A CONTRIBUTION TO THE PHYSIOLOGY OF THE FRESH-WATER SPONGES (SPONGILLIDAE) BY H. VAN TRIGT.

Oct - Dec
1924.

CONTRIBUTION TO THE PHYSIOLOGY OF THE FRESH-WATER SPONGES (SPONGILLIDAE).

Gee.

A CONTRIBUTION TO THE PHYSIOLOGY OF THE FRESH-WATER SPONGES (SPONGILLIDAE).

PROEFSCHRIFT TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE PLANT- EN DIERKUNDE AAN DE RIJKS-UNIVERSITEIT TE LEIDEN, OP GEZAG VAN DEN RECTOR-MAGNIFICUS, DR. P. C. T. VAN DER HOEVEN, HOOGLEERAAR IN DE FACULTEIT DER GENEESKUNDE, VOOR DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP VRIJDAG 28 MAART 1919, DES NAMIDDAGS TE 3 UUR, DOOR **HERMAN VAN TRIGT**, GEBOREN TE AMSTERDAM.

BOEKHANDEL EN DRUKKERIJ
VOORHEEN E. J. BRILL, LEIDEN 1919.

Met beide handen wil ik deze gelegenheid, mij bij mijn promotie geboden, aangrijpen om U, Hoogleeraren en Oud-Hoogleeraren mijner Faculteit te Leiden en verder U allen, die tot mijn wetenschappelijke vorming hebt bijgedragen, mijn hartelijken dank te betuigen!

Deze dank geldt U, Hooggeleerde FRANCHIMONT, vóór de heldere, systematische wijze, waarop Gij de Organische Scheikunde voor onze oogen placht op te bouwen, U, Hooggeleerde LORENTZ voor Uwe onvolprezen colleges en Uw Leerboek der Natuurkunde, en U, Professor MARTIN, voor Uwe onderhoudende wijze van doceeren; doch vooral U, Professor JANSE, die mijn eerste schreden op het pad der Physiologie hebt geleid, voor Uwen vriendelijken raad en belangstelling in mijn werk en mijn plannen.

U, Doctor DE GRAAF, ben ik erkentelijk voor de goede microscopisch-technische leiding, welke Gij den studenten door hulp en voorbeeld geeft.

Hooggeleerde PEKELHARING, Hooggeleerde VAN RIJNBERK! Bijzonder grooten dank ben ik U verschuldigd voor de gelegenheid, die Gij mij op Uwe gastvrije laboratoria te Utrecht en Amsterdam zoo langen tijd en in zoo ruime mate hebt geboden, om onder Uwe leiding en met de door mij dankbaar aanvaarde hulp Uwer Assistenten, Professor RINGER en Doctor DE BOER, in de gebieden der Physiologische Chemie en der Physische Physiologie wegwijs te worden. Ik zeg niet te veel, wanneer ik verklaar, dat de jaren op Uwe voortreffelijke laboratoria doorgebracht voor mij altijd tot de aangenaamste en leerzaamste van mijn leven zullen blijven behooren. Ook de belangstelling en aanmoediging, welke ik in de latere jaren van U heb mogen ondervinden, stemmen mij zeer dankbaar!

Hooggeleerde VAN KAMPEN, zeer geachte Promotor, verplicht voel ik mij jegens U voor de groote welwillendheid, waarmede Gij deze functie op U hebt genomen op een tijdstip, dat het onderzoek voor mijn proefschrift reeds vrijwel voltooid was. Uwe critische opmerkingen bij het doorlezen dezer verhandeling zijn door mij zeer gewaardeerd.

Niet eindigen wil ik mijn proefschrift, mijn leerjaren wil ik niet afsluiten, alvorens nog een woord te hebben gewijd aan de nagedachtenis van mijn betreurden Leermeester en oorspronkelijken Promotor, Professor VOSMAER. De groote, ik mag wel zeggen de algeheele vrijheid in mijn Zoölogische studiën, mij van den beginne af door hem veroorloofd, zal bij mij in dankbare herinnering blijven voortleven, evenlang als mij zijn beeld voor den geest zal blijven zweven als dat van den volhardenden Werker, die zich niet door ziekten of pijn van zijn studiën wilde laten scheiden ..... tot het moest.

Het past mij een woord van dank uit te spreken aan de Faculteit der Wis- en Natuurkunde voor hare toestemming dit mijn proefschrift in een vreemde taal te laten verschijnen. De groote bezwaren in deze bijzondere tijdsomstandigheden verbonden aan het uitgeven eener verhandeling in het Hollandsch en in een vreemde taal, beide, hebben mij — zij het ook noode — doen besluiten vergunning te vragen van de Hollandsche uitgave te mogen afzien.

Ten slotte wil ik niet nalaten U, Mejuffrouw VAN DOBBEN, mijn oprechte erkentelijkheid te betuigen voor Uwe zorgen aan het Engelsch dezer studie besteed.

STELLINGEN.

I.

Ten onrechte wordt de „symbiotische" alg der zoetwatersponzen tot het geslacht Chlorella gerekend.

II.

Ten onrechte verklaart men het verschijnsel, dat de groote meerderheid der „symbiotische" algen in zoetwatersponzen in donker kleurloos zijn (worden), door het voor de hoogere planten bekende feit, dat in duister geen chlorophyl gevormd kan worden.

III.

Het nut der „symbiotische" vereeniging van zoetwaterspons en groene alg (in licht) is voor de alg niet gelegen in een gunstiger voedingsmedium, doch in het feit (in beperkte mate) beschermd te worden.

IV.

Van het standpunt der zoetwaterspons zijn de voortdurend uit het omgevende water in haar weefsels binnen gevoerde „symbiotische" algen uitsluitend als voedsel te beschouwen, bestemd om verteerd te worden, tenzij de mogelijkheid verwezenlijkt mocht blijken, dat de door deze algen in licht afgescheiden O_2 een aanmerkelijken, gunstigen invloed heeft op het stofwisselingsproces der spons.

V.

Ten onrechte beschouwt men de flagelbeweging van de choanocyten der sponzen als geheel afwijkend van die der Flagellaten.

VI.

Ten onrechte beschouwt men de wijze van voedselopvangen door de choanocyten der sponzen als geheel afwijkend van die der Choanoflagellaten.

VII.

Ten onrechte zegt BIEDERMANN (WINTERSTEIN's Handb. d. Vergl. Physiol.), „dass die Kragenzellen" (der sponzen) „wirklich die einzigen direkt nahrungsaufnehmenden Elemente sind".

VIII.

Het dieet heeft bij den mensch invloed op de werkzaamheid van het ptyaline. (Zie o. a. VAN TRIGT, Ztschr. f. Physiol. Chemie, Bd. 85, 1913.)

IX.

De mogelijkheid bestaat, dat het organisme zich bij verandering van dieet naar de veranderde eischen schikt door, zonder de concentratie van het enzym te veranderen, aan het digestiemilieu een geschiktere samenstelling te geven, b. v. met betrekking tot de concentratie der H-, OH-, Cl- en andere ionen. (Zie o. a. RINGER en VAN TRIGT, Versl. Kon. Ak. v. Wet., Nov. 1912 en Ztschr. f. Physiol. Chemie, Bd. 82, 1912.)

X.

De electrogrammen met behulp van den snaar-galvanometer verkregen van het pulseerende caudale lymph-hart van den aal laten zich op eenvoudige wijze uit zijn bouw verklaren. (Zie VAN TRIGT,

Ztschr. f. Biologie, Bd. 62, 1913 en Ned. Tijdschr. v. Geneesk. 1913 en 1914.)

XI.

De dermatomerie van Lacerta viridis levert ons den sleutel ter verklaring van de dermatomerie der tot nu toe onderzochte hoogere dieren (kat, macacus, mensch), daar de factoren, welke vorm en ligging der dermatomen beheerschen, bij Lacerta in den meest primitieven toestand aanwezig zijn. (Zie VAN TRIGT, N. Verh. Bataafsch Genootschap, 2de Reeks, Dl. 7, 1917 en Ned. Tijdschr. v. Geneesk. 1918.)

XII.

Het door LIEBERKÜHN en WELTNER beschreven verschijnsel, dat levende gemmula-cellen van zoetwatersponzen in water gebracht tijdens de opzwelling hunne dooierkogels uitstooten, kan niet alleen beschouwd worden als een reactie tot zelfbehoud, doch ook als een inleiding tot celdeeling, welke door de dooiermassa zoolang is tegengehouden. (Zie VAN TRIGT, Arch. Néerl. d. Physiol., T. 2, 1918.)

XIII.

Het is zeer waarschijnlijk, dat het zetmeel in de plantaardige cel niet alleen dienst doet als voedsel, doch tegelijkertijd een „reservoir van ademhalings-energie" is. (Zie JANSE, Jahrb. f. wiss. Botanik, 1918.)

XIV.

De door STRASBURGER en BOWER gegrondveste antithesen-theorie der generatie-wisseling wordt door de bekende gevallen van apogamie en aposporie niet noemenswaard aangetast. (Zie BOWER, The Origin of a Land Flora, 1908.)

XV.

ABEL's uitspraak (Palaeobiologie, 1912), dat „der Daumen eine

Neuerwerbung der Reptilien ist und bei den Stegocephalen und Amphibien überhaupt ursprünglich nicht vorhanden war", is — in verband met de wet van DOLLO — niet vereenigbaar met de opvatting, dat de pentadactyle (voorste) extremiteit der hoogere vertebraten direct van het Ichthyopterygium af te leiden zou zijn.

XVI.

Tegen de opvatting van EPSTEIN (Naturw. Wochenschr., 29 April 1917), dat Spirula niet als een afstammeling der Belemniten beschouwd moet worden, doch als de laatste levende vertegenwoordiger der echte Clymeniden, zijn groote bezwaren aan te voeren.

This paper will also be published in the Tijdschrift der Nederlandsche Dierkundige Vereeniging, 2de Serie, Dl. XVII (1919).

CORRIGENDA.

Page 80, line 21, read: .... not *only* to be ....
„ 80, „ 22, „ : .... but *also* in ....
„ 83, „ 19, „ : .... not *only* expl..... but *also* by ...
„ 106, „ 12, „ : .... by *themselves* alr. ....
„ 110, „ 26, „ : .... less *completely* ox. ....

A CONTRIBUTION TO THE PHYSIOLOGY OF THE FRESH-WATER SPONGES (SPONGILLIDAE).

PREFACE.

A paper as follows must necessarily be established by a detailed statement of observations as well as by illustrations taken from life. As my investigations have led to so many subjects, this paper has become rather extensive; and it would have lost its clear survey, had I not taken special precautions to prevent this.

1. For a survey of the questions and the results, I refer to the various points of the Introduction and to the adjoined points in the Summary at the end of this paper. This Summary gives the chief results, and the pages of the text, the tables and illustrations concerning them.

2. In the text itself the questions and results are always printed *in italics*.

My principal results have already been published in the Proceedings of the Kon. Akademie van Wetenschappen at Amsterdam, meeting 24 Nov. 1917.

CONTENTS.

INTRODUCTION.

If I venture to treat here at large, with the help of my own research, the problem of the chlorophyll, of the current of water, of the ingestion and the defecation in fresh-water sponges, it is not, because there is but little known about these problems. On the contrary; investigators have already studied them since decennaries. We even possess a great number of papers on these subjects; some of which are considered in literature as standard researches giving decisive results. Nevertheless, I think it necessary to treat those problems once more; in the first place, because during my investigations, which I have continued as exact as possible for at least 4 years, several of these results obtained and generally acknowledged — some of them being of the greatest interest — proved to me absolutely inexact; and in the second place, because I will be able to confirm by new and better proofs some of the results, which have not yet been generally acknowledged in consequence of a less complete argumentation.

I will mention in short the chief points (see also VAN TRIGT, 57b):

1. In 1882 BRANDT (8, 9) came to the conclusion, that the chlorophyll corpuscles of the fresh-water sponges were unicellular algae (Zoochlorellae), morphologically and physiologically independent of their hosts. RAY LANKESTER (35) and GEDDES (22), however, got to quite the opposite view: the chlorophyll corpuscles of Spongilla are identical to those of the plants, the investigators who think them to be parasitic algae are misled. BRANDT's proofs are good enough, but not yet quite sufficient; he did not succeed in convincing LANKESTER and GEDDES. Nevertheless, BRANDT's

opinion is almost generally acknowledged — eg. by BEIJERINCK (4) 1890, DELAGE (16) 1899, HERTWIG (29) 1903, OLTMANNS (47) 1905, WELTNER (68) 1907 and BIEDERMANN (6) 1911 —; SOLLAS (53) 1906 only — and apparently MINCHIN (45) 1900 too — holds the view of LANKESTER, though in a note he indicates the possibility of the chlorophyll corpuscles being algae. I will show by decisive proofs that BRANDT was right (see Summary, point **1—5**).

2. The investigators, who agree with BRANDT, unanimously declare that the symbiotic alga of the Spongillidae belongs to the genus Chlorella — eg. BEIJERINCK (l. c.), OLTMANNS (l.c.), SCHENCK (56) 1908, WILLE (69) 1911 —. But having examined the mode of reproduction of those algae, I have been able to state that, at least in my sponges, we do not meet with a member of the genus Chlorella at all, but with a form probably closely related to the genus Pleurococcus (see Summary, point **6**).

3. By lack of sufficient daylight green fresh-water sponges become colourless, viz. creamy-white, and colourless sponges remain colourless. In such sponges LANKESTER (l. c.) found the chlorophyll corpuscles, which used to be green, now colourless; and he concludes, that these colourless forms may either pass directly into (green) chlorophyll corpuscles by the action of sunlight, or that during their development, by sunlight, in stead of yielding the colourless form they pass into the green type. In the same way BRANDT (l. c.) says, when speaking about these colourless corpuscles: „eben so gut wie die Chlorophyllkörper von höheren Pflanzen, können doch aber auch die Chlorophyllkörper von Algen bei mangelhaftem Lichtzutritt blasser werden"; and elsewhere: „dass die Chlorophyllkörper der Zoochlorellen ihre grüne Farbe im Dunkeln einbüssen, ist selbstverständlich". So both authors suppose conformity of the behaviour in darkness of the chloroplasts of the higher plants and of the chlorophyll corpuscles of the Spongillidae; and so they explain the fact mentioned above, viz. that in darkness green sponges grow colourless and colourless ones remain colourless, by analogy to the fact known from the Angiospermae: that chlorophyll can not be produced in darkness. This, now, proved to me quite inexact. The

lack of light is really the cause of the green (colourless) sponges becoming (remaining) colourless in darkness, but for quite a different — much more complicate — reason than BRANDT and LANKESTER think of (see Summary, point **7—15**).

4. The general conception (except of LANKESTER c. s.) of the symbiotic relation of fresh-water sponge and alga is, as a relation probably based on mutual use. So Spongilla is almost considered to be a „classic" example of symbiosis next to the Lichens. We possess however but a few proofs, very little decisive, concerning this mutual relation of host and guest (BRANDT 8). Now, with my experiments I have come to the conclusion that, instead of a classic example of symbiosis in the sense of the mutualism of the Lichens, the association of sponge and alga may be called at best a transition of a process of nutrition (of the sponge) into a still very imperfect symbiosis (see Summary, point **16—19**).

These were the original points I intended to examine. But, as is usually the case, when seeking we find by chance quite new ways leading to other territories. So I found out a method, which enabled me to observe wholly intact, normally living tissue of sponges with oil-immersion for many hours, on several consecutive days. In this way I got an insight into the following points:

5. As for the cause of the current of water in the canal-system of the sponges, the research and theory of VOSMAER and PEKELHARING (62) 1898 are almost generally acknowledged — eg. by MINCHIN (45) 1900, BIEDERMANN (6) 1911, BABAK (3) 1912 and partly by JORDAN (32) 1913 as well —: By the irregular strokes (to and fro) of the flagella of the choanocytes the pressure of the water on the inside of the wall of the flagellated chambers is continually changing; one time it is positive, next time negative. In the first case the choanocytes acting as valves will prevent the water from flowing out through the prosopyles. If, on the contrary, the pressure is lessened, water can easily flow into the chamber by these openings. The sponge will thus suck in water through the incurrent canals, which flows out again by the osculum. The current in the flagellated chambers is not regularly,

continually streaming but irregularly whirling. This is, in short, the theory of VOSMAER and PEKELHARING. Now, I have been able to establish in my living sponge-preparations that the movement of the flagella, as described here, is not the normal one, but abnormal, and caused by exhaustion. The real movement takes place, just as in the Flagellata, in a spiral- or undulating line, while the current of water in the chambers is decidedly regular and quick (see Summary, point **20**).

6. In close relation to the problem of the water-current in sponges is that of the ingestion of food — already a very old matter of dispute. Here too we owe the last and principal researches to VOSMAER and PEKELHARING (l. c.), by whom was shown once more, that the flagellated chambers, c. q. the choanocytes, would be the real „eating-organs" (CARTER) of the sponge; although both these investigators think it possible, that now and then food-particles can be captured by cells lining the canals. In literature this view is almost generally accepted — eg. by DELAGE (16) 1899, SOLLAS (53) 1906, BIEDERMANN (6) 1911 and JORDAN (32) 1913. MINCHIN (45) 1900, however, thinks [1]) that in the simplest forms of sponges indeed the collar-cells represent the chief „eating-organs", but that in the higher organized ones the function of ingestion may be usurped more and more by cells in the parenchyma (amoebocytes or porocytes). Now, I myself have observed in my living preparations, that in Spongilla ingestion certainly takes place by the choanocytes, but that there exists still another mode of ingestion — for larger bodies only — at the exterior of the flagellated chambers at the entrance of the prosopyles.

It stands to reason, that the mode of capturing food by the choanocytes was conceived in perfect agreement with the theory of the water-current, as mentioned by VOSMAER and PEKELHARING. So these investigators declare that the motion of the flagella, by the whirling movement of the water it produces in the chambers, causes, that food particles arrive easily within the collars of the choanocytes and thus come in contact with

1) Here MINCHIN follows METSCHNIKOFF (44).

their protoplasm. Now, as I have been able to observe in my living preparations quite another way of movement of the flagella and the water-current as being the normal one in the flagellated chambers, the way in which the choanocytes capture their food was also bound to prove wholly different. The food-particles are by no means captured within, but at the outside of the collars or at the outside of the collar-cells themselves; so exactly in the same way as in the Choanoflagellata (cf. DOFLEIN (17)). Already JORDAN (32) said: „Den Vorgang der „Phagocytose" durch die Kragenzellen mit demjenigen zu analogisieren, den wir bei den Choanoflagellaten kennen lernten, ist sehr verlockend, aber das vorliegende Beobachtungsmaterial reicht nicht hin, uns ein Recht zu solchem Analogisieren zu geben". COTTE (12) 1902, namely, seemed to have observed something as a capturing of food at the outside of the collars, although he said the capturing within the collars to be the chief manner of ingestion.

Quite separate from the problem of ingestion of solid food remains PÜTTER's theory (48, 49) 1909, 1914, which says that sponges do not feed so much with micro-organisms as with organic substances in solution, present in the water.

(See Summary, point **21—23**).

7. Finally the problem of defecation and excretion; up to now it has been studied but little, and the few results we possess, and which we owe amongst others to MASTERMAN (42) 1894 and COTTE (13) 1903, are only accepted in literature with reserve — BURIAN (10) 1910, JORDAN (32) 1913 —. There are mentioned, for instance, an expulsion of cells loaded with waste solids at arbitrary points of the inner or outer surface of the sponge-body, and a process of defecation by the choanocytes. Now, I have been able to observe in my living preparations, that defecation (and probably excretion at the same time) takes place on a large scale by means of vacuoles. And that probably this process should be considered partly in direct relation to the above (point 6) mentioned second way of capturing (larger) particles. So we get to know a — very necessary — quickly working system of cleansing of the sponge (see Summary, point **24—25**).

These are the chief points of my investigation. I want to declare emphatically that for all my microscopic examinations I used no other than living preparations. Observing them has been an inexhaustible source of enjoyment to me, also beyond the problems I was about! This makes me think: Those zoologists, who devote themselves exclusively to morphology, should consider that, when studying biological problems, they generally commence by depriving the organism to be examined of the most interesting phenomenon, it ever may show — even the most interesting phenomenon on earth! —: LIFE.

METHODS.

The capturing of sponges. The sponges were collected in the lakes near Leyden (Brasemermeer). Both forms, Spongilla lacustris and Ephydatia fluviatilis, are to be found there in great quantities; of course, such an unlimited quantity of proof-material is of the greatest importance for physiological investigations. When loosened from their supporting layer — stone or wood — the sponges were immediately cleaned from adhering parts of mud etc., and transported to the laboratory as quickly as possible.

The conditions of life in their habitat as to light, purity of the water etc., in connection with the peculiarities of the freshwater sponges, were studied by me in all seasons for several years. So I got many data, concerning the sponges living under normal conditions, on which I could test the exactness of the results of my proofs.

The culture of the sponges. After many experiments the following arrangement of the aquaria has proved to be the best. The glass aquaria (capacity 50 and 100 litres), and the other vessels used for the experiments, were standing in an unwarmed apartment of the laboratory (windows on S. W.), while daylight was tempered by partly closing the shutters and the air refreshed

by an open window. The aquaria contained water from the conduit, which was continually flowing in; as I may conclude from my experiments, the water was entirely renewed in this way in 5—10 hours. Plants, nor any nutriment for the sponges were ever added — pure, streaming water proved to be best. In the aquaria the sponges were in (tempered) daylight, not too many together, in their natural attitude lying on wire-gauze, 10—20 centimeters from the bottom and 15—20 centimeters from the surface of the water; in this way they were safe from the influence of anything that might sink to the bottom. The aquaria were cleaned once in 1—2 months. For culture of sponges in darkness aquaria arranged in the same manner but surrounded by perfectly light-tight wooden cases were used. The Spongillae generally kept alive for 1—1$^1/_2$ months, in the beginning they often grew strongly; the Ephydatiae, however, sometimes lived even for 5 months, but they did not grow so quickly as Spongillae. Sometimes sponges were cultivated separately in glass vessels (capacity 3 or 5 litres), also containing conduit-water, though not streaming; this water was renewed once a day.

The culture of the isolated chlorophyll corpuscles. The cultures were obtained in the following way: A green sponge was rubbed and pressed, the parts of the skeleton removed, and the remaining dark green liquid mixed with some water. Then this liquid was kept quiet for about an hour, during which time a thick green mass settled at the bottom — formed, as appeared, by amoebocytes and other sponge-cells and by detritus — while a still green liquid remained. The latter almost exclusively contained the isolated green chlorophyll corpuscles. Next this liquid was divided into equal quantities over many little glass vessels; these vessels were filled with the culture-media wanted to the same volume (50 cM^3), covered with glass plates, and placed either in (tempered) daylight, or in complete darkness in the same room as the aquaria. In the beginning the culture media were renewed every 2 or 3 days (as long as there were processes of rotting of sponge-rests), afterwards once in 1 or 2 months; to which it was of much use, that the chlorophyll corpuscles, once having

been isolated, used to settle down to the bottom in one day's time, then forming, together with sponge-rests, a continuous membrane rather firmly attached to the bottom.

