THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Emmett Rowles final oral examination for the degree of

Master of Arts

We recommend that the degree of

Master of Arts

be conferred upon the candidate.

E. L. Lund
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Date_____

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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Emmett Rowles for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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Date _____

THE RELATION OF THE RESPIRATORY EXCHANGE TO DIFFERENTIATION, DEDIFFERENTIATION, AND POLARITY IN OBELIA SP.

A THESIS

PRESENTED TO THE FACULTY

IN THE

GRADUATE SCHOOL

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF ARTS.

BY

EMMETT ROWLES

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I. General.

The phenomenon of regeneration in organisms has been studied extensively, and some of the external conditions attending such structural changes or necessary to their consummation have been noted from time to time. However, the work done has been mostly rough measurements of rates of differentiation, size of regenerated parts, etc.

The purpose of the experiments reported in this paper was to obtain some information regarding the nature of the metabolic processes which underlie and are associated with the morphological changes of differentiation and dedifferentiation in the hydroid, Obelia. Perhaps the most fundamental or general index of the nature of metabolic processes in cells are the oxidation, represented by respiratory exchange. And since these are amenable to accurate measurement it was considered most desirable to make a study of the relation of respiration to the processes of differentiation and dedifferentiation.

The work was carried out during the summer of 1921 at the Puget Sound Marine Biological Station at Friday Harbor, Washington.

I wish to acknowledge here my great indebtedness to E. J.Lund of the Department of Animal Biology of the University of Minnesota, and Professor of Physiology at the Station, who first suggested the

problem to me and under whose direction the work was carried on. I also wish to express my thanks to Professor Nachtrieb, Head of the Department of Animal Biology of the University of Minnesota, and to Professor T. C. Frye, Director of the Station, for the use of rooms and equipment, and also for encouragement in my work.

The process of differentiation of the polyps of the hydroid, Obelia, has been very carefully studied in its morphological aspects, while the morphological changes occuring in the polyps during the process of dedifferentiation have as yet been but poorly and incompletely worked out.

Space shall not be given in this paper to a discussion of the former, since the reader has at his disposal many complete and accurate descriptions of the process.

Typical stages in the process of dedifferentiation as it occurs in the Obelia colonies that were used in the experiments are given in Plate I.

These stages represent the approximate stages at which the determinations of the rate of O_2 consumption of the dedifferentiating colonies were made. The drawings were made from preserved samples of material from the same lot as the experimental colonies used in the experiments, the results of which are tabulated under Table XV, having the same treatment and history as the latter. The stages shown in Plate I. are then typical of the experimental colonies used.

Tests were made to determine the rate of O_2 consumption during the process of differentiation and the process of dedifferentiation of polyps in the hydroid, Obelia, in order to contribute some data as to the points of comparison in the intimate nature of the two processes.

Figure one in the plate represents a normal, fully differentiated polyp of Obelia. The well defined ectoderm and endoderm will be noted in both the tentacles and the body of the polyp. The endoderm is very thick in the latter. In the second figure several changes are observed. The tentacles have become notably shorter and relatively thicker. The mouth has closed completely over, a solid ring of ectoderm and endoderm appearing around the hypostome. The latter structure is somewhat shorter and the endoderm of the body cavity is so drawn and retracted as to give the appearance of having undergone a process of contraction. The cells in this stage are apparently being compressed and the nuclei appear as darker, larger blotches. In figure three the hypostome has almost entirely disappeared; the lumen of the body cavity is full of cells and cell masses that are evidently in a process of transfer into the stem. The endoderm as such has almost entirely disappeared and from all indications the endodermal cells have been squeezed from their normal position and relations by a process of contraction. The endoderm is still present in apparently unchanged form and the tentacles are essentially the same as in the preceding stage with the exception of a slight increase in thickness. From the clean-cut outlines of these stages of dedifferentiation it does not appear that the process is one of complete"degeneration" or break-down of cell structure.

In stage four the tentacles have almost entirely disappeared although still distinct and not fused as Loeb maintains, as given below, and the lumen of the body cavity is so completely filled with cell masses as to be almost indiscernible. The process of absorption of the cellular elements is still plainly observed and the ectoderm of the oral surface appears to have also started to be ab-

sorbed. The ectoderm of the tentacles and of the sides and bottom of the body cavity is still intact however. In the next stage the situation is not far different from that just described except that the process of absorption has progressed a little farther. In the sixth stage, however, all traces of the tentacles have disappeared, the ectoderm of the tentacles and the body cavity have lost their individual differentiation and been largely absorbed and only a small knob of undifferentiated cells and cell masses remain. The process of absorption of the cellular elements is plainly visible in all the stages. In stage five a remarkable phenomenon is noted. Here the dedifferentiating tissue has retreated entirely into the stalk and the protoplasm, responding to the antagonistic conditions has secreted a perisarcal partition between itself and the exterior where marauding protozoa and bacteria may be present. The nuclei of the ectodermal and endodermal cells is plainly visible here. In the last stage the dedifferentiating material is seen to have retreated still farther into the stem leaving the protecting partition standing out clear and distinct in the stem above it. This phenomenon of secretion of a membranous partition was observed in a large number of cases.

There can be no doubt from the figures shown that the process observed is a true dedifferentiation rather than a process of "degeneration" as Thacher (1) claimed for other hydroids, as will be explained below.

The phenomenon of dedifferentiation as related to the morphological changes occurring during the process has been studied in many types of organisms by various authors. Schultz (2) has shown that such a phenomenon occurs in Clavellina when kept in unfavorable conditions and in hydra when starved. Driesch (3) was the first to

note the process of dedifferentiation in Clavellina where he performed experiments on half animals, cutting the animal into two parts and using the half containing the branchial sac in his experiments. He found that the whole organism regenerated from this half. Two methods of regeneration were noted in this instance. Sometimes a bud containing the missing part was formed, and sometimes the adult half totally dedifferentiated into an opaque mass which later differentiated into a complete individual. Thus the process of dedifferentiation in this case was a preparation for differentiation, and the stimulus for the dedifferentiation or loss of organs was injury. Driesch (3) also obtained dedifferentiation in whole individuals by keeping them confined in the laboratory. In the process of dedifferentiation noted here, the walls of the organs are reduced to an embryonic character, the organs themselves being reduced to a series of sacs, each separate. Schultz (2) confirmed these results and obtained dedifferentiation in hydra and other forms. Loeb (4) was the first to note dedifferentiation in the marine hydroids.

Loeb (4) showed that the material of the polyps of campanularia withdraws into the stem if the polyp is placed in contact with a solid object such as the sides or bottom of a containing vessel. This withdrawal is preceded by the transformation of the material of the polyp into a shapeless mass. This begins with a shortening of the tentacles, followed by their folding together. Very soon all the tentacles begin to fuse into a homogeneous mass. This process usually begins at the peripheral ends of the polyps. Soon no tentacles can be recognized as individuals. At this stage the dedifferentiated material begins to flow back into the stem. The part nearest the stem creeps with almost an amoeboid motion while

the rest follows more slowly. To explain his assumption that the contact with a solid body was the direct cause of the dedifferentiation, Loeb refers the reader to Duclaux' work on the causes of the coagulation of the blood. According to this work of Duclaux' (5), the nature of the object with which the leucocytes of the blood come into contact, determines whether the enzyme of coagulation, the plasmase, is to become active or not,

Loeb (4) also showed that the polyps of antennularia were lost when the relation of the colony to gravity was changed.

Thacher (1) worked on these same two forms and others and has the best paper that has yet appeared on the morphological changes attending the processes of dedifferentiation in the hydroids. He found that the hydroid Eudendrium sheds its polyps on being brought into the laboratory, but he also obtained real dedifferentiation in the same hydroid under the same circumstances. The same results were obtained for pennaria by Cerfontaine. Thacher found that Tubularia never absorbs its polyps on being brought into the laboratory but sheds them soon after being collected. New polyps are regenerated after a day or so.

Pieces of Campanularia were cut by Thacher (1) and placed in dishes in contact with the sides and bottom as described by Loeb. At first the polyps remain expanded but soon irregular, transverse contractions take place in the polyps driving the digestive current with great force, sometimes ejecting the contents of the gastrovascular cavity through the newly formed stolon or through the mouth. The current becomes more and more heavily laden with granules of all sizes. Then the polyps contract into the cup, becoming shorter and shorter, until they disappear. Their cells pass into the gastrovascular cavity, and at the same time the cells of the hypostome

begin to be absorbed. The usual time for complete absorption is six to twelve hours, but it may be delayed for one or two days. The protoplasmic threads extending from the coenosarc to the perisarc just below the cup are not broken until the last stage.

The endoderm first dedifferentiates in the wall of the body of the polyp. The tentacles started later and at the very last the lamellae breaks and the endoderm of the tentacles passes into the body cavity. The ectoderm of the tentacles is very thick at this time, but the tentacles are not fused as Loeb maintains. Then after the endoderm of the tentacles has passed into the body cavity, the ectoderm, containing nettle and ectodermal cells, is poured into the body cavity. Some degeneration of the ectoderm also takes place, the cells being sloughed off to the outside. The great majority of the absorbed cells are taken into the endodermal cells that line the gastro-vascular cavity. Some of them pass to the outside.

Both Eudendrium and Pennaria show this process of degeneration, followed by absorption. Gast and Godlewski (6) confirmed Thacher's results on Pennaria. According to Thacher the phenomenon is one of degeneration followed by absorption rather than one of true dedifferentiation of cellular and organismic structure. Quoting Thacher, (1), " The constant position of the endodermal gland cells near the beginning of the stalk throughout the degenerative changes shows that there is no drawing of the cells into the stem until the final stages".

An interesting observation was made by Perkins (7) on what he called "Degeneration phenomena in the larvae of Gonionemus". Yet the phenomenon was one of true dedifferentiation. He observed that the hydra-like larvae of Gonionemus settled to the bottom of aquaria and became ameba-like in form. The cell walls disappeared, the

tentacles were retracted, and the animal assumed various shapes. The nidocils of the nematocysts were always present in unchanged form, sticking out of the homogeneous protoplasm as unchanged spicules. No conditioning causes were given for such results other than that the animals were kept in the laboratory and were, therefore, probably at room temperature.

Greeley (8) working on hydra was able to secure true dedifferentiation by reducing the temperature. Developing buds were forced to lose their germ layers, return to a homogeneous mass, and be absorbed. He did some other work which will be discussed later.

Quite different in method yet essentially the same sort of experiments are those of Muller (9), H.V.Wilson (10), Huxley (11) and others on the regeneration of dissociated cell masses in sponges and coelenterates. Wilson first tried this method and with signal success.

Lieberkuhn (12) first noted in 1856, as quoted by Wilson (10), "That the choanocytes of freshwater sponges give up during the winter their distinctive features and assume the characters of mesenchyme elements". In 1879 Metschnikoff (13) observed the same phenomenon in marine sponges kept in foul water.

There has been much doubt in the past as to the exact nature of the stages in the metamorphosis of the sponges, especially regarding the history of the cells involved. Evans (14) has shown by very complete work that the choanocytes are derived from the ciliated ectodermal cells of the larvae. The origin of the sponge gemmules has also been much in doubt, although the work of Maas (15) and others tends to show that they are derived from certain cells of the adult sponge in the process of dedifferentiation. Maas maintains that the formation of the gemmule itself is preceded by a process of

"regressive differentiation", in which the mother cells from which the gemmules are to be derived return to a more unspecialized type in a more or less ameboid condition. This same theory may be applied to the dedifferentiation of the whole sponge when kept in confinement or in otherwise unfavorable conditions.

Thus Wilson (16) showed in 1907 that sponges kept in confinement give up their differentiated structure and return to a more simplified, embryonic state. Maas (15) showed in 1906 that abnormal chemical environment would do the same thing. Wilson (10) found in the monaxonid, Stylotella, that, "The oscula and the bulk of the pores close, much of the canal system is repressed, the skeletal arrangement is simplified, and the flagellated chambers broken up for the most part into their constituent elements which are scattered through the mesenchyme". At any time differentiation may be secured by returning the animal to normal conditions. As Wilson (10) says this is analagous to such changes as the dedifferentiation of the spongillidae during the winter months and the metamorphosis of such totipotent regenerative mass as the sponge gemmule. Wilson (10) states that both the latter and the dedifferentiating sponge mass, the result of confinement, are composed of a layer of flat epithelial cells on the outside, and of indifferent amobecytes on the inside. In other words, the two types are very similar in structure and are probably the result of the same kind of process. Thus dedifferentiation repeats an embryonic stage.

Since in the later stages of confinement the sponge mass divides into several masses and in no case is the process of reduction the exact reverse of the process of development of the sponge, to assume here the theory first expressed by Eugene Schultz (2), that the reduction process is a reversal of the process of development, it

becomes necessary, as Wilson (10) states in his paper, to assume that the tissues of the sponge return to a total totipotent state from which they may develop into the adult sponge without the necessity of passing through the larval and metamorphic stages. The characteristic method that Wilson used in his later experiments on dedifferentiation was one whereby he was able to produce dedifferentiation almost instantly by mechanically disintegrating the sponges. He accomplished this by forcing the sponge body through fine gauze in sea water. By this process he divided the sponge tissue into isolated cells and cell masses. The latter he removed. These masses could divide and also fuse. Wilson sees in this dividing and fusing an inherited characteristic from remote ancestors whereby they were able to survive disadvantageous conditions. From these dedifferentiated masses Wilson was able to secure regenerated masses. His work includes experiments on both Coelenterates and Porifera.

Wilson worked on the behavior of dissociated cells in Eudendrium and in Pennaria of the hydroids and in Alcyonaria and Asterias. He cut the hydroid into fine fragments and passed them through fine gauze. From the dedifferentiated masses thus secured restitution bodies were formed and in a few cases he obtained normal, fully developed hydranths from the masses. In most cases,however, he was unable to secure more than a differentiation of the germ layers and in some cases not even that. He attributed the failure to get a higher percentage of full regeneration to faulty technique, listing the following as especially weak points in the various experiments: (1) The technique allows parts which must die, such as tentacles and gonophores, to get into the cultures. (2) It subjects tissue which needs the best environment to the quiet water of a laboratory dish.

Small gauze floats used on the tops of running aquaria were used to no effect. Only clean colonies should be used and preferably colonies without gonophores. The stem tissue was found to be better than the whole colony for these experiments. The sea water should be well aerated and filtered.

Histology of Eudendrium Restitution Masses.

The 24 hour masses showed a syncytial arrangement with a few individual cells scattered throughout the protoplasm. Some varied from this in that they showed a cleft like space separating two layers in the protoplasm. The outside layer was composed of four or five layers containing smooth cells of an embryonic type and a few cnidoblasts. In some cases only the nuclei in a vaguely outlined reticular matrix were discernible. The inner layer was syncytial, containing many large nuclei and a few large vacuoles containing inclusions.

The three day old masses had a definite ectoderm and endoderm and a central yolk mass. In places the "yolk" mass was continuous with the endoderm. There were a few small nematocysts in the "yolk" mass.

Histology of Pennaria.

Restitution masses.

The material was squeezed out of pieces of the stem onto a cover glass immersed in water. After five minutes strong formalin was added and the masses mounted on a slide. Cell masses were found corresponding to the endoderm cells in structure and appearance. Some of the masses showed pseudopodia and no nuclei could be seen in the restitution masses but the masses were not stained. Other smaller and paler cells were present doubtless of ectodermic origin. Cnidoblasts with included nettle cells were also present.

Besides the latter restitution masses, granules and small particles were also present; these latter often times fused into larger restitution masses. No data is given as to whether these regenerate into differentiated tissue. The same aggregation of cells is present regardless of whether the stem alone or the polyps alone or the whole colony is used. These separate cells and masses were all fused within ten minutes. The structure of these masses throughout was that of a syncytium. Cell boundaries were present here and there. Some of these cells could be identified by the shape and included granules as endoderm cells. The ones that could be identified as such are few, however, and this leads one to the conclusion that most of the endoderm cells undergo a transformation which prevents their recognition later. Most of the original nematocysts are still present at the end of 20 minutes but soon disappear entirely. What becomes of the cnidoblasts themselves? Do they share in the formation of new tissue? This would be a case of dedifferentiation of a highly differentiated structure. No data is given on this point however.

The mass was highly vacuolated and contained relatively large nuclei. From Wilson's preparations it seems highly improbable that the ectodermal cells became segregated to the outside to form new ectoderm, and the endodermal cells to the inside to form new endoderm.

After 17 hours well-defined ectodermal and entodermal layers were present in the form of cellular syncytia. Large nematocysts were present in both layers evidently carried over from the parent but some of them are in a process of dissolution. In one day new perisarc was secreted. In two days the entodermal cells had assumed adult shape and form and contained spheroidal granules. The

ectoderm was still a cellular syncytium.

At four to five days the masses were still spherical with perisarc, ectoderm, endoderm, and central yolk mass. As outgrowths developed both ectoderm and endoderm assumed the character of columnar epithelia, especially in the outgrowths themselves.

Thus Wilson was able to secure dedifferentiation in a very short space of time by mechanically disintegrating the tissue. Child (17) states that when part of an organism becomes physically or physiologically isolated, it has a tendency to develop into a new whole if it has the power of complete regulation, that is, if it is made up of totipotent material. By physiological isolation Child means being cut off from the exchange of stimuli that maintains the organism as an individual. Wilson was able to produce this isolation by mechanical disintegration.

Huxley, working on Sycon species of sponges was able to obtain restitution masses that were composed entirely or almost entirely of collar cells and others composed entirely of dermal cells. He also noted the formation of so-called choanocyte or dermal cell blowouts, spherical segments jutting out from the restitution mass. He was unable to obtain good results with homocoelous sponges. He used four methods in procuring his disintegrated masses .1. The method used by Wilson, passing the sponge tissue through fine gauze. 2. So-called choanocyte isolation, by teasing transverse sections of the sponge by means of a needle. 3. Teasing the whole sponge mass. 4. Squeezing without gauze.

In one experiment he noted that the restitution mass divided into a large number of small spherical masses. These spherical masses were surrounded by a gelatinous envelope and each spherule was composed of a one layered epithelium covering a central mass.

No cell outlines were visible in the central mass which was of a yellowish color. Broken spicules were present in some. The outer gelatinous membranes if present were surrounded by numerous bacteria. Later the spherules showed a separation of the cells that were on the outside and an acquisition of a surrounding gelatinous mass. Eventually the spherules all degenerated; no recovery was noticed. Huxley states that the cause of the formation of such structures was to be regarded as unfavorable conditions such as stale water. Important points noticed in connection with this study were as follows:

1. The size of the spherules varied within considerable limits. 2. The covering of epithelium could be evidently formed from either dermal cells or choanocytes, unless the dermal cells dedifferentiate into cuboidal cells such as were sometimes found. 3. The separation of the clear, epithelial covering cells, similar to a process preceding dedifferentiation in Clavellina and Perophora. 4. The production of a dermal epithelium late in the history of such spherules may be regarded as related to the fact that restitution bodies with dermal epithelium are more viable than those without, that is composed of choanocytes alone. 5. The formation of cell outlines with later their distinct separation, when at first no cell outlines were visible at all. This is analagous to the formation of syncytia in coelenterate restitution masses and their subsequent resolution into cells as observed by Wilson (10), and Drew (18).

Huxley also observed that if any larvae were present in the sponges that were disintegrated they would adhere to the masses and eventually be absorbed in to the mass with the flagella still beating. They would soon be an undistinguishable part of the restitution mass.

