REGENERATION IN RHIZOSTOMA PULMO.

BY
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WITH 6 FIGURES.

I. INTRODUCTORY.

The several experiments, of which this paper presents a resumé, were conducted during the early summer of 1903, at the Naples Zoölogical Station, while occupying the table of the Smithsonian Institution, for the courtesy of which it is a pleasure to express my obligations.

The primary object of the experiments was to test the regenerative capacity of the Scyphomedusae and to institute certain comparisons between these results and those obtained by similar experiments previously made upon the Hydromedusae. So far as I am aware no similar experiments have been made upon the Scyphomedusae with the definite purpose of testing this particular aspect of their physiological constitution. Romanes in his experiments upon "Primitive Nervous Systems," '85, has recorded incidentally the fact that certain mutilations of medusae are promptly healed, but gave no details. Eimer, '78, has also carried on similar experiments and with the same general purpose of testing the character and distribution of nervous centers, but makes no reference to the matter of regeneration. And quite recently Uexküll, '00, has likewise reviewed these experiments of Romanes and Eimer and carried them somewhat farther than they had done. But while arriving at somewhat different conclusions, drawn from a series of experiments in some features coincident with those to be described now, he makes no reference to any regenerative processes, devoting attention almost exclusively to the movements, specially those of rhythmic character, and seeking physical explanations of them.

The earlier references of Haeckel to the capacity of larvae of certain medusae to regenerate entire organisms are likewise indefinite. Morgan in referring to the subject in his recent book on "Regeneration," '01, merely remarks that among Scyphozoa 'the jelly-fishes belonging to this group have a limited amount of regenerative power."

I very much regret that an unusual scarcity of material compels me to leave several points somewhat less fully considered than is desirable, but I trust they are not of sufficient gravity to seriously mar the general value of the results as a whole.

In one respect this scarcity of material, making necessary successive experiments on the same specimen in many cases, proved fortunate rather than otherwise, since facts of importance were thus brought to light which might otherwise have been overlooked. Some of these will be referred to specifically in another connection.

II. EXPERIMENTAL.

The experiments were performed upon Rhizostoma pulmo, one of the most common of the Mediterranean medusae. Both in size and vigor this medusae affords one of the most satisfactory forms for experimentation which has come under my observation. It seems likewise to suffer less under the somewhat artificial conditions of the aquarium than any other which I have had occasion to use. As compared with Aurelia and Cyanea of New England waters it is incomparably superior in every way, but particularly in its ability to thrive for weeks in an environment which would prove fatal to the others in as many days. With the single exception of Gonionemus I know of no other medusa which affords so good a type for this sort of observation and experimentation. It was not unusual to have specimens under direct observation in the ordinary aquaria of the laboratory rooms for from four to six weeks and without apparent deterioration, even in some cases under the severe tax of extensive mutilation made necessary by the experiments to which they were subjected. It should be stated however that as a rule younger and smaller specimens proved much better than those of larger size; the latter, on account of

their greater mass, are inclined in most cases to sink toward the bottom of the tanks, where after a time certain disorganizing influences appeared to set up pathologic conditions which seemed to deplete their vigor and at the same time render their regenerative processes less satisfactory.

The experiments were directed to three ends, namely to determine: 1, The capacity of the medusae to reproduce lost parts, or to recover from such injuries as might ordinarily happen to them in a state of nature, such as the battering effects of waves, the injuries inflicted by enemies, etc.

2, The comparative powers of the various regions to regenerate, or in other words, the relation of the regenerative capacity to liability to injury.

3, The capacity to regenerate such highly specialized organs as rhopalia, or other sensory structures.

The experiments included specimens of sizes from about 20 m/m to 125 m/m in diameter, and while all proved to have unexpected powers of regeneration those of medium size, from 40 to 70 m/m, proved very much more satisfactory than those of larger size both in convenience and in their promptness in responding to the several sorts of operations, and they apparently were more healthy and vigorous during the progress of the experiments than were those of larger size. Those having a size of 100 m/m or more in diameter proved to be much less prompt in regeneration and, as will be seen in the records of experiments, were much more liable to deteriorate or utterly collapse than were the smaller specimens. This is only what might be more or less expected, and is quite in keeping with observations on other classes of organisms. The same tendency was more or less evident in specimens on exhibition in the public aquarium in which of course no mutilations or similar injuries had occurred. In this connection may be noted a somewhat anomalous pathological phenomenon observed in large specimens both in the exhibition aquaria and in the small aquaria during the course of experimentaton, namely, the appearance of whitish blotches, or patches of disintegrating tissues at various places on the exumbrella of the animal which sooner or later affected its health and general behavior.

The matter will be referred to in further detail in another connection and some reference made as to its probable significance and cause.

In all cases the primary experiments were made as soon as possible after the medusae were brought into the laboratory. I have said the *primary* experiments. This refers to the fact already alluded to, that in several cases experiments were variously repeated upon the same specimen. This was in part for the purpose of testing the conclusiveness of preceding experiments, and in part owing to the fact that there was an insufficient supply of material to serve the demands of the course of experiments under way. Details as to these aspects will be given in connection with the several experiments described.