The cultures of colourless chlorophyll corpuscles (from colourless sponges) were obtained in the same manner.

The culture-media were the following: **1.** water from the conduit, **2.** solution of inorganic food, **3.** solution of organic food, either diluted or concentrated, **4.** liquid from a pressed sponge, also diluted or concentrated.

The following solutions of inorganic food were used:

I. Solution of OEHLMANN (from DOFLEIN (17)).

999	Gr.	water
0.2	„	$MgSO_4$
0.4	„	NaH_2PO_4
0.4	„	KNO_3

II. Solution of BEIJERINCK (from GERNECK (23)).

100	Gr.	water
0.05	„	NH_4NO_3
0.02	„	KH_2PO_4
0.02	„	$MgSO_4$
0.01	„	$CaCl_2$
traces		$FeSO_4$

And the following solutions of organic food:

I. Solution of ARTARI (2).

0.5	%	peptone (WITTE)
1	„	glucose
0.3	„	KH_2PO_4
0.1	„	$MgSO_4$
0.1	„	$CaCl_2$
traces		Fe_2Cl_6

II. The same, but containing 6% glucose.

III. Diluted solution of organic food; obtained by mixing 50 cM^3 of the solution of inorganics I and 3 drops of the following solution of organics (of ZUMSTEIN, from DOFLEIN l. c.):

100	Gr.	water
10	„	peptone (WITTE)
10	„	glucose
4	„	citric acid
0.4	„	$MgSO_4$
1	„	KH_2PO_4
1	„	NH_4NO_3

The liquid from a pressed sponge (for culture-medium) was obtained in this way: The superfluous water was pressed out of some green sponges; then the sponges were rubbed and the remaining liquid was filtered through a cloth, in order to separate it from the intact sponge-cells (but not from the chlorophyll corpuscles etc.). It was my intention to obtain in this way a medium (without the living sponge) for the chlorophyll corpuscles, differing as little as possible as to composition and concentration from that in the living sponge. When this medium had to be renewed, I could not make use again of pressed juice of a green sponge — for the chlorophyll corpuscles cannot be held, not even by filtering paper —; I then used the juice of a colourless sponge.

In all these cultures appeared among a colourless, granular ground-substance formed by sponge-rests, besides a great many chlorophyll corpuscles, of course also some other organisms (algae, protozoa, bacteria); so they were not pure cultures. To this fact I owe many interesting data concerning the influence of such organisms on a culture of my chlorophyll corpuscles.

Nevertheless, I have tried in several ways to get really pure cultures; but always in vain. In the first place in the way indicated by BEIJERINCK (4) for algae in general, the so called gelatine-method. As I did not succeed in applying this method to the chlorophyll corpuscles — as little as BEIJERINCK — I will only mention it in short: Before little colonies of the green chlorophyll corpuscles could be detected, the gelatine was always entirely liquefied. I tried to suppress the development of bacteria and mould in the originally sterile, neutrally reacting gelatine by adding citric acid or sugar to it and by infecting with traces

of a culture of chlorophyll corpuscles; but I did not succeed. Next I tried to pick up a single chlorophyll corpuscle from a drop of water by means of SCHOUTEN's apparatus, in order to transport it to the sterile gelatine. The apparatus, which proved good for bacteria, was of no use to me: in water the corpuscle sticks to the needle, but it immediately loosens when taken out; and my efforts to get a more appropriate form of needle-point were unsuccessful.

So I did not get a pure culture of the chlorophyll corpuscles of the Spongillidae. But this was of no consequence to my research; the ordinary cultures procured all results I wanted.

Microscopic preparations. As I stated already in the Introduction, I did not use any other than living preparations; namely in two forms:

1. Ravel preparations of a sponge tissue, or preparations of chlorophyll corpuscles from a culture, made on a glass-slide with a drop of the original liquid; the coverglass surrounded by vaseline in order to entirely separate the preparation from the outer world.

2. Sponges grown on coverglass. These preparations, in which I observed many important phenomena in sponge life, were made in the following way: A branch of a Spongilla — Ephydatia is not appropriate for the experiment because of its slow growth — is cut at full length in two, and each half is divided again into short pieces of 1 c.M. Each piece is then put on its long flat side on a large coverglass into an aquarium. When kept quiet, these little sponges will then soon attach themselves with this side (the wound side) to their glass; while a thin membrane of newly formed sponge-tissue will even extend from each old sponge piece as a centre over the coverglass (Fig. 3). This membrane thickens according as it is growing older. In a week's time we then possess in this membrane a wholly intact, normally living sponge preparation, fit for microscopic examination even with an oil-immersion! To that purpose we put the coverglass, the sponge at the underside, on glass feet into a glass vessel filled with water, so, that the upperside of the coverglass remains

on the surface of the water and the whole can be placed on the table of the microscope. If we renew the water now and then, and if, after examination, we put the preparation back into the aquarium, we can observe this living tissue under wholly normal conditions for many hours, on several consecutive days.

Microscopising. All my microscopic examinations were made in an ENGELMANN case, with the help of ZEISS's eye-piece n°. 4 and a most excellent specimen of a LEITZ $^1/_{12}$ oil-immersion. This last lens gave even better results than the precious apochromatic system of ZEISS (with compens. eye-pieces).

The estimating of the number of chlorophyll corpuscles, etc. in a preparation. I often had to know — for the sake of mutual comparison — the absolute number of, for instance, the various green and colourless chlorophyll corpuscles in a same volume of several sponges, or in a certain volume of several cultures. If I had wished to count these corpuscles, it would have required a suspension — adequate to counting as for exactness — of equal volumes of sponge-tissue (or culture) in the same quantities of water; otherwise the exact method of counting would have been worthless. But of course, we cannot obtain equal volumes of sponge-tissue or culture, nor a really equal suspension; while, also, counting would have proved a very tiresome work.

I therefore always took, by means of pincers from a sponge or with a pipette from a culture, quantities of the material as equal as possible, and spread it out on a glassslide, while parts of the skeleton were removed. Then the coverglass was pressed in an always equally strong way, the superfluous liquid sucked up, and the coverglass surrounded by vaseline; next I estimated under oil-immersion the number of the various chlorophyll corpuscles present in the whole microscopic preparation, consequently, present in an almost equal volume of each sponge or of each culture. This method, always applied in the same manner, gave rather good results, as Table n°. 4, 6, 8 show; besides, the differences, I wanted to know, were generally considerable enough. So this method of estimating was in all respects preferable to counting, which would have led to an imaginary exactness only.

The degrees of numerousness, used for estimating, are the following (arranged according to the increasing number):

I = chlorophyll corpuscles absent in the preparation.
II = „ „ very rare „ „ „ ; viz. 1—4.
III = „ „ rare „ „ „ ; in some fields 1.
IV = „ „ rather rare „ „ „ .
V = „ „ here and there „ „ „ .
VI = some chlorophyll corpuscles present in the preparation.
VII = several „ „ „ „ „ „ ; in every field
VIII = somewhat more „ „ „ „ „ „ . [± 3.
IX = rather numerous „ „ „ „ „ „ .
X = numerous „ „ „ „ „ „ .
XI = very numerous „ „ „ „ „ „ .
XII = a mass of „ „ „ „ „ „ ; the fields filled up with them.

It goes without saying, that the limits between these different degrees may not be considered as having been strictly indicated.

In the same way were examined for many sponges: the number of oildrops — present in a preparation or in an amoebocyte —, the number of globules of carbohydrate (coloured by I) — present in an amoebocyte —, and the number of amoebocytes — present in a preparation —; here too the same degrees were used.

One should, however, not try to compare these numbers (I—XII) mutually in the different groups (that of the chlorophyll corpuscles, of the oildrops, of the globules of carbohydrate, and that of the amoebocytes) too exactly. — Besides, there will never be any reason to do so. — So, for instance, one should not consider a number of chlorophyll corpuscles in a preparation, indicated by X, as being exactly the same as a number of amoebocytes also indicated by X. These degrees of numerousness may only be directly compared within each group; while for the groups mutually this only counts in a very limited way. This is inevitable, when using the method of estimating the number: for our estimation is involuntarily influenced by the extent of the corpuscles the number of which must be estimated, and by the

space in which they occur, as well as by their greatest number, we know as normally occurring within that space. But, as mentioned above, we will never have to compare mutually direct the numerousness in the different groups (that of the chlorophyll corpuscles, that of the oildrops, etc.), but only the changes of number — i. e. the increase or decrease — in the different groups mutually. That can always be done, of course.

RESEARCH.

A. THE CHLOROPHYLL OF THE FRESH-WATER SPONGES.

I. How and where the green colouring-matter occurs.

Spongilla lacustris and Ephydatia fluviatilis — the only fresh-water sponges I am going to treat in this paper — occur in our country in a green and in a colourless form; between which, however, exist many intermediate tints from emerald-green to creamy-white. But a newly caught specimen never shows any of these colours purely; for, in consequence of its growth in the more or less dirtied water of our canals and lakes, the sponge tissue is so overloaded with particles taken from the water, that the colour may have taken a dirty-brownish tint. The green sponges do not show this very clearly, but creamy-white specimina are even never to be found: they are always gray-brown in different variegations. I therefore think that all the different colours of the fresh-water sponges mentioned in literature (emerald-green, green, brown, yellow-brown, flesh-coloured, gray, dirty-white, white) are simply to be reduced to the principal colours (grass-)green and creamy-white with their intermediates, while the others can be explained as having been caused by the dirtied water, in which the sponges were living.

I conclude this from the following facts: **1.** I collected my sponges in a moor-lake, with pale brown coloured water, contain-

ing numerous brown particles. **2.** Generally not the whole (green or colourless) sponge had such a dirty tint — at least not Spongilla. As one knows, this sponge grows on stones or wood as a thin crust, from which long (10—20 c.M.) finger-shaped branches proceed; contrary to Ephydatia, which generally forms flat cushions with but little elevations. Of course, the growth of Spongilla chiefly takes place at the end of the branches, and is rather quick. But then it is obvious that the tissue of such a region, where the cells are rapidly dividing, can not possibly have the same degree of dirtiness (by particles from the water) as an older not growing region. And besides, in young tissue there are no flagellated chambers; so there the possibility of taking particles from the water is, of course, considerably reduced. This proved to be the case. In summer, when Spongilla is growing quickly, it generally shows brightly (not dirty) coloured tops, $^1/_2$—$^1/_4$ c.M. long; which, however, in specimina from cleaner water contrasted much less distinctly with the older tissue. **3.** When such a sponge (with bright coloured tops on dirty-brownish branches) was put into an aquarium filled with water from the conduit, all the difference in colour between the tops and the older tissue had disappeared within a few days; also this last tissue had got a bright colour, whether it was green, creamy-white, or an intermediate. This tissue, originally loaded with brown particles, then proved under immersion to contain almost none. **4.** Something the like can be said of Ephydatia.

So the two chief forms of Spongilla lacustris and Ephydatia fluviatilis are a grass-green (Fig. 1) and a colourless (creamy-white) one (Fig. 2).

I am going to treat the green form first.

The sponge owes its green colour to numerous little green corpuscles present in its tissues, especially in amoeboid cells (Fig. 69), which for shortness' sake I shall indicate with the common name of *amoebocytes*. (As for the different cell-forms in the fresh-water sponges, see Weltner (68) 1907). *These amoebocytes crowded with the green corpuscles form the greater majority of the sponge cells,*

spread all over the body in the parenchyma, so among the mesogloea (intercellular substance, ground subst. [1])). (As for their great importance in sponge life, see WELTNER (l. c.) and MINCHIN (45) p. 57—60). The green colour grains are mostly lying free in the protoplasm (of the amoebocytes), only seldom in a vacuole. In fig. 4 such an amoebocyte is represented in the form I so often observed, when wholly isolated from the sponge-tissue and attached to the coverglass in my living preparations of tissue. It then seemed entirely „vacuolized". However, I don't think these open spaces to be real vacuoles belonging to the cell. (See Appendix, III).

Besides in these amoebocytes, the green corpuscles also occur — but much less numerous — in the pinacocytes of the inner and outer surface of the sponge body, in the choanocytes of the flagellated chambers, and finally in the intercellular groundsubstance.

II. The behaviour of the green colouring-matter.

We now have to examine of what the green corpuscles of the sponge cells consist; in other words, we have to prove that the material of which the corpuscles consist is morphologically and physiologically identical to the chlorophyll, that we know from the plants. SORBY (54), LANKESTER (35) and BRANDT (8) have already studied this problem. SORBY and BRANDT compared the spectra of solutions of the green sponge colour, obtained in different ways, with the corresponding solutions of vegetable chlorophyll; in this way the identity of the spectra was stated. LANKESTER compared the structure; and concluded, that the similitude in form and structure proves the absolute identity of the green corpuscles of the sponges to that of the plants.

As regards *the physiological identity*, we don't possess any decisive proofs at all; though this only should give the decision! In the first place we have to show that the green corpuscles produce O_2 when exposed to light, and in the second place, that in

1) For this, see Appendix, I.

light they also photosynthesise (produce carbohydrate or oil). This having been established, we are justified in declaring — in connection with the points of similitude already known — that the green colouring matter of the sponges is chlorophyll. I have been able to procure both proofs.

1. In order to prove the production of O_2 in light, I proceeded in the following way:

a. I cut two equal pieces from a branch of a green Spongilla, and an equally large piece from a colourless one. These pieces were put separately — without having been taken out of the water — into glass vessels, filled with water from the conduit, under inverted funnels and tubes, etc. (see Table 1c). One green piece and the colourless one were placed into bright day-(evt. sun-)light, the other green piece, however, on the same spot in darkness. At first no gasbubbles were to be found anywhere in the vessels; but at the end of the experiment the green sponge piece, exposed to light, showed numerous rather large bubbles, as well at the outside as w i t h i n its tissue, and the funnel contained a lot of them. The green sponge in darkness and the colourless one in light, on the contrary, didn't show any; except a few bubbles inside and outside their funnels, evidently formed by air from the water. I have repeated this experiment several times, always with the same result (Table 1a, b): only the green sponge, when exposed to light, formed gas-bubbles, the green sponge in darkness did not, nor did the colourless one in light. The quantity of gas produced, however, was always too little for a determination. Yet it is almost beyond doubt, that it has been O_2 [1]).

b. Wilson (70) and Müller (46) stated the fact, that rubbed sponge material can regenerate to new sponge globules, able to develop themselves almost in the same way as the gemmulae of the fresh-water sponges. Now, I put equal quantities of such globules originating in one green Spongilla, into 12 little glass vessels of the same capacity, filled with water and covered with

1) According to Brandt, Hogg stated already in 1840 the rising of gasbubbles from a Spongilla exposed to sunlight.

glass plates. One half of these vessels was exposed to daylight, the other kept in darkness (at the same temperature). After a week's time the sponge globules in all 6 vessels, which had been exposed to light, appeared to be still intact, while those kept in darkness proved to be intact in 2 vessels only, but wholly destroyed in the other 4. These observations make us think of a production of O_2 by the globules in light. This is the more evident, when we consider that entering of O_2 from the air into the vessels was almost completely prevented by their glass covering.

c. To ENGELMANN (18) we owe the bacteria-method, that enables us to detect the production even of traces of O_2. It is based, as one knows, on the fact, that many bacteria are brought to violent motion by the mere presence of but traces of O_2, while in lack of O_2 they get at rest. To prove this production of O_2 by the green corpuscles of the Spongillidae, I proceeded as follows: On the surface of an algal-culture I found a membrane of moving bacteria, from which I grafted a little on peptone-gelatine. So in a few days I got a sufficient quantity of the bacteria. I then ravelled a piece of green Spongilla on a glass slide, etc., etc. (see Table 2). After the preparation had been kept in darkness for $^1/_4$ of an hour, the bacteria were accumulating and violently moving round the air bubbles, but elsewhere they had almost got at rest. This proved that here we had to do with bacteria sensitive to O_2, as we wanted. After another period of darkness of $^3/_4$ of an hour, the movements round the air bubbles also almost had stopped. When, next, the green sponge preparation was exposed to light, we could observe under the microscope that, little by little, the movements of the bacteria were resumed all over, till the original intensity was reached. We might repeat this experiment numerous times, always with the same result (see Table 2): in darkness the movements stopped, in light they were resumed. Accordingly, the preparation proved to contain many amoebocytes crammed with green corpuscles, and numerous of those corpuscles isolated. So these last ones produced in light the O_2, necessary for the movements of the bacteria.

I made this experiment with several preparations, also from

Ephydatia and always with the same result. But now the contra-experiment (Table 2)! When I proceeded with a colourless sponge in the same way as described for a green one, I found that, after exposure to light, the movements were n o t resumed by the bacteria. Accordingly, the preparation of the colourless sponge proved to contain n o green corpuscles at all, neither isolated nor in amoebocytes. I repeated this experiment also several times.

I think to have given in these experiments *the decisive proof, that the green corpuscles of the Spongillidae produce O_2 in light, but not so in darkness.*

2. Now we have to show the photosynthesis (production of carbohydrate or oil) of the green corpuscles in light [1]). In the first place I should mention that in fact most corpuscles show one — sometimes more — drops of oil, but never any carbohydrate (pag. 25). To prove their production, I proceeded as follows (Table 3): Preparations were made from a light-green Spongilla (grown in twilight and afterwards kept for some time in darkness) in a darkroom by candle-light. One half of them was immediately put into complete darkness, while in the other ones the percent of the isolated green corpuscles containing an oildrop was examined (Table 3 c.). This proved to be 42°/₀. These preparations were then exposed to tempered day-light. Now we see in the table that in two days the percent rose from 42 to 79, but that in the preparations in darkness it remained 35. Then being exposed to day-light for two days, the latter too showed a rising, namely from 35°/₀ to 78°/₀; while at the end a percent of ± 90 was reached in all preparations. I could also observe that the oildrops of the corpuscles, which had been exposed to the light for a longer time (so in culture n°. 396 a—d) were larger. *So it is clear, that the green corpuscles of the Spongillidae photosynthesise oil in light, but not so in darkness.* I have re-

1) It seems that BRANDT (l. c.) has observed this for Spongilla; he at least mentions that the isolated corpuscles remained alive for weeks, „according to their producing assimilates". But he doesn't give more about it; and where he should have treated the phenomenon more extensively, he does not mention it at all.

peated this experiment several times (Table 3 a. b.); always with the same result.

It appeared to me, that the oildrops are formed much more quickly, when the green corpuscles are exposed to light after having been kept — as a preparation — for some weeks in darkness: in that case the percent rose in half an hour from 38 to 70. This short exposure to light seems to have extended its influence also to later on; at least in darkness the percent rose up to 88. This is not inconceivable. The oildrops, once having been produced, seem to be used but very slowly by the corpuscles — even in darkness — (Table 3 a, n^0. 163; 3 b n^0. 210). These two cases are by no means the only ones; I have sometimes been able to state that most green corpuscles of a culture, after a period of 6 months in darkness, still contained a drop of oil.

Finally I have been able to directly observe the forming of an oildrop in a green corpuscle, which at first contained none at all. The colour grain had not been kept in darkness; so the forming did not proceed so quickly: the first appearance was made after 3 hours of exposure to light, a real oildrop only to be seen after 21 hours.

So we have proved the identity of the green colouring-matter of the Spongillidae to the chlorophyll of the plants by physiological arguments. We may now speak of the chlorophyll, the chlorophyll corpuscles of these sponges.

III. The nature and structure of the green chlorophyll corpuscles.

As I stated in the Introduction, the results of Brandt (8, 9) concerning the nature of the chlorophyll corpuscles of the freshwater sponges are quite different from those obtained by Ray Lankester (35) and Geddes (21, 22). Brandt concluded that these corpuscles were algae, Lankester and Geddes however, that they consisted of chlorophyll formed by the sponge itself as an inherent part of its cells.

I am now going to treat in short the arguments of BRANDT. In the following points he gives the chief differences between chlorophyll corpuscles and algae:

chlorophyll corpuscles	algae
parts of cells	cells by themselves
consisting of groundsubst. + chlorophyll	consisting of chlorophyll + uncoloured protoplasm.
never a nucleus present	always a nucleus present
no cellulose membrane	generally a cellulose membrane
not able to live by themselves	able to live by themselves

Then BRANDT examines, which of these two series may be applied to the chlorophyll of the Spongillidae (and of the other chlorophyll containing animals). That is to say, he extensively discusses the investigation of Hydra viridis, and for Spongilla he refers to the results obtained in Hydra; in his way however, it remains somewhat doubtful what BRANDT exactly stated for Spongilla. For the corpuscles of Spongilla he especially describes: **1.** Generally a through-shaped chloroplast and hyaline protoplasm. **2.** A nucleus, that could be recognized very distinctly by means of haematoxylin or magdala. **3.** A diameter of 1.5—3 μ. **4.** After having been isolated from the sponge-cells, they remained normal and alive for 3—4 weeks. **5.** Sometimes they then seemed to have multiplied (but this required closer examination). **6.** Green corpuscles of Spongilla, added to a culture of uncoloured specimina of Stentor coeruleus were ingested by these, but not digested nor ejected; so it proved to be possible to graft these corpuscles.

I have some remarks on these points: **1.** The nucleus. As said before, BRANDT discusses Spongilla very briefly only and for details he refers to the description of Hydra. In that description is mentioned, that in all corpuscles (after having been stained with haematoxyline) one or two, sometimes more, violet, round or somewhat irregular spots could be distinguished; BRANDT declares them to be nuclei. They were solid corpuscles, but their

more detailed structure could not be examined because of the small dimensions. Now I can not deny the possibility of their being really nuclei; but to me it seems rather premature to simply declare one, two or more, somewhat irregular spots to be nuclei, on the mere account of being stained violet by haematoxyline. One will agree with me, after having read my own observations (pag. 25—26). **2.** The grafting. BRANDT's words are: „Die Stentoren nahmen die grünen Körper alsbald in grosser Menge auf und stiessen sie weder aus, noch verdauten sie dieselben. Sie blieben auch dann grün, als Hr. K. sie für mehrere Stunden in reines Wasser setzte". I must remark that, if the Stentores have not been observed for more than some hours, this experiment does not sufficiently prove the possibility of grafting the green chlorophyll corpuscles.

Next we get to LANKESTER's quite opposite view. The latter says to be convinced (by his morphological investigations), that in Spongilla the chlorophyll is present in corpuscles which are entirely identical to those of the plants, and formed, just as these, by the protoplasm of the cells in which they occur: **1.** A nucleus can never be shown. **2.** When the containing sponge cells are destroyed the isolated chlorophyll corpuscles remain intact (also in plants). **3.** It then appears that some protoplasm adheres to every corpuscle; a fact which can be explained in this way, that, when the amoebocytes are destroyed, a lump of protoplasm sticks to each chlorophyll corpuscle; accordingly, there is no differentiation of a mass of protoplasm belonging to every chlorophyll corpuscle to be seen in the intact amoebocyte. **4.** It was not possible to discover amylum (by means of I.) within the corpuscles, but it could be done in other parts of the amoebocytes. **5.** By lack of sunlight Spongilla remains colourless; it proves that in the otherwise green cells there are then colourless grains, which appear to be chlorophyll corpuscles in a somewhat abnormal condition. LANKESTER thinks it impossible to consider these colourless grains parasitic algae, ready to change into green ones by the action of sunlight. (Finally LANKESTER critisizes BRANDT's conclusions, which criticism BRANDT (9) treats in his turn.)