Huxley tried various cultures in an endeavor to secure a medium in which the restitution masses would live and grow but without success.

He also observed that powdered carmine was ingested by a culture of choanocytes. This ingestion was entirely intra-choanal. When Nitzchia was added very few were ingested but were seized by the flagella and lashed to and fro. Mechanical and chemical stimuli served to cause a retraction of the collars and flagellae in choanocytes in restitution masses.

Huxley differs from Wilson,in believing that the position of the cell is a function of its character. He maintains that ectodermal cells always return to be ectodermal and that endodermal cells are always endodermal cells. At the same time he admits that he did not get the entire dedifferentiation in the sponges that Wilson did in the coelenterates. He takes refuge in the fact that Wilson was not able to state definitely that the ectodermal and endodermal cells in the hydroids did not again return to their original form since their individuality was lost in the formation of cellular syncytium in the restitution masses. Also he relies to a certain extent for his position on the fact that in masses consisting of a large proportion of choanocytes, a large proportion of the regenerated masses were choanocytes. Likewise when a small number of dermal cells were present in the masses they always migrated to the outside of the mass to form a dermal covering.

This secretion of a fluid as noted by Huxley in the formation of choanocyte or dermal blowouts is an interesting phenomenon. Mr. Gray as quoted by Huxley, has found the formation of similar spheroidal masses in fragments of the gills of Mytilus. Huxley concludes

that it may involve changes of the same nature as those taking place in the formation of a blastocoele.

All of the workers in this field seem to be agreed that either too small or too large masses do not live long and differentiate to a limited degree only. Huxley assumes that it is due in the small masses, in part at least, to a relatively larger surface which interferes in some unknown way with the gaseous exchange. In the large masses he assumes that the interior of the mass is poorly oxygenated, with resulting degeneration.

Huxley (11) also did some work in dedifferentiation on the social ascidian, Perophora. He attempted in this work to determine some of the underlying causes for or factors influencing the process of dedifferentiation. He finds what he calls two kinds of dedifferentiation in perophora but processes seem to differ only in degree. Or rather the process of dedifferentiation is the same in both cases but is followed in one case by a process of absorption, analagous in its elemental aspects to the process of absorption of the hydranths of a hydroid as noted by Thacher.

In the ascidian, perophora, under certain conditions the Zooids are seen to undergo a reduction or dedifferentiation process similar to the process described for Clavellina by Driesch. The ascidian on being taken into the laboratory maintains itself without food by the resorption of the stolon into the zooids. This resorption is preceded by a process of dedifferentiation similar to that described by Driesch for the zooids of Clavellina. Huxley explained this fact that the zooids maintained their normal size at the expense of the stolon by assuming that, since the zooid was more differentiated and more highly organized than the stolon that it was the dominant part and therefore that its existence was maintained under unfavor-

able conditions at the expense of the less highly organized and therefore less important stolon. He also assumed that if by some means he could experimentally cause the zooid to lose its dominance that the zooid would then dedifferentiate under unfavorable conditions and be absorbed. Therefore, since Child (17) has shown that in a great many cases the more highly organized parts of an organism are more susceptible to sub-lethal or lethal solutions of KCN, Huxley used dilute solutions of KCN to produce a loss of dominance on the part of the zooids. He was able to secure in this way a dedifferentiation of the zooids analagous to the process described by Driesch in Clavellina, and after the dedifferentiation a resorption of the material of the zooids into the stolon.

In order to determine something of the relation of the process of dedifferentiation to that of resorption, Huxley caused a cessation of the circulation by subjecting the organisms to solutions of KCL. He found that although KCN caused dedifferentiation in the zooids very little, if any, resorption took place. He logically concluded that the process of resorption in this case was dependent to a very large extent on the circulation.

In this process of resorption the cells apparently migrate from the tissues directly to the circulation.

Huxley endeavors to explain the higher organization and the resulting dominance of the zooids over the stolon by assuming that such a dominance would take expression in a higher metabolic rate in the dominating part. He then endeavors to prove that the zooids have a higher metabolic rate than the stolon by using the method so long used by Child, that of susceptibility to KCN. It is assumed that the higher the metabolic rate the greater the susceptibility to KCN. However, since the work of Lund, Allen, and others shows

that the rate of metabolism in a great many organisms is entirely independent of the rate of susceptibility, the method is open to grave objections and any results based on this method cannot be adjudged final.

At 5° C. Huxley finds that dedifferentiation occurs to a small degree but very little resorption occurs, probably due to a slowing or cessation of the circulation.

Huxley also obtained partial dedifferentiation in Amaroucium and in Botryllus.

Undoubtedly the most profitable and definite work on the actual steps in such a process as dedifferentiation either morphological or otherwise can be performed using the simpler forms of animal life as experimental material. Thus most of the work done so far has been done on porifera and coelenterates and to a lesser extent on the protozoa. The latter form of course is much more desirable as experimental material since no extra-cellular material is involved. However, in the protozoa the phenomenon of encystment is met with and thus the process of dedifferentiation is modified to that extent. Therefore most of the work on dedifferentiation in protozoa has been done with regard to the causes of, history of, and nature of encystment. Rhumbler and Cienkowsky (19) in 1866 isolated infusoria in small, loosely covered dishes and allowed the water to exaporate from them. Cysts were formed. In 1902 Doflein (20) obtained spore formation in the same way. In 1888 Maupas found that lack of food caused certain carnivorous protozoa, notably Oxytricha, to encyst. Hertwig (21) in 1896 found that either lack of food or excess of food caused Actinosphaerium to encyst. Klebs (22) in 1896 found the formation of zoospores in Vaucheria filaments when they were exposed to darkness after being in the light.

He found that hypertonic or hypotonic solutions caused zoospore formation regardless of the presence or absence of light. Low temperature aided the formation of the zoospores. Greeley (8) in 1901 found that by lowering the temperature a cyst could be secured with Stentor. No spore formation could be observed in this case. These results could not be obtained by raising the temperature. By lowering the temperature to 4° C. for a few hours cysts could be obtained with Monas. No such cysts were formed with control animals at room temperature. The organisms returned to normal form at room temperature within 24 hours. If the cysts were kept at 1° C. on ice for a few days they broke up into spores, from three to twenty-five in each cell. These spores were discharged from the resting cell as soon as they were formed. They had thick cell walls and were non-motile. They could be kept indefinitely at a temperature below 8° C. and withstood dessication perfectly. They returned to the motile form at room temperature especially in the presence of fresh-water algae. Some of these spores stay in the cell and develop there. Greeley in further studies on the cause of dedifferentiation of which the process of encystment may be regarded as one form, found that dedifferentiation could be induced in hydra by subjecting it to a temperature of 6° C. Even a growing bud is absorbed back into the parent stalk at this temperature. A lowering of the temperature or extraction of water have an effect on the protoplasm of the Cecropia Chrysalidas to cause accelerated development and metamorphosis. All of these results point to the fact that in unfavorable conditions living tissue undergoes certain regressive changes which may allow it to survive the unaccustomed conditions.

A very important paper on the morphological changes occuring in a single cell during the process of dedifferentiation was contrib-

uted by Lund (23) working on the ciliate protozoan Bursaria sp.

In such an organism which does not possess a plane of symmetry any cell division would result in the formation of dissimilar daughter cells if not preceded by a process of dedifferentiation. The author found that such a process of dedifferentiation occurs in Bursaria as a precedent to division, encystment, or regeneration following cutting. However, he also found that dedifferentiation may occur without any apparent conditioning causes, and without being followed by division, encystment, or regeneration. Differentiation to the normal form followed later. This is an important point as showing that dedifferentiation is a normal process of the organism, occurring without the stimulus of division or regeneration. The relation of these results to the other work in dedifferentiation and a discussion of the authors theories of dedifferentiation will follow later in the paper.

Passing now to the factors that govern the process of regeneration, we find that the work done, while large in volume, is neither complete nor conclusive.

Regeneration was first noted and spoken of by Aristotle and Pliny. Trembly (24) did the first good work on regeneration, on hydra, in 1740. Reamur in 1742 and Bonnet (24) in 1745 did some further work on regeneration. Trembly found that it takes less time for a hydra cut into parts to regenerate in warm weather than in cold weather. Thus we have here the first note of any of the factors that influence this process of regeneration. Reamur and Bonnet worked on freshwater worms and on earthworms. Bonnet assumed that the capacity of a part to regenerate depended on its liability to injury, in the normal state.

Spallanzani, working on the earthworm, found that if cut the

posterior end regenerated a head and the anterior end a tail. He also found that young tadpoles regenerate tails faster than older ones. Here we have note of another factor influencing the rate of regeneration. He also noted that the legs and tails of salamanders are regenerated if removed. Starved animals regenerated missing parts. He also noted that toes regenerate, if removed, slower than the whole appendages do if they are removed. The tentacles and the whole head of snails will regenerate if removed. It is a noteworthy fact that only as much of an organ or part will regenerate as is removed. The missing part is regenerated to a smaller size than the normal and then grows to the normal size. In other words, the differentiation itself is completed before the part has reached full size. The process of differentiation is followed then by a period of rapid growth until the part has reached normal size in relation to the other parts of the animal. Sometimes as in the earthworm all of the missing part is not regenerated. Thus if more than a certain number of segments are removed from the anterior end of an earthworm only part of the normal number will be regenerated although the worm will have a perfect anterior end. In the earthworm, hydra, and planaria lateral regeneration is possible. In some animals a small piece will regenerate the whole animal. Such a process is possible in the sponges, hydroids, protozoa, etc.

Regeneration can take place physiologically without the stimulus due to injury, such as the process of moulting of feathers, loss and growth of horns, loss of the peristome in protozoans, etc.

There are many theories as to the causes of regeneration and the nature of the process. Roux (21) thinks that regeneration is brought about by the rearrangement of the cells present, since no new material is added to the organism. Roux assumes that a re-

differentiation of the cells takes place. Roux distinguishes between rearrangement and redifferentiation of the cells present and a proliferation of the cells present. Regeneration may take place without the latter process but never without the occurrence of the first. Regeneration is defined by Driesch (3) as "the reawakening of those factors that once more bring into play, by means of division and growth, the elementary processes that had ceased to act when the embryonic processes were finished". Driesch uses the word reparation to describe such a process as the regeneration of the hydranth of tubularia. The hydranth is assumed to be formed from material from the old tissue. Roux and Driesch used the word regulation to explain as Morgan (24) says, "the readjustment that takes place by means of which the typical form is reached or maintained". The word is used also to express changes in metabolism or such a process as adaptation. Morgan (24) prefers the term regeneration and for changes in metabolism, the word regulation. According to Morgan the "word regeneration has come to mean in general usage, not only a replacement of a lost part but also the development of a new, whole organism, or even a part of an organism, from a piece of an adult, of an embryo, or of an egg".

Morgan calls those processes of regeneration which are preceded by a proliferation of material "epimorphosis". Those in which a part is transformed directly into a new organism, he calls "morphallaxis".

There are many factors that affect the rate or the amount of regeneration. They may be roughly classified as, effect of changes in temperature, effect of food, effect of gravity, effect of contact, effect of chemical changes, effect of light, effect of electrical changes.

Trembley, Spallanzani, Bonnet (26), Lillie and Knowlton (27), and Peebles (28) show that there is a certain temperature at which regeneration has a maximum rate for various organisms.

Bardeen (29) has shown that worms may regenerate more quickly when in a starving condition. In this connection Zeleny (30) working on crayfish and on brittle stars has found that the greater the amount of the injury, the greater the rate of regeneration. That is, if two of the appendages of the crayfish are removed they will regenerate faster than one would if removed. Morgan endeavored to find out if such a phenomenon were caused by a difference in the amount of the reserve food material in the whole organism. He did this by ascertaining the rate of regeneration of the legs of salamanders some of which had been starved while others were normal. He found that the rate of differentiation and therefore the rate of regeneration were the same in both sets of animals, but in the starved animals the regenerated appendages were much smaller than in the normal animals. He calls this conclusive proof that the rate of regeneration is not affected by a change in the amount of reserve food material in the organism.

Goldfarb (31) has contributed the latest work on the effect of light on regeneration. He experimented on a hydroid, Eudendrium ramosum. The polyps and also the gonads were removed. He found after careful experimentation that regeneration following the removal was not affected by the presence or the absence of light. Goldfarb found that light was required for the normal regeneration of the polyps of pennaria.

The polyps appeared in eudendrium about 48 hours after the cutting. Three types of regeneration were noted:

1. The direct rays of the sun were found to be injurious to the

colony, preventing regeneration. There is no reference as to the possible cause of such an effect as the above but it is highly probable that the ultraviolet rays which are filtered by glass caused the disturbing effect.

2. Poor regeneration was noted in colonies that were left in unchanged water.

3. Colonies left in the room under diffuse sunlight with the sea water changed daily showed the best regeneration.

Loeb (4) found for antennularia that the hydranths could be turned into stolons by orienting them in respect to gravity. Loeb also maintained that the hydranths could be forced to lose their differentiation and to be absorbed by placing them in contact with a solid object such as the bottom or sides of a containing vessel. However, Thacher (1) showed that these results could be obtained without contact with any solid material and were caused by other unfavorable conditions, such as stale water, room temperature, etc.

Loeb (32) found that maximum growth from pieces of tubularia is obtained in sea water with a much less osmotic concentration than normal sea water. Turbidity is thus necessary to growth. Loeb showed that regeneration takes place only when salts of potassium and of magnesium are present. More than a small amount prevents regeneration. Lack of O_2 or in other words a lower O_2 concentration than normal will prevent regeneration. If one end of a stem of tubularia is placed near the bottom or just in contact with the bottom, no regeneration is possible. Jacobson (33) showed that the layer of water just above the sedimentary layer is poor in O_2.

As brought out in the above review, the regenerating part will assume the original form and orientation if unaffected by any of the external forces that might be brought to bear on the differentiating

member. However, if some external force, experimentally or otherwise is brought to bear on the regenerating part this part will be altered to a greater or less degree from the normal form. Thus in organisms that have an axial or polar orientation, such as the hydroids, the regenerating ends of a cut piece have normally a definite polarity. This polarity sometimes is manifested by a growth of a stolon at one end of a cut piece of the stem of a hydroid while at the other end a normal hydranth is regenerated. At other times both ends of a cut piece regenerate a hydranth but the time of the appearance of the hydranths, and according to most of the authors, the rate of the development of the two hydranths is different. The end corresponding to the apical end of the stem shows the first regeneration. This polarity in the hydroids is without doubt occasioned by physico-chemical processes within the hydroid. These processes take expression in one form by the presence of a distinct gradient in electric potential as brought out by Mathews(34). Thus it would seem that electrical forces might play a part in the control and the orientation of parts in the development of regenerating hydranths in cut pieces of the stem of a hydroid. That such is actually the case has been demonstrated by Lund (35) in some recent work on the control of organic polarity experimentally by means of direct electric current. By using a current of exactly the right density, after careful experimentation he was able to control the time of the appearance of the hydranths on the two ends of the cut internodes of the stem of Obelia. Thus he was able to reverse the original polarity of the organism by proper orientation of the piece in the electric current. From the results that he secured in a large number of experiments he logically concludes that electrical forces or differences in electrical potentials play a large part in

the determination and control of organic polarity. Later work by the same author shows definitely that not only can the polarity of a regenerating hydranth be predetermined by the proper orientation in an electrical field of the proper density but also that the direction of the growth can be determined by the manner of orientation in an electric field likewise of exactly the right density. These contributions are among the most important of any work done in this field since for the first a controlling force for the determination of polarity and the direction of orientation of a differentiating, as well as a growing part, of a living organism, has been found which can be accurately measured as to amount and direction.

This work throws much light on the nature of the forces that are internally directing the processes of differentiation.

The only work that has been done directly on the effect of the process of regeneration on the respiratory exchange of organisms is a paper by Schonborn (36) on the O_2 consumption of internodes of the stem of tubularia during the regeneration of new hydranths.

This work does not give much definite information on the subject. Nine sets of internodes only were used and some of the experiments on these were incomplete. The experiments are not comparable between themselves since the experimental conditions in each of them varied. The determinations were carried out for the rate of O_2 consumption, using the Winkler method. Determinations of the rate of the O_2 consumption were carried out for pieces of the stem about three centimeters in length, the pieces having the normal polyps in the first determination, having the polyps removed in second determination, and having the polyps regenerated in the third determination. However, the author does not explain the method of cutting the polyps off, nor does he determine the effect of the injury

due to the cutting on the rate of O_2 consumption of the regenerating stem. His experiments do show,however, that the rate of the O_2 consumption in the normal colony does not vary from day to day, unless the experimental period is so long as to cause a lack of O_2 in the latter parts of the period. As was perhaps to be expected, he got in most cases a little less rate of O_2 consumption in the stems with the hydranths removed than in the ones that had normal hydranths. However, in the determinations made after regeneration of new hydranths had started he finds in the 9 experiments cited an increase in the rate of O_2 consumption in 6 cases and the opposite in the remaining three. Also in 2 of the 6 that showed a decrease no original rate of the O_2 consumption for the normal stem is given due to errors. These facts combined with the fact that the differences between the rate of consumption of the stems with hydranths and the stems with hydranths removed, and the differences in turn between these latter and the rate of consumption of the same stems with regenerating hydranths, are very small in most cases, the data is worth very little; in fact, it must be disregarded.

The results of his experiments, in resume, are then of very little importance since the results are contradictory among themselves and there is no large preponderance of results of any one type to settle definitely whether the rate of the O_2 consumption of stems of tubularia from which the original hydranths have been removed, increases or decreases with the regeneration of new hydranths. Any data which we may contribute will be, therefore, of great value in arriving at a fundamental understanding of the processes that take place during regeneration.

Among the fundamental properties of living organisms, as outlined above, are the marvellous powers of correlation and of symmetry.

This takes the form of axial symmetry in such forms as the hydroids.

One of the objects of this investigation was to endeavor to discover whether or not a difference exists in the rate of metabolism between the apical and the basal ends of the Obelia colonies. The question of the existence of an axial gradient in the rate of metabolism in various organisms has been the subject of a large amount of investigation, especially by Child and his co-workers. Practically all of the work that has been done, however, has been based on differences in the susceptibility to lethal solutions of KCN. It has been assumed by Child that the organism or part of an organism that had the greatest rate of metabolism was the most susceptibile to KCN. Later Tashiro (37) using his biometer made some direct determinations of the rate of CO_2 production of nerves and correlated this with the rate of susceptibility to KCN. The more central portion of the nerve had the higher rate of CO_2 production.

Hyman (38), working on microdilous oligochaetes, found a double axial gradient in susceptibility to KCN corresponding to the double gradient in electrical potential described by Morgan and Dimon (39) in earthworms. Hyman and Galigher (40) found that there is an axial gradient in Nereis and lumbriculus that is also double in nature, a higher rate of O_2 consumption being found in the posterior than in the anterior end, which in turn had a higher rate of O_2 consumption than the middle third of the body. A modification of the Winkler method was used.