The first experiment was made upon a large specimen, and in order to determine at the outset whether the earlier observations of Romanes and others, that complete removal of the marginal sense organs resulted in complete paralysis of the medusa, these organs were carefully removed by means of triangular incisions as indicated in Figure 1, *a*. The results were substantially confirmatory of the earlier records, the medusa becoming more or less passive, except for an occasional single contraction at very irregular intervals. This experiment was made on May 11, and the following series of observations will suffice to show the general course of events. It should be added in this connection that along with the excision of the rhopalia several other marginal excisions were made, and that three of the oral arms were cut off close below the region of the gastric enlargement. The aspect of the specimen on the next day was practically the same. While there was an occasional contraction of the bell accompanied by certain movements of the body, there were no indications of rhythm.

May 13th.—The medusa, while apparently in perfect health and vigor of general functions, was still unable to originate any definitely rhythmic movements, though responding to various mechanical stimuli, such as a strong current of water from the tap, or the touch of a glass rod. At various times during the day there was evident a rather marked tendency toward spontaneous movements, and occasionally something very like a rhythm,

several contractions following each other in regular succession, though never continuing beyond three or four pulsations.

May 14th.—The medusa, while still more or less passive as before, was yet apparently recovering more of the power of spontaneity, several pulsations occurring at more frequent intervals, but these were not of sufficient vigor to produce any locomotion.

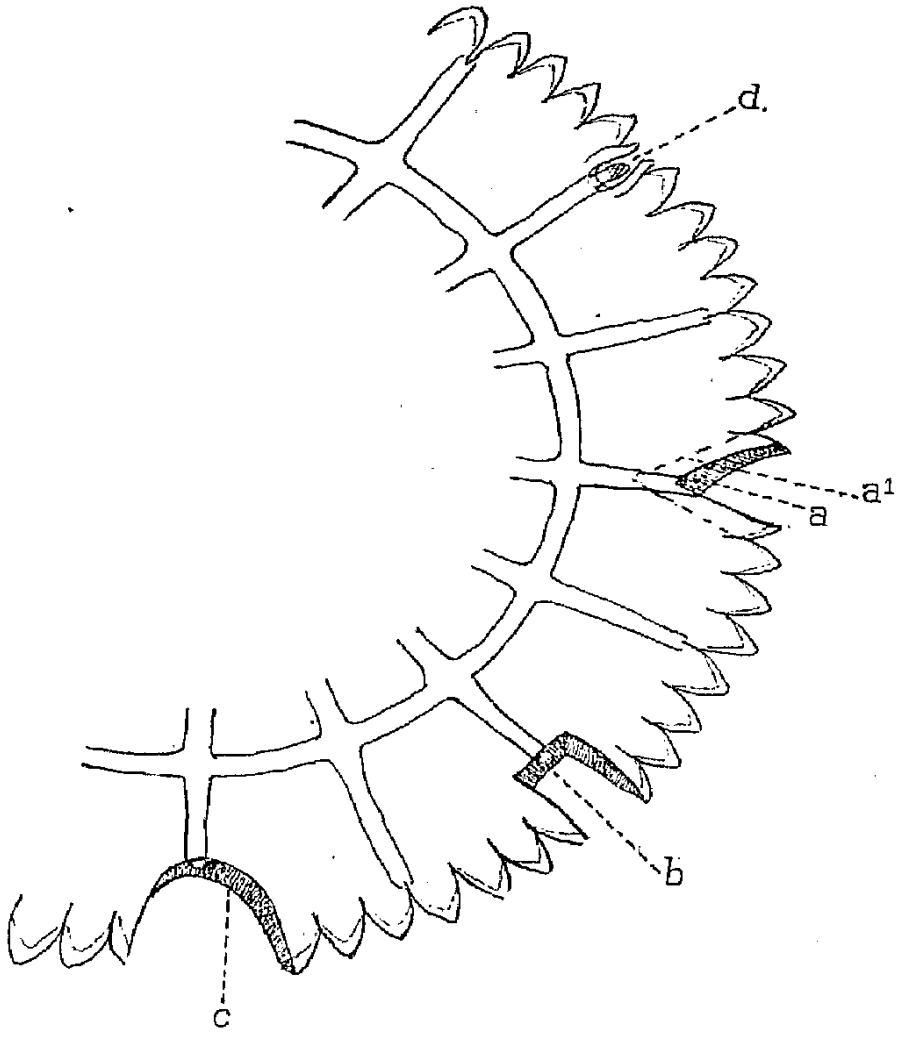

FIG. 1.

Diagram showing methods of excising rhopalia.

a, usual triangular excision; a^1, excision of larger mass; *b*, rectangular form of excision; *c*, circular form of excision; *d*, form of rhopalium and lappetts.

May 15th.—During this and the following day there was an apparent relapse of the medusa to the condition of the first day. There was also less vigor apparent, such stimuli as those referred to above producing but slight effects. This condition continued during the 18th, 19th and 20th.

May 21st.—The medusa seemed to have recovered the vigor or tone to which reference has been made above. There was also a very evident rhythm in the contractions, often as many as ten or more regularly recurring pulsations occurring at irregular intervals during the day. As before, however, they were not of sufficient force to secure the locomotion of the animal. The same condition was observable during the following day.

May 23d.—There was again a marked decline in both vigor and general tone of the body, which showed evident signs of degeneration. This condition continued during the following day, and on the morning of the 25th the medusa was found to have died during the preceding night.

Upon careful examination it was found that wherever tissue had been mutilated or excised there had been a definite healing of the wounds and in the case of the oral arms there were indications of new growth. I was not able to distinguish that there had been any regeneration of the sensory organs, and this will appear somewhat surprising in the light of the following experiments. Whether there had really been no regeneration at all, or that I had overlooked the new organs, or whether they may have disintegrated during the night following the death of the medusa I am unable to say. Certain it is, however, that if regeneration had gone forward as markedly as in the following cases one could hardly have failed to distinguish it. I am inclined to believe that the paralysis following the total removal of these organs may have served to delay or inhibit active regeneration.