Also on some of these points I have a few remarks: **1.** A nucleus could never be found. But we should consider — BRANDT mentions it too — that LANKESTER never used BRANDT's staining method but always picro-carmine only. **2.** No differentiation of protoplasm belonging to each chlorophyll corpuscle could be seen in the intact amoebocyte. That is absolutely inexact; to me the contrary proved to be the case.

I am now going to show, by decisive proofs, that the chlorophyll corpuscles of the Spongillidae are real algae associated in „symbiosis" to the sponge, just as BRANDT declared. For that purpose I will make use of two sets of proofs, one being morphological, the other physiological.

a. Morphological proofs; description of the structure of the green chlorophyll corpuscles.

I am going to treat the living green corpuscles of Spongilla only, as those of Ephydatia are quite the same.

1. *Protoplasm and chloroplast.* The shape of the corpuscles is round or somewhat oval; the diameter 1.7—3.8 μ, generally 2—3 μ. Under oil-immersion we observe that in most corpuscles the green chloroplast takes exactly one half of the body, the other half consisting of uncoloured protoplasm (Fig. 5). The separating line between chloroplast and protoplasm, which in oval bodies is always situated along the longest axis, may be bent a little. But there are also numerous corpuscles with differently shaped chloroplasts (Fig. 12—16, 23—24, 30), generally taking more than one half of the corpuscle; or even two chloroplasts in one corpuscle. The mutual relation between these different forms will be discussed afterwards. It is a matter of course that one same chlorophyll corpuscle, seen from different sides, will show different aspects; so Fig. 24 and 30 give an aspect of Fig. 13, and Fig. 23 an aspect of Fig. 5 and 12—15. The protoplasm of the corpuscles appears to be more or less hyaline, not completely homogeneous, but usually containing some diffuse, darker spots (Fig. 5). The chloroplasts, on the contrary, appear to be completely homogeneous; their

colour is green. All this concerns the isolated chlorophyll corpuscles as well as those lying in amoebocytes.

2. *Enclosures.* Very often the protoplasm contains one, sometimes more, refractive globules, 0.4—1 μ in diameter, which appear blue-green when the microscope is well adjusted (Fig. 5). Sometimes one can also find them within the chloroplast. These globules are not stained by I (in KI sol.); but by sudan III (in alc. sol.) they become red, and gray-black by osmic acid. So they are fat-globules, better: oil-drops [1]). No other enclosures were ever to be found within the chlorophyll corpuscles (except, of course, what is mentioned sub **3** and **4**); so no carbohydrates: I (in KI sol.) caused only a diffuse brown-colouring of the whole corpuscle, but nowhere any special colouring.

3. *The nucleus and pyrenoide.* As I have mentioned already, the chloroplasts are entirely homogeneous; a pyrenoid was never to be found. But now the nucleus! If one stains chlorophyll corpuscles, killed with formol-alcohol (1 p. form. 40 % + 9 p. alc. 64 %) and which have lost their green colour, with methylene-blue, one will often find in them one or more sharply outlined, refractive, blue globules and sometimes a more diffuse blue spot. The sharply outlined blue globules (Fig. 38, 39) are doubtless the originally blue-green oildrops, still occurring in the unstained matter as well. By their refraction they simply concentrate the pale-blue light in the field of the microscope, in the same way as drops of ceder-wood oil do in a solution of methylene-blue in water. Perhaps the more diffuse blue spots might be nuclei; they are smaller than the preceding ones and are always situated near the middle of the corpuscle (Fig. 40, 41). When the same material is stained with haemateine-eosine (DE GRAAF), the oil-drops remain uncoloured, viz. blue-green; but sometimes

1) In consequence of the very small dimensions of those globules it may sometimes be difficult to detect their red or gray-black colour; when comparing them however with normal (not stained) globules, the difference is always distinctly marked out. For comparing one should also stain other fat-globules, for instance milk, with the same substances; one will see then, that only the larger globules have a bright red or a dark gray-black colour, but that the colouring of the globules, having the same diameter as our oildrops, is just as weak.

a corpuscle may be found containing a dark violet-red spot in the same situation as the spots coloured by methylene-blue. I stained the material, killed with formol-alcohol, also with haematoxyline, just as BRANDT did; then the oil-drops appeared to be coloured somewhat violet — in this case not by concentration of the light in the field of the microscope —, while sometimes chlorophyll corpuscles were te be found containing a violet spot in the same place, where it was in the other stainings. But there were also specimina containing numerous spots, even irregular violet lines (Fig. 42). The same result was obtained by staining of the material (with haematoxyline) when killed with ZENKER's liquid.

These results prove, that it is not impossible that those more diffuse, centrally situated, little spots are real nuclei. Against this view, however, speaks the following: **1.** these spots were but seldom to be found in a corpuscle; **2.** in other cases the corpuscle contained numerous of those spots and even irregular lines with exactly the same (violet) colouring. One might rather think them to be accidental colourings of arbitrary enclosures of the protoplasm (conf. the oildrops). Consequently, I do not venture to settle the question of the presence of a nucleus in the chlorophyll corpuscles.

When now we compare my results with those of BRANDT, there is very much conformity in the facts stated; and only a difference on the chief point of the frequency with which the so called nuclei occur. BRANDT found them in all corpuscles. Now I suppose that BRANDT, who never mentions the oildrops in the corpuscles, evidently does not know them, simply has always considered these also somewhat violet coloured oildrops as nuclei, as well as the diffuse spots. In this way it is possible that he found all corpuscles containing a nucleus.

4. *The cell-wall.* Only one decisive way exists for demonstrating a cell-wall: by means of plasmolysis. I therefore put the isolated green corpuscles for many hours into a solution, which I had accidentally at hand, viz. that of pag. 10, I (inorg., in concentration × 50). The plasmolysis could be observed very distinctly on many

specimina; the cell-wall appeared as a very thin line without any perceptible thickness (Fig. 6—11).

Herewith the description of the structure is finished. *We have stated that the green chlorophyll corpuscles of Spongilla are round or oval bodies, 1.7—3.8 μ in diameter, surrounded by a cell-wall, and consisting of protoplasm and a chloroplast; while perhaps a nucleus is present, but a pyrenoide is absent. They inclose oildrops, but carbohydrates were never to be found within them. From these data we may conclude that, very likely, these chlorophyll corpuscles are vegetable cells.*

The structure of the green chlorophyll corpuscles of Ephydatia completely agrees with that of the corpuscles of Spongilla. So they too are vegetable cells.

b. Physiological proofs.

1. The *green chlorophyll corpuscles* of Spongilla as well as those of Ephydatia, *isolated* from the sponge tissues, *can remain normal and alive* for 6 months, and even longer. They were isolated and cultivated in the way mentioned above on pag. 9—11. I am not going to treat these cultures here, but I refer to the extensive culture-tables (Table 4) at the end of this paper.

We know that, on the contrary, chlorophyll corpuscles isolated from plant-cells are not able to live on; as for instance they swell and are destroyed, when put into water (see Brandt (8), Hugo de Vries (63), Jost (33) Kny (34), etc.).

2. The isolated green corpuscles of both sponge types *multiply* rather strongly in cultures, especially during the first 2 months; but stages of division are also to be found in cultures of 6 months, even in those of 9 months (Table 4, 9, 10). I shall treat these stages afterwards.

3. Green chlorophyll corpuscles, as we know them from the sponge tissues, *also occur free in nature* — viz. in the waters in which the sponges are living — so, not inclosed by other organisms, but quite independant. I found their number changing from at least 200 per litre in the beginning of March up to at

least 3700 at the end of July. *Also stages of division occur free*, quite identical to those of the corpuscles of the sponges (Table 10). The diameter of the single corpuscles may sometimes be somewhat smaller than that of those in the sponges, viz. 1.4—3 μ; the oval shaped body may be lengthened. One may find the same forms, however, in old cultures of the isolated corpuscles of sponges too. Finally I will mention the fact that, free in nature, the corpuscles often stick together in masses of 10—120 specimina. I will return to this subject later on.

4. *When the sponge dies its green corpuscles survive.*

5. *It proved possible to me to durably transmute colourless Spongillidae into the green form, by infecting them with green corpuscles isolated from a green sponge.* Lateron I will treat the method, in which this infection is brought about, more extensively (Table 7). Now I will mention only, that for that purpose the colourless sponges were placed for 3—72 hours into a diluted suspension of isolated green corpuscles in water; after that they were transported, either colourless or already light-green, into an aquarium filled with water from the conduit only and placed into day-light. The green colouring then proved, in the course of some weeks, not decreased at all; on the contrary, it was strongly increased; many sponges had obtained rather a normally green colour in a month's time (see Table 8 N° 103, 207, 246, 248, 254, 257, 258, 326, 327), and their amoebocytes proved to be normally laden with normal green chlorophyll corpuscles (pag. 16—17). At the same time such colourless sponges, but without having been in a suspension of chlorophyll corpuscles, were — as a contra-experiment — also exposed to daylight in water from the conduit. These sponges got a faint green tint (colourless sponges always grow green in daylight; I will treat this afterwards), but in intensity their colour remained far behind that of the infected specimina (Table 8 n° 102, 206, 259, 328); proof, that the latter really owed their normal green colour to the infection with chlorophyll corpuscles, followed by the rapid multiplication of these corpuscles.

From these experiments we may deduce, that the green chlorophyll

corpuscles are organisms, on the one hand capable of living by themselves without the sponge body; on the other hand of accommodating themselves to the life within the sponge tissues, when taken from the surrounding water by the sponge.

This result together with that of our morphological investigation justifies the conclusion: that the green chlorophyll corpuscles of the Spongillidae are algae, associated to the sponge in „symbiosis".

IV. The genus of the „symbiotic" alga; its mode of reproduction.

As I have mentioned already in the Introduction, the symbiotic alga of the fresh-water sponges is generally considered to belong to the genus Chlorella — e.g. by Beijerinck (4) 1890, Oltmanns (47) 1905, Schenck (56) 1908 and Wille (69) 1911 —. I am going to discuss the investigation of Beijerinck. This investigator says:

„Es dürfte nicht überflüssig sein, bevor wir weiter gehen, an dieser Stelle einen Rückblick zu werfen auf die durch die Culturversuche festgestellten Eigenschaften unserer Gattung Chlorella, sowie auf die dazu gebrachten Arten:

Chlorella: Einzellige, grüne, zu den Pleurococcaceeen gehörige Algen, mit kugeligen, ellipsoidischen oder abgeplatteten Zellen von 1—6 μ Mittellinie, gewöhnlich mit nur einem Chromatophor von der Gestalt einer Kugelsegmentschale; Pyrenoid undeutlich oder fehlend. Im Lichte entsteht unter Sauerstoffentwicklung aus Kohlensäure *Paramylum, welches sich mit Iod braun färbt.* Zellkern meist einfach, bisweilen in Zweizahl, von wechselnder Grösse, nur aus Chromatin bestehend. *Die Vermehrung beruht auf freier Zellbildung durch successive Zweitheilung. Die Theilproducte kommen frei durch Platzen der Wand der Mutterzelle;* sie können sehr verschieden sein in Grösse ($^1/_2$—4 μ.). Schwärmsporen fehlen vollständig. In süssem und salzigem Wasser, wahrscheinlich auch auf dem Lande."

Next Beijerinck describes the different species belonging to

this genus: **1.** Chlorella vulgaris, **2.** Chl. infusionum, **3.** „*Chlorella* (*Zoochlorella*) *parasitica* BRANDT; *Chlorophyll von Spongilla fluviatilis*, vielleicht identisch mit Chl. infusionum und wahrscheinlich während des individuellen Lebens durch Spongilla von aussen aufgenommen. Isolirungsversuche nicht gelungen". And at last: **4.** „Chl. (Zoochlorella) conductrix BRANDT; Chlorophyll von Hydra, Stentor, Paramaecium und wahrscheinlich von vielen anderen grünen Thieren".

In the first place I should mention the fact, that in botanical literature of modern times the Chlorellae are no longer reckoned among the Pleurococcaceae, but that Chlorellae and Pleurococcaceae are considered to be two entirely separated groups (see OLTMANNS (47) 1905, WILLE (69) 1911, and this paper pag. 33—34).

But when we compare the definition BEIJERINCK gives for Chlorella, and consequently for the symbiotic alga of the Spongillidae as well, with my observations concerning this alga, we are struck by the fact, that BEIJERINCK mentions paramylum, coloured brown by I, to be a product of the photosynthesis, while, on the contrary, I found oildrops and never any carbohydrate that could be stained by I. A thing of much more importance, however, is the entirely different mode of reproduction I stated.

The mode of reproduction of the green algae of the Spongillidae. When studying the different forms of chloroplasts I immediately observed, that in cells with double chloroplast both halves of the latter were always symmetrically situated with respect to an imaginary axis of the cell (Fig. 16, 17a). I also often found two cells, each with a single chloroplast, which cells were connected one with the other along a short distance of their wall, while their chloroplasts were situated in the same way, now symmetrically with regard to the common cell-wall (Fig. 22a). Consequently, the last couple of cells seemed to be a stage of division of the first cell with double chloroplast. This proved to be really the case: once that my attention was drawn to this fact, I have observed numerous different stages of division (Fig. 17—22). Sometimes I have also been able to follow the division of an alga —

at least for a part (Fig. 17a-c, 22a-c). This division always proceeded very slowly; hours, even days passed before one could observe the slightest change in the division-stage. And this does not only count for specimina in ravel-preparations, but also for those that remained in absolutely normal conditions either within or without the tissues of a living sponge grown on cover-glass. It also proved probable to me that the algae, with the different shapes of the single chloroplast (Fig. 12—15) mentioned above (pag. 24), are all transitory stages from the alga with the primitive and most occurring shape (in which the chloroplast takes exactly the one half of the body, Fig. 5) into forms with double chloroplast (Fig. 16, 17), so into stages of division.

Knowing this, we may compose the following cyclus of development of the symbiotic algae of Spongilla; we place the primitive and most occurring form at the beginning and at the end: Fig. 5, 12—16, 17a—c, 18, 19, 20a—b, 21, 22a—c and Fig. 5 again.

All illustrations of the symbiotic algae have been made with great care from living specimina, with oil-immersion and in ENGELMANN's case. All these stages were found in cultures of the isolated algae as well as within the tissues of the sponges.

I have still got to mention a few points somewhat more at large: **1.** The stages of division were by no means always larger than the single ones; this is clear, for the single forms may greatly differ one from the other as to their dimensions.

2. I call attention for the eccentric way, in which the separating wall in a mother cell is formed (Fig. 17b—c, 18, 29c). As one knows, the separating wall in algae, for instance in Spirogyra, is formed by a regular, concentric growth out from the existing cell-wall; the ring, formed in this way, closes more and more towards the middle of the cell, till at last it divides the cell body in two parts. I saw this but once in the symbiotic algae (Fig. 25). In all the other cases the forming took place by eccentric growth out from one side of the mother-cell-wall, while the separating wall penetrated more and more into the cell till at last it reached the opposite side (of the mother-cell-wall). It is a phenomenon comparable to the one stated by TREUB (57a.)

on cell divisions in seed-buds of Epipactis palustris. Probably in direct relation to this eccentric manner of cell division are the facts, that the separating wall is generally thicker at the base than at the top, and that both halves, the chloroplast has already divided into before, diverge more on the side where the separating wall will start (or started) than on the opposite side (Fig. 17a—c, 18, 29). In the one case of concentric division which I observed these phenomena, accordingly, did not occur (Fig. 25).

3. I want to mention a phenomenon that is only to be seen on very accurate observation. From the apical end of the separating wall, when growing, a very thin line is extended to each of both halves of the chloroplast (Fig. 26—29). These lines are thinner than the separating wall itself, and evidently move on through the whole cell with the growth of this wall. In connection with the facts in Spirogyra, one might be inclined to consider these lines as threads of the nuclear spindle; which spindle will then move from one side of the cell-wall to the other, according as its forming of the separating wall proceeds; therefore, something like TREUB stated in Epipactis. But I can not say this for certain, for I don't possess any observations as to the nuclear division.

4. When studying these stages of the algae in division, one should take care not to confound simple figures caused by the diffraction of the light with real cell structures; one is apt of doing this on account of the very small dimensions of the chlorophyll corpuscles. These figures, however, are easily to be recognized, as they are always parallel to the cell-wall or outline of chloroplast.

5. I could never detect any trace of a membrane surrounding — as if it were a mother-cell-wall — a stage of division as for instance Fig. 20, 22 and 27 show; although I have always accurately examined.

The chlorophyll corpuscles in the amoebocytes are nearly always turning slowly; one time one can observe such an alga from this side, next from that side; a thing of much importance when studying stages of division. In this way the following

aspects of one and the same dividing alga were observed: Fig. 29 *a—d*. In *a* the alga is seen exactly at the least bent part of the wall (= *b* seen from aside); in *b* the alga is seen on the top (= *a* on top); in *c* in a position between *a* and *b*; and in *d* more or less from below (= *a* in slanting upward direction). This alga with its surrounding amoebocyte has been observed for $1\frac{1}{2}$ hour.

It is a matter of course that I observed a great many of those stages of division, much more than have been drawn here; they were all like these; I will only add Fig. 31.

Besides these double-stages, there also occur plural-stages of division, but much less numerous (Fig. 32—34).

What I have mentioned here about the cell-division for the chlorophyll corpuscles of Spongilla concerns also the symbiotic algae of Ephydatia as well as the similar algae, which occur free in nature.

As I stated above, free in nature these algae often occur sticking together in groups of 10—120 specimina, sometimes subdived in small groups of 4 specimina. Evidently we have to do then with the result of 2 successive divisions of a single cell. Moreover, one may sometimes find the algae of one group (of 10—120) in almost the same stage of development; so, probably, all of them originating in one cell. Neither the smaller groups of 4, nor the groups of 10—120 pieces are ever to be found surrounded by a common membrane or wall. Nevertheless, the (round) corpuscles often obstinately stick together, when we try to separate them; so, apparently, they are kept together with a kind of slimy substance.

So we have stated, that the green symbiotic alga of the Spongillidae multiplies by simple, vegetative division of the whole mother-cell (into two), the new separating wall sticking to the mother-wall and, consequently, also dividing the latter (into two). So there exists here no cell-division within, and independent of the mother-cell-wall, therefore no „freie Zellbildung".

Thus the symbiotic alga does not at all answer the definition given by Beijerinck *for Chlorella*; neither the definition given for Chlorella in modern literature — Grinzesco (24) 1903; Olt-

MANNS (47) 1905; WILLE (69) 1911 —: *Chlorella possesses a bell- or ball-shaped chloroplast and multiplies by „freie Zellbildung"*, the daughter-cells surrounding themselves within the mother-cell each with its own membrane arisen quite independently from the mother-cell-wall. Consequently, they are lying free within this old wall and get at liberty by its bursting or early dissolving. So they are aplanospores; zoospores and sexual reproduction are absent. A nucleus is present. A pyrenoide may be absent. The product of photosynthesis is starch, oil or glycogen.

On the contrary, the symbiotic alga answers exactly the definition given in literature — GAY (20) 1891; ARTARI (1) 1892; CHODAT (11) 1894; OLTMANNS (47) 1905; WILLE (69) 1911 — for the Pleurococcaceae: *The Pleurococcaceae possess a disc-shaped chloroplast and multiply by simple vegetative division of the whole mother-cell (into two)*; the new wall, forming a partition in the mother-cell and sticking to the existing wall, divides this one as well (into two). So they do not multiply by „freie Zellbildung". Zoospores, aplanospores, and sexual reproduction are absent. A nucleus is present; a pyrenoide may be absent (Pleurococcus vulgaris). The wall is not so very thick, or thin (Pleurococcus vulg.). A jellied envelope is present, but in the genus Pleurococcus rather indistinct. The product of photosynthesis is starch or oil (the latter in Pleurococcus vulg. and others). The algae live in the air or in fresh-water. The diameter of Pleurococcus vulg. is 3—7 μ.

Now, my description of the symbiotic algae of the Spongillidae I examined (pag. 24—27, 30—33) *entirely corresponds with this definition of a Pleurococcus* (except that the nucleus has not yet been sufficiently demonstrated in the symbiotic algae). *I therefore consider these algae to be a form closely related* — if not identical — *to Pleurococcus vulgaris* NAEGELI. It is only in dimension that both algae differ, the symbiotic one being 1.7—3.8 μ, Pleurococcus vulg. (according to ARTARI) [1]) 3—7 μ. One might call the first Pleurococcus parasiticus.

1) ARTARI calls it Pleurococc. vulg. MENEGH.

I should mention, however, that it might be possible, that our fresh-water sponge will associate with quite different unicellular algae in other countries, as I will point out afterwards (at the end of chapt. VIII and chapt. IX).

V. Green and colourless fresh-water sponges; under what circumstances they occur; the nature of their „symbiotic" algae.

When we examine under what circumstances the fresh-water sponges occur, we find that, generally speaking, the *green sponges grow on places exposed to bright day-light* — for instance on the wooden lining of the lake bank —; *the colourless sponges*, on the contrary, on places *in darkness or in twilight* — for instance under bridges and landing places of steamers —.

One might ask if the green and the colourless form could not be two separate varieties of the sponge. This is not the case; for, as I will mention afterwards, *green sponges prove to grow colourless in darkness and colourless ones prove to grow green in light* (Table 8, p. 66), while moreover one may often find in nature specimina partly green and partly colourless.

I analyzed on a large scale the number and nature of the symbiotic algae in fresh-water sponges by means of ravel preparations (Table 6 A, C). In that table (column 1 and 3) we see:

1. *In the green sponges in light as well as in the colourless ones in darkness green as well as colourless chlorophyll corpuscles (algae) occur.*
2. In the green sponges the green corpuscles are much more numerous than in the colourless ones.
3. In the green sponges the colourless corpuscles are somewhat less numerous than in the colourless ones.
4. *In the green sponges the colourless corpuscles are much less numerous than the green ones; in the colourless sponges they are just as numerous or still somewhat more numerous.*

I am speaking here about colourless chlorophyll corpuscles, in other words, about a colourless form of the symbiotic algae. This requires further explanation.

As mentioned in the Introduction, LANKESTER (35) already has found in colourless sponges the otherwise green amoebocytes now overladen with colourless grains, which appeared to be chlorophyll corpuscles in a somewhat abnormal condition, viz. irregular and angular. LANKESTER concluded that there had to be some relation between those two (green and colourless) forms.

I have examined this more exactly and it proved to be the case. But I must not only declare that — contrary to LANKESTER's observations — the colourless chlorophyll corpuscles may be perfectly similar to the green ones as to their structure, but also that their mutual relation is quite different from what LANKESTER thought (Introduction pag. 4). On account of its great importance, I will treat this question in a new chapter.

