ridgemount Bassett, Southampton* £20
1911 Davies, Humphreys, 6, *Southwick Place, London, W.* .... Ann.
*1908 Dendy, Prof. A., F.R.S., *Firsdene, Oatlands Drive, Weybridge* .... Ann.
1910 Devonport Education Authority .... Ann.
1884 Dewick, Rev. E. S., M.A., F.G.S., 26, *Oxford Square, Hyde Park, W.* .... *C.*
1885 Dixey, F. A., M.A. Oxon., *Wadham College, Oxford* .... £26 5*s.* *and* Ann.
1906 De Morgan, W. C., *c/o National Provincial Bank, Plymouth* .... Ann.
1910 Dobell, C. C., M.A., *Imperial College of Science and Technology, South Kensington, S.W.* .... Ann.
1910 Drew, G. H., B.A., *The Laboratory, Plymouth* .... Ann.
1890 Driesch, Hans, Ph.D., *Philosophenweg 5, Heidelberg, Germany* .... *C.*
†1889 Ducie, The Rt. Hon. the Earl of, F.R.S., *Tortworth Court, Falfield, R.S.O.* £50 15*s.*
1910 Duncan, F. Martin, *Spring Cottage, Oxted, Surrey* .... Ann.
1884 Dunning, J. W., 4, *Talbot Square, London, W.* .... £26 5*s.*
1884 Dyer, Sir W. T. Thiselton, M.A., K.C.M.G., F.R.S., *The Ferns, Witcombe, Gloucester* .... *C.*

*1898 Eliot, Sir C. N. E., K.C.M.G., C.B., *Endcliffe Holt, Endcliffe Crescent, Sheffield* .... Ann. £5

1906 Elliott, Sir Thomas H., K.C.B., *Board of Agriculture and Fisheries, 4, Whitehall Place, London, S.W.* .... Ann.
1908 Elwes, Maj. Ernest V., *Glendower, St. Albans Road, Babbacombe* .... Ann.
1893 Enys, John Davies, *Enys, Penryn, Cornwall* .... Ann.
1885 Ewart, Prof. J. Cossar, M.D., *University, Edinburgh* £25

1894 Ferrier, Sir David, M.A., M.D., F.R.S., 34, *Cavendish Square, W.* .... Ann.
1884 Fison, Sir Frederick W., Bart., 64, *Pont Street, London, S.W.* .... *C.*
1897 Foster, Richard, *Windsworth, Looe, R.S.O.* .... Ann.
1885 Fowler, G. Herbert, B.A., Ph.D., *The Old House, Aspley Guise, Bedfordshire* .... Ann.
1884 Fry, George, F.L.S., *Carlin Brae, Berwick-on-Tweed* £21
*1907 Gamble, Prof. F. W., D.Sc., F.R.S., 38, *Frederick Road, Edgbaston, Birmingham* .... Ann.
1906 Gardiner, Prof. J. Stanley, M.A., F.R.S., *Caius College, Cambridge* .... Ann.
1907 Garstang, Prof. W., D.Sc., 2, *Ridge Mount, Cliff Road, Headingley, Leeds* Ann.
1885 Gaskell, W. H., F.R.S., *The Uplands, Shelford, Cambridge* .... *C.*
1901 Giles, Col. G. M., *Looseleigh, Crownhill, Plymouth* .... *C.*
1910 Gooding, H. C., *Ipswich Street, Stowmarket* .... Ann.
1910 Goodrich, E. S., F.R.S., *Merton College, Oxford* .... Ann.
1885 Gordon, Rev. J. M., *St. John's Vicarage, Redhill, Surrey* .... Ann.
1899 Guinness, Hon. Rupert, *Elveden, Thetford* ....£35 15*s.*
†1884 Günther, Dr. Albert, F.R.S., 2, *Lichfield Road, Kew Gardens* .... Ann.
1900 Gurney, Sir Eustace, *Sprowston Hall, Norwich* .... Ann.