The next series of experiments differed materially from the former, particularly in that care was taken to retain certain of the rhopalia in order to insure continued activity of the organisms during the progress of the experiment. The number of rhopalia retained varied from one to eight, the latter case serving as a means of testing the relative influence of these bodies on the behavior of the animals and the rate of regeneration.

On May 12th several specimens, averaging only about half the size of the preceding, namely, about 50 m/m in diameter, were experimented upon. In the first one all the rhopalia were retained, but marginal notches were made of varying sizes between the

sensory bodies, and several of the oral arms were excised. In other specimens a varying number of the rhopalia were excised, and in one case all the oral arms were cut off close to the gastric enlargement and on one side including a portion of this organ itself.

I shall not undertake to transcribe in detail the records of each day, but give rather summaries of results as briefly as is compatible with clearness, trusting that nothing of importance may be sacrificed in the attempt to bring the records within as brief compass as possible.

One of the first effects distinguishable in these and following experiments was the evident quickening of the pulsations of the medusae by the process of excision of the organs, or similar operation. Not only was the rate of the rhythm greatly increased, passing from about seventy pulsations per minute as an average for medusae of this size, to ninety, or even one hundred per minute. And this rate continued during the entire day, or at every observation, which was quite frequent, and well on into the second day, when the rate fell to ninety and later to eighty; but it was not till the third day that the rate had fallen to the normal of seventy per minute. An examination at this time showed an evident healing of the wounds and some signs of regeneration. Had this been restricted to the sensory bodies it might have been interpreted as signifying some important relation of these organs to rhythmic activity, but the fact that similar effects were produced upon specimens which had not been deprived of their rhopalia would sufficiently negative such an inference.

Eimer, '74, had noted such an effect following a division of medusae, particularly those which had been divided into halves or fourths, and had undertaken to show that it was chiefly an expression of the reduced size of the organism due to its division, citing the normal rhythm of specimens of varying size as strongly suggesting such an inference.

Romanes, '85, however, was not able to confirm Eimer's contention either in reference to matter of fact or the cause assigned. Romanes, while citing the variation as to the rate of rhythm in

specimens of similar size, is inclined to emphasize what he terms the prepotent influence of certain of the lithocysts (rhopalia) in coördinating the rate of movement, and the presence or absence of such prepotent organs in the portions of medusae under examination.

Forbes, '48, had long previous called attention to the fact of these quickened movements under the influence of various stimuli, citing particularly a result of an experiment which he had made of a similar character to those which I have cited above. In an experiment in which he had, as he expresses it, "paralyzed one half of the animal" by cutting out the rhopalia from one side, he finds "that the other half contracted as usual, though with more rapidity, as if the animal were alarmed or suffering." He remarks farther that "all medusae when irritated become much more rapid in their movements and contract or expand their disks or bodies in a hurried and irregular manner, as if endeavoring to escape from their persecutors." (Naked Eyed Medusae, p. 3.)

While in certain details the conclusions of Forbes may be questioned, of his general observations as to matters of fact there can hardly be doubt. Furthermore, whether the suggestions of either Eimer or Romanes are more than approximate guesses, the later observations of Uexküll have rendered doubtful. So far as my own experiments have gone they hardly touch the problem of the cause of such reactions. We may safely conclude that, in any case, they are of the nature of responses to any continued physical stimulus, such as the experiments under consideration certainly were. With the healing of the wounds there would of course ensue a decline of the irritation, which in turn would be followed by a return to the normal rate of rhythm.

On May 26th, or two weeks following the operation, the medusae had measurably regenerated all the excised organs. The notches cut in the umbrella margins had grown out to complete the normal symmetry and there had been developed in the areas the characteristic purple pigment, differing from the color of the uninjured portions only in its intensity. The new rhopalia were apparently normal in everything save size and pigmentation.

It is rather noteworthy that in these experiments certain of the

organs which among the Hydromedusae are most promptly regenerated are here among the most slow to develop; such, for example, as the oral arms and gastric lobes. The fact that in the rhizostomous medusae these organs have no very active function in the capture of food might apparently afford some plausible reason for this difference in the rate of regeneration. In Gonionemus the gastric and oral organs are among the most prompt in regeneration, and are, of course, also among the most important in the functional activities of the animal. That this, rather than liability to injury, should be a predisposing factor in regeneration would seem to be confirmed in the case of Rhizostoma, for as will appear in later experiments there seems to be no good reason to suppose that the liability to injury, to which these organs are constantly exposed, has anything to do with the capacity for rapid or perfect regeneration.

Additional experiments were begun on May 28th and 30th. In this series the specimens varied in size from 20 to 60 m/m in diameter. As remarked above there was in these cases the same degree of promptness in the responses, which was markedly in contrast with that shown by specimens of considerably larger size, but in the present cases there was also apparent a somewhat less favorable response in the very small specimens. This fact considered in connection with the difficulty of operating easily upon small specimens, emphasizes the value of animals of medium size for such experiments. This conclusion was emphasized throughout the entire course of experimentation.