VI. THE STRUCTURE OF THE COLOURLESS „SYMBIOTIC" ALGAE; HOW THEY ARISE FROM THE GREEN ONES.

I will treat now only the pure *structure of the colourless chlorophyll corpuscles*, as we can observe it from the well preserved specimina in which it is still clearly showing. This structure *is perfectly similar to that of the green ones*, as shown in the illustration (Fig. 35); the diameter is the same. The colourless corpuscles may also contain an oildrop and generally occur free in the protoplasm of the amoebocytes, just as the green ones do.

What then is the relation between these colourless symbiotic algae and the green ones? Did the former arise from the latter or the latter from the former?

In relation to the facts, that green sponges occur in light and colourless ones in darkness (p. 35); that green sponges grow colourless in darkness and colourless ones grow green in light (p. 35); and to the fact, that we know the same to be the case for the higher plants, one might be inclined to conclude that all these facts are based on one same *phenomenon, known for the higher plants, viz. that chlorophyll can not be produced in darkness.* LANKESTER and BRANDT (l.c.), indeed, did explain these facts in this way, as I mentioned in the Introduction (pag. 4). And also

Oltmanns (47) supposes that in the colourless gemmules of the fresh-water sponges the algae would be colourless for lack of light.

But is it correct to suggest this analogy of algae to higher plants; is it not possible that the symbiotic algae do produce chlorophyll in darkness?

When comparing the amount of algae in green and colourless sponges (p. 35, Table 6) one is inclined to declare, on account of the presence of green algae in colourless sponges in darkness and of colourless ones in green sponges in light, that evidently lack of light can not be the cause of the algae being colourless. But this conclusion would not be right; for it is possible that it is dark within the tissue of a green sponge in light — therefore the colourless algae —; and on the other hand we know, that a sponge, c. q. a colourless one, is able to capture green chlorophyll corpuscles from the surrounding water (pag. 27—28).

In order to decide whether the symbiotic algae can produce chlorophyll in darkness or not, we have to cultivate them isolated from the sponge tissues. So I did on a large scale (see Table 4 B, cultures in water, except column 6), with the following results: **1.** During the first two months a rather vigorous multiplication of the green material took place, together with an inrease of the number of green chlorophyll corpuscles (and not caused by a propagation of other algae). **2.** Even in cultures of 4 months and older such multiplication occurred, or green stages of division were to be found. **3.** In old cultures the normal green corpuscles were still present in a great number. **4.** On the contrary, the colourless corpuscles (with structure) in general did not increase, but even decreased in number; in this way: the original number disappeared rather quickly, lateron some new ones might arise, but also these disappeared after some time.

So the fact known for higher plants, viz. that chlorophyll can not be produced in darkness, proved not at all appliable to our *symbiotic algae*. On the contrary, these algae *can produce chlorophyll in darkness very well indeed.*

It proved then to me to be a well-known fact in botanical literature since Schimper (51) 1885, that algae can produce chloro-

phyll in darkness (Heinricher (27) 1883, Etard and Bouilhac (19) 1898; Matruchot and Molliard (43) 1900; Oltmanns (47) 1905). Moreover, the same is known about the seedlings of some Gymnospermae, about ferns and mosses (Stahl (55) 1909).

So we have to find another explanation for the facts, mentioned above (p. 35), than the simple one given by Lankester and Brandt (p. 36—37).

Consulting the botanical literature I found, that in algae the producing or not-producing of chlorophyll — in darkness and even in light! — depends for a great deal on the nature of the feeding-milieu (Beijerinck (4) 1890; Artari (2) 1902; Grintzesco (24) 1903; Radais (50) 1900). But a rule in general appliable cannot be given for it, one kind of alga behaves in this way, another in that way. By combining the results of the investigators mentioned we may, for instance, distinguish the following 3 types of algae:

A. Scenedesmus.

a. when cultivated in light.

1. in water + salts + NH_4NO_3 without organic substances no growth takes place (Sc. acutus) or vigorous growth of green algae (Sc. caudatus).

2. in water + salts + little organic substances growth of green algae takes place.

3. in rich organic feeding solution the algae become colourless.

b. when cultivated in darkness.

1. when glucose is present in the solution growth of green algae takes place.

2. in rich organic feeding solution the algae will probably also become colourless.

B. Chlorella vulgaris, Stichococcus bacillaris.

a. when cultivated in light.

1. in poor or in rich feeding solution growth of green algae takes place.

b. when cultivated in darkness.

1. in poorer feeding solution (water + salts + KNO_3 + glucose) the algae become colourless.

2. in rich feeding solution (water + salts + peptone + glucose) vigorous growth of green algae takes place.

C. Chlorococcum infusionum from the Lichen Xanthoria parietina.

a. b. when cultivated in light or in darkness and in all kinds of feeding solutions the algae remain green.

So the algae prove to become colourless under very different conditions as to light and food. Therefore we ought to examine, if perhaps our symbiotic algae can lose their green colour and pass into the colourless form by a combination of darkness (or light) and a certain feeding milieu. And at the same time we should examine, if the colourless form of the symbiotic algae may in its turn pass into the green one by a certain combination of light (or darkness) and feeding milieu.

For the present I will treat the first question. We have to examine then, to which of the following types of algae — the only ones possible and partly hypothetical — our symbiotic alga belongs:

A. when cultivated in darkness.

type I (Scenedesmus).
in poorer feeding solution the algae remain green.
in rich feeding solution the algae become colourless.

type II (Chlorella, Stichococcus).
in poorer feeding solution the algae become colourless.
in rich feeding solution the algae remain green.

type III (Chlorococcum from Xanthoria).
in poorer feeding solution } the algae remain green.
in rich feeding solution }

type IV (hypothetical).
in poorer feeding solution } the algae become colourless.
in rich feeding solution }

B. when cultivated in light.

type I (Scenedesmus).
in poorer feeding solution the algae remain green.
in rich feeding solution the algae become colourless.

type II (hypothetical).
in poorer feeding solution the algae become colourless.
in rich feeding solution the algae remain green.
type III (Chlorella, Stichococcus, Chlorococcum from Xanthoria).
in poorer feeding solution } the algae remain green.
in rich feeding solution }
type IV (hypothetical).
in poorer feeding solution } the algae become colourless.
in rich feeding solution }

A poor or a rich feeding solution here always means a solution poor or rich in organic feeding substances.

To decide to which type the green symbiotic alga of our sponges belongs, it was isolated from the sponge tissues and cultivated in light and in darkness in various feeding solutions. I will not treat these cultures here at large; one can find a detailed description in Table 4 A, B.

The result we infer from these cultures is, among others, that the symbiotic algae remain green and multiply by means of green descendants under all circumstances. However, we had not yet examined every imaginable combination of feeding in the inorganic and organic feeding solutions used here; on the contrary, some chief combinations only. So it might be possible still, that a fresh-water sponge offered exactly such a combination of food to its algae, that they did lose their green colour in darkness or in light. So we had to study this possibility as well, for which a feeding solution was required, differing as little as possible as to composition and concentration from the one surrounding the algae in the living sponge. I got this solution (the so called diluted and concentrated liquid from a sponge) in the way described on pag. 11. The result inferred from these cultures is quite the same as that from the preceding cultures, as Table 4 A, B shows:

1. *The isolated green symbiotic algae of the Spongillidae, whether cultivated in light or in darkness in poor or in rich organic feeding solutions, even in liquid pressed from a sponge, remain*

normal (green, for instance) and alive for months, and multiply by normally green descendants.

2. In general the number of isolated colourless symbiotic algae (with structure) does not increase in these cultures, but decreases; they disappear from the culture.

So the green symbiotic algae of the sponges belong to type A, B, III, to the same type as the symbiotic algae of Xanthoria.

Next I wanted to examine (pag. 39) if the colurless form of the symbiotic algae may pass into the green one by a certain combination of light (or darkness) and feeding milieu. I cultivated therefore the isolated colourless algae in light and in darkness in the same feeding solutions, as the green ones were cultivated in. I refer to Table 4 C, D. The result was:

The isolated colourless symbiotic algae of the Spongillidae (n.b. those with structure), whether cultivated in light or in darkness in poor or in rich organic feeding solutions, even in liquid pressed from a sponge, disappear from the culture after some time; they never pass into the green form.

We may now conclude: *It is impossible, that the green symbiotic algae pass into the colourless ones, nor can the colourless algae pass into the green ones, by the combined influence of darkness or light and a certain feeding milieu* — at least not in those cases, which regard the condition of the sponge. This also proceeds from the following facts:

As I stated above, green sponges generally occur in light and colourless ones in darkness or twilight. In nature however — as exception to the rule, but by no means very seldom — colourless sponges also occur in light and — rather seldom — green ones in twilight. Sometimes one may even find, close together, some green and colourless sponges in bright daylight, or in twilight. It goes without saying that — as in each of these two groups of sponges (that in light and that in darkness) the algae live under the same conditions as to light and food — one may not attribute the fact of the majority of the algae being green or colourless in one case or in the other to the combined influence of those two factors.

What indeed may be the relation between these green and colourless algae?

I refer again to Table 4 A, B. There we see that, when the green algae decrease in number, the colourless ones increase (in order to disappear after some time). Therefore it is probable, that the green ones can become colourless.

Next we should consider that, as green sponges grow colourless in darkness (pag. 35), which goes together with a considerable decrease in number of the green algae and a considerable increase of colourless ones, so, we might say, together with a transition of green algae into colourless ones, we are now able to state, that this transition is not caused by the combined influence of darkness and the feeding milieu in the sponge tissues but that it must be closely related to the life of the sponge; in other words, we want a living sponge to perform this transition.

I am going to prove, that the green symbiotic algae of the freshwater sponges can only pass into the colourless ones by dying.

I. Analyzing the nature and number of the symbiotic algae in green and colourless Spongillidae (Table 6), one will find the already mentioned (pag. 35) green and colourless algae. But examining these colourless algae more exactly, we see that there exist 3 different forms of them: **1.** the colourless ones with clearly marked out internal structure (Fig. 35) mentioned on pag. 36; this form is the least numerous; **2.** the colourless algae, the internal structure of which is only visible as a shade (Fig. 36); this form is somewhat more numerous (**1** and **2** together are the group of the „colourless ones with structure"); **3.** the colourless ones, the internal structure of which is no more visible at all, but which appear internally either homogeneous (Fig. 37) or somewhat granular, and wich are the most numerous of the 3 forms (the group of the „colourless ones without structure"). But closely examining one can still detect a 4th group, viz. of „vague shades" of colourless algae, in which there is of course neither any internal structure to be seen. All these forms of symbiotic algae generally occur — as mentioned for the green ones and

the colourless ones with structure (p. 17, 36) — free in the protoplasm of the amoebocytes; so they are not lying in vacuoles. Nevertheless, sometimes one may also find them in food vacuoles (Chapt. VIII).

It is very likely now, that the 3 last groups, the colourless algae with shade of structure, the colourless ones without structure, and the vague shades of colourless ones, are only successive stages of „solution" of the colourless ones with clearly marked out structure; for this reason it would be rather evident, that the latter are dying.

II. I have been able to prove by numerous experiments, that in fact the isolated green symbiotic algae can change in this way only: first into colourless ones with clear structure, then into colourless ones with shade of structure, next into colourless ones without structure, then into vague shades of colourless algae, in order to finally disappear. The green algae were killed purposely in many of these experiments, for instance by heating or more or less intense lighting. (Table 5, n°. 68, 94, 95, 290 n, 290 p, 307 l, 308 l, 318, a. o.)

In this table also many cultures taken from Table 4 are recorded. In them it is striking to notice, that through infection of mould, common algae or diatoms the green symbiotic algae are changed into colourless ones with structure, while the latter, passing via the other colourless stages mentioned above, will disappear from the culture (N°. 84, 85 l, 113, 141, 188, 189, 190, 194, a. o.). Bacteria, on the contrary, don't seem to do any harm to these green algae at all (Table 4).

As, when purposely killing the isolated green algae, we get a succession of quite the same stages of colourless algae which we also meet in sponges, it is indeed very likely, that in the latter too the colourless algae with structure arise in no other way than by dying of the green ones, in order to pass also after that into the various successive stages of „solution" — mentioned above — to finally disappear entirely from the sponge tissues. And the more so, because the same succession of stages can be found in cultures infected by mould, common algae and diatoms.

III. On pag. 41, treating the cultures of isolated colourless algae, we concluded that these algae (n.b. those with structure) always disappeared from the cultures, and never passed into the green ones. The same proves to be the case (Table 4) for the isolated colourless algae without structure. Which was already self-evident.

We have proved now, that the green sponge algae may change into the colourless ones by dying. We shall have proved incontestably, that the green symbiotic algae within the sponge tissues become colourless exclusively by dying (in order to disappear afterwards), only when we have shown that all colourless algae present in these tissues are really dying. The following are the proofs:

IV. The general rule given under III; while, on the contrary, the green algae remain alive for months.

V. When a sponge dies, its colourless algae do not remain intact — as the green ones (p. 28) — but they disappear.

VI. Colourless stages of division of symbiotic algae are but seldom to be found in sponges — on the contrary several green ones (Table 6) —; therefore the former must probably be explained as having been originally green.

VII. As known, living protoplasm is generally not stained, or less quickly stained than dead one. By means of this fact I could definitively decide whether the colourless algae were alive or not. I therefore made ravel preparations of living, green and colourless sponges, and added equal quantities of a solution of eosine or methylene-blue in water, with the following results:

1. *Green Spongilla material* containing numerous green symbiotic algae, a few colourless ones with structure, and rather numerous colourless ones without structure.

A. In eosine. After 25 hours the numerous green algae have as a rule not been coloured red, only very seldom one meets with a green specimen with red tint. Colourless algae are but very seldom to be found, almost all of them are red: the few with structure — they have no green chloroplast! — as well as the rather numerous ones without structure.

B. In methylene-blue. After 25 minutes the numerous green algae are not yet blue at all. Colourless algae, however, are nowhere to be found, all of them are blue: the few with structure (they have no green chloroplast!) as well as the rather numerous ones without structure.

2. *Colourless Spongilla material* containing several green symbiotic algae, a few colourless ones with structure and rather numerous colourless ones without structure.

A. In eosine. After 25 hours the (several) green algae are not yet red at all. Colourless algae are but seldom to be found, almost all of them are red: the few with structure (without green chloroplast), as well as the rather numerous ones without structure.

B. In methylene-blue. After 35 minutes the (several) green algae are not yet blue at all. Colourless algae, however, are nowhere to be found, all of them are blue: the few with structure (without green chloroplast) as well as the rather numerous ones without structure.

The same counts for the algae of Ephydatia.

By these last experiments we have got the decisive proofs, that all colourless symbiotic algae in sponges are dead, at least are dying. *So we have incontestably shown, that the green „symbiotic" algae in the tissues of the Spongillidae become colourless exclusively by dying (of the algae, namely), in order to gradually pass from colourless algae with clear structure into the successive stages of „solution", colourless algae with shade of structure, colourless ones without structure, vague shades of colourless ones, and to finally disappear.*

For completeness' sake I want to mention that the colourless sponge algae, arisen from green ones in a culture infected by diatoms (pag. 43), were also quickly stained with methylene-blue. This proves that they too were dying.

VII. The intrinsic amount of the various green and colourless stages of the „symbiotic" algae in sponges. The factors ruling this amount. How green and colourless sponges keep up their „colour", and how they arise from each other.

α.

We now have stated the nature and origin of the colourless symbiotic algae, and resume the discussion started in chapter V. We put the questions: What is a green sponge, what a colourless one; how do they keep up their „colour", and how do they arise from each other?

For the present we might answer the first two questions as follows (pag. 35, Table 6): Green sponges are sponges containing a great number of living green algae and a smaller number of dead colourless algae; colourless sponges are sponges containing a small number of living green algae and a greater number of dead colourless ones. *But we should now compare the green sponges — usually occurring in light*, as we know (p. 35) — *and the colourless ones — usually occurring in darkness — more accurately as to their intrinsic amount of the various (green and colourless) stages of the symbiotic algae; especially newly caught sponges.*

To that purpose I analyzed, as mentioned on p. 35, a great number of those Spongillidae, green and colourless ones, Spongillae as well as Ephydatiae (Table 6 A, C); the amount of algae in Spongilla was examined separately in tissues in different stages of development: in very young tissue, in full-grown, and in tissue at rest (gemmulae). The number of the algae mentioned always concerns the amount present within an equal volume of each sponge. *The results of the analyses are as follows:*

1. *Green as well as colourless symbiotic algae occur in the green sponges in light as well as in the colourless ones in darkness.*

2. *In the course of the development of the tissues of the green Spongillae the number of the green algae remains constantly con-*

siderable, decreasing a little in the gemmule-stage. The number of the green stages of division is comparatively great, but in gemmules they are absent; hardly the gemmules have germinated, however, and the number increases again. The number of the colourless algae with structure is small and remains rather constant during development, showing a considerable decrease during and after the gemmule stage. The number of the colourless ones without structure is not great at the beginning, but increases rather considerably afterwards, also showing a strong decrease (as far as we may judge) during or after the gemmule stage.

3. *In the course of the development of the tissues of colourless Spongillae the number of the green algae remains constantly small, in order to disappear completely in the gemmule stage; when, however, the gemmules have germinated the number immediately increases. The number of the green stages of division is constantly very small; as a rule they are even missing. The number of the colourless algae with structure is not great and remains rather constant during development, showing a considerable decrease during and after the gemmule stage. The number of the colourless ones without structure is rather great at the beginning and increases considerably afterwards, also showing a great decrease during and after the gemmule stage.*

4. *In this way one observes in the tissues of the green Spongillae as well as in those of the colourless ones a gradual change of number of the various stages of algae: from very young tissue to full-grown, from full-grown tissue to gemmules, and from gemmules to very young tissue again.*

5. *During or immediately after the gemmule-stage the tissue of a Spongilla contains a minimum of green and colourless algae.*

6. *In the green sponges the number of the green algae is usually much greater than that of the colourless ones (with, and without structure) together; only in some cases this number is equal.*

7. *In the colourless sponges the number of the green algae is always much smaller than that of the colourless ones together.*

8. *In the green sponges the green algae are always much more numerous than in the colourless sponges.*

9. *In the green sponges the colourless algae are generally less numerous than in the colourless sponges — of course one must compare tissues in the same stage of development —; this concerns the colourless algae with structure as well as those without. For the first ones this difference in number is smaller than for the last ones; and for the latter it still increases rather considerably during the development of the sponge tissue, in order to probably disappear almost entirely during or immediately after the gemmule stage.*

10. *In the green and in the colourless sponges the number of colourless algae with structure is always much smaller than that of those without structure.*

11. *The total number of symbiotic algae present in the green sponges surpasses that in the colourless ones.*

Having stated this, we want to know, what is the reason of all this (point 1—11). In short, why in nature a (green) sponge in light contains an excess of green living algae and a smaller number of colourless dead ones, a (colourless) sponge in darkness, on the contrary, an excess of colourless dead algae and a smaller number of green living ones; how both sponge types keep up their „colour"; and how they arise from each other (pag. 35).

β.

For that purpose we have to examine the factors ruling the number of the symbiotic algae in the sponge tissues. These factors are 6 in number, viz.:

A. the import of the algae from the surrounding water into the sponge (the factor of import).

B. the export of the algae from the sponge-tissues into the surrounding water (the factor of export).

C. the increase of number of the algae in certain parts of the tissues by the reduction or death of other parts (the factor of reduction).

D. the decrease of number of the algae in a certain sponge-volume by the growth of the sponge (the factor of growth).

E. the multiplication of the green algae within the tissues (the factor of multiplication).

F. the mortality of the green algae within the tissues (the factor of mortality).

I shall now treat each factor separately.

A. *The factor of import.*

As I mentioned above (pag. 28), it is possible to durably transmute colourless sponges into the green form by placing them into a diluted suspension of isolated green symbiotic algae in water. I give a more detailed description in Table 7. There we see, that a living colourless Spongilla (volume 3—10 c.M^3) is able to make perfectly clear within a few days 3 litres of a grayish suspension of algae, by which the colourless Spongilla itself grows light-green; and that the rapidity of this process is directly related to the number of oscular tubes, the sponge shows. As we know from the research of VOSMAER and PEKELHARING (62), these tubes accelerate the rapidity of the water current through the sponge body — serving as lengthening pieces of the draught canals —. Therefore we may conclude that, the quicker the current in the canals of the colourless sponge, the more quickly the sponge will get clear — so to say by filtering — the troubled suspension, and itself will grow light-green by capturing the green algae. The oscular tubes are not indispensable, of course — as was proved —; the current of water can also go on, when they are lacking. One might think it possible, that the sponge does not only catch the algae within its tissues, they have got into by the current, but also at its outer surface. This possibility might be realized; but it can hardly come into consideration in comparison with the first method of capturing, as I will point out afterwards in chapter B and C (on the current of water and the ingestion of food). I will only mention now that the algae are soon joined quite normally within the sponge amoebocytes (not vacuoles!), and that the light-green colour of the sponges, after they have been transported into daylight into aquaria filled only with water from the con-

duit, does not at all decrease in the course of some weeks but even strongly increases, as stated already on pag. 28. All this concerns Spongilla as well as Ephydatia. (See Table 8.)

In this way the import of the green algae proved possible. One might ask, however, if this factor really exists in nature under normal conditions. This is certainly the case. For we know that the symbiotic algae occur free in nature in exactly the same waters as the sponge is living in (pag. 27—28); namely to the amount of at least 200 per litre in March and at least 3700 in July; but these numbers give a minimum only, probably very much too low. Moreover, one should not consider the intensity, with wich a sponge filters the surrounding water clear, to be but weak. As to this, we saw in Table 7 that a little sponge (of but 3 c.M^3 vol.) is able to make clear 3 litres of a troubled suspension within 3 days, while the filtered water was again and again mixed with the suspension. So, how many times had not the same water to pass through the sponge body before all was clear at last; how many litres has the little sponge really filtered in those 3 days! Another proof of this really enormous „filtering power": sponges as large as a finger proved able to make clear 3 litres of water mixed with 2 c.M^3 of milk within a day's time; but exclusively when oscular tubes were present. (This result was not caused by sponge-enzymes, as one might think; for such liquids mixed with material from a pressed sponge remained troubled for days. Nor did the milk sink to the bottom).

Having stated now the presence of green symbiotic algae free in the water and the enormous „filtering power" of a sponge, we may conclude that in nature the import is a vigorously and continually acting factor of increasing the number of green algae in sponge-tissues. And this must be the case! For we stated (p. 47) in colourless sponges from darkness that green algae are constantly present in a small number, that stages of division are generally absent, that the colourless algae (without structure) increase considerably in number, in other words, that green algae are continually dying; so it is absolutely necessary, that new

green algae are constantly imported from the surrounding water in order to keep up their (small) quantity within the sponge tissue (the factor of reduction is normally not acting). Therefore the import is the only factor of increase of the number of green algae in colourless sponges in darkness.