1884 Halliburton, Prof. W. D., M.D., F.R.S., *Church Cottage*, 17, *Marylebone Road, London, W.* .... Ann.
1909 Hamilton, Dr. G. C., 21, *Finsbury Circus, London, E.C.* .... Ann.
1884 Hannah, Robert, 82, *Addison Road, Kensington, W.* .... *C.*
*1885 Harmer, S. F., D.Sc., F.R.S., *British Museum (Natural History), Cromwell Road, S.W.* .... *C.*
1889 Harvey, T. H., *Cattedown, Plymouth* .... Ann.
1888 Haselwood, J. E., 3, *Lennox Place, Brighton* .... *C.*
1884 Haslam, Miss E. Rosa, *Ravenswood, Bolton* £20
1884 Head, J. Merrick, F.R.G.S., J.P., *Pennsylvania Castle, Isle of Portland, Dorset* .... Ann.
1884 Heape, Walter, *Greyfriars, Southwold, Suffolk* .... *C.*
1910 Hefford, A.E., B.Sc., 43, *Parliament Street, London, S.W.* .... Ann.
*1908 Hepworth, Commander M.W. Campbell, C.B., R.N.R., *Meteorological Office, South Kensington, London, S.W.* .... Ann.
*1884 Herdman, Prof. W. A., F.R.S., *The Zoology Department, The University, Liverpool* .... Ann.
1884 Herschel, Col. J., R.E., F.R.S., *Observatory House, Slough, Berks.* .... *C.*
1889 Heywood, Mrs. E. S., *Light Oaks, Manchester* .... *C.*
1910 Hicks, F., *Zoological Laboratory, King's College, London, W.C.* .... Ann.
1884 Hickson, Prof. Sydney J., M.A., D.Sc., F.R.S., *Ellesmere House, Wilenslow Road, Withington, Manchester* .... Ann.
1910 Hill, Dr. Alex., *Royston, Herts* .... Ann.
*1907 Hill, Prof. J. P., *The Zoological Laboratory, University College, London. W.C.* .... Ann.
1897 Hodgson, T. V., 54, *Kingsley Road, Plymouth* .... Ann.