In part of the specimens of this series only three rhopalia were excised, in others four, in others five. In some the rhopalia were all removed from one side, while in others only alternate organs were removed. In some specimens the same order was observed as to excision of mouth arms and other similar operations. One of the specimens of the series had only one full-sized mouth arm, while the others were in what seemed to be various stages of regeneration. As is well known these organs among medusae of this type are among the most open to accident from attack of fishes or other predatory enemy. The specimen under consideration would seem to confirm the results of these experiments that

these organs are readily regenerated, and that in a state of nature as well as under the artificial conditions of the laboratory. An examination made with the hand lens on June 2d, or only four or five days following the operation, showed the first indication of regenerating rhopalia. As the organ first makes its appearance it is a very minute papilla-like body, and in these cases at the inner, or upper edge of the notch made by the incision. Examined under the compound microscope the papilla appears as a minute, solid bud growing out from the terminal region of the radial canal, though it does not at first seem to be a direct outgrowth of that organ. Very soon, however, there is established a direct connection with the canal, and it is quite easy to distinguish the circulation of the gastric fluid in the little bud, which becomes definitely vesicular, as shown in Figure 2. The growth of the organ, after its vesicular stage is established, is quite rapid and there can soon be distinguished the thickening of the terminal portion to form the lithocysts. Coincident with this stage of development there is discernible the development of the new hood and lappets, accessory organs, and as will be shown in connection with a study of the histology of these organs, the corresponding development of the so-called olfactory and ocellar pits.

In connection with the present series the following experiments were made with a view to demonstrate that, not only in form but in function, the new rhopalia were perfect organs. From one of the specimens just described in which three rhopalia had been originally excised the other five were excised on June 5th, or seven days after the original experiment. If the three regenerated organs had not yet attained to functional utility the effect of removing the others would, of course, result in the typical paralysis, as in the first experiment already described. As was anticipated, the careful removal of all the rhopalia except the three regenerated ones did not in the least interrupt the normal rhythm or activity of the creature, save to act as a stimulus to quicken it, as already cited in connection with a previous series. This experiment was repeated upon several others of this as well as subsequent series, and always with the same results, except in a single case which may as well be cited in this connection, though coming under later experiments.

In this case the original operation had removed six rhopalia, leaving but two. Soon after the appearance of the new rhopalia, but before they had begun to approach complete development, or before there was any indication of the presence of lithocysts or pigment, the two original organs were carefully removed, and in this case with what might likewise have been anticipated, namely, the complete inhibition of the normal rhythm and the consequent paralysis of the organism. This inhibition continued during the

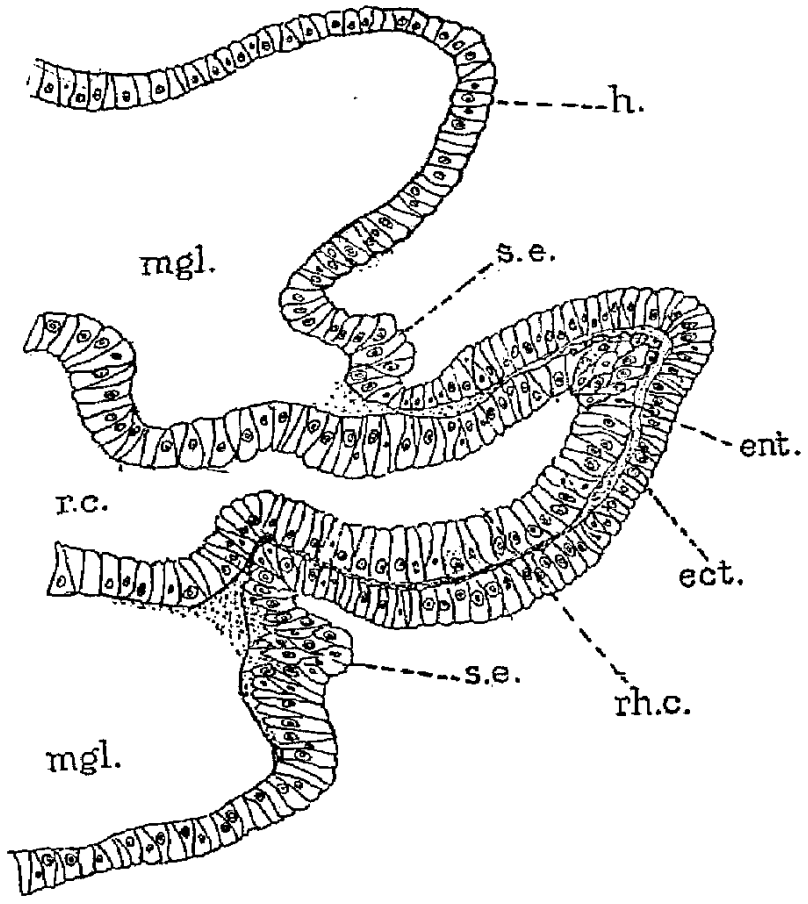

FIG. 2.

Section of rhopalium in early stage of regeneration. ect, ectoderm; ent, entoderm; h, hood; mgl, mesogloea; r. c., radial canal; s. e., sensory epithelium.

following two days. With the continued development of the new rhopalia activity was recovered, though, owing to the interposition just at this juncture of an unhealthy condition of the medusa, it failed to entirely recover the usual vigor or tone which the others had shown.

These experiments, abundantly corroborated by subsequent ones, leave no shadow of doubt, it seems to me, as to the capacity of

these organisms to regenerate in the last detail one of the most highly specialized organs known among Coelenterata. This will be shown more fully in connection with the later account of the histology of the regenerated organs.