Galigher (41) working on Nereis found that the portions of the body showing the higher rates of metabolism were more susceptible to KCN than the parts having a lower rate. In other words, they endeavored to show that Child's idea of a metabolic axial gradient

corresponds to the gradient in electric potential found by Morgan and Dimon. They also endeavored to show that the susceptibility method for measuring the rate of respiratory metabolism was entirely trustworthy. However, in the light of work done by Lund (42) and Allen (43) independently the method cannot be regarded as being trustworthy especially since the results of the experiments mentioned above cannot be accepted free from objections, for no attempt was made to define the meaning of the word metabolic rate. True metabolic rate must be a measure of the rate of the cell metabolism. The above workers made no attempt to determine the amounts either absolutely or relatively of the cell tissue in the anterior and posterior ends actually taking part in metabolic activities. Until that is known there is no way by which the value of the results may be judged.

So at the present time there is almost a total lack of direct evidence of a conclusive nature as to the existence of a gradient in the rate of metabolism between the anterior and posterior ends of an organism. In order to furnish some data that might throw some light on this important subject several experiments were performed in connection with the work reported in this paper to determine the difference, if any, in the rate of O_2 consumption between the apical and the basal ends of main stems of Obelia colonies.

In addition to the intended work on the rate of O_2 consumption during the processes of differentiation and dedifferentiation of polyps in the hydroid Obelia, as outlined above, it was necessary to determine the exact effect of cutting on the rate of O_2 consumption in Obelia colonies since otherwise I would have no index as to whether an increase or decrease in the rate of O_2 consumption during such structural changes as differentiation and dedifferentiation

was the result of new polyp formation or was some after effect of the cutting. At the same time the experiments, the results of which are given in Table V., show definitely the effect of injury on the rate of metabolism in a normal organism as indicated by a change in the rate of O_2 consumption. The question has been much in doubt as to whether injury to a tissue stimulates the rate of O_2 consumption or whether injury is followed by a retardation in the rate of the metabolic processes, due to some attempted reorganization of the distrubed tissue.

Experiments were also performed to determine what percentage of the total rate of O_2 consumption in the whole colony was found in the stem alone after the removal of the polyps. The rate was found to be much lower in such cases. Was this lowering entirely due to injury? What changes occur in the rate of O_2 consumption during the regeneration of new polyps in cut internodes of Obelia colonies? Is the apical end of a colony by virtue of its position the dominating part and is this dominance expressed by a difference in the rate of O_2 consumption between the apical and basal ends of the same colony?

Is this difference in the rate of O_2 consumption, if found, still present if based on equal volumes of coenosarc of the two ends of the colony? These are some of the questions concerning which the experiments on fully differentiated Obelia colonies given in this work will, it is hoped give some data that will be of value in an understanding of the actual processes taking place during such changes.

Some work has been done on the loss of differentiated structure by certain organisms, as stated above, and the process has been variously called dedifferentiation, involution, and reduction. The

first term shall be used in the references made to such a process in this paper.

I endeavored, during the course of my experiments, to determine the changes, if any, in the rate of O_2 consumption during the dedifferentiation of the polyps of the Obelia colonies. However, I made no attempt to follow closely the morphological changes taking place during the process of dedifferentiation of the polyps, or to make a cytological study of the history of the cells involved. Mounts were made as described above to show the approximate stages in the process of dedifferentiation.

A study of the methods, material, and actual experiments performed follows as well as an analysis of the results and a review of the literature.

(b). Materials and Methods.

In order to measure such amounts of O_2 as were absorbed by hydroid colonies, it was necessary to have a method that could accurately measure dissolved oxygen. For this purpose I used a modification of the Winkler method for the determination of dissolved oxygen. The adaptation of this method to my experiments is as follows.

The burettes used were graduated to read in twentieths of a cubic centimeter. The amounts of reagents taken were modified in the following way. The flasks were calibrated accurately for 25 cc. content and the amounts of reagents needed were thus much less than given in the ordinary Winkler procedure: 0.1 cc. of 4N-solution of MNCl; 0.1 cc. of 4N-solution of KI;0.1 cc. of 4N-solution of NaOH; 0.3 cc. concentrated HCl solution; N/40 solution $Na_2S_2O_3$were the amounts used in these experiments.

Obelia was selected for these experiments because the normal rate of O_2 consumption of suitable colonies, was of a magnitude to

correspond to about one-half of the quantity of dissolved oxygen in 25 cc. of sea water at air saturation and at approximately 10° C. temperature, within 10 hours, and because it would regenerate quickly following cutting. Since it is axially symmetrical it permits the determination of the rate of respiration along the axis of the main stem of the colony. It was also very abundant.

The material was collected in several different places and was of two different species, but the material for any one experiment was always collected from the same place, and had the same history and treatment. The material was always placed in the live box after collecting and left there until taken out for experimentation. No colonies were used that had been in the live box for more than 48 hours. Only clean colonies were used. Those that were covered with debris or diatoms that could not be removed by gently shaking in the water were discarded. For the most part young and actively growing material was used; the exceptions are noted under the specific experiments.

All colonies that were to be used in experimentation were removed from the live box immediately preceding the experiment, placed in a bucket of fresh sea water and carried to the laboratory. On reaching the laboratory the colonies were removed from the large volume of sea water as required. If more than a few minutes were required for manipulation the water in the bucket was changed so that the temperature of the water in the bucket varied within the limits of two degrees.

The colonies were carefully examined under the microscope and clinging organisms removed before placing in the flasks. If internodes were to be cut the colony was carefully placed in a flat crystalizing dish containing fresh sea water and the internodes cut by

means of a small, sharp scalpel, using a dissecting microscope. Each colony was taken in turn and as soon as all the internodes were cut for any one experiment they were placed in their respective flasks containing fresh, filtered sea water. The flasks were tightly corked, all air bubbles being excluded, placed in a bucket of sea water at sea temperature, carried to the dock and suspended for the duration of the experiment in the sea. If the whole colonies were to be used the same procedure was followed with the exception of cutting the internodes. The temperature of the sea water was usually 10° C. and varied within the limits of 1.5° C. during the season. In order not to permit growth and production of O_2 by diatoms or other small plant growths that may have escaped the examination under the microscope, on account of small size or for other reasons, the experimental flasks were kept in the dark during the course of the experimental period by covering the bucket.

At the end of the experimental period the flasks were removed from the bay and carried in a bucket of sea water to the laboratory. The colonies or internodes, as the case might be, were removed from the flasks in turn and the flasks analyzed for O_2 content. Three blanks were analyzed at the beginning of each experimental period for the O_2 content of the water as controls. The experimental colonies were kept between experiments in labeled dishes of fresh sea water, in most cases. In some of the experiments they were kept at room temperature and in others at sea temperature during and between the experimental periods. The temperature for any one period was kept constant.

In studies of the respiration of isolated internodes from the stems, the colonies were removed from the bucket as they were used in the cutting of the internodes. The colony was placed in the flat

dish containing fresh sea water and the internodes cut by means of a small, sharp scalpel. Care was taken to cut all the internodes evenly and squarely. When sufficient internodes for one flask had been cut they were transferred to a small dish containing fresh sea water, and the next set of internodes was cut. When all the internodes for any one experiment were cut they were transferred to their respective flasks, containing fresh, filtered sea water. The flasks were tightly corked, placed in the bucket, and carried to the dock. The internodes were handled very carefully to avoid injury. A smooth mouthed pipette was used in transferring them to the flasks where they were placed one at a time. At the end of the experiment the flasks were carried to the laboratory, the internodes were removed, and the water in the flasks analyzed for O2 content.

The internodes were removed from the flasks by a combination of two methods. A wire spatula curved to suit the purpose was used at first. But it was found that this method was too slow and was, therefore, subject to too much error if used alone.

It was, therefore, necessary to find a method that would remove the internodes quickly, without injury to the pieces, and without stirring the water to any great extent, and allowing any appreciable error. After much experimentation a method was found which would satisfy all these requirements. A small glass tube with very thin walls was selected so as to fit snugly in the mouth of the flasks, allowing a small rubber jacket to be placed around the tube as shown in figure I to prevent leakage. A shorter tube of somewhat larger diameter was fused into the side of the first tube just above the end used for insertion into the flask, figure I. e. A small ball of glass of a diameter just large emough to fit in the large tube but not small enough to go in the smaller tube was placed

in the larger tube, figure I.f. The two ends of the tubes were fitted with corks which were made to fit tightly by means of small glass rods inserted through the cork. In using this apparatus it was first necessary to fill the tubes with water of the same temperature as that which was contained in the flask from which the internodes were to be removed. Then the cork was quickly and carefully removed from the flasks and quickly replaced by the tube. All this was done in such a way that the water in the flask was not disturbed so as to cause currents and result in the mixing of the water in the flask with that in the tube. To remove the internodes the flask was inverted as shown in figure II. in such a way that the small glass ball in the tube was allowed to remain in the side tube so as not to interfere with the free fall of the internodes into the tube, as in figure II. After the internodes had fallen to the bottom of the straight tube against the cork, then the apparatus was inverted, care being taken that the glass ball would fall into the straight tube and prevent the internodes from falling back into the flask. The internodes were then allowed to flow out of the tube by placing in a dish of sea water and then removing the corks in the tubes. Any internodes that remained accidentally after this procedure were removed by means of the spatula. It will be seen that in this method the internodes were not subjected to any treatment whereby they would be liable to any injury. The water in the flask was now immediately analyzed for 02 content. The use of this apparatus to avoid error needed careful preparation and practice in manipulation. But after I became accustomed to its use the error was almost negligible.

The following table, I, shows tests made to determine the changes in 02 content, if any, of flasks containing fresh, filtered sea water

after disturbing the water with a spatula precisely as done during the process of removing the internodes or whole colonies from the flasks. In the first column the O_2 content of the control flasks which were not disturbed are given and in the next column in the same file are given the O_2 content of flasks containing sea water taken at the same time and from the same lot as the controls but which had not been disturbed prior to analysis by a spatula. The differences are given in the third column. The results show that any error from this source is so small as to be negligible.

In table V. tests are given to determine the change, if any, in the less than air saturation by boiling, after disturbing with a spatula precisely as done in removing internodes or whole colonies from the flasks.

As in table I. the first column shows the O_2 content of flasks containing water not disturbed by the spatula, the next column shows the O_2 content of flasks containing water from the same lot that had been disturbed by the spatula, and the differences are given in the third column. Again any error due to disturbance by the spatula is negligible.

In table II. tests are given which were made to determine if the O_2 content of the control flasks or blanks would remain constant over a period which would embrace the longest experimental period used in the course of the work. A knowledge of any such variation in the O_2 content of the water in the experimental flasks without the presence of experimental organisms is, of course, essential to a correct calculation of the O_2 consumption of the organisms by means of this method. If the water would vary in O_2 content due to other causes than consumption by the organisms, of course, the method could not be used. Tests were made with twelve different flasks

containing fresh, filtered sea water, six of which were allowed to remain standing for four hours, and six for 24 hours. At the end of that time the flasks were analyzed for O_2 content and the decrease or increase computed from the O_2 content of controls analyzed at the beginning of the tests. It will be seen that again the variations are almost negligible, the highest variation not exceeding 0.05 of a cc. of thiosulphate. This variation, due probably to bacterial action, although very small, will be taken into account in the evaluation of the results of the work reported in this paper.

In table III. tests are given to determine the amount of variation in O_2 content of the experimental flasks due to the use of the special apparatus described above for the removal of the internodes from the flasks at the end of the experiments. Tests were made with twelve flasks and the error computed from controls which were analyzed at the beginning of the tests. The highest variation is 0.07 cc. thiosulphate or about 1 percent.

In table VI. the same tests were made as given in table III. using water that had a lower concentration of O_2 than the normal sea water. The differences are given in column three and are again practically negligible. Thus the change in the O_2 content of water in the flasks which had a lessened O_2 concentration due to consumption by the internodes of Obelia, that might be due to the use of the apparatus described above for the removal of the internodes, is also practically negligible.

In table IV. are given results of tests which show what is the degree of constancy of the rate of O_2 consumption by different Obelia colonies over different periods of time. The table shows tests made immediately and at the end of 24 hours. The experimental period and all experimental conditions were exactly similar in the two de-

terminations. The difference between the two tests are given in column five. The greatest difference is 0.10 cc. thiosulphate. Since six different typical colonies were used the tests should provide ample evidence that the experimental material has a rate of O_2 consumption of sufficient constance to permit experimentation. The only work that I have been able to find reported in the literature that has been done in determining directly the rate of O_2 consumption of hydroids, is that by Schonborn on the O_2 consumption of the stem of Tubularia during the regeneration of new polyps. He also used a modification of the Winkler method for the determination of the amount of dissolved oxygen. He found in tubularia that the rate of O_2 consumption did not vary from day to day unless the experimental periods were so long as to cause a lack of O_2 to the organisms. For the two hydroids, Obelia and Tubularia, then it would seem that the rate of O_2 consumption is constant from day to day in normal adult colonies.

II. Experimental.

(a) Experiments on normal, fully differentiated Obelia colonies.

(1) The effects of mechanical injury on the rate of O_2 consumption.

As stated above one of the primary reasons for this research was a desire to determine the changes in the rate of O_2 consumption during the regeneration of new polyps in cut internodes of Obelia. Now in order to determine such changes it was necessary to start with the initial consumption of the cut internodes as controls. But before we could be sure that the rate of O_2 consumption of the regenerating internodes was different from the normal rate of O_2 consumption of the stem it was necessary to know the effect of the mechanical injury of the cutting as such on the rate of O_2 con-

sumption of a normal colony.

Therefore, several experiments were performed to determine the effects of cutting, if any, on the rate of O_2 consumption of normal, fully differentiated Obelia colonies.

The material for these experiments was the best that could be found. The branches of the species used were not very numerous but the polyps were large and normal. However, some of the colonies were rather old; the perisarc was dark over much of the main stalk. The colonies to be used in the experiment were carefully selected from the material and carried to the laboratory in a bucket containing fresh sea water at sea temperature. They were examined as usual, all foreign material removed, and were placed in turn in the experimental flasks containing fresh, filtered sea water. The flasks were tightly corked, all air bubbles being excluded and carried to the dock in a bucket of sea water where they were suspended in the water at sea temperature for the duration of the experimental period. The flasks were covered to prevent the access of light to the colonies. Previous to the experiment controls were run to determine the approximate rate of O_2 consumption of the colonies in order to select an experimental period of the proper length. A period long enough to provide a workable basis and at the same time not so long as to cause any lack of O_2 and resulting asphyxiation was selected. A four hour experimental period was decided upon in this instance.

Three duplicate blanks were analyzed at the beginning of the experiment for controls. Three duplicate experimental colonies were used in the experiment. At the end of four hours the experimental flasks were removed from the bay, carried to the laboratory in a bucket of fresh sea water, the colonies removed by means of a

small, curved spatula, and the water in the flasks analyzed for the O_2 content. Then the colonies were immediately placed in turn in a small, flat crystallizing dish and cut into small pieces by means of a small, sharp scalpel. The pieces were placed in their respective flasks containing fresh, filtered sea water, the stoppers inserted, and were again suspended from the dock for the duration of the experimental period.

At the end of that period they were again carried to the laboratory, the pieces removed in the same order in which they were placed in the flasks and the contents of the flasks analyzed for the O_2 content. Since controls had been analyzed at the beginning of the experiment, the O_2 consumption of the cut colonies could be determined. The difference between the rate of O_2 consumption of the normal colony, and of the same colony when cut into pieces is then calculated.

The results of these experiments as well as a typical experiment, similar to these but performed on totally dedifferentiated colonies, are given in Table VII. The part of the table dealing with the effect of injury on the dedifferentiated colonies will be discussed and analyzed later.

The most striking thing about the results of these experiments is the fact that in every case as shown in the table VII, an injury to the colony by cutting results in a decrease in the rate of O_2 consumption. That these results are well beyond the limits of experimental error can easily be shown by comparing the total possible experimental error with the difference between the rate of O_2 consumption by the whole colony and by the same colony when cut. The total variation in the O_2 content of the flasks due to other

reasons than consumption by the colony is, as shown above, approximately 5 percent. But the smallest decrease in the rate of O_2 consumption in all the experiments performed on this phase of the problem was 27.02%. This figure will be found in table VIII, A, in the last column and third file. This table is a condensed resume of several experiments performed to test this question. The results all show the same thing, a decided decrease in the rate of O_2 consumption of Obelia colonies following cutting. Since these same results were secured over and over again there can be no doubt that the results are correct. Eighteen different colonies in six different experiments showed the same results. However, the results were not able to show a constancy in the percentage falling off of the rate of O_2 consumption. This varies from 27.02% to 81.81% as shown in the last column of table VIII.

Having determined the effect of the mechanical injury due to cutting on the rate of respiratory exchange in internodes of Obelia, I next endeavored to determine the changes in the rate of O_2 consumption of normal Obelia colonies due to the removal of the polyps by cutting. In order to have an index as to the magnitude of the rate of O_2 consumption to be expected in the stems and cut internodes of Obelia it was necessary to determine, if possible, the amount of the respiration of the whole colony that took place in the stem alone. In order to determine this, several normal colonies were selected and the O_2 consumption over an experimental period of four hours, was determined. Then these same colonies were taken and the polyps removed by means of a small, sharp scalpel. The stalks of the colonies were then placed in their respective flasks and the O_2 consumption over the same time period, four hours, was

determined for the colonies without polyps, at sea temperature. At the end of the experimental period the polypless stems were removed from their respective flasks and the contents of the flasks analyzed for O_2 content. Taking the difference between the O_2 consumption of the whole colonies for four hours and the O_2 consumption of the same colonies without polyps for the same time period, we find that there is a large decrease in the rate of O_2 consumption after the removal of the polyps. Such a result might be expected since the polypless colonies contained much less volume of coenosarc than the whole colonies.

Precautions were necessary during the removal of the polyps to prevent an appreciable increase in the temperature with resulting changes in the rate of respiration, which could not be ascribed as entirely due to the removal of the polyps. The average time period for the removal of the polyps from one colony was one hour. The colonies were, therefore, kept in a large volume of sea water at sea temperature during the process of removal of the polyps. The water was changed at small intervals, ten or fifteen minutes. The temperature of the water bath varied within the limits of two degrees.

The polypless colonies were kept for a few days in fresh sea water at room temperature to follow the course of regeneration of new polyps. Tests were now made on these colonies to determine the effect of the regeneration on the rate of O_2 consumption.

The results of two of the experiments performed are given in Tables IX and X. The results of each of these tables are the averages of two experiments in which the experimental conditions were identical. Together they embrace all the experiments performed on this phase of the problem. All the tests on any one colony are

given consecutively and appear in order of the number of the colony as given in the first column. Thus the colonies are numbered, 1, 2, 3, and etc. and the tests made appear in the succeeding columns, the time of any particular test being found in the second column. The condition of the colonies, that is, whether before or after the removal of polyps is given in column three and the O_2 content of the control or blanks are given in columns four and five. The two succeeding columns show the content of the experimental flasks and the difference between the latter and the controls; this difference represents the consumption of the Obelia colonies. The difference between the first and the second rows or files is the difference, for each colony between the O_2 consumption of the normal colony and the same colony without polyps. The ratio of the two are given in the last column with the O_2 consumption for four hours of the normal colony as the numerator of the ratio.

In table IX three determinations were made after the initial one following the removal of the polyp, to determine the changes in the rate of O_2 consumption during the regeneration of new polyps. The percentage change due to this cause is given in the last column, also, for each colony, below the ratio of the whole and cut colonies.

The increase in the rate of respiration as given in the tables will be seen to be constant and gradual with the exception of the fourth test, 35- 45 hours after the beginning of the experiment, for colony number three. It will be noted that in this test a decrease is found from the previous test of 1.025 cc. thiosulphate. This decrease may be due to a temporary cessation of the process of regeneration, or it may be due to some accidental experimental error.