*1905	Holt, E. W. L , 46, *Lower Baggot Street, Dublin*	Ann.
1909	Hoyle, W. E., M.A., D.Sc., *National Museum of Wales, City Hall, Cardiff*	Ann.
1891	Indian Museum, *Calcutta*	Ann.
1888	Inskip, Capt. G. H., R.N., 22, *Torrington Place, Plymouth*	Ann.
1885	Jackson, W. Hatchett, M.A., D.Sc., F.L.S., *Pen Wartha, Weston-super-Mare*	Ann.
1910	Jenkinson, J. W., D.Sc., 27, *Polstead Road, Oxford*	Ann.
1911	Kirkpatrick, R., *British Museum (Natural History), Cromwell Road, S.W.*	Ann.
1897	Lanchester, W. F., B.A., 19, *Fernshaw Road, Chelsea, London, S.W.*	C.
1885	Langley, Prof. J. N., F.R.S., *Trinity College, Cambridge*	C.
1895	Lister, J. J., M.A., F.R.S, *St. John's College, Cambridge*	Ann.
1910	Liversedge, Prof. A., F.R.S., *Hornton Cottage, Hornton Street, Kensington*	Ann.
1910	Lucas, Keith, M.A., *Trinity College, Cambridge*	Ann.
1885	Macalister, Prof. A., F.R.S., *St. John's College, Cambridge*	Ann.
1884	MacAndrew, James J., *Lukesland, Ivybridge, South Devon*	Ann.
*1910	MacBride, Prof. E. W., M.A., D.Sc., F.R.S., *Royal College of Science, South Kensington, S.W.*	Ann.
1900	Macfie, J. W. Scott, *Rowton Hall, Chester*	C.
1902	Major, Surgeon H. G. T., 24, *Beech House Road, Croydon*	C.
1889	Makovski, Stanislaus, *Saffrons Corner, Eastbourne*	Ann.
1885	Marr, J. E., M.A., F.R.S., *St. John's College, Cambridge*	C.
1902	Martin, C. H., *The Hill, Abergavenny*	Ann.
1906	Masterman, A. T., D.Sc., *Board of Agriculture and Fisheries (Fisheries Division)*, 43, *Parliament Street, London, S.W.*	Ann.
1910	Matthews, D. J., *The Laboratory, Plymouth*	Ann.
1910	McClean, W. N., 63, *Evelyn Gardens, South Kensington, S.W.*	Ann.
1884	McIntosh, Prof. W. C., F.R.S., *Nevay Park, Meigle, N.B.*	C.
1884	Michael, Albert D., *The Warren, Studland, nr. Wareham, Dorset*	C.
1909	Midgley, J. H., *Grange-over-Sands, Lancs.*	Ann.
1903	Mill, H. R., D.Sc., 62, *Camden Square, London, N.W.*	Ann.
1899	Minchin, Prof. E. A., 4, *Tennyson Mansions, Cheyne Row, Chelsea, S.W.*	Ann.
*1905	Mitchell, P. Chalmers, D.Sc., F.R.S., Secretary Zoological Society, *Regent's Park, London, N.W.*	Ann.
1906	Morford, Rev. Augustin, *The Friary, Saltash, Cornwall*	Ann.
1910	Müller, Prof. Hugo, F.R.S., 13, *Park Square East, Regent's Park, London, N.W.*	Ann.
†1896	Murray, Sir John, K.C.B., F.R.S., *Challenger Lodge, Wardie, Edinburgh*	Ann.
†1884	Norman, Rev. A. M., M.A., D.C.L., F.R.S., *The Red House, Berkhamsted, Herts*	Ann.
1910	Orton, J. H., B.Sc., *The Laboratory, Plymouth*	Ann.
1910	Pennell, Lieut. H., *Awliscombe, Honiton, Devon*	Ann.
1906	Plymouth Corporation (Museum Committee)	Ann.
1910	Plymouth Education Authority	Ann.
1906	Port of Plymouth Incorporated Chamber of Commerce	Ann.
1910	Porter, Horatio, 16, *Russell Square, London, W.C.*	Ann.

1910	Preston, H. B., F.Z.S., 53, *West Cromwell Road, London, S.W.*	Ann.
1884	Pye-Smith, P. H., M.D., 48, *Brook Street, W.*	*C.*