When treating the factor of import I should mention another remarkable fact. In winter, when the sponge tissue is reduced to the gemmules fixed quite free in the skeleton, several normal green symbiotic algae prove to be sticking to that skeleton; probably caught there in the course of the winter from the flowing water. The gemmules, now, when germinating on their place in the old skeleton — which happens very often — will immediately find some green algae to their disposal. So the rapid increase in number of the green algae in newly germinated gemmules, I mentioned on p. 47 (**3**), has to be explained.

Next one might ask if in nature the factor of import does only concern the green algae, or the colourless ones too. The latter would be realized, when also colourless algae occur free in nature. This is certainly the case. Their number, however, always proved to be but very small, compared to that of the green ones; what stands to reason (conf. pag. 42—45). *Therefore the factor of import may be neglected as far as the colourless algae are concerned.*

Finally *we may accept that the factor of import is equally active in (green) sponges in light as in (colourless) sponges in darkness*; for the number of the algae in the surrounding water will be equal for both of them in consequence of the continual water circulation, and both sponges will probably possess the same „filtering power" [1]).

---

1) From an experiment it seems to result that the „filtering power" of green sponges in light is greater than that of colourless sponges in darkness. In this case also the import would be greater in the first sponges. We want, however, many experiments to answer this question, and for the moment it is of no importance to us (see the note at pag. 68). It will, however, be much more important when considered in relation to a lack of O_2, which possibly exists in (colourless) sponges in darkness (chapt. VIII).

B. *The factor of export.*

I treat this factor as theoretically possible and the reverse of the import; for a sponge laden with algae might continually eject some of them; though the above stated eagerness with which a sponge captures algae does not make this supposition very likely. But I should mention that I have really observed sometimes (not often!) the ejecting of green symbiotic algae together with feces by vacuoles in the process of defecation, I shall treat afterwards (chap. E). The export can not be experimentally stated in another way; for a sponge in captivity may always show some small parts of its tissues dying (at least this possibility can never be excluded), whereby algae are set at liberty. *I think we may consider the export of algae from the sponge tissues as an uncertain, but probably not important factor.*

C. *The factor of reduction.*

After some time in captivity the fresh-water sponges show a reduction of their tissues, which originally filled the whole skeleton but then begin to reduce to the inner parts. This may be the consequence of a progressive dying of the outer layers, followed by their destruction. At least this is often the case. A same phenomenon of reduction, however, might also be caused by the tissues growing more and more compact, by the internal canals and lacunes being filled. It seemed to me that in a sponge in reduction always both processes were going on. *Their influence on the remaining part of the tissues would be the same: the number of the algae present within the unit of volume of these tissues would increase.* When the reduction is caused by the tissue growing more compact, this result is quite evident. In the other case we may distinguish two possibilities: **1.** The dying amoebocytes might be (partly) ingested by the remaining ones, with preservation of the green algae. **2.** The destruction of the dead amoebocytes would set numerous green algae at liberty in the neighbourhood of the remaining sponge parts; thus the factor of import would be increased. One can often observe this last phenomenon: a dying sponge is surrounded by a green cover of algae, settled to the bottom.

In nature, however, the process of reduction occurs in autumn only. *We may therefore neglect this factor for sponges living free in nature (except in autumn)*; but we should prevent that in our experiments (in aquaria etc.) this reduction becomes efficient, and we should take care to limit its troubling consequences (the increase of the import) as much as possible by a continual vigorous circulation of fresh-water through the aquaria.

D. *The factor of growth.*

As a very young sponge forms but a minute corpuscle, when full-grown, however, a large crust with long branches (10 c.M. and even longer) — Spongilla — or a thick cushion — Ephydatia —, it stands to reason that *the growth of the sponge must be (not in a short, but in a long-space of time) a very active factor of decreasing the number of algae present in the unit of volume of its tissues.*

One should consider, however, that growth takes place almost exclusively at the top of the branches in Spongilla, as I mentioned already on pag. 16. So especially here the factor of growth will act, although it might be possible that this influence is spread all over the sponge tissue by means of an accumulation of amoebocytes from all parts of the sponge body to the branch tops. But it is remarkable to notice that these tops are exactly the parts of a sponge, which become green first of all and remain so for the longest time, as I stated repeatedly; so some other factors must act here as well. Nevertheless, I have once found in a very warm season a great number of quickly grown, green Spongillae with indeed very light-green coloured tops.

Green Spongillae (from light) proved to be larger than colourless ones (from darkness). This difference can not always be observed, of course; nor is it always so prominently marked out as in Fig. 1 and 2; but I have been able to state it in general and in different months of several years. Later on I will treat the cause of this phenomenon (chap. VIII). *So we may conclude the factor of growth to be more active in green sponges in light than in colourless ones in darkness.*

E. *The factor of multiplication.*

We stated above (p. 27, 31, 47, and Table 4, 6) that the green symbiotic algae multiply, when isolated in cultures, as well as within the tissues of the sponge.

We have to examine now the rapidity of the multiplication under different outer conditions (Table 9, 10). We will do this by counting the number of the stages of division present in 100 green algae at a certain moment. Before we proceed to this investigation we should ask, what factors rule this number. It will increase by 2 factors: **1.** by the number of the algae (per 100) which divide within a certain space of time, **2.** by the time in which the division of an alga is accomplished. We may say that the 1st factor depends on the state of feeding of the algae, the 2nd on the temperature. The latter was always the same in my experiments of one series; therefore we may consider the time, in which the divisions of the algae took place, to be equal also for every experiment of a same series. Consequently, we possess in the number of stages of division per 100 algae (the percent of stages of division), which I examined in my investigations, a direct measure, true for every series of experiments, of the number of algae (per 100) which divide within a certain, equally long space of time; in other words, a measure of the intensity of multiplication of these algae.

In this way we will have examined this intensity always calculated per 100 green algae, which are present in a culture or in a tussue of a sponge. The other factors (import, export, reduction, growth and dying), however, have been studied or will be studied per unit of time and per unit of volume of sponge tissue. Consequently, we have to bring also our results concerning the intensity of multiplication in accordance with these units, in order to combine all results afterwards.

Proceeding now to my investigations we see:

1. *Periodicity does not occur in the intensity of multiplication*, neither for the algae in the sponge tissue, nor in cultures; neither within 24 hours, nor within some days (Table 9). I therefore believe that the algae continually multiply each in its turn,

without any mutual regularity; while the process of division proceeds but very slowly (pag. 31).

2. The weaker the concentration of the algae present in a culture or in a sponge tissue in light, the higher is their intensity of multiplication (Table 9, 10; in sponges one should consider the concentration at the beginning and at the end of the experiment; the abnormal nos 336 l, 341, 365 I, 375, 376 should be neglected); in darkness, on the contrary, the intensity is always the same (viz. ± 0) in sponges (Table 10, conf. Table 6). I will not examine, now, the cause of these phenomena; one may ascribe them to the quantity of food (eg. CO_2), the algae can dispose of. All this concerns the intensity calculated per 100 green algae. Now we have to remould these results and to calculate them per unit of sponge volume. We therefore should know the mutual proportions of the various concentrations, which we can only calculate roughly (p. 13). In this way we find in light (Table 10 and 8, n°. 337 l, 347, 338 l; 333 l, 334 l, 343) on the one hand an intensity (per 100 algae) of 13 with an average quantity of algae (in the unit of volume) of VII, on the other hand an intensity (per 100 algae) of 1 with an average quantity of algae (in the unit of vol.) of XI. Now I stated that XI = ± 70 × VII. Consequently, the same sponge volume in light, containing 100 algae in the weak concentration and therefore possessing an intensity of multiplication of 13, must contain in the strong concentration 7000 algae and an intensity of multiplication of 70. Thus: *in light the intensity of multiplication of the algae in sponge tissue, calculated per unit of sponge volume, in a strong concentration of the algae surpasses the intensity in a weak concentration; in darkness they are the same, viz. 0.*

3. The intensity of multiplication of the algae in the sponge tissues as well as of those in cultures is much larger in light than in darkness; in a sponge in darkness it is ± 0 (Table 10, cf. Table 4 and 6). The concentration of the algae in every two experiments belonging together (one in light, the other in darkness) was generally almost the same in the cultures; in the sponge tissues, however, in light (necessarily) even stronger than

in darkness. Consequently, if this concentration had been equal, the intensity of multiplication (calc. p. 100 alg.) in the sponge in light would have surpassed that in darkness even more. The cause of this phenomenon is, of course, again the state of feeding of the algae.

This rule concerns the intensity calculated per 100 green algae; while the concentration of the algae was then supposed to be the same in light as in darkness. From this follows: *the concentration of the algae being the same, their intensity of multiplication in sponge tissue in light, calculated per unit of sponge volume, largely surpasses the intensity in darkness.*

F. *The factor of mortality.*

In chapter VI we have stated that all colourless algae present in sponge tissues are dead or dying green ones; and in chapter VII α, that *generally rather a considerable quantity of the green algae is continually dying in those tissues, first changing into colourless algae with structure and then into colourless ones without structure* (p. 45). As besides we see from Table 5 (n°. 68 p, for instance), that there the stage of colourless alga with structure is passed much more quickly than that of colourless one without structure, we might in general consider through analogy also *in the sponge tissue the number of colourless algae with structure to be a direct measure for the intensity of dying of the green algae during a short period preceding our analysis; and the number of colourless algae without structure, on the contrary, to be the total amount of the algae, that died (in the same sponge volume) during a much longer period preceding our analysis.* This conclusion agrees with the results from the analyses of Table 6; for there we have stated (pag. 48, **10**) that the number of the colourless algae without structure is always much larger than that of the colourless ones with structure. This is only possible, if the first mentioned stage of colourless ones (that without structure) is passed less quickly than the second.

Knowing this, we may conclude from the analyses of Table 6 (pag. 46—47, **2**, **3**) that *in green sponges in light as well as in colour-*

less ones in darkness: **1.** *the intensity of dying of the green algae remains constant in all stages of development of the sponge tissue, showing however a considerable decrease in the gemmule-stage;* **2.** *the total amount of dead algae increases (in the unit of sponge volume) during the development of the tissues*, which is of course a matter of fact. Nevertheless, it is important to see that the latter shows from the analyses; for *it proves that these colourless algae without structure must hold in the tissues for rather a long time.* Is is even probable, that in full-grown tissue (at least in the beginning) the same colourless algae without structure would still be present, which were already there at the time the tissue was young and growing; for their continually increasing number can not be explained in another way (as the intensity of dying is constant, and one should admit also that the number of dead algae, passed at the same time from colourless ones with into colourless ones without structure, will also disappear at the same time from this last stage). That *the total amount of dead algae should considerably decrease during or after the gemmule stage* is quite conceivable, for then the intensity of dying proved much smaller, while on the other hand colourless algae are continually disappearing.

Next we want to compare the mortality of the green algae in sponge tissue in light and in darkness. For that purpose we can make use of both groups of colourless algae mentioned here. First we should ask however, if in the dying of the green algae within a sponge the stage of colourless one with, as well as that of colourless one without structure is passed in light just as quickly as in darkness. One might answer that this question does not come into consideration, as we concluded above: **1.** that in both cases the first stage is passed so quickly, that we may consider it to be a measure of the intensity of dying during a short period, and **2.** that in both cases the algae can not possibly disappear from the second stage before the sponge tissue is full-grown. This is exact. Yet I shall also answer the question experimentally, at least as far as the second stage is concerned.

When asking this question, one thinks of a possible difference

of rapidity, with which the colourless stages are gone through, as caused by: *a.* a changing influence produced by the green algae on the sponge tissue (production of O_2 and assimilates in light, but not in darkness), *b.* some other influence independent from those green algae. I will treat both possibilities in connection with my experiments. In order to get a pure result, the factors of import, (export), growth, reduction and of dying should be excluded as much as possible in these experiments; which may be obtained by cultivating colourless sponges (so sponges containing a small number of green and a large number of colourless algae) in flowing water from the conduit, and to continue the experiments for not too long a time. These experiments belong to a large series of similar ones, made for different purposes (Table 8). My material consisted in Spongillae and Ephydatiae; generally of each sponge a piece was cultivated in light and another piece under the same conditions in darkness. In the beginning of the experiment both pieces of each pair possessed an almost equally small quantity of green and of colourless algae with structure, and an almost equally large quantity of colourless ones without structure. Table 8, n°. 336, 365 I, 365 II, 375, 376 give an answer to the possibility mentioned sub *b.* For at the end of these experiments: **1.** the quantity of green algae proves to be just as small as at the beginning, and therefore to have been of no influence, **2.** the large quantity of colourless algae without structure in each sponge piece in light proves just as much increased, decreased or remained equal during the same time as in the partner sponge piece in darkness; *proof, that in sponge tissue, when the number of green algae is small, the stage of colourless alga without structure is passed in light just as quickly as in darkness.* Table 8, n°. 263, 264, 298, 299, 337, 338, 341—342, 347—346 give an answer to the question mentioned sub *a.* For at the end of these experiments: **1.** the quantity of green algae proves to have increased rather much in light, therefore perhaps of much influence, **2.** the large quantity of colourless algae without structure in each sponge piece in light proves to have changed just as much, or more increased in the same time than

in the partner sponge piece in darkness; *proof, that in sponge tissue, when the number of green algae is important, the stage of colourless alga without structure is passed in light either just as quickly or less quickly than in darkness, but by no means more quickly.* Besides, this is logical (cf. chapt. VIII). Something the like will evidently be the case, when one compares a sponge in light containing many green algae and one containing but a few; for the latter will behave almost like a sponge in darkness.

Now we proceed to the comparison of the mortality of the green algae in sponge tissue in light and in darkness. Are there more, or less algae dying in a sponge in light than in a sponge in darkness?

From the results obtained through analyzing the number of algae in sponge tissue (Table 6, pag. 46—48) we may immediately answer this question, if we consider that **1.** in colourless sponges in darkness the import is the only factor keeping up the number of green algae (pag. 51), and that **2.** the import is equally active in green sponges in light as in colourless ones in darkness (pag. 51). *We may thus conclude that, although the colourless sponge in darkness possesses much less green algae than the green sponge in light (pag. 47, 8), still more green algae die in the colourless sponge in darkness* — as the intensity of dying (the number of colourless algae with structure) as well as the total amount of dead algae (the number of colourless ones without structure) is at its largest in the colourless sponge (pag. 48, **9**). Consequently, much more specimina of a same quantity of green algae die in a colourless sponge in darkness than in a green one in light.

As to this fact, one might object that it might be possible that a sponge (in light, for instance) regularly wants — it may contain many or but a few green algae — a same quantity of these algae to feed upon; that therefore it would not be of any use to calculate the mortality per same number of green algae, as then one would find, of course, a large relative amount of dead algae in a colourless sponge, even if it did not grow in darkness. This reasoning might be exact, certainly (see below); but in any

case we have stated here, that even the absolute amount of dead algae in the colourless sponges in darkness surpasses that in the green ones in light.

I will prove also by direct experiments, that of a same quantity of green algae in sponge tissue — in other words, the concentration of the green algae being the same — more algae die in darkness than in light. Also here the factors of import, (export), reduction and growth should be excluded as much as possible in the experiments; that of multiplication can not be excluded, but it will not be of influence on the results. For that purpose we cultivate green sponges in flowing water from the conduit for not too long a time. See Table 8, n°. 100, 260, 261, 292, 293, 294, 333, 334, 343—344, (357, 359) — (348, 356, 358), 366 I, 366 II, 371, 372; all of them green Spongillae and Ephydatiae. Generally of each sponge a piece was cultivated in light and another piece under the same conditions in darkness. In the beginning of the experiment both pieces of each pair possessed an almost equal and large quantity of green algae, an almost equal and small quantity of colourless ones with structure and an almost equal and moderate quantity of colourless ones without structure. Now, during the experiments the number of green algae generally proved to have more decreased and the number of colourless algae with structure and that of colourless ones without to have more increased in the sponge pieces in darkness, than in the partner sponge pieces in light during the same time. *So it is clear, not only from the intensity of dying (number of colourless algae with structure) but also from the total amount of dead algae (number of colourless ones without structure), that in sponge tissue, when the concentration of the green algae is the same, much more algae die in darkness than in light (calculated per unit of time and of sponge volume).* (In some experiments — n°. 344, 348, 358, 366 I, 366 II — the number of colourless algae with structure has decreased at the end in the sponge in darkness, or has remained the same. Evidently this is caused by the already very much progressed decrease of the green algae (by dying), for the number of the colourless ones

without structure proves in all these cases, that also here more algae died in darkness than in light).

I want to point out emphatically that we got this result by means of experiments in which the sponges in light contained a large quantity of green algae. We therefore know (pag. 59) that in these experiments the stage of colourless alga without structure can not have been passed more quickly in the sponges in light than in those in darkness, but that it has been passed just as quickly or even less quickly in light. When we suppose — to get a pure comparison — this stage to have been passed just as quickly in both cases, the above obtained result must be binding, perhaps even a fortiori.

One should also examine, if there is any difference in the intensity of dying of the green algae in the tissue of a sponge with a strong and in that of a sponge with a weak concentration of green algae. To answer this question we may mutually compare the number of colourless algae with structure in green and in light-green sponges, after these sponges have been cultivated for some time in water from the conduit, in light or in darkness (Table 8). At first after culture in light. We find this number in 27 green sponges: 1 II + 2 III + 7 IV + 10 V + 4 VI + 3 VII and in 18 light-green (or colourless) ones: 1 I + 1 II + 2 III + 3 IV + 4 V + 4 VI + 3 VII. So it follows that *in light the intensity of dying of the green algae in sponge tissue in a weak concentration is almost just as large as in a strong concentration of the algae (calculated per unit of sponge volume).* Then the same for sponges cultivated in darkness. In 8 green sponges the number of colourless algae with structure proves to be: 1 II + 1 III + 1 V + 3 VI + 2 VII, and in 37 light-green (or colourless) ones: 2 I + 5 III + 8 IV + 4 V + 10 VI + 8 VII. Consequently, *in darkness the intensity of dying of the green algae in sponge tissue in a weak concentration is somewhat smaller than in a strong concentration of the algae (calculated per unit of sponge volume).*

When we consider both these facts in all experiments of Table 8 (with respect to the concentration of the green algae in the

beginning and at the end of the experiments), the above obtained result (p. 60) — that more algae die in darkness than in light — remains valid; then there are even some more experiments leading to the same result: n°. (246, 248) — (255, 256), 339, 340, 367, 368, 369, 370, 374, 378—377. Moreover, the exactness of this result — not only for a same strong concentration of the green algae, but also for a same weak one — also follows very clearly from the last two argumentations.

Finally I should mention that we have realized the fact in these cultures of green sponges in aquaria in darkness, that of all 6 factors, normally ruling the number of algae in sponge tissue, only the factor of dying has (practically) remained.

Afterwards I will treat the cause of the dying of the algae in sponge tissue, when speaking about the symbiotic relation of sponge and alga.

7.

Having studied the 6 factors ruling the number of symbiotic algae in sponge tissue separately, we are now going to examine, if some combinations of those factors can be realized. We will not take into consideration the uncertain factor of export, the abnormal one of reduction and the but slowly working factor of growth, in order to combine the 3 principal factors of import, of multiplication and of dying:

I. Import + multiplication can not be realized, of course.

II. Import + dying is realized, together with growth, in nature in colourless sponges in darkness. See Table 6.

III. Import + multiplication + dying is realized, together with growth, in nature in green sponges in light. See also Table 6.

IV. Multiplication + dying can be realized by cultivating green and colourless sponges in flowing water from the conduit in light and in darkness for not too long a time; in this way the factors of import, (export,) reduction and of growth will be excluded as much as possible. These experiments are very important! *For we will succeed in this way to transmute green sponges into colourless*

ones by culture in darkness and colourless sponges into green ones by culture in light, with the smallest number of active factors possible. Consequently, we must succeed in explaining these phenomena, the interpretation of which has been sought for ever so long in quite a wrong direction (pag. 36—41), as being caused by the combined action of the multiplication and the mortality of the green algae in the sponge tissues, in light and in darkness. See Table 8, all nos (all green and colourless Spongillae and Ephydatiae); generally of each sponge a piece was cultivated in light and another piece under the same conditions in darkness. In each piece the colour and mostly also the amount of algae in its tissues was accurately examined at the beginning and at the end of the experiment.

In general we distinguish 3 types of sponges in respect to their behaviour during the experiments. In type I the green colour, so the number of green algae (as the experiments show), increases; in other words the increase of green algae surpasses the decrease. In type III the green colour and the number of green algae decrease, in other words the increase of the green algae is smaller than the decrease. In type II the green colour remains constant as well as the number of green algae, or the green colour or the number of green algae changes a little by increase or decrease; in the last case but one we may speak of a type II^{I}; in the latter of a type II^{III}.

We see from the table:

A. **1.** From 92 Spongillae, cultivated in light, behaved like

type I 60 } = 63 = ± 68 %
„ II^{I} 3 }
„ II 22 = 22 = ± 24 %
„ II^{III} 2 } = 7 = ± 8 %
„ III 5 }

If we consider, however, that under type II 21 already originally green coloured sponges occur — in which some decrease of the green colour could probably have been observed very well, some increase but very difficultly — we are justified in neglecting those 21 specimina. Than we would find:

From 71 (= 92 — 21) sponges in light behaved like

type	I + II^{I}	63	= ±	89 °/$_{o}$
„	II	1	= ±	1 °/$_{o}$
„	II^{III} + III	7	= ±	10 °/$_{o}$

Consequently, in the greater majority of Spongillae, when cultivated in light, the increase of the green algae proves to surpass the decrease. Accordingly, we saw that in light: *a.* most colourless to light-green Spongillae (61) became greener (or at least increased in number of green algae); but once this did not happen; *b.* most green Spongillae (23) maintained their colour; but in 7 sponges the green colour diminished (so here the decrease of the algae surpassed the increase).

2. From 32 Spongillae cultivated in darkness behaved like

type	I	0	= 4 = ±	13 °/$_{o}$
„	II^{I}	4		
„	II	3	= 3 = ±	9 °/$_{o}$
„	II^{III}	2	= 25 = ±	78 °/$_{o}$
„	III	23		

Consequently, in the greater majority of Spongillae, when cultivated in darkness, the increase of the green algae proves smaller than the decrease. Accordingly, we saw that in darkness: *a.* most green to light-green Spongillae (25) lost, or at least diminished, their green colour; only once it remained constant; *b.* the colourless Spongillae (6) remained colourless, though in 4 pieces the number of green algae appeared to have somewhat increased.

B. 1. From 34 Ephydatiae cultivated in light behaved like

type	I	14	= 18 = ±	53 °/$_{o}$
„	II^{I}	4		
„	II	4	= 4 = ±	12 °/$_{o}$
„	II^{III}	4		
„	III	4	= 12 = ±	35 °/$_{o}$
„	I, III,	4		

Consequently, in the majority of the Ephydatiae also, when cultivated in light, the increase of the green algae proves to sur-

pass the decrease. Accordingly, we saw that in light: *a.* most colourless to light-green Ephydatiae (15) became greener (or at least increased in number of green algae), that but few specimina remained colourless (3) or even decreased in number of green algae (3), while others became greener in the beginning in order to lose their colour entirely afterwards (4); *b.* the green Ephydatiae sometimes kept up (3), sometimes even increased (2), several times however considerably decreased (4) their green colour. So it proves that in general Ephydatia in light behaves like Spongilla; but that even under these circumstances it possesses a strong tendency to become colourless.