In table X only one test was made to determine the effects of

the regeneration of new polyps on the rate of O_2 consumption. Here there is very little increase in the rate of O_2 consumption and in colonies 1 and 5 there are slight decreases. However, the time of this test was only 26-30 hours after the beginning of the experiment and the indications of regeneration of new polyps were very slight. The polyps that had started to regenerate were very few and some of the cut ends showed a slight beginning of dedifferentiation. For that reason the tests were discontinued after the first. The results of these experiments then show definite decrease in the rate of O_2 consumption after the removal of the polyps from a colony.

However, since the largest decrease in the rate due to the removal of the polyps is a little over 86 percent and the largest decrease due to injury of cutting alone is 81.81 percent as shown in table VIII., we cannot be sure that the decrease found in the rate of O_2 consumption of Obelia colonies due to the removal of the polyps may not be due to the effect of the mechanical injury of cutting off the polyps.

As shown above the effect of cutting the Obelia colony into pieces is to cause a decrease in the rate of O_2 consumption, and as also shown, in the experiments on the effect of polyp removal on the rate of O_2 consumption, there is a tendency for an increase in the rate of O_2 consumption following the cutting if sufficient time is allowed to elapse for recovery processes to set in. An increase could only be expected in view of the fact that the rate of O_2 consumption as a result of the cutting is much lower than the rate of O_2 consumption of the normal, uncut colony.

To test this question thoroughly tests were made on several

different colonies to determine if there was an increase in the rate of O_2 consumption during the regenerative processes following cutting.

The results of three of the experiments performed are given in tables XI, XII, and XIII. All the experiments showed the same results but lack of space prevents the tabulation of all of them.

In each of the tables named above the plan of arrangement is essentially the same as in tables IX and X, with the exception of the last two columns. The last column in tables XI, XII, and XIII gives the total percent increase in the rate of O_2 consumption from the time of the beginning of the experiment till the end. The next to the last column in the same tables gives the total percent of internodes showing a capacity for regeneration. A beginning of regeneration of a new polyp at either end was taken as an index of a capacity for regeneration.

In order to secure a large amount of regenerating tissue the internodes of the stems of the colonies were cut by means of a small, sharp scalpel. In the experiment reported in table XI only the internodes of the main stem were used and the internodes were cut so that each of the three duplicate experimental flasks would contain the same number of internodes, and each set of internodes would have the same relation to the apical and basal ends of the colony as the other sets. In this way any difference in the rate of O_2 consumption between the apical and basal ends of the colonies would be eliminated from these experiments. Four successive tests were made on the internodes. The material for these experiments was collected immediately prior to the experiment at the docks at Friday Harbor. The usual precautions were taken in handling the material and in cutting the internodes. The internodes were kept at sea temperature 10.5° C., during and between each test.

The tables show a gradual increase in the rate of O_2 consumption during the process of regeneration of new polyps. The O_2 consumption for each test of each set of internodes is given in the seventh column of the table and the total percent increase for each set of internodes is given in the ninth column of the table. XI, The only test that does not show an increase in the O_2 consumption is the last test of the third flask in column seven. Here there is a slight decrease but this was attended by no increase in the amount of regeneration that was noticeable. The increase varies in this experiment from 70.129 percent to 90.69 percent. This increase is far beyond the limits of the experimental error.

In the second experiment given in table XII, the colonies from which the internodes were cut were collected from a different source than in the preceding experiment and were of a different species. They were removed from the live box for experimentation about fifteen hours after collecting and were handled in the usual way. Four flasks containing sixty internodes each were used in this experiment. Three tests were made on each flask. The experimental periods were twelve hours long and the internodes were kept at sea temperature during and between each test. The plan of the table is the same as table XI. Again an increase in the rate of O_2 consumption during the regeneration of new polyps is noted. However, in flasks 2 and 3 it will be noted that the percent increase is comparatively low, 20 and 10 percent, respectively. This is undoubtedly due, however, to a delay in the process of regeneration as shown by the condition of the internodes in these flasks at the time of the second test and by the amount of O_2 consumption of these two sets of internodes during the second test. Observation

showed no regeneration and the tests showed no increase in the rate of O_2 consumption. In the succeeding test, however, the third, when regeneration had begun there is also an increase in the rate of O_2 consumption.

From these results there seems to be little doubt that regeneration of new polyps is accompanied by an increase in the rate of O_2 consumption. The only increase in this experiment that is not well beyond the limits of experimental error is that obtained with the internodes in the flask number four. This increase of 10 percent, might possibly have been due to error; the other results are beyond any possible error due to the method. In flask number two of this experiment the percent of internodes showing a capacity for regeneration were counted but the notes were accidentally lost and the space is left blank in the table.

The results of the third experiment are given in table XIII. The material for this experiment was collected from the same place as the preceding experiment. The colonies were clean and in good condition but were not quite so young as those in the previous experiments. Three duplicate flasks containing ninety internodes each were used. To secure a greater amount of O_2 consumption the internodes were kept at room temperature, 14-16 degrees C., during and between each test. The temperature was kept as constant as possible by means of water baths.

It will be noted that there is an enormous increase in the rate of O_2 consumption following the process of regeneration in these internodes. It is again significant that in the second test of the third flasks where no regeneration is noted that there is no increase in the rate of O_2 consumption, but a decrease. A point of

interest in this table is the small initial rate of O_2 consumption as compared to the initial rate as given for the internodes in the preceding table, XII. This small initial rate of O_2 consumption may be due to the condition of the material that was used in this experiment. The material was rather old, the perisarc being dark over much of the main stem. The lower parts of the main stem of some of the colonies showed beginning dedifferentiation. This small initial consumption was secured in spite of the fact that ninety pieces were used in each flask and that the tests were carried out at room temperature. The total percent increase in the rate of O_2 consumption due to the regeneration of new polyps is undoubtedly connected with the total amount of regenerated tissue but it is very hard to demonstrate such a relation. In the experiments given in tables XI, XII, and XIII, the total number of internodes showing a capacity for regeneration were counted and the percent calculated from this, but no direct relationship between this capacity for regeneration and the total increase in the rate of O_2 consumption can be established since some sets of internodes with a greater capacity for regeneration than some other sets, as shown by the beginning of a regenerated polyp or stolon, might have a much smaller volume of regenerated tissue than the set which had the smaller total percentage capacity for regeneration.

However, the tables show that there is an undoubted increase in the rate of O_2 consumption due to the regeneration. The differences are far beyond the limits of experimental error.

After having determined the effect of regeneration on the rate of O_2 consumption of internodes of Obelia, several experiments were performed to determine the difference, if any, between the basal and

the apical ends of the stems of Obelia colonies. Several experiments were performed as a preliminary to the experiments cited here in order to test the technique, determine the proper number of internodes to use, the proper length of the experimental period, etc. When all of these had been determined an experimental period of ten hours was decided upon for the first experiment, and fifty internodes were placed in each flask.

Internodes were used instead of the whole stems because a more accurate measure of the amounts of protoplasm taking part in the metabolism could be made, and also for convenience in handling.

In the first experiment reported here, table XIV, the internodes were cut from the best material possible and the colonies were carefully examined under the microscope prior to the cutting. Only the main stalks were used in cutting the internodes and any basal internodes that were not entirely differentiated and healthy were rejected. The internodes on the main stalk were counted and divided into three equal parts. If the number of internodes on the main stalk was not divisible by three the one or two extra internodes were removed from the basal end of the stalk. The apical third of the internodes were placed in one flask, marked "Apical Pieces", the middle third of the internodes in another flask, "Middle Pieces", and the basal third of the internodes in a third flask, "Basal Pieces", in table XIV.

The internodes were kept at room temperature during and between each test. The temperature was kept as constant as possible, 14.5° - 16.5° C., by means of water baths.

The internodes were carried through three successive tests after the first to follow the changes in the rate of O_2 consumption

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attendant on the process of regeneration of new polyps.

In a comparison of the rates of O_2 consumption in the different parts of the main stalk it will be necessary to compare the first determinations of the respective flasks only. Doing this, we find that the largest total rate of O_2 consumption is in the apical pieces. (Figures are given in the first test for each flask in column seven.) However, this is scarcely a correct comparison since no account of the amounts of the protoplasm in the various sets of internodes has been taken. In order to make these results more comparable, the internodes were preserved and the volume of the coenosarc within the perisarc in each internode measured by measuring the intraperisarcal volume. Since in all the fresh and living internodes the coenosarc adhered to the perisarc, the measurements were a correct measure of the volume of the coenosarc at the time of the first test. These measurements were made by the use of an ocular micrometer with the low power of the compound microscope. From these measurements the total volume of the coenosarc in the different sets of internodes was calculated and from these the rate of O_2 consumption per cubic millimeter of coenosarc and, therefore, of living tissue in each set.

The measurement of the volume of the coenosarc has the advantage that the perisarc is eliminated from the calculations which could not have been done by weighing the internodes. On the other hand, the density of the coenosarc and, therefore, the amount of non-respiring water or other liquid parts are not as well taken into account by measuring the volume as by weighing. In addition the differences in the volume of the gastro-vascular cavity in the apical and the basal ends is not taken into account by the measurements made. Taking the values for the O_2 consumed per unit volume of

initial rates of consumption we find as before that the apical internodes show the largest total amount of O_2 consumption, but in calculating the rate of O_2 consumption per unit of coenosarc, we find that there is a much higher rate in the basal internodes.

In turning to the next experiment on the differences in rate of O_2 consumption between the apical and basal ends of Obelia colonies, table XVI, we find that here the same results are secured. Ninety pieces were used in this experiment over an experimental period of ten hours. The differences between the initial consumption of the respective sets of internodes is far beyond the possible experimental error. The difference between the total amounts of O_2 consumption for this period is 0.50 cc. thiosulphate. This difference exceeds the amount of possible variation by experimental error which would amount in this instance to a little less than 0.20 cc. thiosulphate. Thus there can be no doubt that the difference is real.

In each of the three experiments given above in tables XIV, XV, XVI, one or more tests were made after the first to add to our data concerning the changes in the rate of O_2 consumption during the process of regeneration of new polyps. The total percent increase in O_2 consumption during regeneration is given for each flask in the last column in each table. In tables XV and XVI the increases are not large. This may be partly due to the high initial consumption of the freshly cut internodes. Material for these two experiments was younger and in much better condition than the material used in the experiment the results of which are given in table XIV.

Having made determinations to add some data on the changes in the rate of the O_2 consumption of internodes of Obelia colonies during the process of regeneration as outlined above, later tests

were made to determine the changes in the rate of O_2 consumption attendant to the process of dedifferentiation of the polyps of normal, fully differentiated Obelia colonies. As outlined above, many workers have studied the morphological changes occuring during the dedifferentiation of various organisms or parts of organisms, but no one has made any direct determination of the rate of metabolism during the process of dedifferentiation.

Huxley, working on Perophora, assumed that the rate of metabolism of a dedifferentiating zooid decreased during the process of dedifferentiation of the zooid, using as an index a change in the susceptibility to KCN. Such results are very inconclusive, however, and some direct evidence is needed on the subject.

The process of dedifferentiation of the polyps of Obelia has been described above. Several experiments were performed to determine the changes in the rate of O_2 consumption during this process of dedifferentiation. All the experiments were carried out under similar experimental conditions and are, therefore, comparable among themselves. All of them show the same results, a gradual decrease in the rate of O_2 consumption during the process of dedifferentiation.

Taken as a typical experiment, one of the experiments performed is given in table XVII. In this experiment six normal Obelia colonies, colony 1, colony 2, etc., were taken from freshly collected material and the O_2 consumption for the period of four hours determined at room temperature, 14° - 15° C. The colonies were then kept at room temperature in sea water changed every four hours and allowed to dedifferentiate. Determinations of the O_2 consumption over a four hour period were made every 20 hours, given in

column 2, at room temperature. In colonies 1,2, and 5 the process of dedifferentiation, as far as could be observed, had not started at the time of the second test, 24 hours after the beginning of the experiment. As shown in column seven for the second test for these colonies the decrease in the amount of O_2 consumption is either very slight or not present at all.

In colony three the process of dedifferentiation was not noticeable by examination under the microscope until the time of the third test, 72 hours after the beginning of the experiment. In the first two tests made there is no decrease in the rate of O_2 consumption but an increase, column seven. This increase is probably due to the fact that the colonies were kept at a temperature four to five degrees above that to which they were accustomed and until the process of dedifferentiation had set in the rate of O_2 consumption might be expected to be higher than when first taken from the sea. If we accept this explanation then we may interpret the very small decrease found in the second test of colony 2, column seven, as due to processes of dedifferentiation which were not at that time manifested in external changes in the morphology of the polyps.

It will be noted in the last column of the table that the lowest rate of O_2 consumption for each of the colonies is found at the time of the sixth test. By referring to the condition of the colony at the various tests given in the third column, it will be noted that the point of most complete dedifferentiation was reached at the time of the sixth test and at the time of the last test, each of the colonies showed more or less evidence of regeneration of new polyps. As shown in the seventh column, the largest decrease in the rate of

O_2 consumption in percent of normal, 80.60 percent, is found in the sixth test of the fourth colony, and the least decrease, 46.30 percent, is found in the sixth test of the third colony. This means a decrease in every case of approximately 50 to 60 percent of the normal rate of O_2 consumption.

We have in this experiment very good corroboration of the results obtained in the experiments to determine the changes in the rate of O_2 consumption occuring during the regeneration of new polyps in cut internodes of Obelia, tables XI, XII, and XIII. The last determination for each colony shows an increase in the rate of O_2 consumption, columns seven and eight. This increase is associated with a noticeable regeneration of new polyps. The increases, with the possible exception of colony six, are beyond the limits of experimental error. The method shows itself here sensitive to small changes in the rate of O_2 consumption and brings out clearly in this instance the reverse nature of the processes of dedifferentiation and of differentiation as related to the rate of O_2 consumption. The colony showing the most regeneration of new polyps, colony four, showed the largest increase in the rate of O_2 consumption, proof of the correctness of the results.

There can be no doubt then that the process of dedifferentiation of the polyps of Obelia, which is the same as the process noted in other hydroids by Loeb and Thacher, is attended by a decrease in the rate of O_2 consumption.

Although we cannot give an exact quantitative relation between the increase in the rate of O_2 consumption during the process of regeneration and the decrease during the process of dedifferentiation, since no measurement of the actual amount of protoplasm tak-

ing part in the metabolic processes was attempted, yet we can be certain from the results that the changes are dependent on and proportional to the amount of regeneration or of dedifferentiation.

Since all the experiments performed to determine the changes in the rate of O_2 consumption during the process of dedifferentiation gave the same results, only the results of one experiment are given, as it is typical of all the others.

The objection might be raised to the results of this experiment, table XVII, that during the process of dedifferentiation the colony is especially open to the activity of bacteria that occur to a greater or less extent in sea water. This activity would result in an increase in the number of bacteria and would cause, therefore, a more or less large error in the determination of the rate of O_2 consumption. Such an error would, however, tend to nullify the decrease in the rate of O_2 consumption due to the process of dedifferentiation. Since no such results were obtained, but a large decrease in the rate of O_2 consumption due to dedifferentiation was obtained in every experiment, we can only conclude that the activity of bacteria in the experimental flasks was negligible for not only these experiments, but all the others reported here, since these experiments on dedifferentiation would be the most likely to be affected by the activity of bacteria.

The objection might also be raised that the presence of protozoa in the experimental flasks would influence the results. The only experiments in which protozoa were observed at all were in the tests made during the later stages of the process of dedifferentiation. Yet the increase in the rate of O_2 consumption due to the presence of the protozoa was not enough to offset the decrease in

the O_2 consumption due to the dedifferentiation. Since these results were not vitiated it is safe to assume that the other experiments on colonies that were not in a process of dedifferentiation where no protozoa were observed, were not affected to any appreciable extent by the presence of unicellular organisms.

No attempts were made to determine the factors that caused the occurrence of the process of dedifferentiation, beyond the fact that the dedifferentiating colonies were kept at room temperature, 14 - 15°, C. in water that was not always fresh. However, several important observations were made. It was noted on a large number of colonies that the process of dedifferentiation is naturally occuring in the normal state of the colonies in the sea. Many colonies, especially those taken from eel grass where a more or less large amount of debris, both organic and inorganic, was found, showed dedifferentiation of the polyps on the basal branches. A few polyps were noted in the apical regions that were in a state of dedifferentiation. It is hard, of course, to determine the factors that were the cause of the dedifferentiation. It may have been adverse chemical constitution of the water due to the presence of the debris, or it may have been due to contact with some solid objects. Some of the dedifferentiated polyps were in contact with solid debris when found and apparently had been for some time.

Another fact which was observed and which I have not seen referred to in the literature, was the presence of dedifferentiating and of regenerating polyps on the same stalk, sometimes side by side. Such a condition seems to indicate that the stimulus for the dedifferentiation of the polyps in the case observed came from within the organism itself and that the process of dedifferentiation may be

inaugurated by changes in the physiological state of the organism, without the stimulus of an external force.

Having determined that the rate of O_2 consumption of dediff-erentiated Obelia colonies is much lower than the rate in fully differentiated colonies and knowing also that the effect of mechanical injury on the rate of O_2 consumption of differentiated colonies was such as to cause a large decrease in the rate of O_2 consumption, it became interesting to know what the effect of mechanical injury would be on the rate of O_2 consumption of fully dediff-erentiated colonies.

Six different experiments were performed to determine this question, the results of which are given in tables VII and VIII,B. In the table VII B one of the experiments is given in detail.

The results of these experiments are very important. They show that the effect of injury on a colony that is already dediff-erentiated is such as to cause an immediate rise in the rate of O_2 consumption, and not a decrease as was found in the case of differ-entiated colonies. The percentage increases are given in the last column of the tables. They are far beyond the limits of experimental error.

IV. Significance and critical evaluation of this study.

In evaluating any results of work done on such processes as the recovery from mechanical injury, regeneration or differentiation, and dedifferentiation in living organisms it is necessary to keep in mind the fundamental properties of living matter if we expect our data to throw any light on the nature of those processes.

Living organisms are distinguished from nonliving things by the presence of certain properties which are essential for the main-

tainance of the living condition.

These properties are usually classified as follows:

(1) The power of movement.

The power of movement is possessed by all living organisms both plant and animal. In the former it generally takes the form of direct movement. This movement takes place as the result of stimuli which may be either external or internal in origin. The energy required for growth has, however, a direct internal origin, and the direct movement may also although not necessarily be initiated by an energy change taking place within the organism, which acts as a stimulus. In attempts to explain the processes attending movement they are translated into terms expressing the energy changes which occur in the nonliving world. Thus we endeavor to translate a process defined in unknown, vague terms, into those that are known and definite.

(2) The power of growth.

All living organisms have the inherent power of growth. This process of growth is the result of additions to the structure from the inside of the organism by changes in the internal mechanism of the organism.

(3) The power of reproduction.

The property of reproduction is always associated with living forms. It is easily demonstrated in the very smallest even of living organisms.

(4) The process of respiration.

This property requires the use of special apparatus for detection and measurement but it is very fundamental in living organisms. Prior to the discovery by Lavoisier in 1775 of the source of the production of heat in the animal body many authors ascribed to

a special psychical force the transformations of energy that take place within the body. From that time on there has been a universal attempt by biologists to determine the nature of the socalled vital processes in terms of the known method of measuring the energy transformations that occur in the nonliving world.