Other series of experiments, continued to June 20, while varied in some aspects of detail, were of substantially the same character and with results quite similar to the preceding.

In several of the experiments care was taken to so modify the form and extent of the excised portions as to secure evidence as to the influence of contiguous tissues or parts upon the regenerating organs. In Figure 1 is shown, for example, several aspects of the mode of excising the rhopalia. For the most part the excision was in the form of a triangular cut from the margin inward toward the radial canal, as shown in the figure. The dotted line a^1 will show also in the same connection the occasional extension of the cut to include twice the usual mass. In Figure 1, *b*, will be seen another form of operation. In this case the portion cut out was

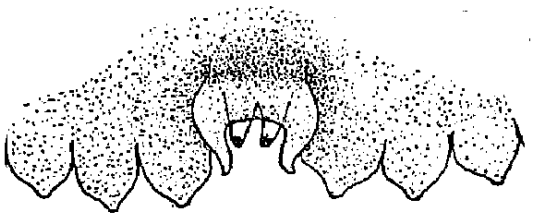

FIG. 3.

Twin rhopalia regenerated in place of the single original one.

rectangular instead of triangular, as in the former. The mass excised in the operation also varied as before. At *c*, in the same diagram, may be seen another form of excision in which the cut was circular instead of angular, as in the former cases. It is interesting to note that, so far as I was able to determine, the form of the excision had no perceptible effect upon the form or rate of regeneration. In the case of the rectangular or circular excisions the new organ appeared in its typical place at the median position of the upper portion of the notch. In the case of the large or small portions excised in the triangular cuts not the slightest difference could be distinguished. With the exceptions of some two or three cases to be considered, there was not the

slightest evidence of any deviation from the exact position occupied by the original organ.

The apparent exceptions referred to are as follows: First, that in at least two cases twin rhopalia were developed instead of the single original one which had been excised. This is well shown in Figure 3. Second, that in one case two rhopalia were regenerated instead of the one originally excised, but unlike the preceding, they appeared at different points — one in the usual position at the upper angle of the notch, the other at the lower, or marginal portion of the notch, as shown in Figure 4.

The mere fact of the occurrence of double rhopalia during regeneration instead of single ones is not of itself particularly remarkable, for the occurrence of such features is not an unusual one in a state of nature, both ephyrae and adult medusae being occasionally found with such double organs. Some further inquiry should, however, be directed to the peculiar position in which the organ noted in Figure 4, at *a*, occurs, namely, at one side of the notch and near the margin instead of the usual position. On the assumption that these organs are of sensory function and correlated with marginal nerve centers it might be

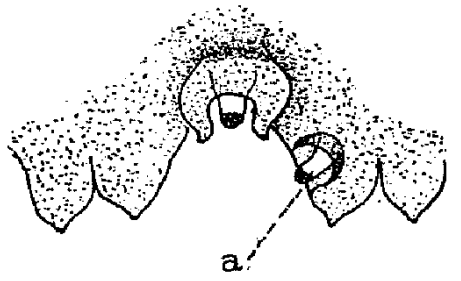

FIG. 4.
Two regenerated rhopalia; a, near the margin.

thought that in regeneration they would be likely to occur in close relation with such centers, and that the case under consideration might be thus explained. The fact is very clear, however, that such is not the case with the vast majority of the experiments where apparently the relation of nerve centers had nothing whatever to do with their position in regeneration. And when furthermore we reflect that these are not nervous organs in any true sense, either in their origin or development, though possibly correlated with

some sensory function, it must be more or less evident that such an explanation of the single case cited would hardly hold.

Nor would it perhaps be more satisfactory to appeal to what has been designated as polarity in explaining either series. The occurrence of the organs in conjunction with the radial canals and their apparent differentiation from terminal portions of these structures would seem to afford a much more probable explanation of their regeneration at these apparently predetermined positions. And may we not find in this view a simple explanation of the occurrence of the anomalous case referred to in Figure 4, *a*, for we find near the margins a more or less complex network of anastomosing canals, the presence of one of which may have been the inciting cause of the development of a sensory body at this particular point.

It is interesting to note in this connection that no appearance of heteromorphism occurred during the entire series of experiments. This feature I have referred to in a previous paper, '97. in connection with similar work on Hydromedusae. On the assumption that these organs are metamorphosed tentacles we migh naturally look for heteromorphic phenomena similar to that recorded among the crustacea, in which occasionally instead of an eye an antenna develops. Nothing of the sort, however, occurred. There seems in every organ and tissue a remarkably inflexible physiological constancy. This is the more remarkable when contrasted with the highly flexible character of the polyp phase of the group among which are found the widest range and variety of heteromorphism.

The fact is not overlooked that Rhizostoma is devoid of tentacles, which might be assumed as sufficient reason why heteromorphism of this sort was not manifested. The fact remains, however, that its polyp has the typical tentacular equipment, and that in its metomorphism they are resorbed and possibly take the usual course, some of them contributing toward the formation of rhopalia. It might be an interesting problem to determine in detail just the extent of this supposed metomorphism of the polypal tentacles into rhopalia. May it not be possible that the supposed metamorphism is in reality a resorption and that only,

and that the rhopalia are essentially independent developments such as are found during the process of regeneration? I merely raise the suggestion as it has been forced upon my attention in course of these experiments. It seems worth farther investigation.