2. From 37 Ephydatiae cultivated in darkness behaved like

type	I 0	= 2	= ± 5%
„	II^{I} 2		
„	II 13	= 13	= ± 35%
„	II^{III} 9	= 18	= ± 49%
„	III 9		
„	I, III 4	= 4	= ± 11%

If we consider, however, that under type II 12 already originally colourless sponges occur — in which some increase of the green colour could probably have been observed very well, but no decrease can possibly have been observed — we are justified in neglecting those 12 specimina. Then we would find:

From 25 (= 37—12) sponges in darkness behaved like

type	$I + II^{I}$	2 =	8%
„	II	1 =	4%
„	$II^{III} + III$	18 =	72%
„	I, III	4 =	16%

Consequently, in the greater majority of Ephydatiae, when cultivated in darkness, the increase of the green algae proves smaller than the decrease. Accordingly, we saw that in darkness: *a.* most green to light-green Ephydatiae (11) lost their green colour, but 1 kept it up; *b.* the colourless Ephydatiae generally remained colourless (12) or even lost green algae (7);

but in 2 specimina the number of these algae increased, while 4 other ones became greener in the beginning in order to lose all colour afterwards. So Ephydatia in darkness proves to behave like Spongilla.

C. Finally 8 sponges of unknown genus, cultivated in light; 4 behaved like type I and 4 like type II. Such a sponge cultivated in darkness behaved like type III.

The results of our experiments may be resumed as follows: **1.** *Green Spongillidae remain green in light.* **2.** *Colourless Spongillidae remain colourless in darkness.* **3.** *Colourless Spongillidae become green in light.* **4.** *Green Spongillidae become colourless in darkness.* **5.** *When Spongillidae are cultivated in light, the increase of the green algae surpasses the decrease.* **6.** *When Spongillidae are cultivated in darkness, the decrease of the green algae surpasses the increase. I want to point out emphatically that these results were obtained by cultivating the sponges under such circumstances, that the factors of import, export, reduction and of growth were almost entirely excluded. Consequently, the facts stated here must necessarily be explained by the two remaining factors: that of multiplication and that of mortality. Therefore the results, given in these factors, run in the following way:*

1, 2 *for green sponges in light and colourless ones in darkness:* $mu = mo$ (I)
3, 5 *for light-green and colourless sponges in light:* $mu > mo$ (II)
4, 6 *for green and light-green sponges in darkness:* $mu < mo$ (III)
(mu = factor of multiplication, mo = that of mortality)

Proceeding from formula I, we will now prove the exactness of the other formulae by means of the above obtained data (p. 55—56, 60, 61) concerning the factors of multiplication and mortality. This is quite simple. We proceed from a green sponge grown in an aquarium in light; for this formula (I) $mu = mo$ is binding. What happens, when we transfer this sponge into an aquarium in darkness? Then this green sponge must pass into a colourless one — according to our data. We may symbolize this transition in this way:

green sponge in light:	$mu = mo$
	∨ ∧
green sponge transferred into darkness:	$mu <$ [1] mo
↓	‖ ∨
light-green sponge in darkness:	$mu <$ mo
↓	‖ ∨
colourless sponge in darkness:	$mu = mo$

Now the reverse: proceeding from a colourless sponge grown in an aquarium in darkness (so: $mu = mo$). What happens when we transfer this sponge into an aquarium in light? Then the sponge will become green:

colourless sponge in darkness:	$mu = mo$
	∧ ∨
colourless sponge transferred into light:	$mu >$ mo
↓	∧ ‖
light-green sponge in light:	$mu >$ mo
↓	
green sponge in light:	$mu = mo$

I am not going to treat this question at large now, for I will soon come back to it, when treating the behaviour of the sponges in nature.

δ.

Having examined the 6 factors, ruling the number of symbiotic algae in sponge tissue, as well as some of their chief combinations, we will try to answer the above (p. 48) *mentioned questions, concerning Spongillidae in free nature, by means of the data we have obtained in the mean time.*

---

1) $<$ is stronger than $\lessdot$, $\lessdot$ is stronger than $<$.

I. The question, *why green as well as colourless algae occur in sponges in light as well as in darkness.* Because in sponges in light import, multiplication and dying of green algae takes place, import and dying in sponges in darkness.

II. The very important question, directly corresponding with that of the foregoing pages, *why in nature a sponge in light contains an excess of green algae, consequently it is green; why a sponge in darkness contains a small number of green algae, consequently it is colourless; how a green and a colourless sponge arise from each other.*

The data, we will make use of, concerning the factors of import [1]) (*i*) (pag. 50, 51), export (*e*) (pag. 52), reduction (*r*) (pag. 52—53), growth (*g*) (pag. 53), multiplication (*mu*) (pag. 55—56), and of mortality (*mo*) (pag. 60, 61) are printed *in italics* on the pages mentioned here.

I want to point out emphatically that all these data have been calculated per unit of time and per unit of sponge-volume, while the facts resulting from Table 6 (pag. 46—48, point 1—11), which we have got to explain here, are also calculated per same units.

We now imagine a sponge (containing an arbitrary quantity of green algae) keeping constant its colour, therefore its number of green algae. For this number the following formula would be binding:

$$i + r + mu = e + g + mo$$

An equation of balance: the number of green algae added per unit of time in the unit of sponge-volume continually counterbalances the number which is substracted. Now we know that in nature a (green) sponge in light as well as a (colourless) sponge in darkness really keeps up its colour for a long time (pag. 35). Consequently, this formula is binding for their quantity of green algae.

---

1) If the import in green sponges in light might prove greater than that in darkness (p. 51, note), the following argumentations would be binding a fortiori.

A. If in nature such a sponge containing an arbitrary number of green algae and growing in light — for its green algae then we have $i + r + mu = e + g + mo$ (I) — is transported into darkness, what is going to happen then? The multiplication of the green algae must then become much smaller ($= \pm 0$), the mortality much more intensive; while the import remains the same, also the export (at least in the beginning), but the growth of the sponge will probably decrease immediately and the reduction remains the same ($= 0$). The original equation $i + r + mu = e + g + mo$ passes into $i + r + mu < e + g + mo$ (II). The balance is broken, the sponge must continually lose green algae, therefore become more and more colourless. In consequence of this decrease of the concentration of green algae the import, the reduction and the multiplication do not change, the mortality however decreases as well as the export, while also the factor of growth will be diminished; but the formula $i + r + mu < e + g + mo$ remains binding (III). Consequently, the end of this process must be that all green algae disappear from the sponge tissues, the sponge itself becoming perfectly colourless. Then, of course, also the second part of the formula $e + g + mo$ must decrease automatically till it has become equal to $i + r + mu$, so till a balance $i + r + mu = e + g + mo$ is re-established (IV); but now another than the original one. So the mortality must have diminished; nevertheless it is then still stronger than in the beginning (p. 59), and rather considerable. When this state of perfectly colourless sponge in darkness is reached, the import is still rather considerable, the factor of growth has diminished still more and the multiplication, the reduction, and the export are ± 0. In stead of $i + r + mu = e + g + mo$ we may put $i = g + mo$; in fact even $i = mo$. In other words, in such a colourless sponge in darkness the whole import is always counterbalanced by the mortality (and the growth).

We may symbolize the transition mentioned, in the following way:

(I) green sponge in light:

(II) green sponge transported into darkness:

(III) light-green sponge in darkness:

(IV) colourless sponge in darkness:

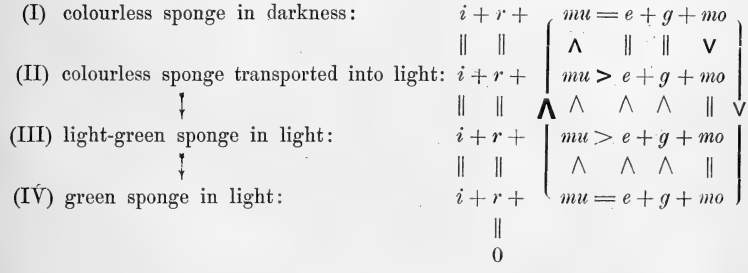
$$
\begin{array}{l}
i + r + mu = e + g + mo \\
\quad \| \quad \| \quad \mathbf{V} \quad \| \quad \vee \quad \mathbf{\Lambda} \\
i + r + mu \mathbf{<} e + g + mo \\
\quad \| \quad \| \quad \| \quad \vee \quad \vee \quad \vee \\
i + r + mu < e + g + mo \\
\quad \| \quad \| \quad \| \quad \vee \quad \vee \quad \vee \\
i + r + mu = e + g + mo \\
\quad \| \quad \| \quad \| \quad \| \quad \| \quad \| \\
i + 0 + \ 0 = 0 + g + mo \\
\quad \| \qquad\qquad \| \quad \| \\
i \qquad = \quad g + mo \\
\quad \| \qquad\qquad\quad \| \\
i \qquad = \qquad\quad mo
\end{array}
$$

From this we see that, if in nature a sponge containing an arbitrary number of green algae, so a more or less green sponge, which grew in light and kept up its colour, is transported into darkness, it must unavoidably lose all its green algae and accordingly become colourless itself, especially in consequence of a very much decreased multiplication and a strongly increased mortality of those algae in darkness. When all green algae have disappeared, the sponge is able only by continually importing new ones from outside to keep up a very small number of green algae in its tissues, as these too continually disappear by dying (conf. Table 6 and p. 47, **3**).

B. Now the reverse. What will happen, when a sponge in nature containing an arbitrary small number of green algae, which grew in darkness and kept up its colour, is transported into day-light? In darkness the formula $i + r + mu = e + g + mo$ (I) was binding for its green algae. Now, in light the multiplication of the algae must become much more intensive, the mortality much lower, the import remains the same, also the export (at least in the beginning), while the factor of growth (in the beginning) as well as the reduction ($= 0$) remain the same. The original equation $i + r + mu = e + g + mo$ passes into $i + r +$

$mu > e + g + mo$ (II). The balance is broken, the sponge keeps more and more green algae, therefore becomes green itself. In consequence of this increase of the concentration of green algae the multiplication increases, the mortality, the import and the reduction remain the same, but the export and certainly also the factor of growth must increase little by little. The formula $i + r + mu > e + g + mo$ however remains binding (III). The end of this process must be, that the sponge lodges an excess of green algae in its tissues, it therefore becomes dark-green itself; even there would not seem to come an end to the increase of the number of green algae in this way. That is impossible, of course; in such a dark-green sponge the factors must change in such a way, that at last this perpetual increase ceases. This changing is again the consequence of this great increase of concentration of the green algae: although the multiplication has still increased — it is considerable and much more intensive than in the beginning (Table 10) — and the mortality has remained the same — it is moderate and much lower than in the beginning (pag. 59) —, the factors of growth and export have certainly strongly increased, while the import has remained the same (rather considerable) as well as the reduction (= 0). In this way a new balance $i + r + mu = e + g + mo$ must arise in the dark-green sponge in light (IV) — of course another than the originally existing one.

We may again symbolize the transition mentioned, in the following way:



In this way we find that, if in nature a sponge containing an arbitrary small number of green algae, so a more or less colourless sponge, which was growing in darkness and kept up its colour, is transported into daylight, it must unavoidably accumulate a large quantity of green algae in its tissues and therefore become dark-green itself, especially in consequence of a strongly increased multiplication and a much decreased mortality of those algae in light. When the whole tissue is crammed with green algae, the various factors ruling their number change in such a manner, that a new balance is established; these changes are an increase of the growth of the sponge and perhaps also of the export of green algae.

(I have always put the export to account. It is, however, an uncertain factor, as I have stated. One may therefore leave it out entirely. That would be of no consequence to the argumentation).

Up to now my argumentations have set forth from sponges, which kept up their colour and, therefore, showed the equation of balance for the number of their green algae (p. 68). But the same can be proved for sponges changing their colour: as we proved that a dark-green sponge from light becomes colourless in darkness, it is a matter of course that a sponge, which grew green or colourless in light (therefore, a still more or less light-green sponge) must also become colourless in darkness; while the reverse will be the case for sponges, which were growing colourless or green in darkness, and were then transported into daylight. The questions, why in general a green sponge in light remains constantly green, and a colourless one in darkness constantly colourless ($i + r + mu = e + g + mo$), have in fact been answered already above under B and A (pag. 69—72). Also other questions may be proposed and answered in the same way.

We have stated now, that (and why) generally in nature **1.** *sponges in darkness must become colourless and sponges in light green,* **2.** *green sponges in light and colourless ones in darkness must keep up their colour.*

(I want to point out emphatically that, up to now, I have exclusively spoken about the normally occurring cases; I will treat the exceptions below.)

III. Now we come to the question, *why in nature a sponge in light — so a green sponge — contains a moderate number of colourless algae and a sponge in darkness — so a colourless one — rather a large number of these algae.*

Also here we can make use of an equation for the number of dead (colourless) algae:

$$i + r + mo \gtrless e + g + d$$

(The letters stand for the same as in the formulae above; d means the number of colourless algae disappearing (by solution) in the unit of time from the unit of sponge-volume).

We know, however, that $i = 0$ (pag. 51), $r = 0$ (pag. 53), $e = \pm 0$ (pag. 52), and $d = \pm 0$ (at least before the sponge is full-grown: pag. 57). So we get:

$$mo \gtrless g$$

of course it is (p. 57),

eg. for a green sponge in light: $mo > g$

consequently for a colourless one in darkness: $mo \gg g$

as will show from the data given on pag. 60—61 and 53. So we see that in the same time the number of colourless (dead) algae must increase much more in the sponge in darkness than in that in light.

Lateron I will treat, why the number of colourless algae with structure remains constant during the development of the sponge tissue (chapt. VIII). Why that of the colourless ones without structure increases is mentioned on pag. 56—57.

IV. *Why does the number of the various (green and colourless) algae always show a decrease in the gemmule stage?*

As for the green algae, we may symbolize the transition of a colourless sponge in darkness into the gemmule stage as follows:

colourless sponge in darkness:	i	$+ r +$	mu	$= e +$	$g +$	mo
	‖	‖	‖	‖	‖	‖
	i	$+ 0 + 0$		$= 0 +$	$g +$	mo
	∨	∧	‖	‖	∨	
young colourless gemmule in darkness:	0	$+ r + 0$		$0 +$	$0 +$	mo
	‖	∨	‖	‖	‖	∨
old colourless gemmule in darkness:	0	$+ 0 + 0$		$<$	$0 + 0 +$	mo
	∧	‖	‖	‖	∧	∧
germinated gemmule in darkness:	i	$+ 0 + 0$		$0 +$	$g +$	mo

Consequently the green algae must entirely disappear from old colourless gemmules in darkness, in order to re-appear immediately in small numbers, when the gemmules have germinated (conf. pag. 47, **3**).

The transition of a green sponge in light into the gemmule stage may be symbolized:

green sponge in light:	i	$+ r +$	mu	$= e +$	$g +$	mo
	‖	‖	‖	‖	‖	‖
	i	$+ 0 +$	mu	$= e +$	$g +$	mo
	∨	∧		∨	∨	
young green gemmule in light:	0	$+ r +$	mu	$0 +$	$0 +$	mo
	‖	∨	∨	‖	‖	∨
old green gemmule in light:	0	$+ 0 + 0$		$<$	$0 + 0 +$	mo
	∧	‖	∧	∧	∧	∧
germinated gemmule in light:	i	$+ 0 +$	mu	$e +$	$g +$	mo

Consequently, the number of green algae must decrease in old green gemmules in light, but probably increase immediately after the gemmule has germinated (conf. pag. 46, **2**). The fact, that the multiplication of the algae stops in old green gemmules, is stated on pag. 46, **2**. There are several reasons possible for it; none of them can be proved, however.

The decrease of the number of colourless algae with structure in the gemmule stage will be explained lateron, when speaking about the cause of the dying of the algae in sponge tissue. But I want to remind that we concluded (pag. 42) that this dying is closely related to the life of the sponge; and we know that the gemmules are stages of rest of the Spongillidae. So it is evident that probably herein is the cause of the decrease of the mortality during that period. That the total amount of dead (colourless) algae (without structure) decreases in the gemmule stage, has been explained already on pag. 57.

In this way we have proved with the help of the data we obtained, when studying the 6 factors (of import, export, reduction, growth, multiplication and mortality) ruling together the number of the symbiotic algae in sponge tissue: **1.** *Why in nature the Spongillidae must contain such an amount of the various green and colourless stages of the symbiotic algae in light and in darkness, as we have experimentally stated on pag. 46—48, point 1—11.* **2.** *How in nature these sponges keep up their „colour" (green or colourless), and how both „coulour"-types arise from each other (pag. 35).*

As mentioned above, up to now I have exclusively treated the cases normally occurring in nature, viz. those of green sponges growing in light and colourless ones growing in darkness (pag. 46). We stated, however, that sometimes green sponges might be found in nature in darkness and colourless ones in light (p. 41); and in Table 8, pag. 63—66, that as an exception to the rule green sponges may become less green in light and colourless ones more green in darkness. How is that to be explained?

We saw that the amount of green algae, so the colour of the sponge, is ruled in nature by the proportion $(i + r + mu) : (e + g + mo)$ and in my experiments by $mu : mo$. We must consider, however, that each of these factors is depending on numerous circumstances. These circumstances proved in general rather con-

stant — for we could deduce several generally appliable rules concerning these factors, by which could be explained the normally occurring cases of changing or remaining constant of the number of green algae in sponge tissue (in light or in darkness). But this fact does not at all exclude the possibility, that these circumstances do change — as an exception. In such cases our rules, deduced for normal circumstances, would not be binding of course.

In such a way one will have to explain the abnormalities mentioned. When analyzing now the amount of algae present in these cases (Table 6 B), we find: in green sponges in darkness the multiplication to be normal — very slow — but the mortality abnormally low; in colourless sponges in light, on the contrary, an abnormally slow multiplication and a normal mortality. By these facts the abnormalities become already more conceivable.

VIII. The nature of the „symbiotic" association of sponge and green alga; its use to the alga and to the sponge.

This part of my investigation has indisputably been the most difficult one. Only after much groping, I succeeded in finding a way out from the labyrinth of facts, which bear upon these problems; not by lack of points of issue, but even more by their great number, however partly leading — at first sight — in opposite directions.

α.

I will begin with the use which the „symbiotic" association offers to the alga.

In the first place this question: *How shall we state whether the algae are in better conditions in one milieu — eg. a sponge — or in another?* We want to state the use of the „symbiosis" to the alga, in other words: if the alga is in a better condition in the sponge tissue than in the water. Which phenomenon on the alga shall we take as criterion?

Only two criteria are to be considered, as being most self-evident: 1st the intensity of the multiplication of the algae, 2nd the total increase of an algal-culture within a certain time. It appears less desirable to me to accept a criterion in the appearance of the algae, viz. in the more or less normal or degenerate condition in which they are, as it must be very difficult to apply.

So there remains: *the intensity of multiplication* (the number of stages of division per 100 green algae, see p. 54) and *the total increase of an algal-culture.* Perhaps one will suggest that both these criteria come to the same in fact; but this is not the case at all. *The intensity of multiplication gives a way of measuring the favourableness of the conditions under which every alga individually lives, for instance of the feeding milieu* (apart from factors which, in short, destroy those algae). *The total increase (or decrease) of an entire culture, however, is a measure for the favourableness of all factors, which possibly can be of any influence to the algae.* So it may be that, though an alga finds somewhere a favourable feeding milieu, by which it multiplies rapidly, the whole culture is exposed to such a degree to the gluttony of protozoa, that after all the number of algae is decreasing instead of increasing.

So it is most important to apply both criteria in our investigations.

Comparing a sponge (in light) and lake water (in light) with regard to the question we are treating presently, we notice first and premost the enormous difference in concentration of the green algae. The sponge is grass-green or dark-green, the water from the lake never has a green tint; the sponge contains numbers of green algae in its tissue (one could take 3×10^{11} green algae per litre), the water from the lake only 4000 per litre (p. 28), at the end of July.

I have stated in chapter VII, that sponges in nature constantly increase their number of green algae by import. So at any rate the accumulation of algae in a green sponge is already partly explained by this mechanical process. But import only is not

sufficient — just think of the colourless sponges in darkness with an import just as large (but with a much smaller multiplication and a much greater mortality) — also a rather considerable multiplication is required and low mortality.

What about this multiplication and this mortality of the green algae in a sponge, compared with those phenomena in algae free in the water? *Is in light the intensity of multiplication in a sponge larger, the same or smaller than in water?*

For this see Table 10, to which I have already referred several times. In this table there is given every time in column 1 the intensity of multiplication of the green algae for a sponge in the light (and in the dark) and for a culture in water from the conduit or from the lake in light (and in darkness). From that we see that in most experiments the intensity in the water is ever so much larger than in the sponge; in the latter it is generally below 15, in water it amounts to 20—35!

Let us, for the present, not ask how that phenomenon in caused; at any rate it goes without any doubt for these experiments. *Now what about the total number of green algae when cultivated in light in sponge and in water?* See Table 10, column 3. That number proves, notwithstanding the much larger intensity of multiplication in water, in general in water less increased than in the sponge. So it cannot be otherwise than that the algae, which have much more multiplied in water, must also have been more destroyed there, in some way or other, than in the sponge. (With all these experiments the factors of import, export, reduction and of growth were excluded as much as possible). *So all things together, the algae in the light are in more favourable conditions in the sponge than in the water; really the sponge must protect its algae against destruction* [1]).

This fact must also inevitably appear, when we notice that, according to Table 8, entirely colourless sponges with only few

1) To get this result purely, we must compare the algae with equal begin-concentration in sponge tissue and in water from conduit, of course, after they have been cultivated for the same time and at the same temperature. Then consider what is stated in the following pages (p. 82—83).

green algae, cultivated in light in water from the conduit (so excluding import), soon show a larger amount of green algae than the water of the lake ever possesses.

It is rather well conceivable, although not quite sure, against what the sponge protects its algae. A priori one supposes this protection to be against protozoa and other small fresh-water organisms which will swallow the algae. Also in my cultures I have got an indication for this, as shown in Table 9 B. There one sees, among others, an algal-culture in light in small concentration in water from the lake, which the 2nd week consisted of a thin green membrane surrounded by a (newly formed) darker green rim. During the 3rd week, however, first the rim and next also the membrane suddenly begins to disappear. Now, numerous protozoa prove to have grown in the culture; among which there are some filled with the green sponge algae. About a week later the whole algal-culture has almost been destroyed; numbers of degenerate algae remained, while the protozoa disappeared for the greater part.