(5) The property of irritability.

It has been demonstrated that most animals possess the property of irritability and it is rather generally assumed that the ones in which it has not been demonstrated possess this property as one of the fundamental properties of living matter.

Since in this work an attempt has been made to find out the relation of such processes of living organisms as, response to mechanical injury, regeneration following injury, dedifferentiation under certain unfavorable conditions, etc. to the rate of metabolism as an index of the processes underlying such changes, it is highly pertinent to outline the importance of such knowledge. It is understood that living matter does not create energy but merely transforms energy from one form to another and transfers energy from one point to another. Therefore, it is logical to assume that such changes as above mentioned in the life course of an organism are attended by certain causative, internal changes or reactions. It has been well demonstrated in the past that the source of practically all energy in the organism comes either directly or indirectly from the oxidation of food material within the organism. However, oxidations are not the only form of energy change that may or do occur in living matter. There are reducing reactions which are always a corollary to oxidizing reactions. For a substance to be oridized it must be able to reduce another substance to secure the oxygen or else the reducing bodies may unite together, forming

synthetic products. Thus there is a continual reaction in living organisms between the reducing and the oxidizing substances.

Another type of reaction is the hydration processes. Certain substances take up water with resulting decomposition. Many of the disintegration processes are of this kind. All digestive processes are of this kind. Somewhat the same, but the reverse, are the dehydration processes whereby many of the more complicated parts of living protoplasm are synthesized. In addition to all these forms of reaction there is the important phenomenon of correlation of all the processes, chemical, physical, or otherwise in the living organism which until very recently has been the most fruitful source for the argument of the vitalists. If the parts of a cell are so thoroughly mixed that all organization is lost and cannot be reformed then the cell dies. There is then a definite arrangement of all the parts of the cell. This arrangement as well as the correlation of reactions in the various parts of the cell is determined by the delicate controlling influences which have only lately been intelligently investigated. These investigations show that these processes may be either electrical or physical or chemical in nature. One phase of this system of correlation embraces the whole process of nerve control and stimulation, the whole field of irritability. Thus we must have recourse to the sciences of physics and chemistry for an accurate understanding of the various processes of living matter which have in the past been defined and even described in terms that were arbitrary, vague, and misleading, without reference to any of the modern, fundamental phraseology and standards of measurements of energy transformations and transfers which occur in such processes of living matter.

Therefore, following the first statement that most of the energy change in living organisms are the result either directly or indirectly of oxidation of food material it will be recognized that it is of the highest importance to have a knowledge of the changes in the rate of O_2 consumption of an organism during such vital processes as those described above.

For a critical analysis of the preceding experiments it must be first understood that the results are beyond the limits of experimental error. This was shown to be the case in the discussion of the individual experiments. Assuming then that this point has been adequately demonstrated we shall then pass to an evaluation of the results of these experiments in the light of present day work in this field and of modern interpretation of living processes.

In the first place most modern biologists admit that vital phenomena are the result of processes taking place within a colloidal complex, having as a ground substance water, in which are suspended certain substances in various states of aggregation. Living protoplasm has all the properties of a true colloid which has undoubtedly a large variety of components and phases with their attending multiplicity of possible changes and variations of form, structure, and constitution. It is highly probable then that numerous changes, chemical, physical, and electrical are taking place continually in the colloidal complex that constitutes the organism. It is highly important to know then what effects such disturbances as mechanical injury have on such systems, and what changes are taking place in such a complex during the processes of regeneration, dedifferentiation, etc.

Taking the first problem that we set ourselves to give some data on, we find that it was clearly demonstrated that there is a

large decrease in the rate of O_2 consumption due to the mechanical injury of Obelia colonies by cutting. What does such a decrease signify? We have seen in some of the experiments that such cutting of the colony is always followed by the regeneration of new polyps on the cut ends, tables XI, XII, and XIII. Thus recovery from the injury takes the form, in its later phases, of regeneration. We have seen that many authors have the conviction that any process of differentiation is preceded by a process of dedifferentiation or a return to a more embryonic state of the cells and tissues involved. What is the nature of such a return to a more embryonic state? Is it a hydrolysis of the complex radicals that make up the protoplasm? Since there is a decided decrease in the rate of O_2 consumption we should expect that the oxidative energy that was formerly used in the normal colony was here in the dedifferentiating colony replaced by energy from another source. This energy might come from the process of hydrolysis which might logically be the cause of the break down of the dedifferentiating tissues. However, to cause such extensive changes that are evident in the dedifferentiating tissue, the process would necessarily be the hydrolysis of large masses. If such were the case then the same hypothesis would hold for the decrease in the rate of O_2 consumption due to the injury of the normal colony. This decrease seems to indicate that there is indeed a process of dedifferentiation immediately following the injury and that this dedifferentiation is then a preparation for the process of regeneration of new polyps which follows. All of the results on the rate of O_2 consumption attending a state of dedifferentiation seems to indicate that tissue in a dedifferentiated or embryonic state has a lower rate of O_2 consumption than the older or

more fully developed tissue. Also that since the rate of O_2 consumption is very low for dedifferentiating tissue and for tissue that has just been mechanically injured, tissues in such a state are undergoing a process similar to hydrolytic cleavage, only that the cleavage is affecting large masses instead of single molecules.

The effect of injury on the dedifferentiated Obelia colonies, tables VII and VIII (B), is such as to cause an increase in the rate of O_2 consumption. This result, the reverse of those obtained as the result of injury to fully differentiated colonies, is further evidence that mechanical injury to differentiated colonies causes a dedifferentiation of the injured part. These results would indicate that a state of dedifferentiation is necessary to the beginning of a process of differentiation.

Although various workers have contributed to the literature relating to the effects of injury, and various types of stimulation on the rates of respiration, most of the work is inconclusive.

There has much controversy as to the effects of various kinds of stimuli on the rate of the respiratory exchange in animals. One of the indices of the living tissue is the ability to respond in various ways to stimuli, external and internal.

The external stimuli may be classified as mechanical, thermal, chemical, electrical, or light. The response may take the form of a physical, chemical, thermal, light, or electrical response. All others may be grouped under these.

The respiratory exchange is an index of the chemical or metabolic processes taking place within the organism. Any response that the organism might make to any of the above forms of stimuli, is probably connected in some manner with the rate of the metabolic

processes. Therefore, in stimulating an irritable system such as a living organism we might expect a change in the rate of the metabolic activity.

Few cases are noted in the literature as to the effect of light on the respiratory exchange of animals. Krogh (33) in his book gives a review of some of the chief work that has been done on this phase of the subject. Moleschott (44) and his pupils working on frogs, Selmi and Piacentina (45) on the dog, and Potts (46) on the mouse, found that light had a stimulating effect on the rate of the respiratory exchange, the spectral colors differing in their effects. Loeb (47) working on the chrysalids of butterflies and Ewald (48) working on curarized frogs were unable to find any such effects. Krogh (33) objects to the results of any of these experiments, however, since the experimental conditions in the former were not favorable and in the latter experiments the cells in which the metabolic processes were going on were not reached by the light rays.

As to the effect of electrical stimuli on the rate of the respiratory exchange, D'Arsonval (49) found in 1891 that high frequency, alternating currents without producing any form of muscular activity increased the rate of the respiratory exchange as high as 100 percent. Krogh (33) thinks that his results are unreliable, however, and accepts as conclusive evidence the work of Loewy and Cohn (50) on man, and of Spaski (51) on rabbits, which shows that there is no real effect of the current on the rate of standard metabolism.

Later work by Tashiro (37) on the nerve tissue of cold-blooded animals tends to show that a direct current stimulates the rate of CO_2 production. However, Moore (52), using a different method, (Haas' indicator method), comes to the conclusion that the respir-

atory enchange of nerve tissue is not affected by stimulation by a direct current.

In the chemical influences on the rate of the respiratory exchange we have a large group of substances that have a more or less marked effect. Warburg (53) and his students have done a large amount of work in this field. He classifies the substances that produce such an effect as either lipoid soluble or lipoid insoluble. The former substances are able to penetrate into the cells, and thus all such substances, according to Warburg have an analagous effect, although chemically different. This assumption has later been shown to be erroneous for the anesthetic substances which are lipoid soluble do not owe their effect to an asphyxiating property. The latter substances, however, are very different both qualitatively and quantitatively in their action on cells and groups of cells.

Those belonging to the first group are either specific or non-specific in their action according to whether they have chemically active radicals or not, Among the specific substances are the aldehydes, the amines, etc. Some of these inhibit the oxidations while others stimulate them. Narcotics are regarded as non-specific in nature and always have an inhibitory effect on the rate of metabolism. Examples of this group are the urethanes.

The lipoid insoluble substances are exemplified by the amino acids, salts, sugars, etc.

Without going into details as to the work done on the thermal influence on the rate of metabolism, it is sufficient to state that the work of many investigators tends to show that the rate of metabolism is greatly increased by a rise in temperature. The best work,

however, has not been able to show that such response follows Van't Hoff's law.

If an animal responds to stimuli in various ways it is said to possess that vague property of life. Just what is the mechanism of response? Does response involve a change in the rate of the metabolic process or are the latter quite independent of the property of irritability? Mechanical stimulus is scarcely a true test of this question since it involves a more or less actual disintegration of the tissue. Such disintegration might well affect the rate of the metabolic processes without being a true index of the effect of a simple stimulus on such processes. This objection is borne out by much of the work done on this problem. Many workers have investigated the effect of injury and of mechanical disintegration on the rate of the respiratory exchange, but in no case has it been definitely decided as to the exact effect of the injury on the rate of intracellular metabolism. The work of Fletcher and Hopkins (54) and of the former independently(55) has shown definitely that the increased production of CO_2 following injury to muscle of warm blooded animals was due to the liberation of CO_2 from the carbonates of the tissue by lactic acid formed in the tissue following the injury. This was definite proof that the increase in CO_2 production was not due to an increased rate of metabolism. Later Tashiro (37), using his biometer, found an increase in the rate of output of CO_2 in nerve tissue, of the frog following injury.

However, later work by Moore (52) showed that this increase in CO_2 production could not be ascribed to increase metabolic rate but was due to the liberation of CO_2 from the carbonates. He demonstrated the presence of acid in freshly crushed nerve tissue by using phenolsulphonthalein as an indicator. He went even farther

and found lactic acid in cooked brain tissue of the frog. He showed that there is no increase in the rate of production of CO_2 by nerve tissue following injury if sufficient time is allowed to elapse to eliminate the production of $\check{C}O_2$ from the carbonates of the tissue. Thus all the results on the effect of injury on the rate of CO_2 production by various organisms are subject to many objections and the only way in which the experimenter can be sure of his results is to use the rate of O_2 consumption as an index of the effect of mechanical injury on the rate of respiratory exchange of organisms and of tissues.

Passing now to the work on the changes in the rate of metabolism in internodes of Obelia during the regeneration of new polyps, we find in corroboration of the previous work on injury that the initial rate of O_2 consumption following the cutting of the internodes is very small. However, in following the process of regeneration in the internodes we find that there is an increase in the rate of O_2 consumption. To what causative agents is this increase due? Assuming, as we have, that protoplasm is a colloidal substance capable of decomposition and also the synthesis of its components from the less complex, we come to the conclusion that in any process of regeneration, as well as in growth, there is the formation of new synthetic products. The low rate of O_2 consumption following the cutting, tables XI, XII, XIII, column seven, would indicate as stated above, that the process of regeneration is preceded by a dedifferentiation of the disturbed area and that this is followed by a redifferentiation of the material of the cells as indicated by the increase in the rate of metabolism following the process of regeneration. But if the process of dedifferentiation is not accompanied by a relatively high rate of the metabolism, why is it assum-

ed that the process of synthesis or differentiation necessitates a relatively high rate of O_2 consumption? The question is pertinent and needs an answer in this connection. Of course, one might assume a difference in the processes of regeneration and of growth and divide the determinations of the rate of O_2 consumption following the cutting of internodes into (1) The effect of the process of regeneration on the rate of the respiratory exchange and (2) The effect of the process of growth of fully differentiated tissue on the rate of the respiratory exchange. But on what basis will it be assumed that the processes of regeneration and of growth are different?

Morgan maintains that the rate of regeneration of the legs of salamander is quite independent of the reserve food supply, while the rate of growth is directly related to a certain degree to the reserve food supply. However, he did not take into account that the process of regeneration in the starved animals necessarily had a food supply from the tissues of the animal itself.

Unless we assume that there is a synthesis of food material from inorganic compounds of the air and water by the organism to supply food to the regenerating part, for which assumption there is no proof, we must assume that the regenerated part is formed at the expense of the older tissue. Then if this be assumed there must be a rearrangement of the parts of the older part of the organism or tissue. As discussed in the review of literature there are many theories as to the nature of this rearrangement, many of them without experimental foundation. However, as stated in the review of literature, most of the workers on the processes of regeneration and of dedifferentiation in various organisms and parts of organisms

have the belief that every process of differentiation or regeneration is preceded by a process of dedifferentiation. This process of dedifferentiation is somewhat generally and universally defined as a return to a more embryonic state. Such a returning process would necessarily involve changes in the structure of the colloidal complex, protoplasm. These changes would involve the breaking down to a more or less degree of the differentiated structure and therefore, the complex radicals of the chemical complex. Then after this breaking down process the process of transfer and differentiation would take place. If we accept the above theories what is the difference fundamentally between a process of growth and one of differentiation following injury? As far as the fundamental and intimate process is concerned it must be admitted that there would be no difference. The only difference would lay in the source of the more simple complexes which were to be synthesized to the more complex protoplasmic radicals such as are present in the fully differentiated tissue. If the two processes are then identical in nature, we should expect to find an increase in the rate of O_2 consumption during regeneration as well as during growth.

The best work on the rate of respiration during growth seems to indicate that the rate is higher during the growing period than after full growth is attained.

The works of Tigerstedt and Souden (56), and Magnus - Levy (57) and Falks (58) on man, as reported by Krogh (33), tend to show that the rate of respiration in young individuals is higher per unit weight than the rate in adults. Work by Miss Reuter, not published, on young and old Holothurian muscles shows very definitely that the rate of O_2 consumption is higher in the younger animals.

According to work by Benedict and Talbot (59), however, the higher rate of O_2 consumption can scarcely be due to greater amounts of active protoplasm.

Having drawn attention above to the fact that the processes of regeneration and of growth both require the presence of more or less large amounts of food supply, it is logical to assume that there may be a relation between presence of the food supply. and the increased rate of O_2 consumption. It is well known that large amounts of food material taken into an organism cause an increased rate of respiration, due to the socalled "specific dynamic action" of the food.

Here, then, it seems to me, is a logical explanation for the increased rate of O_2 consumption during regeneration noted in the experiments reported above, and for the higher rate of O_2 consumption noted by various workers in young and growing animals.

In conclusion, it is the belief of the writer that the increase in the rate of O_2 consumption during the process of regeneration as found in Obelia, is due to the same causes as the high rate of respiration during the growth of various organisms, namely, the specific dynamic action of the reserve food supply which is a necessary attendant to such processes as growth and regeneration.

Also, since a synthesis of simple complexes is necessary during regeneration, this synthesis may require the addition of oxidative energy for their consummation. The increase in the rate of O_2 consumption during the process of regeneration in Obelia tends to confirm a hypothesis put forth by Drechsel, as cited by Matthews (34), that dehydration syntheses may necessarily involve oxidation and reduction energy changes.

According to the best workers on the subject, the process of re-

generation and differentiation is always preceded by a dedifferentiation of the tissue involved.

If this is so one would conclude that differentiated tissue, made up of complex radicals, could be formed only from simpler radicals and not from complex components of perhaps another type.

What, then, is the nature of this dedifferentiation or loss of differentiated structure? Is it the same as autolysis, or tissue disintegration? A brief summary of the literature regarding the process of autolysis is pertinent here.

Autolysis or tissue disintegration may be regarded as one of the most constant properties of living matter. If we regard the use of the cytoplasm of the developing egg by the developing blastomere as disintegration then the first process of the developing organism is attended by disintegration of original structure. It is a fact, of course, that the last phase of living matter is disintegration.

Buchner's work cited by Levene (60) showed that alcoholic fermentation of grape sugar was possible when the yeast cell was dead. Therefore, an inorganic constituent caused the fermentation by an enzymatic process. The source of all animal or vital energy may be regarded as being attributed to tissue disintegration. It has been the prevailing conception that this disintegration was brought about by the burning of the constituents of a living cell. Salkowski showed that a cell in which all signs of life had disappeared still possessed the power of tissue disintegration, of autolysis. In 1871 Hoppe-Seyler (61) wrote: "All organs suffering death within the organism in the absence of oxygen, undergo softening and dissolution in a manner resembling that of putrefaction. In the course of the

process albuminous matter gives rise to leucin and tyrosin, fat to free acids and soaps. This maceration, identical with the pathologic conception of softening is accomplished without giving rise to ill odor, and is a process similar to the one resulting from the action of water, acids, and digestive enzyme".

In organs kept in an absolutely sterile condition Hauser (62) found a general softening and disintegration of structure. He observed the decay of the cytoplasm and disintegration of structure of the nucleus in a process known to pathologists as fatty degeneration. Schutzenberger and Salkowski (63) showed that in organs where only the process of dissolution was allowed, the cells gave up a larger part of their constituent material than before the process of dissolution when subjected to treatment with boiling water. Thus water soluble substances are formed during the dissolution. Similar changes occur in tissues subjected to digestive enzymes either in or outside the alimentary tract. Hoffmeister (64) called this process autolysis.

Thus Salkowski established the fact that tissues, placed in conditions that prevent contamination by putrefying agents, undergo changes similar to those produced by processes of digestion.

It has been shown by Hedin and Rowland (65), Stookey, and Levene (66) and others that this is a phenomenon common to all tissues and that it is due to a constituent of the liquid plasma. Two proteolytic enzymes, pepsin and trypsin, the former from the glands of the stomach and the latter from the glands of the pancreas are found in vertebrates. The former requires acid for its action, the latter is most active in the presence of alkali.

The formation of crystalline products of amino acids is only

possible by trypsin cleavage.

Albumoses and peptone are formed by both enzymes; tryptophan in the presence of bromin is a color test for cleavage by trypsin.

According to Bradley (67) acid facilitates autolysis while alkalies depress it except in muscle tissue where it is unaffected by either. This would indicate that autolysis is not due to trypsin carried in the blood. But Salkowski (60) has found leucine and tyrosin as after products of autolysis. Biondi (67) has shown that there is very little formation of albumoses and peptone by autolytic processes.

Jacoby (69) demonstrated tryptophan among the products of autolysis. The urine of normal individuals contains a proteolytic enzyme. Mathes removed the stomach of a dog and showed that the enzymes disappeared from the urine. He also removed the pancreas from a dog and allowed the animal to recover. No difference could be observed in the proteolytic power of the organs. Thus self digestion seems to be a constant property of surviving tissue.

Jacoby (70) showed that autolysis occurred in a living state by removing the blood supply of the liver of a dog. Schulze, as cited by Levene, found that in germination of plants substances appear which are also apparent in proteolytic digestion of seeds. Levene has made a similar observation on the developing egg of fish and of fowl.