In this connection may be briefly described a phenomenon which only came under critical observation late in the course of the experiments, and which for lack of material it was impossible to follow out to conclusive results. Among the last of the series two large specimens were operated upon as follows: In the first all but one of the rhopalia were excised, while in the second all but two were removed. In both cases there was distinctly noticeable an aberrant, rotary sort of swimming movement, the animal revolving in an irregular circle, instead of directly forward or upward as is usual. Examination showed that this inclination of the body in swimming was constantly in the direction of the remaining rhopalia, which would seem to suggest that perhaps they functioned something after the nature of equilibrium organs. I do not recall that this feature has been referred to by the investigators previously cited, and very much regret that it was not practicable for me to carry out such additional experiments as would have afforded more definite conclusions. It must suffice to merely mention the matter, hoping that at some time someone may be able to secure definite conclusions by extended experiments not only upon this medusa but perhaps on others as well.

III. ABNORMALITIES.

In connection with observations upon several specimens which had become degenerate or perhaps pathologic, resulting from unfavorable conditions of some of the aquaria, or perhaps in some cases due to the depleting effects of the experiments, as in the case of the first experiment cited in this paper, occasion was taken to examine somewhat in detail the observations and experiments of Uexküll and to compare cases coming under my own observations during the course of the experiments.

In one specimen which had shown evident decline of vigor and upon which there appeared certain exumbrellar blotches or cor-

rosion patches, similar to those mentioned in the earlier portion of this paper and comparable in general aspects to cases mentioned by Uexküll, it was found that after all the rhopalia had been removed the specimen yet exhibited certain convulsive contractions which at times simulated an irregular rhythm. I therefore undertook to repeat several of this observer's experiments as to the effects of certain chemical stimuli, specially that of common salt, NaCl. Small crystals of this salt were carefully placed on definite parts of the sub-umbrellar musculature, and I was able thereby to confirm in the main his results. There was a very evident white coloration of the adjacent tissues, and this was followed by a more or less definite, though somewhat irregular, rhythmic contraction of the umbrella which continued for perhaps five minutes. The experiment was repeated several times and upon different specimens and with usually similar results, though differing as to vigor or continuity.

Uexküll had concluded that the recovery of a similar rhythm in specimens upon which he had experimented by excising the rhopalia was due, not to any direct restoration of nervous or other normal equilibrium, but to certain pathologic conditions which had intruded themselves, and among which he was specially impressed by these corrosion abscesses or disease patches, to which reference has been made. Doubting whether an agent of this sort, affecting particularly the exumbrella, could have any very definite importance as a center of stimulus, it occurred to me to vary the experiment by applying the salt to the exumbrellar region instead of the musculature of the sub-umbrella, and though variously repeated the results were uniformly negative in character, no conclusive responses of any sort being obtained. Nor was there observed any of the whitening effects which were so evident in the previous experiments. We may conclude, it seems to me, that the effects produced by the salt in arousing a simulated rhythm of contraction was due to the direct action of the substance on the musculature itself, and not to any general effect produced upon the coördinating centers of the medusa. These stimulating effects of sodium chloride upon muscular tissue are too well known to call for any special mention in this connection.

It would seem, therefore, that in the light of these facts one may well question the validity of Uexküll's conclusions, or rather inferences. The mere presence of whitish blotches on an organism would hardly justify, without the most conclusive demonstration, the inference that the presence of similar effects produced by some reagent proved them identical or even analogous. That there may have been certain pathologic conditions operating upon these medusae of which the whitish blotches were in some respects expressions may have some measure of probability. But that these blotches were in themselves the inciting stimuli giving rise to the simulated rhythm must be regarded as doubtful, if not indeed, highly improbable. Such a conclusion could hardly have been suggested had it been observed that the same whitish blotches are not unusual on specimens which have been for some time in aquaria. Moreover, their presence on such specimens has not in the least, so far as my own observations have gone, served to introduce any variation of the normal rhythm, a condition which might not be unusual on the assumption of these disease patches becoming sources of abnormal stimuli, and thereby introducing erratic or conflicting factors into the physiological processes of the organism. It is well that attention should be directed to disturbing conditions of this character in order that undue weight be not given to a single factor in determining so important a problem. On the other hand it may be quite as important that in discrediting one conclusion there is not substituted another of even less value.

One might be tempted in this connection to go somewhat out of the way to consider Uexküll's conclusions as to the purely mechanical function of the rhopalia in relation to the rhythmic action of the umbrella of medusae. If they might be supposed to act after the fashion of the clapper of a bell, using his figure of comparison, in the case of such medusae as Rhizostoma what explanation shall we have for the identical rhythm exhibited by many other medusae entirely devoid of rhopalia or any equivalent organ? Many other objections will immediately arise when one reflects upon the very different histological conditions of structure found in these organs in various medusae, but to take up any one

of these and other phases of the problem would lead too far afield, and we must satisfy ourselves for the time by the reflection that while such speculations are interesting as well as ingenious they are far from demonstrations.

IV. HISTOLOGY.

A brief study of the histology of the regenerated organs shows the various stages of the process and establishes beyond doubt a true histogeny, though it has not been possible to demonstrate the details of mitosis in the proliferating cells. This may be due in part to lack of just those refinements of technique necessary to bring out these features. Some of the tissues were fixed by means of Flemming's solution, some by corrosive-acetic acid, and still others in 10 per cent. formol in water. I have not been able to distinguish that there was any appreciable advantage in the one over the others, the formalin seeming to afford equally good fixation and preservation. Heidenhain's iron haematoxylin and an aqueous solution of haematein both afforded fairly good differentiation, though they failed as to the nervous tissues, a result which was not unexpected.