1893	Quintin, St. W. H., *Scampstone Hall, Rillington, Yorks*	Ann.

1892	Rüffer, M. A., M.D., *Conseil Sanitaire, Maritime et Quarentenaire, Alexandria, Egypt*	Ann.

1911	Saunders, J. T., B.A., *Christ's College, Cambridge*	Ann.
1888	Scharff, Robert F., Ph.D., *Science and Art Museum, Dublin*	Ann.
1901	Schiller, F. W., *Butterhill, Stafford*	Ann.
*1909	Schuster, Edgar, D.Sc., 110, *Banbury Road, Oxford*	Ann.
1884	Sclater, P. L., F.R.S., *Odiham Priory, Winchfield, Hants*	Ann.
1884	Sclater, W. L., *Odiham Priory, Winchfield, Hants*	Ann.
1885	Scott, D. H., M.A., Ph.D., F.R.S., *East Oakley House, Oakley, Hants*	*C.*
1903	Scott, S. D., *Thirlestaine Villa, Cheltenham*	Ann.
1884	Sedgwick, Prof. A., M.A., F.R.S., *Royal College of Science, South Kensington, S.W.*	*C.*
1888	Serpell, E. W., *Loughtonhurst, West Cliff Gardens, Bournemouth*	£50
1900	Sexton, L. E., 3, *Queen Anne Terrace, Plymouth*	Ann.
1904	Shaw, Joseph, K.C., *Bryanston Square, London, W.*	£13
1908	Shearer, Dr. Cresswell, 30, *Thompson's Lane, Cambridge*	Ann.
1885	Sheldon, Miss Lilian, *High Park, Bideford*	Ann.
*1884	Shipley, Arthur E., M.A., F.R.S., *Christ's College, Cambridge*	*C. and* Ann., £5
1886	Shore, T. W., M.D., *Woodlawn, Kingswood Road, Upper Norwood, London, S.E.*	Ann.
1885	Sinclair, F. G., *Friday Hill, Chingford, Essex*	*C.*
1891	Sinclair, William F., 102, *Cheyne Walk, Chelsea, S.W.*	*C.*
1884	Skinners, the Worshipful Company of, *Skinners' Hall, E.C.*	£42
1889	Slade, Rear-Admiral E. J. W., R.N., M.V.O., H.M.S. *Hyacinth, East Indies Station*	*C.*
*1910	Smith, Geoffrey W., *New College, Oxford*	Ann.
1888	Spencer, Prof. W. Baldwin, M.A., F.R.S., *University of Victoria, Melbourne*	Ann.
1907	Sprague, Thomas Bond, M.A., LL.D., 29, *Buckingham Terrace, Edinburgh*	Ann.
1897	Straker, J., LL.M., F.Z.S., *Oxford and Cambridge Club, S.W.*	*C.*

*1899	Thompson, Prof. D'Arcy W., C.B., *University College, Dundee*	Ann.
1890	Thompson, Sir H. F., Bart., 9, *Kensington Park Gardens, London, W.*	Ann.
1884	Thornycroft, Sir John I., F.R.S., *Eyot Villa, Chiswick Mall*	Ann.
1906	Tims, H. W. Marett, M.D., 8, *Brookside, Cambridge*	Ann.
1903	Torquay Natural History Society, *Torquay*	Ann.
*1897	Travers, J. A., *Tortington House, Arundel*	Ann.
1910	Travers, R. C., *Tortington House, Arundel*	Ann.

1891	Vaughan, Henry, 325, *High Holborn, London*	*C.*

1884	Walker, Alfred, O., *Ulcombe Place, Maidstone*	Ann.
1884	Walker, P. F., 36, *Prince's Gardens, S.W.*	Ann.
1910	Wallace, W., D.Sc., 43, *Parliament Street, London, S.W.*	Ann.
†1884	Walsingham, The Rt. Hon. Lord, F.R.S., *Merton Hall, Thetford*	£20
1906	Waterhouse, N.E., 3, *Fredericks Place, Old Jewry, London, E.C.*	Ann.
1909	Waters, Arthur W., F.L.S., *Alderley, McKinley Road, Bournemouth*	Ann.

1909	Watson, A. T., *Southwold, Tapton Crescent Road, Sheffield*	Ann.
1906	Weldon, Mrs., *Merton Lea, Oxford*	Ann
1910	Willes, W. A., *Elmwood, Cranborne Road, Bournemouth*	Ann.
1900	Willey, A., D.Sc., F.R.S., *McGill University, Montreal, Canada*	Ann.
1908	Williamson, Lieut. H. A., R.N., *H.M.S. Forth, Plymouth*	Ann.
1884	Wilson, Scott, B., *Heather Bank, Weybridge Heath*	C.
1900	Wolfenden, R.N., M.D., *The Grange, Sidcup, Kent*	Ann.
1905	Woolf, M. Yeatman, *Wimpole House, Wimpole Street, London, W.*	Ann.
1893	Worth, R. H., 42, *George Street, Plymouth*	Ann.

IV.—Associate Members.