Protein is a colloid made up of various nitrogenous acids. On heating with strong acids or alkalies it breaks down into the constituents nitrogenous acids: glycoll, alanin, amino-valerianic acid leucin, glutamic acid, phenylalanin, tyrosin, lysin, arginin, histidin, prolin, tryptophan, cystein. Nucleic acid is present in nucleo albumins which occur in great abundance in all nuclei. This

acid contains substances to which considerable role is attributed in the pathogenesis of disease. Its components are: phosphoric acid, carbohyrate, thymin, uracil, cytosin, adenin, guanin, hypoxanthin. Ordinarily very little amounts of the components of the proteins may be found. But during autolysis nothing of the original structure remains, the following components appearing: glycocoll, alanin, amino-butyric acid, amino-valerianic acid, lucin, glutamic acid, aspartic acid, pyrrolidin carbonic acid, tyrosin, phenylalanin.

The products of protein cleavage may be summed up as follows:
(1) Nitrogenous acids containing only one nitrogen in the molecule, monamino-acids.
(2) Acids with more than one nitrogen in the molecule, hexonbases.
(3) Substances resulting from the nuclear degradation, nuclein derivatives or nuclein bases.

On acid cleavage all the amino acids are obtained which are known to appear on the breaking down of protein. Some substances seem to be destroyed to some extent by autolysis since they appear in lesser quantities after self digestion than after acid boiling, especially, alanin, aspartic acid, glycocoll, and prolin.

Knowledge of the further change in amino acids during autolysis is rather meager. Stolte (72) has shown that amino acids exposed to tissue extracts give rise to ammonia, and Magnus-Levy (57) has demonstrated the formation of fatty acids in the course of autolysis. Diamino acids and other basic substances of the protein molecule suffer a similar disintegration. On autolysis of the pancreatic gland or gastric mucosa, the formation of diamines from diamino acids, a process analagous to the transformation of tyrosin into oxyphenylethylmin was observed by Lawrow (73), Langstein, (74) and by Levene (75). Arginin is transformed by a special enzyme into

urea and diamino-valerianic acid, Kossel and Dakin (76).Shiga, as cited by Levene (60) found the same enzyme in the yeast cell.

By what means does the organism, however, build up proteins from the products of digestion? Danilewsky and Okunew (77) showed that in the presence of rennet ferment a substance arises which is insoluble in water, from the soluble albumoses and peptones. Hill, as cited by Levene (60) showed that the starch digesting enzymes could convert sugar into starch and the starch back into sugar. Kastle and Loevenhart (78) showed that the fat-splitting enzymes could change fat to fatty acids and the acids back to fat.

The plasteins have been regarded by many workers as reconstructed native protein. Danilewsky noted their formation by the action of digestive enzymes on primary albumoses and peptones. Kurajeff (70) noted their formation only from the secondary albumoses. Bayer (80) found that plastein is formed from crystalline cleavage products. Stookey and Levene (66) failed to confirm these results which rest on rather flimsy evidence. Coagulation of peptones or albumoses in the presence of rennet ferment is possible but no reversion into coagulable protein is noted. Reversibility of action, so far as is known, is confined to enzymes affecting hydrolytic cleavages.

In nucleicacid, resulting from nuclear breakdown, the components, nuclein and the purine and pyrimidine bases are found in very small amounts in the fresh organs but on autolysed organs they are found to a considerable degree. It has also been shown by Jones (81) and Levene (82), independently, that the purin bases, adenin and guanin, while found to relatively large amounts in fresh organs (spleen) were absent in self - digested spleen, being changed to hypo-xanthin and Xanthin during the process of autolysis.

The pyrimidin bases undergo analagous changes. According to Schittenhelm (83) the entire nuclear destruction is accomplished by three enzymes, one breaking up the nucleic acid into its components, the second splitting off the nitrogen from the nitrogenous constituents, and the third completing the oxidation of the purin derivatives.

It has been shown by Stookey and Levene (66) that tissue extracts contain an antitryptic enzyme. They assume that the autolytic enzyme balances the antitryptic enzyme during the normal, healthy condition of the organism.

Starvation, adverse chemical conditions, phosphorus poisoning, etc. all lead to autolysis. Is autolysis analagous to dedifferentiation? As shown above the process of dedifferentiation also is initiated by adverse chemical conditions, starvation, and unfavorable conditions generally.

We do not know from the literature the changes which the metabolic processes undergo during dedifferentiation. No analysis has been made of the changes taking place, chemical or otherwise. For that reason any data on the changes taking place in the metabolic processes during dedifferentiation are extremely valuable. Does such a process involve merely the transfer of material or is there actually a breaking down of complex substances to less complex ones? In what way may the results of the experiments on dedifferentiation repeated above be interpreted to throw light on the preceding question?

The breaking down of compound radicals to simpler ones in living organisms is accomplished to a large degree by hydrolytic cleavages. A decrease in the rate of O_2 consumption of Obelia colonies

during dedifferentiation as reported above would indicate that perhaps the oxidative energy was here replaced by energy from another source. Such a source might be found in a process of a disintegration , not of simple molecules, but of large masses of protoplasm. The disintegration of such large masses would also account for the large morphological changes observed during the process of dedifferentiation. If we assume then that the process of dedifferentiation is, as the process of autolysis, a result of cleavage of the more complex radicals to form simple ones, what is the difference between the two processes.

It would seem that they are essentially the same type of process. The only distinctions I would make between the two processes are, briefly, the following:

(1) The process of dedifferentiation as it has been observed has not been carried to the degree of cleavage that is found in autolysis.

(2) In the process of dedifferentiation as found in nature, the organization of the cell is not entirely lost, as in the case of autolysis.

Concluding, it is apparent from the experiments reported above, table XVII, that there is a large decrease in the rate of O_2 consumption during the process of dedifferentiation of the polyps of Obelia colonies. From these facts I would draw the conclusion in the form of a hypothesis, that the process of dedifferentiation may partake of the nature of hydrolytic cleavage of large masses, and that this hydrolysis furnishes the energy which replaces the oxidative energy that is lost during the process of dedifferentiation.

Summary.

The following important points were brought out in the above paper :

1) The effect of mechanical injury on the rate of O_2 consumption in Obelia is such as to cause a marked decrease in the rate of O_2 consumption immediately following the injury.

2) This immediate decrease in the rate of O_2 consumption is followed by a gradual increase in the rate of respiration attendant to the process of regeneration of new polyps.

3) The removal of the polyps of Obelia colonies causes a large decrease in the rate of O_2 consumption, due partly, undoubtedly, to the decrease in the amount of protoplasm, and also, probably, to the mechanical injury of cutting.

4) The evidence of an increase in rate of O_2 consumption during the regeneration of new polyps was further strengthened by several especially arranged experiments on regenerating internodes of Obelia, all of which gave the same result , a marked increase in the rate of O_2 consumption during the process of regeneration, and the increase is proportional to the degree of regeneration.

5) Carefully performed experiments show that the rate of O_2 consumption per unit volume of coenosarc is higher in the basal end than in the apical ends of Obelia colonies.

6) The process of dedifferentiation of the polyps of Obelia colonies is accompanied by a decrease in the rate of O_2 consumption, the decrease being proportional to the degree of dedifferentiation.

7) The small rate of O_2 consumption always found just before the beginning of a process of regeneration would indicate, following the work on dedifferentiation, that a dedifferentiation of the tissue

involved precedes the process of regeneration.

8) If a totally dedifferentiated colony is injured, by cutting, a large increase in the rate of O_2 consumption was found. This supports the former assumption that the decrease due to cutting a differentiated colony was due to a process of dedifferentiation, as a preparation for regeneration.

9) Conclusions from the above were as follows:

(a) Mechanical injury to a tissue results in dedifferentiation of the tissue as a preparation for regeneration or differentiation.

(b) If the tissue is already dedifferentiated the process of differentiation sets in immediately following the injury.

(c) The process of differentiation is essentially the same as the process of growth, differing only in the source of the food material.

(d) An increase in the rate of O_2 consumption during the process of differentiation indicates that the building up of new tissue, a process probably of the nature of dehydration synthesis, requires the addition of oxidative energy for its consummation.

(e) The assumption of a gradient in rate of metabolism from the apical to basal end of hydroid colonies on the basis of rate of susceptibility to KCN, is not borne out by the work here reported. The gradient is in the opposite direction, basal to apical end of the colony.

(f) Evidence given above seems to indicate that the process of dedifferentiation may partake of the nature of hydrolytic cleavages of large masses. The energy obtained from these hydrolyses replaces, to a more or less extent, the oxidative energy usually required by a fully differentiated tissue.

(g) Assuming the process of dedifferentiation to partake of the

nature of hydrolytic cleavages, then dedifferentiation is essentially the same sort of process as autolysis or tissue disintegration.

Bibliography.

(1) Thacher,H.F. (1903) Biol. Bull., Vol.V, 297.

(2) Schultz, E. (1908) Vorträge u. Aufsätze über Entwick. Mech. d. Org., Heft. 4.

(3) Driesch, H. (1899) Arch.f.Entwick. Mech., Vol. IX, 103.

(4) Loeb, J. (1900) Amer.Journ.Physiol., Vol.IV, 60.

(5) Duclaux (1899) Traite de Micro-biologie, partie II.,Paris.

(6) Gast and Godlewski (1903) Archiv.f.Entwick.Mech.,Vol.XVI,76-117.

(7) Perkins,H.F. (1902) Biol.Bull., Vol.III, 172-181.

(8) Greeley (1902) Biol.Bull., Vol.V, 42.

(9) Muller, J. Tbid, No. 5.

(10) Wilson, H.V. (1911) Bull. of the Bureau of Fisheries,Vol.30.

(11) Huxley,J.S. (1921) Quarterly Jour.Micro.Science,Vol.54,293-322.

(12) Lieberkuhn (1856) Archiv.f.Anat.u.Physiol., J.Muller.

(13) Metschnikoff,E. (1879) Zeitsch.f.wiss.Zool., Bd. 32.

(14) Evans, R. (1899) Quart.Jour.Micro.Sci.,Vol. XLII, 363-476.

(15) Maas, O. (1910) Festschrift f. R. Hertwig. 1910.

(16) Wilson, H.V. (1907) Jour.of Exp.Zool., Vol. 5, 245-258.

(17) Child, C.M. Senescence and Rejuvenescence. Univ. of Chicago Press.

(18) Drew,G.H. (1911) Jour. of Exp.Zool., Vol. 10, 349-374.

(19) Rhumbler and Cienkowsky (1866) Archiv. für Mikroscopische Anatomie, Vol.I.

(20) Doflein (1902) Archiv.f.Protistenkernde, Vol.I.

(21) Hertwig, R. (1899) Sitzungberichte der Gesellschaft f. Morphologie und Physiologie in München.

(22) Klebs (1896) Die Bedingungen der Fortplanzung bei einigen Algen und Pilzen, Jena.

(23) Lund E.J. (1917) Jour. of Exp.Zool., Vol.24, No.1,1-33.

(24) Morgan, T. H. Regeneration. The MacMillan Co.

(25) Roux (1881) Der Kamf der Teil in Organismus.

(26) Bonnet

(27) Lillie and Knowlton (1897) Zool.Bull.,Vol.I, 179-193.

(28) Peebles, F. (1898) Zool.Bull.,Vol.II, 125-128.

(29) Bardeen, C.R. (1901) Amer.Jour.Phys.,Vol.I, 1-55.

(30) Zeleny,C. (1909) Jour.Exp.Zool.,Vol.7, 477-593.

(31) Goldfarb, A. J. (1906) Jour. of Exp. Zool., Vol. 3, 129-152.

(32) Loeb, J. Regeneration.

(33) Krogh, A. Respiratory Exchange in Animals and Man. Longmans, Green and Co. London.

(34) Matthews (1903) Amer.Journ. of Physiol. Vol. VIII,294-299.

(35) Lund E. J. (1921) Jour. of Exp.Zool., Vol. 34, No. 3,471-493.

(36) Schonborn,E.V. (1912) Zeitsch.f.Biol.,Vol.58, 97-109.

(37) Tashrio, S. A Chemical Sign of Life. Univ of Chicago Press.

(38) Hyman (1917) Jour.Exp.Zool.,Vol.20, 99-163.

(39) Morgan and Dimon (1904) Journ. Exper. Zool., Vol. I, 331-349.

(40) Hyman and Galigher (1921) Jour. Exp.Zool., Vol.XXXIV, 1-16.

(41) Galigher, A.E. (1921) Amer.Jour.Physiol., Vol·LVIII, 301.

(42) Lund E.J. (1918) Amer.Journ.Physiol., Vol.XLIV,No.4,365-373.

(43) Allen, G.D. (1919) Amer.Journ.Physiol., Vol.XLIX,420-473.

(44) Moleschott (1855) Wien,Med. Woch., No. 43.

(45) Selmi and Piacentini (1870) Rendic.Reale Tstituto,Lombardo.

(46) Potts (1875) Habilitationschr., Jena.

(47) Loeb, J. (1888) Pfl.Arch., Vol. 42.

(48) Ewald (1892) Journ. of Physiol., Vol. 13, 847-859.

(49) D'Arsonval (1891) Soc. de Biol., 283-286. Annales d' electrobiologie, I.

(50) Loewy and Cohn (1900) Berl.klin.Wochenschrift, Vol. 37.

(51) Spaski (1900) Physiologiste russe, I.

(52) Moore, A.R. (1918) Proc.Soc.Exper. Biol. and Med.,Vol.XV.

(53) Warburg, O. (1910) Zeitschr.f.Physiol.Chem., 66, 305-341.

(54) Fletcher and Hopkins (1907) Jour.Phys.,Vol.35, 247-310.

(55) Fletcher (1913) Jour.Physiol.,Vol. 47.

(56) Tigerstedt and Souden (1895) Skand.Arch.Physiol.,Vol.6.

(57) Magnus - Levy (1899) Arch.f. (Anat.u.) Physiol., Suppl., 314-381.

(58) Falk (1899) Arch.f. (Anat.u.) Physiol., Suppl.

(59) Benedict and Talbot (1914) Carnegie Publications, No. 201.

(60) Levene Harvey Lectures, Vol. I.

(61) Hoppe - Seyler (1871) Tubinger Med. Chem. Untersuchungen. 499.

(62) Hauser (1886) Arch.f. Exper.Path.u.Pharm., Vol.XX.

(63) Schutzenberger (1874) Bull. de Soc. Chemique, Vol. XXI.

(64) Hofmeister (Levene) Harvey Lecture, Vol.I.

(65) Hedin and Rowland (1902) Zeitsch.f.phys. Chem., Vol. XXXII,341-50

(66) Stookey and Levene (1903) Journ.Med.Research, Vol.X, 212-228.

(67) Bradley (1915) Journ. Biol. Chem., Vol. XXII, 113-123.

(68) Biondi (1906) Virchow's Archiv., Vol. CXLIV.

(69) Jacoby (1900) Zeitsch.f.phys. Chem., Vol.XXX,138-174.

(70) Jacoby (1903) Zeitsch.f.phys. Chem., Vol.XXXIII,128-131.

(71) Schulze (1899) Zeitscg.f.phys.Chem.,Vol.XXVIII,465-471.

(72) Stolte (1904) Hofmeister's Beiträge, Vol. I.

(73) Lawrow (1901) Zeitsch.f.phys. Chem., Vol. XXXIII,111-121.

(74) Langstein (1902) Ibid., Vol.II.

(75) Levene (1904) Amer.journ.Physiol.,Vol.XII.

(76) Kossel and Dakin (1904) Zeitsch.f.phys.Chem.,Vols.XLI & XLII,321, and 181.

(77) Danilewsky and Okunew Maly's Jahresb., Vol. XXV.

(78) Kastle and Loevenhart (1901) Amer.Chem.Journ., Vol. XXIV.

(79) Kurajeff (1901) Zeitsch.f.phys.Chem.,Vol.XXXIII, 197-201.

(80) Bayer (1904) Hofmeister's Beiträge, Vol. IV.

(81) Jones (1904) Zeitsch.f.phys. Chem., XLI, 100-109.

(82) Levene (1902) Zeitsch.f.phys. Chem., Vol.XXXV, 80-84.

(83) Schittenhelm (1901) Amer.Chem.Journ., Vol. XXIV.

Table I.

I. B Blanks. O content of control flasks. Average of three. C.C. Thio.	II. O content of blanks disterbed with spatula. Average of three. C.C. Thio.	III. Difference in O content due to use of spatula. C.C. Thio.
4.00	4.00	-------
4.02	4.00	0.02
4.08 4.08	4.08 4.02	---;-)-- -----

Table II.

I. Blanks. O content of flasks immediately after filling. Average of three. C.C. Thio.	II. Blanks. O content of flasks at end of four hours. Average of three.. C.C. Thio.	III. Blanks. O content of flasks at end of 24 hours. Average of three.. C.C. Thio.	IV. Differences O content. Due to loss or consumption of oxygen. C.C. Thio.
3.92	3.88		-0.04
3.87	3.87		-0.00
4.00		3.95	-0.05
3.72		3.67	-0.05

Table III

I. Blanks. O content of flasks at beginning. Average of three. C.C. Thio.	II. O content of flasks after using apparatus. Average of three. C.C. Thio.	III. Difference in O content due to use of apparatus. Average of three. C.C. Thio.
3.80	3.85	+0.05
3.82	3.85	+0.03
3.95	3.97	+0.02
4.00	4.07	+0.07

I. II. Table IV. III. IV. V.

	Time of test in hours from beginning.	Blanks. O content of controls. average of three.	O content of experimental flasks at end of period.	Total O consumption of colony during exp. period.
		C.C.Thio.	C.C.Thio.	C.C.Thio.
Colony I.	0-4	3.77	1.65	2.12
	24-28	3.82	1.75	2.07
Colony II.	0-4	3.77	1.90	1.87
	24-28	3.82	1.95	1.87
Colony III.	0-4	3.77	2.05	1.72
	24-28	3.82	2.05	1.77
Colony IV.	0-4	3.77	1.85	1.92
	24-28	3.82	1.95	1.87
Colony V.	0-4	3.77	2.10	1.67
	24-28	3.82	2.05	1.77
Colony VI.	0-4	3.77	1.70	2.07
	24-28	3.82	1.85	2.07

I. II. Table V. III.

Blanks. O content of control flasks. Average of three. CC. Thio.	O content of blanks disturbed with spatula. Average of three. CC. Thio.	Difference in O content due to use of spatula. CC. Thio.
2.05	2.07	0.02
2.03	2.05	0.02
2.02	2.03	0.01
2.00	2.00	0.00

I. II. Table VI. III.

Blanks. O content of control flasks. Avedage of three. CC. Thio.	O content of flasks after using apparatus Average of three. CC. Thio.	Difference in O content due to use of apparatus. CC. Thio.
1.97	2.05	0.08
2.00	2.05	0.05
2.02	2.08	0.06
2.03	2.10	0.07

Table VII.