Besides, there exists also a means of protection for the symbiotic algae within the sponge body against other enemies, not enemies which will swallow them, but which cause their destruction in some other way: diatoms and ordinary algae. Above (p. 43, 45 and Table 5) I treated already, how the sponge algae were killed by an infection of diatoms or algae arising in the culture. I do not venture to decide, to what this influence must be ascribed; one might suppose to (lack of food or to) poisoning by products of metabolism, especially as the same thing happens when mould-infection occurs (p. 43). It is obvious, now, that this influence of the infecting algae and diatoms will be stronger in light than in darkness; which, moreover, appears from Table 4. So it might be possible, that a culture of symbiotic algae in water and in light, with of course a high intensity of multiplication, has in fact less increased after some time — by the hurtful influence of luxuriantly growing algae and diatoms — than such a culture in darkness, with but a small intensity of multiplication. In Table 10 there are indeed some of such cultures to be seen.

So here we have such an (apparently) paradoxal phenomenon, as treated above in consequence of the comparison between multiplication-intensity and total increase of the algal-culture in a sponge and in water; indeed, here too we found a smaller total increase with a larger multiplication-intensity of the symbiotic algae (in water).

So one proof more, that this intensity of multiplication and this total increase are independent factors; while, what we treated just now, gives even more support to the above mentioned opinion, that the protection of the symbiotic algae by the sponge is, among others, also meant against diatoms and ordinary algae.

It stands to reason, that this protection must now not be taken, as if there are no algae destroyed in the sponge itself. Of course this does take place, as we saw, eg. on p. 46—48.

On p. 78 we saw that in the cultures of Table 10 the intensity of multiplication of the green algae in light was generally much higher in water than in the sponge tissue. I have purposely omitted till now treating the cause of it, to evade unnecessarily complicating the question we were about.

Very likely that cause is not only to be found in the milieu, in the sponge or the water, but also in the concentration-difference of the algae themselves (see p. 55). It might seem to be very difficult, almost impossible, to directly compare the concentration of the algae in water and in the sponge tissue somewhat accurately. Nevertheless, I believe to have succeeded rather well; and I so came to the conclusion that in fact in the light, in an equal, strong concentration of the green symbiotic algae, their intensity of multiplication in water and that in the sponge are probably the same.

In the first place we see that in Table 10 there are also cases to be found in which the intensity of multiplication in the sponge is just as high as that in water, or just the reverse, that the intensity in water diminishes down to that in the sponge. The first regards sponges with weak concentration of the green algae

— consider both begin- and final concentration — [1]); the latter cultures with a stronger concentration, compared with other cultures. So we see that, when we consider the concentrations, the found intensities of multiplication in sponge and water seem to approach each other. This appears even more clearly, when we compare some intensities in the sponge tissue found in Table 10 with the intensities in water with different concentrations of the algae, stated in Table 9. (Here the concentrations have namely been more accurately established.) We then see that in the sponges 3371, 3381, 347, 3701, 416, etc., all of which are sponges with weak concentration of the algae, a multiplication-intensity ruled, which was equal to, even larger than that of the algae in a strong concentration in the water-cultures of Table 9. So this was an indication the more in the above mentioned direction.

The question was how to compare these concentrations of the algae; so in a culture in water, that is (p. 10) a membrane attached to bottom, and in a more or less green sponge. Of course it is impossible to fix the number of algae in equal volumes of sponge and culture. There only remains the comparison of the colour. Thereto one should consider that one can state the colour of the green membrane on a white and on a dark background. For a colourless or a green sponge one can say, that the background is white (creamy). Therefore the sponge may be compared directly, as regards its colour, with a membrane on a white (creamy) background.

Apply this on Table 9. It then appears, that in A the membrane in the strong concentration on a white background is yellow-green to light-green, the rim (just as the sponge) dark-green; in the weak concentration the membrane greenish, the rim light-green to green. In B the sponge is dark-green, the membrane in the strong concentration on a white background green to light-green; in weak concentration the membrane greenish, the rim light-green. Let us now stick to Table 9 A; this one has been continued longest and is the most accurate, as there was no question about infection, as was in the other one. So we see that with a dark-

1) Nos 3361, 341, 365 I, 375, 376 should be neglected as being abnormal.

green colour, so in a concentration *s.* of the algae, the intensity of multiplication is the same in the sponge as in the water-culture, viz. about 0—6.5 (once 9), on the average **2** in the sponge, in water **2.3**; with a yellow-green to light-green colour, so in a concentration *m.*, in water about 7—20 (once 28), **13** on the average; with a greenish colour, so in a concentration *w.*, in water about 29—39 (once even 45) and **33** on the average.

We should also apply this method to Table 10 and first to the sponges in light, considering again both, begin- and final concentration of the algae. We then find in a concentration *s.*—*s.* of the algae in the sponge a multiplication-intensity (in 22 out of the 23 cases) of about 0—7 (once 9), **2.6** on the average; in a concentration *m.*—*s.* (in 14 out of the 18 cases) of about 5—11 (once higher, 3 times lower), **7** on the average; in a concentration *w.*—*m.* (in 12 out of the 15 cases) of about 9—17 (once lower, twice very low), **10.7** on the average; in a concentration *w.*—*s.* both very low values for the intensity of 3 and 7; and finally in a concentration *w.*—*w.* (in 3 out of the 4 cases) an intensity of about 15—20 (once lower), **15.5** on the average, while the experiments n°. 336 l, 341, 365 II, 375 l, 376 l, with the concentration *w.*—*w.* and their multiplication-intensity of 0, should be neglected as being abnormal (p. 75—76). For the cultures in water from the conduit in light we find: in a concentration *s.*—*s.* an intensity of **1**; in a concentration *m.*—*m.* of 11—23, **15.7** on the average; in a concentration *w.*—*m.* of about 19—28, **23** on the average; and in a concentration *w.*—*w.* an intensity of about 17—37 (once 10), **25.2** on the average. For the cultures in lake water these values are generally lower; as also in Table 9 B.

One will acknowledge that, considering the rather coarse way of comparing the concentrations of the algae in the sponge tissue and in the water cultures, their multiplication-intensities for the strong concentrations (*s.*—*s.* and *m.*—*s.*) in sponge tissue and in water do mutually correspond very well in Table 9 A and 10 [1]);

1) In fact we ought to have stated them separately in Table 10 for each series of experiments (p. 54); the result, however, would have been the same.

while those for the weak concentrations (*w.*—*m.* and *w.*—*w.*) are lower in sponge tissue than in water. But, of course, the correspondence of the intensities for the concentrations *s.*—*s.* and *m.*—*s.* is the most decisive. So we are justified in concluding that *the symbiotic algae multiply in light in equal, strong concentration within the tissue of the sponge about just as quickly as in water; but in equal, weak concentration in the sponge less quickly than in water.* From this we may also deduce (p. 77) that *the feeding milieu for the alga is in fact not more favourable in the sponge than in the water, but about just as favourable or even less favourable.* That it is not more favourable, also very clearly shows from the fact, that the algae multiply in darkness less quickly within the sponge tissue than in water (Table 10, cnf. Table 6, col. 2 and Table 4 B, col. 4).

It is a matter of course that the above proved fact about the protection, which a sponge in light gives to its algae, does not lose anything of its validity, now that we have shown that the greater intensity of multiplication in water, which occurs in these experiments, is not only explained by the milieu but also simply by the concentration-differences of the algae (see note p. 78).

But what about that protection (p. 78) in darkness? It appears from Table 10, col. 3, and also by comparing Table 4 B and 8 that — contrary to the result in light — *the algae in darkness are in much less favourable conditions in the sponge than in the water;* in the sponge all algae are destroyed within short (p. 70).

As final conclusion [1]) *we may give this one: In darkness the*

1) In fact we should have stated this not only by comparing the behaviour of algae when cultivated in water from the conduit and in sponges in water from the conduit, but also by comparing their behaviour in lake water and in sponges in lake water. This, however, is impossible, as we can cultivate sponges only in pure streaming water (p. 9), and as, moreover, the factor of import cannot be excluded in experiments in lake water (p. 11). But in any case we know that the feeding milieu is less favourable to the algae in lake water than in that from conduit (p. 82), and that in light the algae are also in less favourable total-conditions in lake water (than in that from conduit) (Table 10, col. 3). From which perhaps might follow that in light the destruction of the algae in lake water is about just as large as in that from conduit, at least is not weaker.

„symbiotic" association of Spongillides and alga offers much less advantage to the alga than a life free in the water, as in the sponge all algae are destroyed. In light, on the contrary, that „symbiotic" association offers more advantage to the alga than a life free in the water; but that advantage only consists in the fact, that the sponge protects the alga against destruction, by enemies for instance. The milieu — the feeding milieu —, on the contrary, is in the sponge not at all more favourable to the alga than in the water, neither in light nor in darkness, but about just as favourable or even less favourable. When further we know, that also in light algae are constantly destroyed in the sponge — though less than in the water — we must conclude, that from the point of view of the use to the alga that association with the sponge cannot be called at all a symbiosis in the meaning of that of the Lichens. In light the alga, so to say, has chosen the least of two evils; one cannot say more.

β.

We have now come to the still more complicate question of the use of the „symbiotic" association to the sponge.

I will begin with mentioning all the facts regarding this question. After that we shall see, if all these different data can be united into one conception.

1. On p. 25 I have mentioned already that the green symbiotic algae of the Spongillidae lodge oildrops, which they form, as p. 20 shows, by photosynthesis.

2. The colourless algae may also show oildrops (p. 36); but in general not so often as the green algae; while of these colourless ones those, which kept their internal structure, show them more often than the colourless ones, the structure of which has got lost. This may appear from the following analyses of green Spongillae taken from the light, by which the number of algae with oildroplets is given per 100 algae which have been tested.

	1st sponge	2nd sponge	3rd sponge	4th sponge	5th sponge	average
per 100 green algae	72	60	80	94	74	76
„ „ colourless ones with structure	30	22	40	40	30	32.4
„ „ „ „ without „	18	4	10	10	6	9.6

With a view to what we shall treat later on, I want to add, that here almost exclusively algae were examined, which were situated free in the protoplasm of the lodging amoebocytes, so not in vacuoles.

3. Also in the amoebocytes of green sponges, so in amoebocytes overladen with green algae, there occur numbers of refractive oildrops lying free in the protoplasm; see Table 12 and 13. They are not stained by I in I K sol., but they become red with sudan III in alcohol and gray-black with osmic acid (see note p. 25), and are blue-green, when not stained and the microscope is well adjusted — just as the oildroplets of the algae. They also have the same diameter (0.4—1 μ) as these ones, although the larger specimina seem to occur more in the algae than in the amoebocytes. Further the oildroplets of the amoebocytes very often seem to be still much more refractive and sharper outlined than those of the algae. This might show, that the oildroplets from alga and amoebocyte are not identical.

Next I want to mention that, although these „oildroplets" (of alga and sponge) will consist for the greater part of oil, one still has to consider the possibility, that they might possess something as a plasmic stroma or cover, as is also given for instance for the milk-globules. Also the above mentioned (p. 26) colouring of the oil-droplets by haematoxyline makes us think of that.

4. How often I have paid attention to it, I have never been able to see the slightest trace of an oildroplet being ejected by the symbiotic algae. Also the supposition, that such a phenomenon might be possible, does not sound very likely — for we have

found a cell-wall round the alga (p. 26), and even without that it is very unlikely already —. Still I have obtained a number of observations with cultures of isolated green algae, from which seems to follow that sometimes the number of oildroplets, which occur outside the algae c. q. free in the membrane at the bottom of the culture, rises and lowers with the number of oildroplets within the algae, that is to say with the number of algae lodging such a droplet. See Table 11.

In general, namely, the free oildroplets seem to disappear very soon from an algal-culture. Quite intelligible, for instance by the action of bacteria. Further it appears, that these free droplets can only be numerous if there are also numbers of algae containing an oildrop; if the number of the latter lowers — which always happens when the algae are vigorously dividing — that of the former seems to necessarily follow immediately. Is the reverse the case (with rising, namely), this need not absolutely take place. Sometimes however this happens in quite a striking way. I will give an example: A culture in light of numerous green algae, several of which carry an oildroplet, very rarely shows a free oildroplet. After 10 days the number of green algae with oildroplets has increased very considerably, the number of free oildroplets also to rather a great number. Two cultures are made then out of this one, and one of them is placed in light under favourable, the other under unfavourable conditions. After two weeks again the first culture shows a particularly high number of green stages of division of the algae, consequently a small number of algae with oildroplet, and also a small number of free oildroplets; the 2nd culture on the contrary contains no stages of division, numerous algae with oildroplet and rather a great number of free oildroplets. Now follow the first of these two cultures. After a month this one shows very seldom a stage of division and consequently a great number of algae with oildroplet; yet free oildroplets are only present sporadical; but three months later, when dividing-stages lack and thus oildroplets still appear in a great number of algae, the free oildroplets have become rather numerous again.

By these observations one will acknowledge, that there seems to be some relation between the number of oildroplets inside and outside the green algae. What relation — in the experiments under consideration — is not yet quite clear to me. I consider direct ejection of the oildroplets by the algae to be excluded, not only because of what I mentioned above, but even more because of what I am going to say (5). I believe, that this relation is more likely to be in the stages of „solution" of the algae, the colourless ones with and without structure, about which I spoke on p. 42 and 43. There are also some indications for it in Table 11.

5. Also in the amoebocytes of colourless sponges, so in amoebocytes with but few green algae, there are numbers of oildroplets in the protoplasm. In Spongilla their average number in these amoebocytes of colourless specimina from darkness is even somewhat higher than in the amoebocytes of green sponges from the light, i. e. than in amoebocytes filled with green algae (in which the oildroplets are formed). See Table 12. From this follows that, very likely, there is no question at all about ejecting of oildroplets (or their constituent parts) by the green algae; for if this were really the case, the amoebocytes of green sponges from light had to contain ever so many more oildroplets than those of colourless sponges from dark, while on the contrary the latter appeared to lodge even more than the former.

Perhaps one might be inclined to explain this last fact as being caused by a larger consumption of the oildroplets in a green sponge in light than in a sponge in dark. In the first place I have to answer then that, as the amoebocytes are exactly the cells in which the green algae are especially to be found within the sponge body (p. 16) — which cells therefore may be considered as being the place of production of the oildroplets — at any rate at least in them there should still be something to be seen of that larger production in green sponges in light. And in the 2nd place it would be a mere chance, that this supposed increased consumption of oildroplets in a green sponge in light should exactly counterbalance the ever so much larger produc-

tion (which is not at all under the control of the sponge). Besides, what might be the reason for that larger consumption? Perhaps this reminds of the fact mentioned on p. 53, that green Spongillae in light often prove to grow much quicker than colourless ones in darkness. But growth is always only a consequence of, among others, the quantity of food disposable; in other words: if really the lesser growth of the colourless sponges were the consequence of the smaller quantity of oildroplets they can dispose of, it would be a very singular fact that there is still rather a large number of those oildroplets left in the amoebocytes of such a colourless sponge, that there is even more left than in a green sponge in light! Besides, an organism especially wants protein to grow and not so much fat (or carbohydrates). Last not least, I have to propose another question, much more important, in case one sticks to the opinion, that the oildroplets really get directly from the green algae into the amoebocytes; this question namely, how — if this supposition were right — the very few green algae, which are present in a colourless sponge in darkness (or twilight), could possibly procure all those oildroplets, which we find in the amoebocytes of that sponge; while, moreover, those algae can hardly photosynthesise there, even will have to die very soon (p. 70). Consequently these few green algae almost exclusively dispose of the oildroplets, which they already carried when imported from the outside to within the sponge, and which in dark they are certainly not going to give immediately to its tissues! As we are thus obliged to find for the colourless sponge another explanation for the presence of oildroplets in the amoebocytes, the suggestion is very likely, that this (other) explanation will also prove to go for the green sponge. Besides, the whole supposition of oildroplets being ejected by the green algae by itself sounds rather unlikely, as I stated already above under point 4.

But lateron I will mention quite another, much more logical way, in which the amoebocytes do get really their oildroplets from the algae (p. 98—99).

All that is stated sub 5 about ejecting oildroplets by the green

algae counts as much, without any change, for ejecting (dissolved) carbohydrates, from which one might think the oildroplets originating. I shall return to this lateron (**11—13**).

6. From the same Table 12 it also appears, that in the amoebocytes of Spongillae the number of oildroplets in young tissue — the branch-tops (p. 16) — is a trifle smaller than in full-grown tissue — the branch-bases —; that it is however at its largest in young, old and newly germinated gemmulae, but that it strongly diminishes in the stages of development which follow. Consequently, the oildroplets seem to be consumed by the tissue of germinated gemmulae.

7. While on the one side it appears from Table 12 that the number of oildroplets in the amoebocytes is not in correlation with the number of green algae carrying an oildroplet — as shown above under **5** —, on the other side we can conclude from the same table that apparently that number of oildroplets (in amoebocytes) is in some correlation with the number of colourless algae without structure, so with the number of dead algae, in the sponge tissue. This would all at once explain the queer fact, mentioned under **5**, that in the amoebocytes of colourless sponges from darkness there are somewhat more oildroplets than in those of green ones from light; for we know (p. 59), and it appears again from this Table 12, that in a sponge in darkness there are more green algae dying than in light. But also the facts mentioned under **6** could be explained in connection with this: in the course of the development of a sponge the total number of dead algae is constantly increasing, according to p. 57, with a considerable decrease in (or shortly after) the gemmule-stage (this also appears in Table 12); so the same should happen to the number of oildroplets per amoebocyte.

Which, however, is the right connection between the number of oildroplets in the amoebocytes and the dying of the green algae, why this connection must necessarily exist, can only be treated lateron, when I have mentioned all facts concerning it.

8. In the sponge-tissue occurs a lipase, a fat-splitting enzyme. In Table 13 one finds, how I have pointed out its presence.

9. The oildroplets appear to be more numerous in the choanocytes, the collar-cells lining the flagellated chambers, than in the amoebocytes. See Table 14. Perhaps this might make one suggest, that all oildroplets in the sponge have been taken from the surrounding water by means of the choanocytes, the food-capturers par excellence. This suggestion was the more justified, because my sponges were mostly gathered in a by-channel of the lake, along which were numbers of houses which emptied their refuse from the drain-pipes in that canal; even so, that the sponges were often in the middle of the dirt. This supposition, however, proved untenable; for the sponges gathered in the lake itself, in very clean water — far from houses —, contained about as many oildroplets in their choanocytes as those which had grown in the dirty canal water; while this also appeared to count for sponges which had been cultivated for some time in flowing water from the conduit (while no reduction occurred). See Table 14.

10. As we saw above under **7**, that the number of oil-droplets in the amoebocytes was in some relation with the number of colourless algae, we might also expect here for the choanocytes some relation between the number of their oil-droplets and that of their colourless algae. That proves however not to be the case, as follows from this table:

choanocytic layer

green sponge		colourless sponge	
number of		number of	
oildrops	colourless algae	oildrops	colourless algae
rather numerous	some	mass	few
several	»	rather numerous	several
some	few	several	rather numerous

11. I have already mentioned on p. 25, that I have never been able to show any carbohydrate within the symbiotic algae — except the cell-wall, which of course will consist of cellu-

lose. However, carbohydrate (glucose) will probably be formed (but then kept in solution); so also OLTMANNS (47) gives for some algae that, although oil may be found as final product, the prime products of photosynthesis are probably carbohydrates; also see PFEFFER (47a) for plants in general.

12. The amoebocytes of green and colourless Spongillidae and their gemmules show, when stained by I in KI sol., numbers of very small red-brown globules, much smaller than the oil-droplets (Table 14) and especially situated along (the inside of) the wall. On the other hand I have never found vacuoles stained by I in the amoebocytes, as LANKESTER (35) states. One will be inclined now to consider these small brown globules as a solid carbohydrate; but such a colouring by I does not yet give sufficient proof by itself. So I have also tried to prove in a more direct way, that carbohydrates occur in the sponge tissue. And such with the help of the FEHLING solution which, as known, gives a red precipitation, when boiled with a reducing sugar (for instance glucose or an other monosaccharide).

I rubbed a green Spongilla, just boiled the remaining fluid and filtered it, while the symbiotic algae and all smaller particles passed through the filter. The fluid reacted neutrally and was light-green, rather troubled. This liquid, now, showed after having been boiled with some FEHLING solution rather a considerable red precipitation, while a blind experiment (FEHLING boiled without sponge liquid) did not show any reduction. So sugar (probably a monosaccharide) was present in the sponge. Next the same was repeated with sponge liquid, which had in advance been boiled for one hour with some drops of strong HCl (to break down polysaccharides which might be present), then neutralised by KOH and filtered; this liquid showed stronger FEHLING reduction than the original one. From this we may conclude, that the sponge liquid, consequently the sponge tissue, contained a polysaccharide besides the monosaccharide. But this polysaccharide need not exactly have been the carbohydrate which can be stained by I; it may have been exclusively the cellulose of the algal cell-walls (perhaps the increased reduction after hydro-

lysis by HCl might also been explained as caused by the carbohydrates originating from the nucleins?).

The same result, however, was obtained also for the gemmules after I had almost entirely freed their rubbed material of (coarser) solid particles (among others of algae and oil-droplets) by centrifuging and extracting with ether. Moreover, the living cells of the gemmules considerably swell in water, so apparently possess a strongly hypertonic cell-fluid, which corresponds very well with the presence of dissolved sugars.

So we may conclude, that a polysaccharide is present in the sponge-tissue also beyond the (coarser) solid parts of the cells; we now can freely explain the above mentioned small globules stainable brown by I as consisting of the polysaccharide in question.

13. As appears from Table 14, these small globules are present in about the same number in amoebocytes of a green sponge in light, so in amoebocytes filled up with green algae, as in those of a colourless sponge from darkness, so with but few green algae; it may even be, that these globules are somewhat more numerous in the second than in the first cells. From this we may conclude again, that export of carbohydrates from the green algae probably does not take place at all. For if it were the case, these carbohydrates in solution — in **11** namely it appeared, that these will be present in the algae —, which would then evidently be deposited in the sponge tissue partly in the form of reserve food (the little globules), had at any rate to be much more numerous in the green sponge from light than in the colourless one from darkness, while exactly the reverse proved to be the case.

Perhaps one might be inclined to explain this last fact, just as in **5** for the oildroplets, as being caused by a larger consumption of the carbohydrates in a green sponge in light. I then can give exactly the same answer, I have given already (in **5**) for the oildroplets, and which I am not going to repeat.

As we saw that the little globules, which can be stained by I, in the amoebocytes of a colourless sponge from darkness are probably even somewhat more numerous than in those of green

ones from light, it is probable — in analogy with what was stated above under **7** for the oildroplets in amoebocytes — that there must be some direct relation between the number of these little globules and the number of dead algae, which, as known, is also larger in sponges in darkness than in sponges in light (p. 59). I shall mention only lateron, what relation this is.

14. The little globules stainable by I are to be found somewhat more numerous in the choanocytes of the flagellated chambers than in the amoebocytes (Table 14). Also here one could ask the question, which we put above in **9** for the oildroplets, if perhaps not all these globules could have been captured from the surrounding water by the sponge with the help of its choanocytes. But also this question we must answer negatively, as these globules are about as numerous — or only a little less numerous — in the choanocytes of sponges out of the lake-water or the water from the conduit as in those of the sponges from the dirty canal water (Table 14).

