11) *Arbacia* ♀ × *Sphaerechinus* ♂. Es entstehen Blastulae mit Haufen von degeneriertem Chromatin, von denen einzelne gastrulierten und am 6. Tag Gastrulae mit umfangreichem, jedoch unregelmäßigem Skelet lieferten.

In Tab. II sind die Beobachtungen bei den eingehender untersuchten Bastarden zusammengestellt.

Tabelle II.

Ech. ♀ × *Strong.* ♂	ohne Chromos.-Elimination	Entwicklung normal	Plutei
Strong. ♀ × *Ech.* ♂	do.	do.	do.
Strong. ♀ × *Sphaer.* ♂	Elimination auf 21 Chr. während der ersten Teilungen	Erkrankung in frühem Blastulastadium	Plutei. Skelet mütterlich.
Sphaer. ♀ × *Strong.* ♂	ohne Chromos.-Elimination	Entwicklung normal	Plutei. Skelet mit Mischcharakteren.
Ech. ♀ × *Sphaer.* ♂	Elimination auf 21 Chr. während der ersten Teilungen	Erkrankung in frühem Blastulastadium	Plutei. Skelet mütterlich.
Sphaer. ♀ × *Ech.* ♂	ohne Chromos.-Elimination	Entwicklung normal	Plutei. Skelet mit Mischcharakteren.
Strong. ♀ × *Arbac.* ♂	Chromatin-Eliminiation im Blastulastadium	Erkrankung im Blastulastadium	Plutei. Skelet mütterlich

Das vorläufige Resultat, welches sich daraus für die Echinidenbastarde ziehen läßt, ist folgendes: Im Skelet treten dann Mischcharaktere auf, wenn sämtliche Chromosomen die ganze Entwicklung mitmachen. Dagegen sind allem Anschein nach die Skeletcharaktere rein mütterlich, wenn das väterliche Chromatin zum größten Teil elimiminiert wird, sei es in den ersten Karyokinesen oder im frühen Blastulastadium. Danach wird es sehr wahrscheinlich, daß bei der Gestaltung des Skeletes das Chromatin die entscheidende Rolle spielt, wie dies aus andern Versuchen bereits Boveri geschlossen hat.

3. Regeneration in Holothuria.

By Ellen Torelle.

Introduction.

eingeg. 2. Juli 1909.

Few observations have been made on regeneration in *Holothuria*. In order to discover if regeneration takes place under experimental conditions the writer has performed operations on about five-hundred

and fifty (550) *Holothuria*, representing twelve species, three families, and five genera. One species, *Thyone briareus*, was operated upon at the Marine Biological Station at Woods Hole, Mass., during the months of June, July, and August in 1903; the other eleven species were observed at the Zoological Station at Naples from January to June, 1909.

Since some time will elapse before the histological study of the regenerated individuals can be completed in all of the species observed, it is thought best to publish a preliminary account of the results obtained. In two species, *Thyone briareus* and *Cucumaria grubi*, the histology of the regenerated parts has been to a great extent worked out, and the following account, deals principally with these two species.

The complete number of species represented in the experiments include the following:

I. Dendrochirota.

1) *Cucumaria grubi*
2) - *Syracusiana.*
3) - *planci*
4) *Thyone briareus.*
5) - *fusus.*
6) *Phyllophorus urna grubi.*

II. Aspidochirota.

1) *Holothuria stellata.*
2) - spec.
3) - *impatiens.*

III. Synaptidae.

1) *Synapta digitata.*
2) - *inhaerens*
3) - *hispida.*

Methods.

In the experiments upon *Thyone briareus* one-hundred and fifty individuals were operated upon. The entire anterior part of the body containing the ring-nerve, was cut off and thrown away. The anterior part contained, besides the ring-nerve, the calcareous ring, which in this paper will be termed the lantern; the tentacles, mouth, stomach, and ring-vessels. The posterior part, containing the intestine, the reproductive organs, and the respiratory trees, was used for the experiment. In many individuals the intestine was autotomously severed near the cloaca immediately after the operation. In some individuals the reproductive organs were extruded; but the respiratory trees were in every case retained.