1889 Caux, J. W. de, *Great Yarmouth.*
1889 Dannevig, Capt. G. M., *Arendal, Norway.*
1904 Donnison, F., *Deep Sea Fishing Co., Boston.*
1904 Edwards, W. C., *Mercantile Marine Office, St. Andrew's Dock, Hull.*
1904 Freeth, A. J., *Fish Quay, North Shields.*
1904 Hurrell, H. E., 25, *Regent Street, Yarmouth.*
1904 Inskip, H. E., Capt., R.N., *Harbour Master's Office, Ramsgate.*
1904 Johnson, A., *Fishmongers' Company, Billingsgate Market, London, E.C.*
1889 Olsen, O. T., F.L.S., F.R.G.S., *Fish Dock Road, Great Grimsby.*
1904 Patterson, Arthur, *Ibis House, Great Yarmouth.*
1889 Ridge, B. J., *Newlyn, Penzance.*
1901 Sanders, W. J., *Rockvall, Brixham.*
1889 Sinel, Joseph, 8, *Springfield Cottages, Springfield Road, Jersey, C.I.*
1890 Spencer, R. L., *L. and N.W. Depôt, Guernsey.*
1890 Wells, W., *The Aquarium, Brighton.*

OBJECTS

OF THE

Marine Biological Association

OF THE UNITED KINGDOM.

THE ASSOCIATION was founded at a Meeting called for the purpose in March, 1884, and held in the Rooms of the Royal Society of London.

The late Professor HUXLEY, at that time President of the Royal Society, took the chair, and amongst the speakers in support of the project were the late Duke of ARGYLL, the late Sir LYON PLAYFAIR, Lord AVEBURY, Sir JOSEPH HOOKER, the late Dr. CARPENTER, Dr. GÜNTHER, the late Lord DALHOUSIE, the late Professor MOSELEY, the late Mr. ROMANES, and Sir RAY LANKESTER.

The Association owes its existence and its present satisfactory condition to a combination of scientific naturalists, and of gentlemen who, from philanthropic or practical reasons, are specially interested in the great sea fisheries of the United Kingdom. It is universally admitted that our knowledge of the habits and conditions of life of sea fishes is very small, and insufficient to enable either the practical fisherman or the Legislature to take measures calculated to ensure to the country the greatest return from the "harvest of the sea." Naturalists are, on the other hand, anxious to push further our knowledge of marine life and its conditions. Hence the Association has erected at Plymouth a thoroughly efficient Laboratory, where naturalists may study the history of marine animals and plants in general, and where researches on food-fishes and molluscs may be carried out with the best appliances.

The Laboratory and its fittings were completed in June, 1888, at a cost of some £12,000. Since that time investigations, practical and scientific, have been constantly pursued at Plymouth. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council; in addition, naturalists from England and from abroad have come to the Laboratory, to carry on their own independent researches, and have made valuable additions to zoological and botanical science, at the expense of a small rent for the use of a working table in the Laboratory and other appliances. The number of naturalists who can be employed by the Association in special investigations on fishery questions, and definitely retained for the purpose of carrying on those researches throughout the year, must depend on the funds subscribed by private individuals and public bodies for the purpose. The first charges on the revenue of the Association are the working of the sea-water circulation in the tanks, stocking the tanks with fish and feeding the latter, the payment of servants and fishermen, the hire and maintenance of fishing-boats, and the salary of the Resident Director and Staff. At the commencement of this number will be found the names of the gentlemen on the staff.

The purpose of the Association is to aid at the same time both science and industry. It is national in character and constitution, and its affairs are conducted by a representative Council, by an Honorary Secretary and an Honorary Treasurer, without any charge upon its funds, so that the whole of the subscriptions and donations received are devoted absolutely to the support of the Laboratory and the prosecution of researches by aid of its appliances. The reader is referred to page 4 of the Cover for information as to membership of the Association.

CONTENTS OF NEW SERIES, Vol. IX., No. 2.

NOTICE.

The Council of the Marine Biological Association wish it to be understood that they do not accept responsibility for statements published in this Journal excepting when those statements are contained in an official report of the Council.

TERMS OF MEMBERSHIP.

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Annual Members . . .	. per annum	1	1	0
Life Members . . .	Composition Fee	15	15	0
Founders . . .	. . . .	100	0	0
Governors . . .	. . . .	500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, &c.; and have access to the books in the Library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Plymouth.