In Figure 2 is shown a longitudinal section of a regenerated rhopalium at a comparatively early stage, when first distinguishable as a minute papilla. In an earlier part of the paper I have referred to its early appearance as having the character of a solid bud from the upper angle of the notch made in the process of excising the organ. From an examination of this figure, which is among the earliest stages I have been able to satisfactorily section, it would seem that in its origin it probably follows the usual process of the regeneration or development of such organs in the coelenterates, namely, that of budding, involving both ectoderm and entoderm. As shown in the figure, there is here a typical outgrowth from the distal end of the radial canal and, as also mentioned in another connection, it was easy to demonstrate at about this stage of development in the living medusa an active circulation in the bud. The cells of the ectoderm at this stage are of approximately uniform size over the entire organ, and the same is also the case with the cells of the entoderm. There

seems also to be present the middle lamella, though less sharply defined than at a somewhat later period. There appears to be a rapid proliferation of the cells of the entoderm near the terminal portion where they form a mass as shown in the figure, though, as mentioned above, it was not possible to distinguish evidence of mitosis.

It is interesting to note at even this early stage the incipient phases in the regeneration of the sensory areas (Fig. 2, *s. e.*) just above and below the rhopalium. The regeneration of the hood is also shown at *h.*

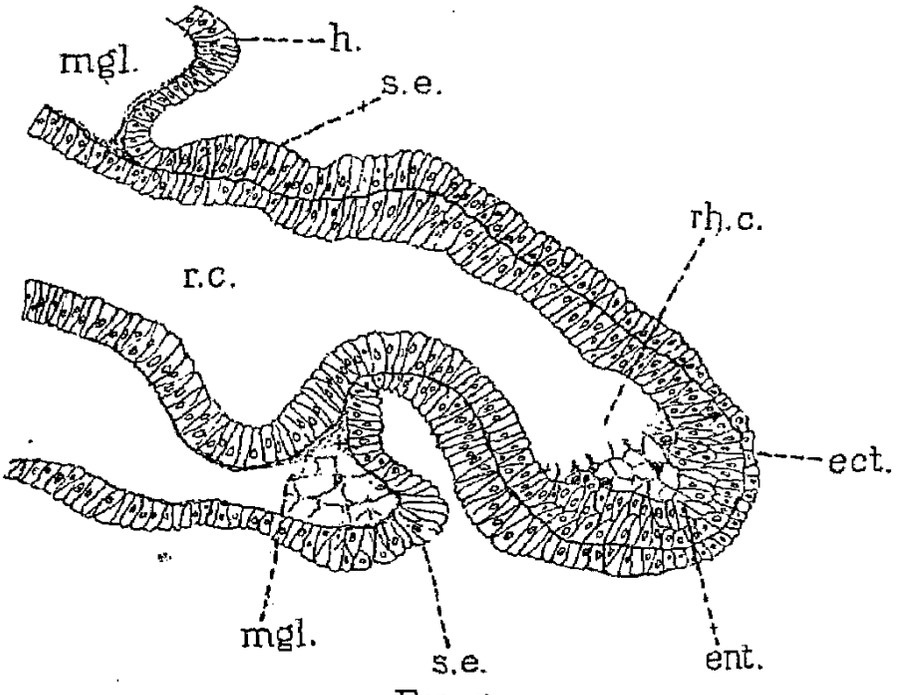

FIG. 5.

Section of regenerating rhopalium. rh. c., rhopalial canal; other letters as in Fig. 2.

In Figure 5 is shown a section taken in the same plane as the former, but at a somewhat later stage of development. The rhopalium has apparently attained nearly full size, but lacking as yet any development of otoliths, though in the network shown at *rh. c.* there is apparently evidence of a differentiation preparatory thereto. There is also shown here the thinning out of the ectoderm of the distal portion of the organ as seen at *ect.*

The sensory areas and epithelium above and below are here seen to have acquired almost their typical form and character as

shown at *s. e.* The hood is also shown at *h,* not having apparently kept pace with the growth of the other organs. Here as before the direct connection of the radial canal with the rhopalium is quite broad and characteristic. The middle lamella, or mesogloea, is shown at *mgl.* above and below, in the latter elements of a loose network being traceable, with the embedded cells, which can also be found indefinitely scattered throughout the jelly.

In Figure 6 we have a section through an almost mature rhopalium, taken in the same plane as the others, only the organ