The amputated animals were placed in traps the sides of which were made of wire-netting permitting the free circulation of water. Mud and ooze from the ocean-bed were put into the traps to a depth of four or more centimeters, and they were immersed in the sea and tied to a wooden pier so that they could easily be drawn out of the water and their contents examined. This precaution was rendered necessary after it had been found that the animals usually died within a week after the operation when kept in dishes under running water in the laboratory. Two animals of each lot operated upon were killed for examination at intervals of three days.

Before the work with *Thyone briareus* was completed, several questions suggested themselves which it was thought the results of further experimentation might answer. When the rich material of the Bay of Naples was placed at the writers disposal it was determined to resolve these questions, if possible.

All the individuals operated upon were given the same conditions after the operation; i. e. they were placed in perforated celloidin boxes or in glass jars and immersed in an aquarium of running water. Normal individuals of each species, were also placed in boxes in the aquarium, in order that the effect upon normal individuals of the conditions under which the regenerating individuals were kept, might be observed.

Among the *Holothuria* observed at Naples, *Cucumaria grubi* was found best adapted to the aims in view, because it survived conditions and operations which caused the death of other species, and because the podia are arranged along the radii. Therefore two-hundred and thirty-nine (239) individuals were operated upon in various ways:

Exper. no. 1. In forty-two individuals the body was divided transversely just posterior to the lantern. Both anterior and posterior parts were given an opportunity to regenerate.

Exper. no. 2. Each of forty-two individuals was divided into three parts in such a way that the anterior end contained the lantern and attached organs; the middle-piece contained the reproductive organs, the greater part of the intestine, and the upper part of the respiratory trees. The posterior part contained a short piece of the intestine attached to the cloaca, and the lower portions of the respiratory trees.

Exper. no. 3. Each of thirty individuals was divided transversely into two equal parts.

Exper. no. 4. Each of fifteen individuals was divided transversely into two parts in such a way that the posterior part contained only the cloaca, and was about one-sixth ($^1/_6$) of the body-length.

Exper. no. 5. Each of fifteen individuals was divided transversely into two equal parts and all organs removed.

Exper. no. 6. Each of fifteen individuals was divided longitudinally into two equal parts and all organs were removed.

Exper. no. 7. Fifteen individuals were cut open along an interradius from oral to aboral end, but no organs were removed.

Exper. no. 8. Thirty individuals were cut into two parts longitudinally in such a way that the lantern was divided into equal or nearly equal parts, and each part contained one-half of the cloaca and one respiratory tree. All parts of organs were left in each of the pieces.

Exper. no. 9. Each of fifteen individuals was first divided transversely into two equal parts; then each part was split longitudinally and all organs except the cloaca removed. The cut edges were then sewed together.

Exper. no. 10. In five individuals 2 cm of the length of a radius was removed.

Exper. no. 11. In five individuals 2 cm of the length of an interradius was removed.

Exper. no. 12. In five individuals a piece of the body-wall 1 cm wide, was removed across one radius and one interradius.

Exper. no. 13. In five individuals, the skin on the anterior end of the animal was cut off so that the calcareous ring projected somewhat but no tentacles, and no internal organs were removed or injured.

Experiments similar to those enumerated above were carried out with other species of Dendrochirota, with the Aspidochirota, and with the Synaptidae. A discussion of the results for these forms is reserved for a later publication.

Results for *Thyone briareus* and for *Cucumaria grubi*.

In *Thyone briareus* and in *Cucumaria grubi*, a contraction of the body-wall immediately follows the operation so that each piece into which the body of the animal has been divided, becomes shorter and thicker. The cut edges draw together from all sides of the circular aperature until the opening is entirely closed. This contraction lasts about twenty-four hours, when a relaxation sets in so that in some cases portions of the internal organs protrude through the opening. When a part of the intestine projects, it sometimes becomes large and bladder-like, as if inflated with air or filled with water. The animal may remain in this condition during several weeks after which the organs are withdrawn into the body; the opening is closed either by the formation of a somewhat circular area of new tissue as in *Cucumaria*, or by having the walls firmly knit together as in *Thyone*. The new tissue can always be distinguished from the old because it is unpigmented.