I.	II.	III. Time of test in hours from beginning of experiment.	IV. Condition of colony before each test	V. Blanks. O_2 content at beginning. Each flask C.C.Thio.	V. Blanks. O_2 content at beginning. Average. C.C.Thio.	VI. O_2 content at end of four hours. One flask. C.C.Thio.	VII. Total O_2 consumed during four hours by one colony. C.C.Thio.	VIII. Total percent change in O consumption after cutting colony.
Normal differentiated colonies. (A.)	Whole colonies. (Controls)	(colony a.) 0-4	Whole normal differentiated.	1. 4.00 2. 4.00 3. 3.95		1.95	2.03	
		(colony b.) 0-4	Whole normal differentiated.	1. 4.00 2. 4.00 3. 3.95	3.98	0.35	3.63	
		(colony c.) 0-4	Whole normal differentiated.	1. 4.00 2. 4.00 3. 3.95		0.45	3.53	
	Same colony after cutting.	(colony a.) 24-28	Colony cut into pieces.	1. 3.70 2. 3.65 3. 3.75		2.50	1.20	40.88% decrease.
		(colony b.) 24-28	Colony cut into pieces.	1. 3.70 2. 3.65 3. 3.75	3.70	1.20	2.50	31.13% decrease.
		(colony c.) 24-28	Colony cut into pieces.	1. 3.70 2. 3.65 3. 3.75		2.05	1.65	53.26% decrease.
Totally dedifferentiated colonies. No polyps (B.)	Whole colony. (Control)	(colony x.) 0-4	Whole dedifferentiated.	1. 4.00 2. 4.00 3. 3.95		3.80	0.18	
		(colony y.) 0-4	Whole dedifferentiated.	1. 4.00 2. 4.00 3. 3.95	3.98	3.60	0.38	
		(colony z.) 0-4	Whole dedifferentiated	1. 4.00 2. 4.00 3. 3.95		4.05	-----	
	Same colony after cutting.	(colony x.) 24-28	Colony cut into pieces.	1. 3.80 2. 3.85 3. 3.75		3.10	0.70	288.88% increase.
		(colony y.) 24-28	Colony cut into pieces.	1. 3.80 2. 3.85 3. 3.75	3.80	3.20	0.60	57.89% increase.
		(colony y.) 24.28	Colony cut into pieces.	1. 3.80 2. 3.35 3. 3.75		3.60	0.20	-----

I. II. III. IV. Table VIII. V. VI

	Experiment Number.	Total O_2 consumed during four hours by whole colony. Each the average of three colonies. C.C.Thio	Total O_2 consumed during four hours by same colonies cut in pieces. Each the average of three. C.C.Thio	Difference in O_2 consumption for four hour period between whole belia colonies and same colonies after cutting. average of three C.C.Thio.	Percent change in total consumption for four hours due to cutting.
Normal Differentiated Colonies. (A)	1.	2.20	0.40	1.80	81.81 decrease.
	2.	1.75	0.50	1.25	77.52 decrease.
	3.	1.85	1.35	0.50	27.02 decrease.
	4.	1.43	0.60	0.83	58.04 decrease.
	5.	3.13	1.48	1.65	52.71 decrease.
Dedifferentiated Colonies. (B)	1.	0.75	2.42	1.67	222.6 increase.
	2.	0.43	1.05	0.62	144.1 increase.
	3.	0.26	0.55	0.29	111.5 increase.
	4.	0.20	0.78	0.58	290.00 increase.
	5.	1.15	1.81	0.66	57.39 increase.

Table IX. V.

I.	II. Time of test in hours from beginning of experiment.	III. Condition of colony before each test.	IV Blanks. O_2 content at beginning. Each flask C.C.Thio.	IV Blanks. O_2 content at beginning. Average. C.C.Thio.	VI. O_2 content at end of four hours. One flask. C.C.Thio.	VII. Total O_2 consumption during four hours by one colony. C.C.Thio.	VIII.	IX
Colony I.	1. 0-4	Whole normal colony.	1. 3.90 2. 4.00 3. 4.075	3.995	2.15	1.845	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps.	2.35/1
	2. 7-11	Same colony without polyps.	1. 3.50 2. 3.625 3. 3.575	3.56	2.775	0.785		
	3. 23-33	Same colony after new polyps had regenerated.	1. 4.20 2. 4.00 3. 4.10	4.10	2.675	1.425	Total percent increase in O consumption during regeneration of new polyps.	141.401
	4. 35-45	Same colony after new polyps had regenerated.	1. 4.225 2. 4.200 3. 4.300	4.235	2.40	1.45		
	5. 47-57	Same colony after new polyps had regenerated.	1. 3.875 2. 3.675 3. 3.750	3.745	1.85	1.395		
Colony 2	1. 0-4	Whole normal colony	1. 3.90 2. 4.00 3. 4.075	3.995	2.65	1.345	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps.	7.2707/1
	2. 7-11	Same colony without polyps.	1. 3.50 2. 3.625 3. 3.575	3.56	3.375	0.185		
	3. 23-33	Same colony after new polyps had regenerated	1. 4.90 2. 4.00 3. 4.10	4.10	3.60	0.50	Total percent increase in O consumption during regeneration of new polyps.	478.37
	4. 35-45	Same colony after new polyps had regenerated	1. 4.225 2. 4.200 3. 4.300	4.235	3.475	0.76		
	5. 47-57	Same colony after new polyps had regenerated	1. 3.875 2. 3.675 3. 3.750	3.745	2.675	1.07		
Colony 3	1. 0-4	Whole normal colony.	1. 3.90 2. 4.00 3. 4.075	3.995	2.175	1.82	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps	3.956/1
	2. 7-11	Same colony without polyps.	1. 3.50 2. 3.625 3. 3.575	3.56	2.10	0.46		
	3. 23-33	Same colony after new polyps had regenerated.	1. 4.200 2. 4.00 3. 4.10	4.10	3.075	1.025	Total percent increase in O consumption during regeneration of new polyps.	-225.00
	4. 35-45	Same colony after new polyps had regenerated	1. 4.225 2. 4.200 3. 4.300	4.235	3.575	0.66		
	5. 47-57	Same colony after new polyps had regenerated	1. 3.875 2. 3.675 3. 3.750	3.745	2.25	1.495		

Table X.

I.	II. Time of test in hours from beginning of experiment	III. Condition of Colony before each test.	IV. Blanks O_2 content at beginning. Each flask.	IV. Blanks O_2 content at beginning. Average.	V. O_2 content at end of four hours one flask	VI. Total O_2 consumption during four hours by one colony.	VII	VIII
			C.C.Thio	C.C.Thio.	C.C.Thio	C.C.Thio.		
Colony I.	0-4	Whole normal colony	1. 3.80 2. 3.775 3. 3.775	3.785	1.30	2.45	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps.	2.33
	10-14	Same colony without polyps.	1. 3.55 2. 3.50 3. 3.45	3.500	2.45	1.05		
	26-30	Same colony after new polyps had regenerated	1. 3.825 2. 3.800 3. 3.850	3.825	2.875	0.95	Total percent increase in O consumption during regeneration of new polyps	---
Colony 2.	0-4	Whole normal colony	1. 3.80 2. 3.775 3. 3.775	3.785	1.565	2.22	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps.	5.94/1
	10-14	Same colony without polyps	1. 3.55 2. 3.50 3. 3.45	3.500	3.425	0.375		
	26-30	Same colony after new polyps had regenerated	1. 3.825 2. 3.800 3. 3.850	3.825	3.150	0.675	Total percent increase in O consumption during regeneration of new polyps.	88.8
Colony 3.	0-4	Whole normal colony	1. 3.80 2. 3.775 3. 3.775	3.785	1.625	2.16	Ratio of total O consumed by whole colony to total O consumed by same colony after removal of polyps.	5.4/1
	0-14	Same colony without polyps.	1. 3.55 2. 3.50 3. 3.45	3.500	3.10	0.40		

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
	26-30	Same colony after new polyps had regenerated	1. 3.325 2. 3.300 3. 3.350	3.325	2.875	0.95	Total percent increase in O_2 consumption during regeneration of new polyps	233.3
Colony 4.	0-4	Whole normal colony	1. 4.00 2. 4.00 3. 4.05	4.02	0.95	3.07	Ratio of total O_2 consumed by whole colony to total of O_2	
	10-14	Same colony without polyps	1. 3.35 2. 3.30 3. 3.40	3.35	2.20	1.15	consumed by same colony after removal of polyps.	$\frac{2.6}{1}$
	26-30	Same colony after new polyps had regenerated	1. 3.75 2. 3.75 3. 3.75	3.75	2.60	1.15	Total percent increase in O_2 consumption during regeneration of new polyps.	---
Colony 5.	0-4	Whole normal colony	1. 4.00 2. 4.00 3. 4.05	4.02	0.25	3.77	Ratio of total O_2 consumed by whole colony to total O_2	
	10-14	Same colony without polyps	1. 3.35 2. 3.30 3. 3.40	3.35	1.60	1.75	consumed by same colony after removal of polyps.	$\frac{2.15}{1}$
	26-30	Same colony after new polyps had regenerated	1. 3.75 2. 3.75 3. 3.75	3.75	2.45	1.30	Total percent increase in O_2 consumption during regeneration of new polyps.	----
Colony 6.	0-4	Whole normal colony	1. 4.00 2. 4.00 3. 4.05	4.02	0.95	3.07	Ratio of total O_2 consumed by whole colony to total O_2	
	10-14	Same colony without polyps	1. 3.35 2. 3.30 3. 3.40	3.35	2.65	0.70	consumed by same colony after removal of polyps.	$\frac{4.38}{1}$
	26-30	Same colony after new polyps had regenerated	1. 3.75 2. 3.75 3. 3.75	3.75	3.00	0.75	Total percent increase in O_2 consumption during regeneration of new polyps.	7.1

Table XX.

	Time of test in hours from beginning of experiment	Condition of internodes before each test.	Blanks. O_2 content at beginning: Each flask	Blanks. O_2 content at beginning: Average	O_2 content at end of ten hours. one flask.	Total O_2 consumption at end of ten hours. 60 pieces.	Percent of internodes showing capacity for regeneration.	Total percent increase in O_2 consumption during regeneration of polyps.
			C.C.Thio.	C.C.Thio.	C.C.Thio.	C.C.Thio.		
Flask 1.	1. 0-10	Freshly cut internodes	1. 3.600 2. 3.650 3. 3.650	3.63	3.20	0.43	86.00	90.69
	2. 12-22	Slight regeneration of new polyps.	1. 3.500 2. 3.600 3. 3.550	3.55	3.00	0.55		
	3. 24-34	More regeneration.	1. 3.750 2. 3.700 3. 3.750	3.73	3.15	0.58		
	4. 36-46	More regeneration.	1. 3.850 2. 3.800 3. 3.800	3.82	3.00	0.82		
Flask 2.	1. 0-10	Freshly cut internodes.	1. 3.600 2. 3.650 3. 3.650	3.63	3.40	0.23	80.00	70.129
	2. 12-22	Slight regeneration of new polyps.	1. 3.500 2. 3.600 3. 3.550	3.55	3.05	0.50		
	3. 24-34	More regeneration.	1. 3.750 2. 3.700 3. 3.750	3.73	3.00	0.73		
	4. 36-46	More regeneration.	1. 3.850 2. 3.800 3. 3.800	3.82	3.05	0.77		
Flask 3.	1. 0-10	Freshly cut internodes.	1. 3.600 2. 3.650 3. 3.650	3.63	3.50	0.13	94.00	82.19
	2. 12-22	Slight regeneration of new polyps.	1. 3.500 2. 3.600 3. 3.650	3.55	3.15	0.40		
	3. 24-34	More regeneration.	1. 3.750 2. 3.700 3. 3.750	3.73	3.00	0.73		
	4. 36-46	Same as above.	1. 3.850 2. 3.800 3. 3.800	3.82	3.15	0.67		

Table XII.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
	Time of test in hours from beginning of experiment.	Condition of internodes before each test.	Blanks O_2 content at beginning. Each flask.	Average.	O_2 content at end of 12 hours. One flask.	Total O_2 consumption at end of ten hours. 60 pieces.	Percent of internodes showing capacity for regeneration.	Total percent increase in O_2 consumption during regeneration of polyps.
			C.C.Thio.	C.C.Thio.	C.C.Thio.	C.C.Thio.		
Flask 1.	1. 0-12	Freshly cut internodes.	1. 2.75 2. 2.80 3. 2.85	2.80	2.35	0.45	61.11	288.88
	2. 14-26	Beginning regeneration of new polyps.	1. 3.45 2. 3.40 3. 3.35	3.40	2.50	0.90		
	3. 28-40n	More regeneration.	1. 3.80 2. 3.75 3. 3.70	3.75	2.05	1.75		
Flask 2.	1. 0-12	Freshly cut internodes.	1. 2.75 2. 2.80 2. 2.85	2.80	1.30	1.50		20.00
	2. 14-26	Same as above.	1. 3.45 2. 3.40 3. 3.35	3.40	1.90	1.50		
	3. 28.40	Regeneration of new polyps.	1. 3.80 2. 3.75 3. 3.70	3.75	1.95	1.80		
Flask 3.	1. 0-12	Freshly cut internodes.	1. 2.75 2. 2.80 3. 2.85	2.80	1.70	1.10	77.77	68.18
	2. 14-26	Slight regeneration of new polyps.	1. 3.45 2. 3.40 3. 3.35	3.40	2.00	1.40		
	3. 28-40	More regeneration.	1. 3.80 2. 3.75 3. 3.70	3.75	1.90	1.85		
Flask 4.	1. 0-12	Freshly cut internodes.	1. 2.75 2. 2.80 3. 2.85	2.80	1.30	1.50	86.66	10.0
	2. 14-26	Same as above.	1. 3.45 2. 3.40 3. 3.35	3.40	2.35	1.05		
	3. 28-40	Regeneration of new polyps	1. 3.80 2. 3.75 3. 3.70	3.75	2.10	1.65		

I. II. III. IV. Table XLI. V. VI. VII. VIII. IX.

	Time of test in hours from beginning of experiment.	Condition of internodes before each test.	Blanks. O_2 content at beginning. Each flask.	Blanks. O_2 content at beginning. Average.	O_2 content at end of test period. One flask.	Total O_2 consumption at end of test period. 90 pieces.	Percent of internodes showing capacity for regeneration.	Total percent increase in O_2 consumption during regeneration of polyps.
			C.C.Thio.	C.C.Thio.	C.C.Thio.	C.C.Thio.		
Flask 1.	1. 0-10	Freshly cut internodes.	1. 3.60 2. 3.50 3. 3.50	3.53	3.40	0.13	91.00	1207.69%
	2. 12-22	Beginning regeneration of new polyps.	1. 3.30 2. 3.30 3. 3.20	3.26	3.10	0.16		
	3. 24-34	More regeneration.	1. 3.45 2. 3.35 3. 3.30	3.40	3.10	0.30		
	4. 36-56	More regeneration.	1. 3.80 2. 3.75 3. 3.70	3.75	2.05	1.70		
Flask 2.	1. 0-10	Freshly cut internodes	1. 3.60 2. 3.50 3. 3.50	3.53	3.35	0.18	86.00	483.33%
	2. 12-22	Beginning regeneration of new polyps.	1. 3.30 2. 3.30 3. 3.20	3.26	3.10	0.16		
	3. 24-34	More regeneration.	1. 3.45 2. 3.35 3. 3.30	3.40	3.00	0.40		
	4. 36-56	More regeneration.	1. 3.80 2. 3.75 3. 3.70	3.75	2.70	1.05		
Flask 3.	1. 0-10	Freshly cut internodes.	1. 3.60 2. 3.50 3. 3.50	3.53	3.25	0.28	78.00	2201.42%
	2. 12-22	No change.	1. 3.30 2. 3.30 3. 3.20	3.26	3.20	0.06		
	3. 24-34	Beginning regeneration of new polyps.	1. 3.45 2. 3.35 3. 3.30	3.40	3.05	0.35		
	4. 36-56	More regeneration.	1. 3.80 2. 3.75 3. 3.70	3.75	2.85	0.90		

Table XIV.

I.	II.	III.	IV.		V.	VI.	VII.	VIII.	IX.	X.
	Time of test in hours from beginning of experiment.	Condition of pieces before each test.	Blanks. O_2 content at beginning		O_2 content at end of 4 hours. One flask 50 pieces	Total O_2 consumed during 10 hours by 50 pieces	O_2 consumed per cu. cm. of coenosarc during 10 hours.	Percent of internodes showing capacity for regeneration.	Total per-cent increase in O_2 consumption during regeneration of new polyps
			Each flask.	Average					
			C.C.Thio.	C.C.Thio.	C.C.Thio	C.C.Thio	C.C.Thio		
Apical Pieces.	1. 0-10	Freshly cut internodes	1. 3.60 2. 3.50 3. 3.50	3.53	3.25	0.28	75.45		
	2. 12-22	Slight regeneration of new polyps.	1. 3.40 2. 3.30 3. 3.20	3.30	2.75	0.55			
	3. 24-34	More regeneration.	1. 3.45 2. 3.35 3. 3.40	3.40	2.25	0.85		92.00	275.0%
	4. 36-46	More regeneration.	1. 3.80 2. 3.75 3. 3.75	3.75	2.70	1.05			
Middle Pieces.	1. 0-10	Freshly cut internodes.	1. 3.60 2. 3.50 3. 3.50	3.53	3.35	0.18	80.89		
	2. 12-22	No regeneration of polyps.	1. 3.40 2. 3.30 3. 3.20	3.30	2.85	0.45			
	3. 24-34	Beginning regeneration of new polyps.	1. 3.45 2. 3.35 3. 3.40	3.40	3.35	0.05		94.00	400.00%
	4. 36-46	More regeneration of new polyps.	1. 3.80 2. 3.75 3. 3.70	3.75	2.85	0.90			
Basal Pieces.	1. 0-10	Freshly cut internodes	1. 3.60 2. 3.50 3. 3.50	3.53	3.40	0.13	99.086		
	2. 12-22	No regeneration of polyps.	1. 3.40 2. 3.30 3. 3.20	3.30	3.30	0.00			
	3. 24-34	Beginning regeneration of new polyps.	1. 3.45 2. 3.35 3. 3.40	3.40	2.90	0.50		62.00	476.92%
	4. 36-46	More regeneration of new polyps.	1. 3.80 2. 3.75 3.70	3.75	2.90	0.85			

I. II. III. IV. Table XV. V. VI. VII. VIII. IX. X.

	Time of test in hours from beginning of experiment.	Condition of pieces before each test.	Blanks. O content at beginning Each flask. C.C.Thio.	Blanks. O content at beginning Average. C.C.Thio.	O content at end of 10 hours One flask 75 pieces C.C.Thio	Total O consumed during 10 hours by 75 pieces C.C.Thio	O consumed per cu. coenosarc during 10 hours. C.C.Thio.	Percent of internodes showing capacity for regeneration	Total percent increase in O consumption during regeneration of ne polyps
Apical Pieces.	1. 0-20	Freshly cut internodes	1. 3.80 2. 3.75 3. 3.75	3.78	2.00	1.75	365.81	64.00	14.28
	2. 22-42	Beginning regeneration of new polyps.	1. 3.70 2. 3.65 3. 3.60	3.65	1.65	2.00			
Basal Pieces.	1. 0-20	Freshly cut internodes	1. 3.80 2. 3.75 3. 3.75	3.78	2.15	1.60	1002.5	90.66	12.50
	2. 22-42	Beginning regeneration new polyps.	1. 3.70 2. 3.65 3. 3.60	3.65	1.85	1.80			