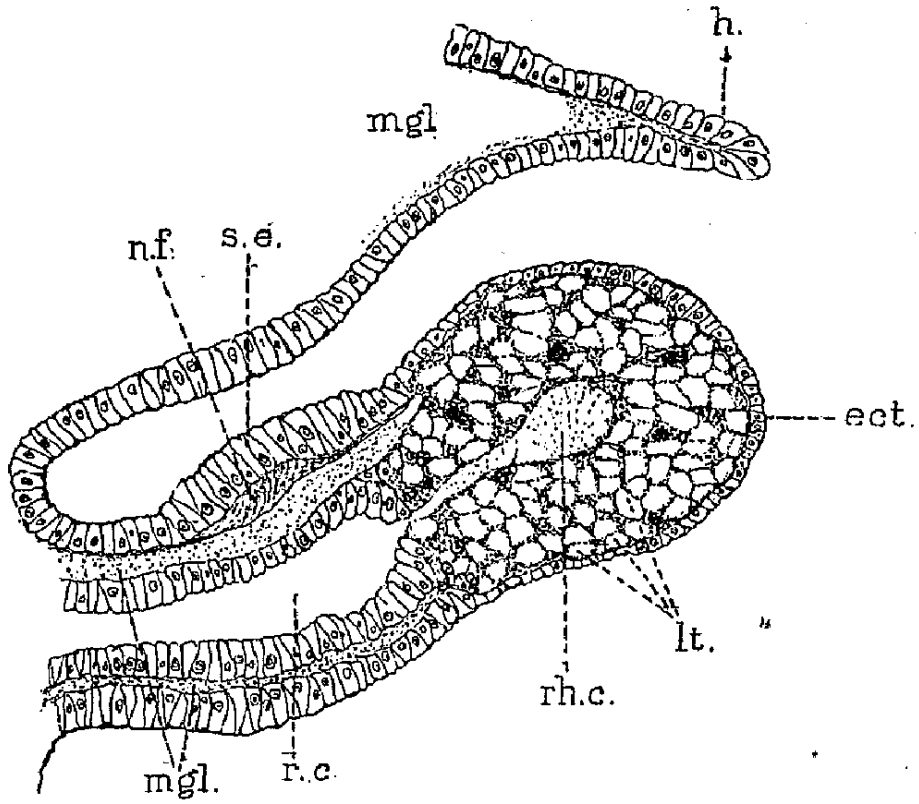

FIG. 6.

Section of regenerated rhopalium, approaching maturity. lt, lithocysts; nf, nerve fibers; other letters as in Figs. 2 and 5.

itself with the terminus of the hood being shown. The ectoderm has become practically uniform over the entire distal portion of the organ, but as it approaches the base of the area of the lithocysts, shown at *lt.* and generally throughout the entire distal part, it becomes columnar. At *s. e.* it forms a definitely arched portion, the sensory epithelium, beneath which at *n. f.* is the so-called nerve fiber area of the nerve center of this region. While it is quite possible to distinguish a more or less fibrous character as

shown in the figure, it has not been possible to trace these fibers into any cellular plexus, or ganglion, such as has been claimed to exist here. Since however my observations in the present instance have been almost entirely restricted to phases of regeneration, it will not be pertinent to discuss the question farther.

As will be seen there is still a continuous connection between the cavity of the distal portion of the organ and the radial canal. This connection Hesse, '95, has shown in figures of normal organs in maturity, but in the present examinations I have found it when fully regenerated to become entirely solid throughout the lithocyst region, the radial canal ending abruptly at its basal end, which is shown almost closed in the figure under consideration.

In the rhopalial cavity, *rh. c.*, which at this time is nearly spherical, there is present a radiating network of delicate fibers, poorly shown in the figure, which seem to diverge from a point on the lower surface and extend entirely across the cavity apparently attaching to the opposite wall. I should consider these fibers of the same nature as those shown in Figure 5 near the terminus of the line *rh. c.*. Though it has not been possible to critically trace the details of the process it seems entirely probable that the entodermic epithelium of this region becomes gradually differentiated into fibers which form the intricate network within which the lithocysts are later deposited. Within this network may be found during the various stages of development the gradual metamorphosis of this entodermic cell mass, the nuclei of the cells often remaining as permanent elements of the organ. Some of the more prominent of these are shown in the figure, and phases of the metamorphosis may be detected near the narrow slit-like canal, just beyond the terminus of the radial canal.

Within the network may also be traced the deposition of the pigment characteristic of the organ.

Concerning the histology of the regenerated oral and gastric organs it has not seemed essential to make special inquiry, since in what has already been shown in connection with the more highly differentiated tissues of the marginal organs it would seem that no serious doubt can remain as to normal histogenic pro-

cesses probably occurring throughout every regenerating organ in this medusa.

It was pointed out in connection with the description of certain experiments that both in form and in function we have among the Scyphomedusae a regenerative capacity extending to the most highly specialized organs. In the subsequent account of the histology of the regenerated organs it has been shown that the process is a perfectly normal and characteristic one, conforming in apparently every detail to the course of development of the embryonic history of the several organs.

LITERATURE CITED.

EIMER, TH.—Über Künstliche Theilbarkeit von Aurelia aurita und Cyanea capillata in Physiologische Individuen. Wurzburg, 1874.

" Die Medusen Physiologisch u. Morphologisch auf ihr Nervensystem. " Tubingen, 1878.

" Organic Evolution. English Translation. Cunningham, 1890.

FORBES, EDW.—British Naked Eyed Medusae. London, 1848.

HARGITT, C. W.—Recent Studies in Regeneration. Biol. Bul. Vol. I, 1897.

" Experimental Studies upon Hydromedusae. Biol. Bul., 1899.

HARGITT, G. T.—Notes on Regeneration in Gonionemus. Biol. Bul., 1902.

HESSE, R.—Uber das Nervensystem u. d. Sinnesorgane v. Rhizostoma Cuvier. Zeits. f. wiss. Zool., 1895.

MORGAN, T. H.—Regeneration. Macmillan, 1901.

ROMANES, G. J.—Jelly-fish, Star-fish and Sea Urchins. 1885.

ÜEXKÜLL, J. VON.—Die Schwimmbewegung von Rhizostoma pulmo. Mitt. Zoöl. Sta. Neapel., XIV, 1900.

Syracuse University,
The Zoölogical Laboratory,
January 20, 1904.