In *Thyone briareus*, the intestine begins to regenerate as soon as the

cut end of the body-wall becomes closed. The new intestine always forms as a bud from one side of the old intestine, at a point near the cloaca. The anterior open end of the old intestine closes forming a blind sac. The new intestine grows forward as a solid rod of cells from one to two millimeters in diameter. The lumen of the new intestine, which becomes continuous with that of the old intestine forms after the solid growth forward has become about one mm in length. As the growth in length proceeds, the diameter of the intestine increases, and continues to increase after the intestine has become attached to the anterior closed end of the body-wall. Until union with the body-wall takes place, the new intestine is a straight tube. As soon as attachment to the body-wall is effected it elongates and turns on itself forming the loops characteristic of the normal animal.

The attachment of the intestine appears to be a stimulus which results in two different processes in the closed oral end of the truncated animal. On the one hand, histolysis of the tissues of the body-wall begins directly above the lumen of the new intestine; on the other, a proliferation of cells at several points near the place where the new intestine has become attached, forms the beginning of a new lantern. In no single case, among the animals examined, had either of these processes begun before the intestine had become united to the body-wall. Histolysis begins in the epidermis and proceeds inwards. After an opening is formed, the oesophagus and stomach are differentiated from the anterior part of the new intestine. The proliferation of cells which form the new lantern takes places in the region of each of the radial nerves. The cut ends of the radial nerves enlarge so that each end has a knob-like appearance. This enlarged end grows downward into the proliferated tissue which projects downward into the body-cavity. The proliferating masses unite thus surrounding the intestine. As the radial nerves grow downward they are accompanied by blood and water-vessels which are differentiated out of the new tissue formed at the extremities of the old vessels, with which the new are continuous.

The »Anlagen« of the various parts of the lantern, such as the tissues destined to form the calcareous plates, are laid down early in its development. Buds from the radial nerves unite to form the ringnerve. Budding and union of the radial vessels leads to the formation of the ring-vessels. The Polian vesicle, the stone-canal, and the vessels which supply the tentacles, are the latest formed of the organs related to the lantern. As a consequence, tentacles do not appear until the regeneration of the lantern is nearly complete. All the tentacles are not formed at the same time; or, it may be that their growth is unequal for one or two first appear, then others are added until the normal number is complete.

If short pieces of the old lantern-muscles have been left attached to the longitudinal muscles at the time of the operation, the ends of these begin to proliferate new tissue about the time that the new lantern forms, although a few specimens examined exhibited regenerated lantern-muscles when no indication of a new lantern could be found. If the animal had been divided just posterior to the lantern-muscles, so that these had been entirely removed, a proliferation of the tissues of the longitudinal muscles takes place, in the form of a bud, at a point homologous with the position of the lantern-muscles in the normal animal. In either case the new muscles grow forward in three or more separate, strand-like parts, which unite into one just before union with the lantern is effected.

The reproductive organs are the last to regenerate in individuals in which these organs have been extruded. This subject will be discussed in a later paper.

In *Cucumaria grubi* as in *Thyone briareus* regeneration takes place readily in the posterior past of an animal when the anterior part containing the lantern and nerve-ring has been removed.

In exper. no. 1, all the posterior parts regenerated but none of the anterior parts.

When the animals were cut into three parts as described in exper. no. 2, thirty-two out of forty-two posterior parts regenerated but none of the anterior parts. Ten middle pieces lived four months, or until killed, but, although the two cut ends were completely healed there was no regeneration of organs evident.

Among the animals which had been divided transversely into two equal parts, all the posterior parts lived and regenerated. Ten anterior parts remained alive until the close of the experiment, and three of these had regenerated missing parts except reproductive organs.

In exper. no. 4, three out of fifteen anterior parts regenerated a new cloaca; all other anterior parts died within six weeks of the operation. Eleven out of the fifteen posterior parts each about 1 cm in length, were living four months after the operation. In each of these pieces the cut end healed, and the anterior body-wall grew forward in the shape of two or more hollow protuberances which formed a body-cavity anterior to the cloaca. Some of these pieces are still being kept alive in order to see if a complete animal will develop from such a small fraction of the whole.

No animal cut longitudinally into two parts lived longer than a week whether the organs were left in the body or removed.

In ten out of fifteen individuals cut open along an inter-radius from

oral to aboral end, new tissue formed along the cut edges so that the wound healed completely.