I. II. III. IV. Table XVI. V. VI. VII. VIII. IX. X.

	Time of test in hours from beginning of experiment.	Condition of pieces before each test.	Blanks. O_2 content at beginning. Each flask. C.C.Thio.	Blanks. O_2 content at beginning. Average C.C.Thio	O_2 content at end of 4 hours. One flask 50 pieces C.C.Thio	Total O_2 consumed during 10 hours by 50 pieces C.C.Thio	O_2 consumed per cu. cm. of coenosarc during 10 hours. C.C.Thio	Percent of internodes showing capacity for regeneration	Total percent increase in O_2 consumption during regeneration new polyps
Apical Pieces.	1. 0-10	Freshly cut internodes.	1. 3.75 2. 3.85 3. 3.80	3.80	1.90	1.90	175.36		18.40
	2. 12-22	Beginning regeneration of new polyps.	1. 3.70 2. 3.65 3. 3.60	3.65	1.40	2.25			
	3. 24-34	Slight dedifferentiation of new polyps.	1. 3.80 2. 3.70 3. 3.80	3.76	1.70	2.06			
	4.36-46	Same as above	1. 3.70 2. 3.65 3. 3.70	3.68	1.55	2.13			
Basal Pieces.	1. 0-10	Freshly cut internodes	1. 3.75 2. 3.85 3. 3.80	3.80	2.40	1.40	842.96		50.00
	2. 12-22	Beginning regeneration of new polyps.	1. 3.70 2. 3.65 3. 3.60	3.65	1.55	2.10			
	3. 24-34	Slight dedifferentiation of new polyps.	1. 3.80 2. 3.70 3. 3.80	3.76	2.00	1.76			
	4. 36-46	Same as above.	1. 3.70 2. 3.65 3. 3.70	3.68	1.95	1.73			

I. II. III. IV. Table XVII. V. VI. VII. VIII.

	Time of test in hours from beginning of experiment.	Condition of colony before each test.	Blanks. O_2 content at beginning. Each flask C.C.Thio.	Blanks. O_2 content at beginning. Average. C.C.Thio.	O_2 content at end of four hours. One flask. C.C.Thio.	Total O_2 consumption in four hours by colony. C.C.Thio.	O_2 consumption percent of normal.
Colony I.	1. 0-4	Normal differentiated.	1. 3.80 2. 3.90 3. 3.85	3.85	2.85	1.00	100.
	2. 24-28	Same as above.	1. 4.15 2. 4.10 3. 4.15	4.13	2.20	1.93	193.
	3. 48-52	Dedifferentiation beginning.	1. 4.05 2. 4.00 3. 4.00	4.02	2.80	1.22	122.
	4. 72-76	More dedifferentiation	1. 3.80 2. 3.80 3. 3.80	3.8	2.70	1.10	110.
	5. 96-100	More dedifferentiation	1. 4.05 2. 4.06 3. 4.05	4.06	3.50	0.56	56.
	6. 120-124	Nearly complete dedifferentiation. (no polyps.)	1. 3.80 2. 3.80 3. 3.85	3.82	3.40	0.42	42.
	7.144-148	Slight regeneration of new polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	3.20	0.62	62.
Colony 2.	1. 0-4	Normal differentiated.	1. 3.80 2. 3.90 3. 3.85	3.85	1.70	2.15	100.
	2. 24-28	Same as above.	1. 4.15 2. 4.10 3. 4.15	4.13	2.25	1.88	87.5
	3. 48-52	Dedifferentiation beginning.	1. 4.05 2. 4.00 3. 4.00	4.02	2.25	1.77	82.4
	4. 72-76	More dedifferentiation	1. 3.80 2. 3.80 3. 3.80	3.8	2.75	1.05	48.9
	5. 96-100	More dedifferentiation	1. 4.05 2. 4.06 3. 4.07	4.06	2.70	1.36	63.25
	6. 120-124	Nearly complete dedifferentiation. (No polyps."	1. 3.80 2. 3.80 3. 3.85	3.82	3.10	0.72	33.5
	7. 144-148	Slight regeneration of new polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	2.85	0.97	45.2

Table XVII (cont'd)

I.	II. Time of test in hours from beginning of experiment.	III. Condition of colony before each test.	IV. Blanks. O_2 content at beginning. Each flask. C.C.Thio.	IV. Blanks. O_2 content at beginning. Average. C.C.Thio	V. O_2 content at end of four hours. One flask. C.C.Thio	VI. Total O_2 consumption in four hours by one colony. C.C.Thio.	VII. O_2 consumption percent of normal.
Colony 3.	1. 0-4	Normal differentiated.	1. 3.85 2. 3.80 3. 3.90	3.85	2.50	0.97	100
	2. 24-28	Same as above.	1. 4.15 2. 4.15 3. 4.10	4.13	3.00	1.13	116.40
	3. 48-52	Same as above.	1. 4.00 2. 4.00 3. 4.05	4.02	3.05	1.35	139.10
	4. 72-76	Dedifferentiation beginning.	1. 3.80 2. 3.80 3. 3.80	3.8	3.00	0.80	82.50
	5. 96-100	More dedifferentiation	1. 4.05 2. 4.06 3. 4.07	4.06	3.30	0.76	80.50
	6.120-124	More dedifferentiation.	1. 3.80 2. 3.80 3. 3.85	3.82	3.30	0.52	53.70
	7.144-148	Slight regeneration of polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	3.20	0.62	63.92
Colony 4.	1. 0-4	Normal differentiated.	1. 3.80 2. 3.85 3. 3.90	3.85	2.20	1.65	100
	2. 24-28	Dedifferentiation beginning.	1. 4.15 2. 4.15 3. 4.10	4.13	2.65	1.48	89.74
	3. 48-52	More dedifferentiation.	1. 4.00 2. 4.00 3. 4.05	4.02	2.65	1.37	83.10
	4. 72-76	Same as above.	1. 3.80 2. 3.80 3. 3.20	3.8	2.20	1.60	96.96
	5. 90-100	More dedifferentiation.	1. 4.05 2. 4.06 3. 4.07	4.06	3.35	0.71	43.10
	6.120-124	Nearly complete dedifferentiation. (no polyps)	1. 3.80 2. 3.80 3. 3.85	3.82	3.50	0.32	19.40
	7.144-148	Marked regeneration of polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	2.60	1.22	80.00

I. II. III. IV. Table XVII (cont'd) V. VI. VII. VIII. IX.

	Time of test in hours from beginning of experiment.	Condition of colony before each test.	Blanks. O_2 content at beginning. Each flask. C.C.Thio.	Blanks. O_2 content at beginning. Average. C.C.Thio.	O_2 content at end of four hours. One flask. C.C.Thio.	Total O_2 consumption in four hours by one colony. C.C.Thio.	O_2 consumption percent of normal.
Colony 5.	1. 0-4	Normal differentiated.	1. 3.80 2. 3.90 3. 3.85	3.85	2.50	1.35	100.
	2. 24-28	Same as above.	1. 4.15 2. 4.10 3. 4.15	4.13	2.55	1.58	177.03
	3. 48-52	Dedifferentiation beginning.	1. 4.05 2. 4.00 3. 4.00	4.02	2.80	1.22	90.38
	4. 72-76	More dedifferentiation.	1. 3.80 2. 3.80 3. 3.80	3.80	2.80	1.00	74.1
	5. 96-100	More dedifferentiation	1. 4.05 2. 4.06 3. 4.07	4.06	3.05	1.01	73.4
	6. 120-124	Nearly complete dedifferentiation. (No polyps.)	1. 3.80 2. 3.80 3. 3.85	3.82	3.15	0.67	48.6
	7. 144-148	Slight regeneration of polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	3.00	0.87	64.5
Colony 6.	1. 0-4	Normal differentiated	1. 3.80 2. 3.90 3. 3.85	3.85	1.00	2.85	100.
	2. 24-28	Dedifferentiation beginning.	1. 4.15 2. 4.10 3. 4.15	4.13	1.95	2.20	77.2
	3. 48-52	More dedifferentiation	1. 4.05 2. 4.00 3. 4.00	4.02	1.90	2.12	74.4
	4. 72-76	More dedifferentiation	1. 3.80 2. 3.80 3. 3.80	3.80	2.40	1.40	49.2
	5. 96-100	More dedifferentiation	1. 4.05 2. 4.06 3. 4.07	4.06	2.80	1.26	44.3
	6. 120-124	Nearly complete dedifferentiation. (No polyps.)	1. 3.80 2. 3.80 3. 3.85	3.82	2.70	1.12	39.3
	7. 144-148	Slight regeneration of polyps.	1. 3.80 2. 3.80 3. 3.85	3.82	2.50	1.32	46.4

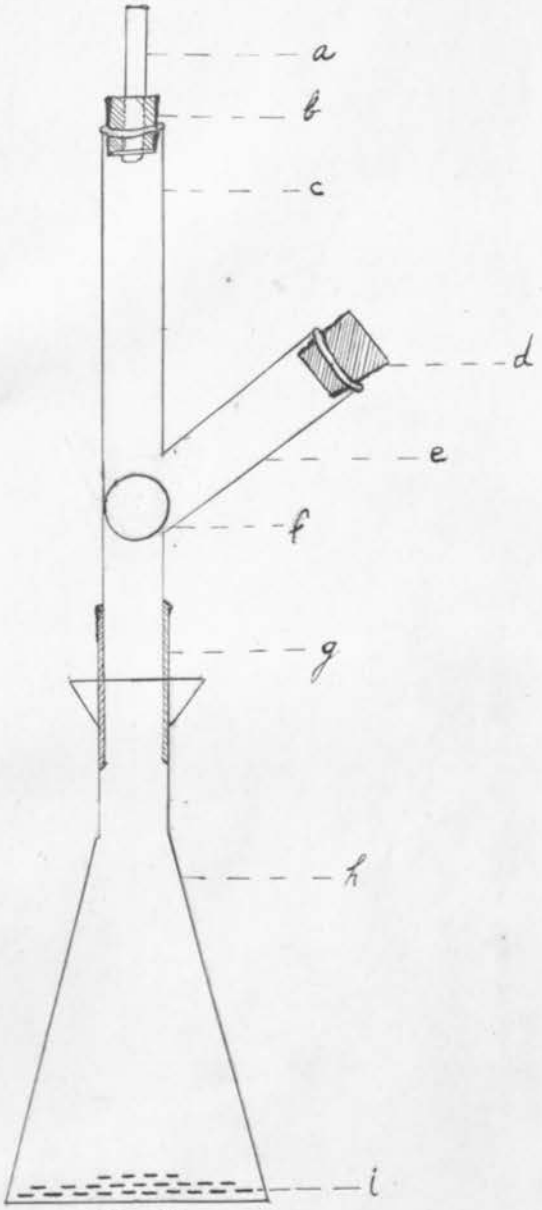


Fig.I.

Apparatus for removal of internodes

a-Glass rod
b-Cork
c-Small glass tube
d-Cork
e-Long glass tube
f-Glass ball
g-Rubber jacket
h-25c.c. flask
i-Internodes

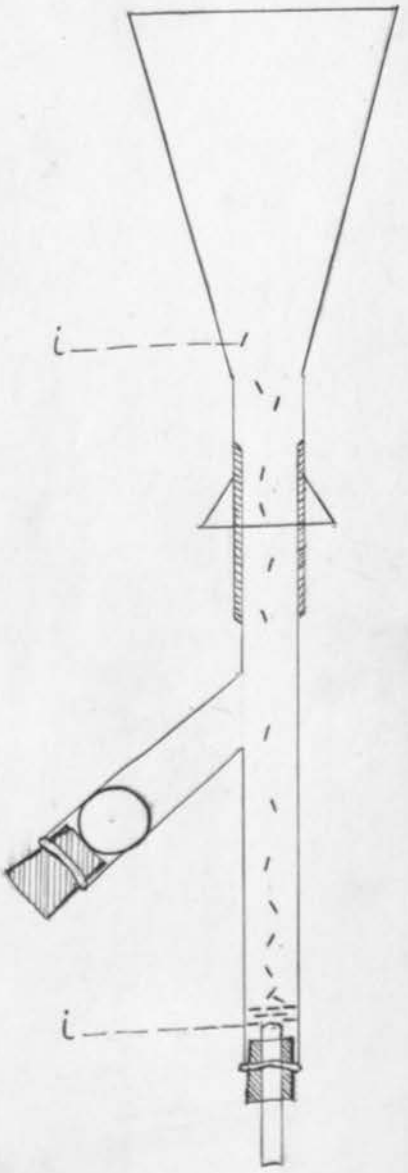
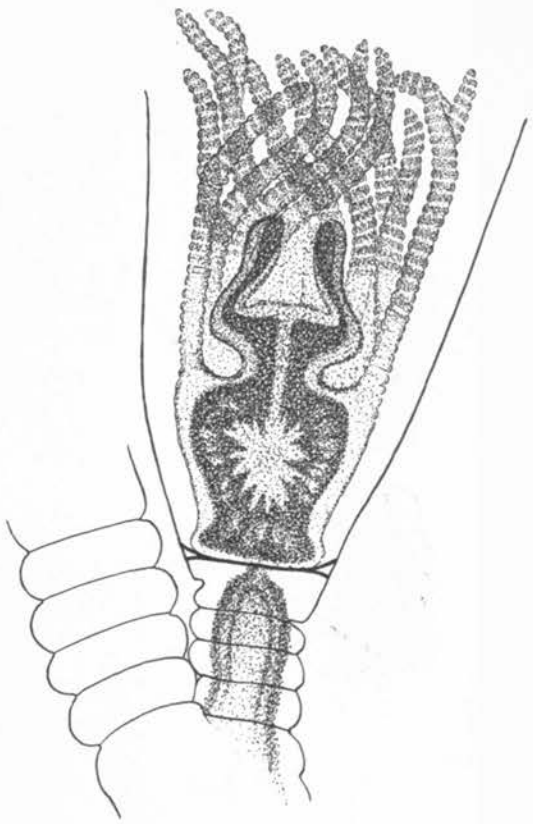
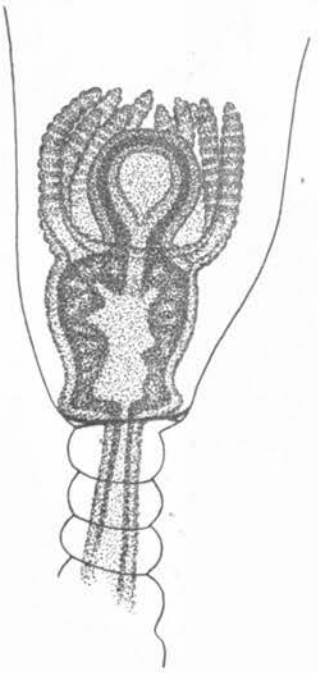


Fig.II.

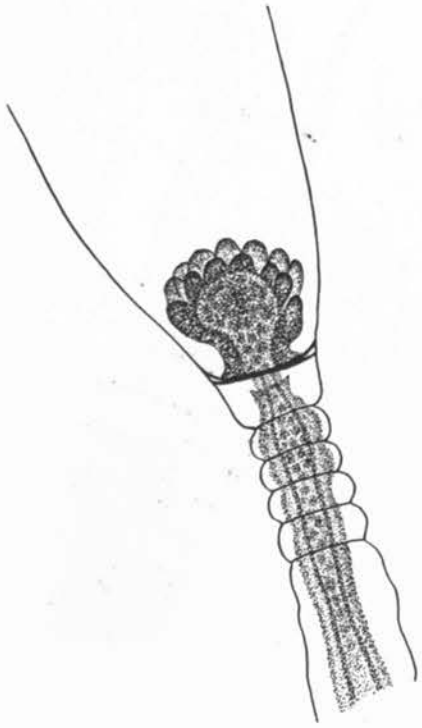
Same apparatus (inverted)



Stage 1



Stage 2



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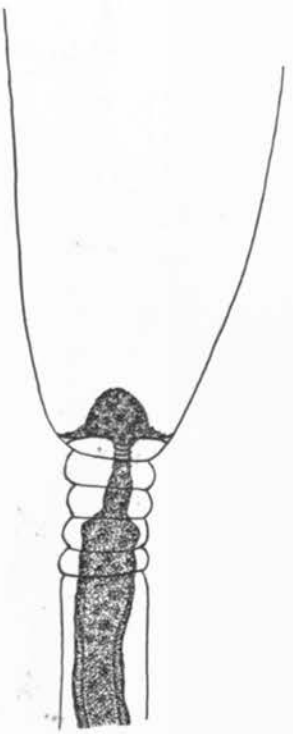
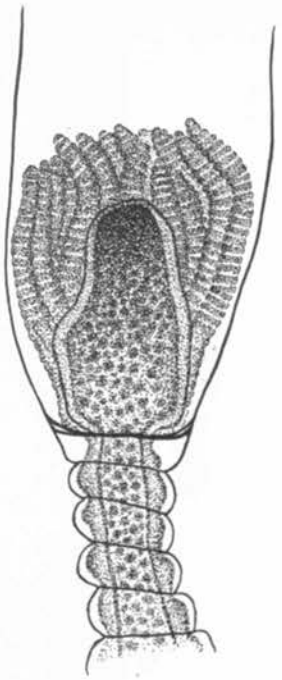


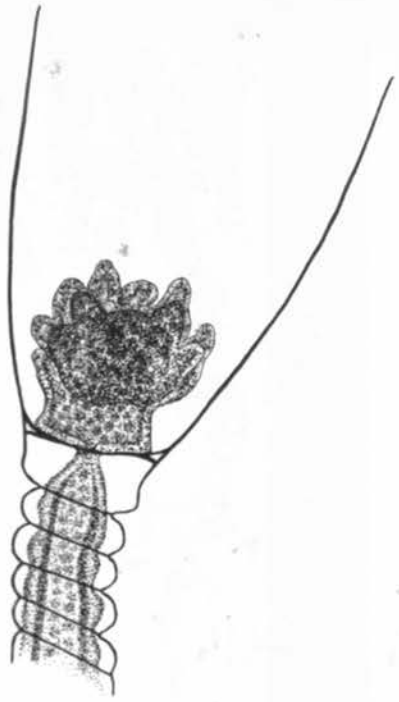
Stage 6

Carol E. Young, del.

Plate I



Stage 3



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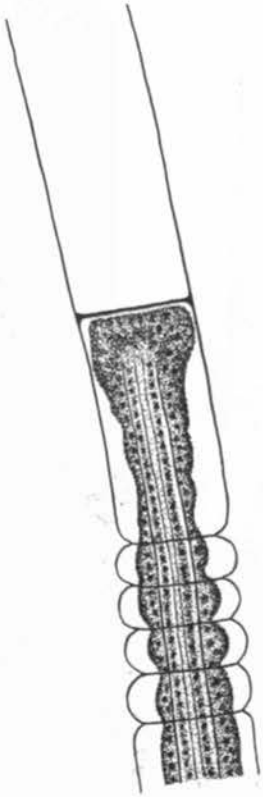
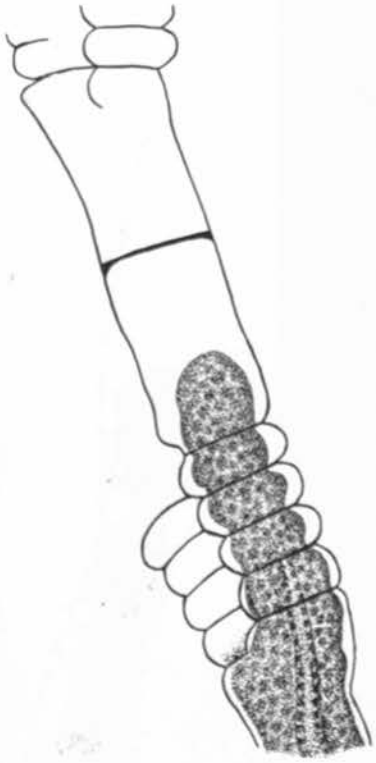


Stage 7



Stage 8