Among the individuals cut into two transversely, then slit longitudinally and all organs removed, one posterior piece, containing the cloaca, healed along the cut edges in two directions, and a new intestine had regenerated when it was killed, June 8th, 128 days after the operation.

Two out of five individuals in which 2 cm of the length of a radius was removed regenerated the lost part; while four out of five of those in which 2 cm of the length of the inter-radius was removed, completed themselves.

In the animals in which a transverse cut was made across one radius and one inter-radius, all regenerated the missing part.

In the animals in which the body-wall was removed anterior to the lantern, but no tentacles or other organs injured, all regenerated the part removed.

In connection with the above account of *Thyone briareus* and *Cucumaria grubi* it may be of interest to note that apparently *Cucumaria planci* regenerates with equal facility, both anterior and posterior parts of the body. But in all other Dendrochirota observed, the operation most favorable to the regeneration of the animal is the one which consists in dividing it transversely just posterior to the lantern. After such an operation, most of the posterior parts complete themselves, but except in the instances cited for *Cucumaria grubi*, no anterior parts regenerate. It is not at this time advisable to attempt an explanation of the apparent inability of anterior parts to regenerate; but the most important factor in the regeneration of the Holothuria seems to be the presence of the cloaca through which respiration principally takes place in the normal individual. This is indicated by the fact that all parts of animals of whatever size, lived four months or more if the part contained the cloaca; but twelve out of fifteen individuals in which only the cloaca was removed, died „within" a comparatively short time. Individuals belonging to the two species *Cucumaria grubi* and *Cucumaria planci* were the only ones who survived the removal of the cloaca.

Among the Synaptidae, no part of the body less than five cm in length and deprived of the lantern and tentacles survived longer than three days.

The Aspidochirota are extremely tenacious of life, the empty body-wall of one-half to one-third of an animal may live two months or more and possesses, the power of movement to an extradinary degree; but regeneration does not readily take place in any of the species observed.

Summary and Conclusions.

1) Parts of the body of species belonging to the family Dendrochirota regenerate more readily than do species observed belonging to the families Synaptidae and Aspidochirota.

2) Parts of the body posterior to the lantern regenerate more readily than does the part containing the lantern.

3) Animals divided longitudinally do not survive the operation.

4) Animals whose body-wall is cut open from oral to aboral end regenerate new tissue to close the wound and replace missing parts autotomously thrown off.

5) The removal of a part of a radius is apparently a more serious injury to *Cucumaria grubi* than the removal of a part of the inter-radius. But it is not clear if this is due to the removal of a part of the radial-nerve or to the removal of a part of the longitudinal muscle.

6) The intestine usually regenerates either from the cloaca, or from a part of the old intestine near the cloaca.

7) The new lantern is formed by a proliferation of the material composing the bodywall. Buds from the old radial nerves grow down into the lantern and ultimately form the ring-nerve.

4. Bemerkungen zur Onto- und Phylogenie der Hydromedusen.

(Erste Mitteilung.)

Von Dr. J. Hadži.

(Aus dem Vergl.-anat. Institut d. k. Universität zu Zagreb.)

eingeg. 8. Juli 1909.

Ohne auf die Darstellung der Geschichte der Frage über die onto- und phylogenetische Entstehung der Hydromedusen hier eingehen zu wollen, was in der ausführlichen Mitteilung geschehen soll, sei hier nur so viel erwähnt, daß bis vor kurzem (1907) über die oben erwähnte Frage eine beinahe vollständige Übereinstimmung aller Zoologen herrschte. Man braucht nur die Lehr- und Handbücher der Zoologie nachzuschlagen, um sich davon überzeugen zu können.

Was die Ontogenie der Hydromeduse anbelangt (ich brauche kaum hinzuzufügen, daß hier in erster Linie die Entstehung der Hydromeduse auf dem Wege der Knospung gemeint ist), so gilt die zuerst von L. Agassiz[1] gelieferte Darstellung, wonach am freien Pole der Medusenknospe sich zunächst eine entodermale Verdickung bilde (Knospenkern, Glockenkern, Entocodon). Der Glockenkern drängt nun das darunter

[1] L. Agassiz, Contributions to the natural history of the United States of America. Boston. III. 1860. IV. 1862.