15. As we saw under **13** that in the amoebocytes there is some relation between the number of globules, stainable by I and the number of dead algae, we might expect the same relation in the choanocytes. This proves, however, not to be the case, as the table below shows.

choanocytic layer

green sponge		colourless sponge	
number of glob. st. by I	colourless algae	number of glob. st. by I	colourless algae
numerous	few	mass	few
mass	some	»	several
»	»	»	rather numerous

16. On p. 18—20 I have proved already, that the green symbiotic algae produce O_2 in light.

17. In the amoebocytes of the Spongillidae food vacuoles are to be found including symbiotic algae in different stages of digestion; so one finds in these vacuoles all normal and „solution" stages of those algae, which I treated repeatedly (p. 42—45), viz.: green ones, colourless ones with clear structure, with a shade of structure, without structure, and vague shades of colourless algae. Besides these symbiotic algae one also finds in the vacuoles: diatoms, ordinary algae, bacteria, all sorts of unrecognisable detritus and the above mentioned oildroplets, mutually combined as well as with the symbiotic algae.

18. In sponges newly caught from nature these food vacuoles are rather rare in the tissues (Table 15). Consequently, the sponge in free nature seems to digest only few symbiotic algae and other food particles in this way by vacuoles.

19. In sponges, which have been for some time in aquaria (with water from the conduit) these food-vacuoles, however, are more numerous (but there does not seem to be much difference then between cultures in the light and in the dark; Table 15); so under these circumstances the sponge proves to digest more food in this way.

20. In newly caught Spongillae food vacuoles are less numerous in young tissue (branch-tops) than in full-grown (branch-bases) (Table 15).

21. In newly caught Spongillae the food vacuoles are somewhat more numerous in the tissues of colourless specimina from darkness than in those of green ones from light; they seem equally numerous in Ephydatiae (Table 15).

22. As I mentioned already on p. 42—43, most of, not only the normal green symbiotic algae but also of the often mentioned colourless dying- and „solution"-stages of those algae (p. 42—45), occur quite free in the protoplasm of the amoebocytes, not in food vacuoles.

23. The amoebocytes with (green or colourless) symbiotic algae form — as was partly mentioned on p. 16 — the greater majority of the cells of the green and the colourless sponges. The

amoebocytes of green sponges lodge, besides their numerous green algae, mostly also one or more colourless algae free in their protoplasm; while, after what was said above, it stands to reason that also the amoebocytes of colourless sponges contain colourless algae free in their protoplasm.

24. In green and colourless sponges the amoebocytes appear to lodge more colourless symbiotic algae than foreign enclosures (unicellular algae, etc.). The same thing counts for the whole sponge-tissue.

25. Particles, which have been taken up by amoebocytes, never come immediately in a vacuole but remain free in the protoplasm; if a vacuole is formed around, this only happens lateron.

26. From Table 8 it appears, that in green sponges cultivated in water from the conduit the number of colourless algae with structure generally also increases in the light.

Now that we have described in the above given 26 points the chief facts, which bear upon the question about the use of the symbiotic association to the sponge, we will now see, if all these various data can be united into one conception; in other words, if we can come to an answer to this question with the help of these data. Probably one will suppose already oneself in which direction the conclusion can be found, by the way in which now the 26 points have been arranged. I must confess, however, that this solution has troubled me a great deal; while on one point it requires still further completion.

As was mentioned under point **17** and **18**, the fresh-water sponge seems to digest free in nature only little nutrition (symbiotic algae and other food particles) by means of food-vacuoles. So the sponge must evidently supply its food in another way. Several ways are imaginable:

First the possibility, that the sponge lives of organic materials in solution, present in the lake water. I am not going to treat

here the question, if this supposition — the PÜTTER theory (48, 49) — should also count more or less for the Spongillidae; for these sponges possess, as we will see presently, another very rich source of food; so that the argument, on which PÜTTER's theory is based, seems not to hold good for the fresh-water sponges. *The dissolved organic materials of the lake water are for them surely not the most important source of food.*

Next one might have thought, that it were the products of photosynthesis of the green symbiotic algae (the oildroplets and carbohydrates) which, after having been ejected by the algae, would serve the sponges as food (point **1, 3, 11, 12**). *We now know, however* (point **5, 13**), *that it is very likely such an ejecting of oildroplets and carbohydrates by the algae does not take place at all.*

There is still another possibility, viz. that the sponge is fed by proteins (or their constituent parts) which the green algae could eject. For the (colloidal) proteins this sounds, however, very unlikely; and also for their parts, the amino acids, will be no question about ejecting by the algae, as we saw above that it is, very likely, already not the case for the primary products of photosynthesis (the carbohydrates), that are still much more numerous. Consequently, nor in this way we escape from the difficulty.

The solution is, that according to point 22—24 (p. 94—95) the sponge has a very important, almost inexhaustable, perhaps even its principle source of food in the green symbiotic algae, which continually die (p. 46—48, 57) free in the protoplasm of its amoebocytes, and which then pass there gradually from „colourless algae with clear structure" into the successive „solution stages", „colourless ones with shade of structure", „colourless ones without structure", and „vague shades of colourless algae", in order to finally disappear entirely (p. 42—45). Consequently, here free in the protoplasm of the amoebocytes necessarily an — although rather slow (p. 57),nevertheless — complete break-down and solution takes place of the substances the symbiotic algae consist of; while the products of the decomposition must come to the disposal of the amoebocytes. How this decomposition is produced, I cannot yet decide; but it is evident, that enzymes of the amoebocytes will take part in it.

So it seems, as if the sponge would dispose of two different methods of digestion, 1st the often appearing slow digestion free in the protoplasm of the amoebocytes, 2nd the less common quicker digestion in food vacuoles of the same cells. But is it not more likely, that these two methods are only apparently different and not really? With a slow digestion only little enzyme will be secreted to the body which must be digested, so no vacuole will be formed around; with a quick digestion on the contrary much enzyme, consequently a vacuole is formed. The whole difference between vacuole digestion and digestion free in the protoplasm would thus only be founded on a difference in the rapidity, with which the amoebocyte desires to digest a body (see also chapt. D). With a normal regular course of the process of life there is no need for a quick digestion, only little enzyme is secreted, consequently no food vacuoles are formed.

But if, for instance, a sponge is taken from its habitat to another place, eg. an aquarium with water from the conduit, where it will miss at any rate all kinds of nourishment — if not the symbiotic algae, yet many other materials (dissolved in lake water?) which probably are not less important — it is very likely, that the sponge will then try to supersede its lack of other materials by a quicker digestion of the food, which is at its disposal; in other words, the sponge will be going to secrete more enzyme and form thus vacuoles round its food. Now, according to point **19**, exactly this thing happens with sponges in aquaria! Next the fact, that in newly caught Spongillae, according to point **20**, the food in young tissue is less quickly digested than in full-grown; it can be explained in several ways. But it is not clear at once why, according to point **21**, a quicker digestion takes place in the tissues of colourless Spongillae in darkness (not of Ephydatiae!) than in those of green ones in the light; for there is probably no question at all about a stronger nutrition of the green sponge from the side of its green algae (p. 96), while in the colourless sponge even some more algae are digested in the protoplasm than in the green one (p. 96, 59, 46—48). So one must come to the conclusion, that the colourless Spongilla

wants more food than the green — once more, this cannot have its cause directly in the algal products of photosynthesis — but in what then? Perhaps the following solution holds good:

In connection with what was mentioned in point **16** (p. 93), one must admit, that the green sponge in the light disposes of a very considerable abundance of O_2 in its tissues, which the (colourless) sponge in the dark cannot have, of course. It is now acknowledged in general physiology, that the katabolic phase of metabolism has quite another course in lack of O_2 than in abundance; in lack of O_2 a much more largely, but much less deeply extending break-down of body-materials (proteins, carbohydrates) takes place than with sufficient O_2 — VERWORN (58), HERMANN (28), HAMMARSTEN (26), BIEDERMANN (6), DE VRIES (63) —; indeed, in lack of O_2 much more material is required for obtaining a certain quantity of energy, as the organism for source of energy is then chiefly dependent on not oxidative splittings which procure but little energy, while then, also by the faulty oxidation, a great deal of the chemical energy of the splitting-products is lost for the organism. In this way one would be inclined to explain the fact, that the colourless Spongilla in darkness wants more food than the green one in the light [1]). For the present this is but a suggestion, to which I shall return however later on.

Now that we have seen that the freshwater sponges for a great deal, perhaps even chiefly, find their food in the symbiotic algae, which continually die and are digested and dissolved free in the protoplasm of their amoebocytes, the connection, which there is according to point **7** (p. 89) between the number of oildroplets in the amoebocytes (point **3**) on the one side and the number of dead (colourless) algae in the sponge tissue on the other side, may be explained quite simply. For the sponge tissue disposes of a lipase (point **8**), and in point **1, 2** (p. 84) we found an always decreasing percent of algae with an oildroplet,

1) That this seemed not to be the case with Ephydatia, might be explained from the fact, that the colourless specimina I examined, which contain always some green algae, were partly originating from the light.

according as the digestion of the algae had progressed. So in other words, *just as the amoebocyte slowly digests the other constituent parts of the algal cell and makes use of their decomposition-products, in the same way and at the same time it also digests the oildroplets of the algae, and with their products of splitting (the glycerine and acid) builds up its own oildroplets.* Indeed, we saw (point **3**), that very likely the oildroplets of alga and amoebocyte are not identical!

From the preceding follows, that we may consider the production of the splitting-products of the oildroplets (the glycerine and acid) — so probably also the production of the oildroplets themselves (see below) — in the amoebocytes to be direct proportional to the number of the algae being digested, in other words, to the sum of the number of colourless algae with and of that without structure.

The same thing will count also for the production of the carbohydrate globules, which can be stained brown by I; as source of the carbohydrate we must of course accept — there are no such globules in the algae (point **11**) — either the dissolved carbohydrates and the wall celluloses, or the nucleins, or again the oildroplets of the algae. (The transmutation of fats into carbohydrates, as well as the reverse, we repeatedly meet in physiology.)

According to point **9, 10, 14** and **15** the oildroplets and the globules, stainable by I, are even more numerous in the choanocytes than in the amoebocytes. From those same points it also appeared, that these oildroplets and globules are not captured from the surrounding water by the choanocytes, and that there is no relation between the number of oildroplets or globules and the number of dead algae in the choanocytes. Therefore it is very likely, that these oildroplets and globules are carried on from the amoebocytes through the „intercellular groundsubstance" to the choanocytes; for the amoebocytes with their constant digestion of algae form, as we saw, the true place of production of those corpuscles. But why are these carried on to the choanocytes in such a great number, that they are even more numerous there than in the amoebocytes?

On p. 50 I have treated already, what an enormous quantity of water a tiny sponge makes circulate through its canal-system. This current of water is kept up by the flagellar-motion of the choanocytes. Consequently in these choanocytes a very strong transformation of energy takes place; *those choanocytes, therefore, must necessarily dispose of a great source of energy. What is more evident than that this large mass of oildroplets and of carbohydrate globules would serve as such!* The more so, as we know from general physiology, that in organisms labour, the so called functional metabolism, exactly takes place at the expense of N-free materials, so of carbohydrates and fats — VERWORN (58), HAMMARSTEN (26).

Next we can say that it is also very likely, that the oil-droplets (and carbohydrate globules?) are used in great numbers for the development of the gemmules; for what we have given an argument above.

Let us just look over, what we have stated in the last pages about the production and the consumption of fat in the sponge; and let us try to make up in this way an explanation of the facts, which we derived from Table 12 for colourless sponges from darkness and green ones from light (point **5**, **6**, p. 87—89).

This must be preceded however by another question. Namely: does the great mass of fat, obtained by the sponge from the digestion of the algae, occur in the sponge as oildroplets — the number of which we can easily state by means of the microscope —, or divided into glycerine and acid — which entirely disappear from observation? In other words: may we see in the number of oildroplets, which has been experimentally stated, the total quantity of fat present in the sponge tissues, or, if not the entire, yet a proportional quantity — or may we not do so at all? This is an important question. The (1st and) 2nd supposition sounds most probable; not the 3rd. This question will soon find its solution in the following.

Now return to our subject. We were going to try to make up an explanation of the facts mentioned under point **5** and **6**, with the help of the data about production and consumption of

fat in the sponge. Our data are (calculated per unit of time and of sponge-volume):

1. The production of fat (p) is always proportional to the number of colourless algae in the sponge (pag. 99). We know this number of algae in the different stages of development of the tissue of green Spongillae from light and colourless ones from darkness, from Table 6, pag. 46—48; so we can immediately fix the course of p: p will increase in green and colourless sponges during the development from very young to full-grown tissue, and in colourless sponges it will always increase more than in green ones; at the end of, or shortly after, the gemmule-stage p will decrease considerably and be about the same in green and colourless sponges.

The consumption of fat is determined by the motion of the flagella in the chambers (f) and by the development of the gemmules (g). From this follows:

2. The consumption (f) is proportional to the number of flagellated chambers (we suppose now, that the average of their flagellar-motions is always equal), the number of which is zero in gemmules, but quickly increases in very young tissue, to remain constant afterwards; f does the same. Next — let us say for simplicity's sake — f will be equal in green sponges in light and in colourless ones in darkness.

3. The consumption (g) is only present and then high in the very young tissue during the development of the gemmules, and the same in green and colourless sponges.

From these data follows: I. As p increases more during the development of colourless sponge tissue than during that of green, while $(f+g)$ remains the same in both, there must be, 1st somewhat more fat in young (N.B. not very young!) tissue of colourless sponges than in that of green ones, 2nd in full-grown tissue of colourless sponges much more fat than in that of green ones (point 5, p. 87). II. 1st. As p increases during the development of green and colourless sponge tissue while $(f+g)$ remains constant, the quantity of fat must increase in that period in both sponges. 2nd. As in both sponges p only considerably lowers at

the end of, or shortly after, the gemmule-stage, but $(f + g)$ diminishes already immediately in that stage to about zero the quantity of fat must be very high (maximal) in that period. 3rd As in both sponges p is a minimum in very young tissue, while here $(f + g)$ reach their maximum, the quantity of fat must lower considerably (be minimal) in that period (point **6**, p. 89).

From this we see: 1st *How perfectly the experimentally stated facts about the quantity of* **oildroplets**, *present in the sponge tissues, correspond with the theoretical calculations of the quantity of* **fat**, *which we could make from the co-operation of the factors that proved to rule the production and the consumption of fat in the sponge.* From this follows again: 2nd That the quantity of oildroplets in the sponge tissue must be in fact proportional to the total quantity of fat that is present (see p. 100).

Undoubtedly, such calculations could be made also for the carbohydrates in the sponge tissue. I do not dispose, however, of sufficient facts to test their result. We might still conclude that the fact mentioned under point **13** (p. 92), that the globules which can be stained brown by I in the amoebocytes of colourless sponges are perhaps somewhat more numerous than in those of green ones, will have to be explained in the same way as we did here for the oildroplets.

At last the question: *What is the reason of the dying of the green symbiotic algae within the amoebocytes?* Probably one will be inclined to see this in the fact, that the amoebocytes digest the algae, as we saw above. Certainly, the amoebocytes are sure to digest the dead algae, but have they also purposely killed all of them — i. e. to make them serve as nutrition? Or does the sponge digest them, because (after) all (or some) algae were already dead for quite a different reason? Perhaps one considers this at first sight as a singular and rather far fetched supposition. After due consideration, however, one will acknowledge, that this supposition is not at all so singular and far fetched; on the contrary, that one certainly must consider this possibility too. For the rest I will immediately admit, that the whole question about

the reason of the dying of the algae is especially of theoretical interest and not so much of practical, as indeed all dead algae come to the benefit of the nourishment of the sponge.

It is rather a complicate question, which I am going to treat more in extenso.

I. In the first place we will answer the question, what causes are theoretically possible for the dying of the green algae in the amoebocytes, and at the same time if those causes should have to behave differently in a sponge in light and in a sponge in darkness: 1st Of course: the sponge cells might actively kill the algae with the aim to digest them, so from want of food. Would there be any reason then to accept, that this cause would be active in light in another degree than in darkness? Certainly! One might expect, that the amoebocytes of a sponge in darkness should kill the algae in a higher degree than those of a green sponge in light. For some pages back (p. 97—98) we had several sound reasons for supposing, that a sponge in darkness would need more food than a green one in light. The more so, as we saw on p. 100, that the sponges are very likely to transform relatively great quantities of energy in the flagellar-motion of their choanocytes, in which transformation in case of lack of O_2 not only more N-free materials, but very likely also the proteins themselves of the choanocytes are broken down and, consequently, must be restituted (p. 98). As 2nd cause of the dying of the green algae in the sponge cells comes into consideration: „poisoning" — so to say — of the algae by products of metabolism of the sponge, so without any relation to the want of food and the nourishment-process but, for instance, more as a reaction of defence of the sponge against a foreign intruder. There is no certain proof for this possibility, but some indication in the direction of poisoning of the algae by products of metabolism can be the behaviour of isolated green sponge-algae, when there appears in their culture a strong infection of diatoms, mould, or ordinary green algae. For, as I have mentioned already on p. 43 and 45, the sponge algae die under such circumstances. (On the other hand the cultures — which, as mentioned before, succeeded well —

of green sponge algae in liquid from a pressed sponge (Table 4), cannot tell against this possibility of poisoning; for constant presence of those hurtful products of metabolism might always be connected with the life of the sponge; though then, of course, they cannot be present in great quantity; what is however not necessary here.) Is there, finally, any reason to suppose, that this „poisonous" influence of the products of metabolism is larger or smaller in a sponge in the light than in the dark? This „poisonous" influence taken by itself does not give way to answer the question affirmatively; but if one considers it as a reaction of defence of the sponge against an intruder, then there **is** a reason to suppose, at least to think it possible, that this influence is stronger in darkness than in light. For the following reason: As we shall see on p. 109—112, the production of O_2 by the green alga in light is in fact the only function of life of the alga, in which the sponge can have any direct interest. Of course this function is stopped in the dark, and then the sponge has no interest in the life of the alga any more, but probably will have a reason to fight against it as a foreign intruder, and such more vigorously than it would have done in light. As **3**rd cause for the dying of the algae in the sponge cells comes into consideration: lack of food, and as **4**th cause: lack of O_2 and accumulation of CO_2 in the algae themselves; all of them only for algae in sponges in darkness, of course.

II. Let us now recall to our memory, what facts we have got to know successively about the dying of the green sponge-algae. **1.** Continually algae are dying in the sponge tissue (p. 56—57). **2.** The intensity of the dying in all stages of development of the tissue is constant, with a considerable lowering however in the gemmule stage (p. 57). **3.** Of the same number much more algae die in a sponge in darkness than in light (p. 60). **4.** The intensity of dying in light is about equally high in a weak concentration of the algae in the sponge tissue as in a strong concentration; in the dark, on the contrary, smaller in the first case p. 61). **5.** In free nature the number of dying algae (colourless ones with structure, p. 56) is always very small in a green

sponge in light with regard to the total number of living (green) algae present (p. 46—48, Table 6). **6.** In nature the number of dying algae (colourless ones with structure) in a colourless sponge in darkness is larger than in the green sponge in light (p. 48); this number (in colourless sponge) is almost equal to that, which is continually imported into the sponge (p. 69—70). **7.** If one cultivates green sponges in light in water from the conduit, the number of colourless algae with structure generally appears to increase (p. 95, point **26**). **8.** Isolated and cultivated in cultures, the algae prove to remain living and green for months in light and in darkness, and even to multiply (p. 40—41).

Let us now see, to what conclusion regarding the cause of the dying of the algae within the sponge — point **I, 1—4** (p. 103—104) — we can come in connection with the facts, mentioned here under **II, 1—8.** And let us then begin with the dying in a sponge in darkness.

As we see on the one side (point **II, 5**), that only so few green algae die in a sponge in light and on the other hand (point **II, 3, 6**) so many more in a sponge in darkness, we must come to the conclusion, that in the last case the lack of light is the reason of the so much increased mortality. So we immediately try to find its cause: on the one side (point **I, 1**) in the sponge cells killing the algae much more considerably from want of food and (point **I, 2**) in the stronger „poisonous" influence of the metabolism-products of the sponge in darkness, on the other hand (point **I, 3, 4**) in lack of food and of O_2 and in accumulation of CO_2 in the algae themselves. However, we also know (point **II, 8**) that without the sponge tissues the algae can live for months in darkness and even multiply. Consequently, that lack of light — with its supposed consequence of lack of food and of O_2 and accumulation of CO_2 in the algal cells — by itself cannot possibly be the direct cause of the dying of the algae in darkness. On the contrary, it proves necessary for that manifold dying of the algae to be in darkness in an actively living sponge (point **II, 6, 3, 2,** cf. Table 10). The only and at the same time most general solution would be therefore, that on the one

side the weakened power of resistance of the algae — in consequence of them being in the sponge in darkness, lacking food and O_2 and with accumulation of CO_2 —, on the other hand the still stronger hurtfull influences from the side of the sponge cells (point **I, 1, 2**) — also in consequence of them being in darkness — are the causes, that such a great number of those algae die in the amoebocytes in darkness.

Why only so few algae die (point **II, 5**) in a sponge in light with regard to the total number present, is also quite clear after the preceding. The power of resistance of the algae will be much higher here, in light, than in darkness. The hurtful influences from the side of the sponge, also by itself already less forcible now, will thus destroy much fewer algae in this case than the sponge, which remains in the dark. But there will still always be a small number of algae, that disposes of less power of resistance for some or other reason; this will have to be destroyed by the sponge (compare **II, 8**).

Also the other facts mentioned sub **II, 1—8** are very well compatible with this solution.

At last we get to the question, what those hurtful influences from the side of the amoebocytes, which proved able to kill the living algae with weakened resistance, are exactly. Whether they are: simply (point **I, 1**) a killing from want of food, so a certain feeding-process of the sponge cells; or (point **I, 2**) a „poisoning" of the algae by hurtful products of metabolism of those cells, consequently something, which in fact would have nothing to do with the direct feeding process of the amoebocytes, but for instance should be more considered as a reaction of defence of the cells against an intruder; or, as we have just formulated above, both causes together. In all three the cases, however, the final result remains the same, namely that the algae killed in one of the 3 ways are digested at last by the amoebocytes. So this question has in fact not much practical use; it is more of theoretical interest, as I remarked above.

Also, no definite answer can be given for the present; there are arguments for both points (**I, 1** and **I, 2**). This can be shown

easiest in consequence of the fact (point **II, 3, 5, 6**), that the green algae in a sponge in darkness die in such a great number, in light on the contrary only in such a small. Against the opinion, that stronger „killing from want of food", so the increased want of food, in darkness (point **I, 1**) would be a cause for the manifold dying, tells:

a. The fact that, as appears from Table 8 and Table 15, several of the (green) sponges, which have become almost colourless in dark, contain only few food vacuoles, so behave as if they did not specially want food, as stated on p. 97—98 (see nrs 333, 334, 340, 344, 366 I, 366 II).

b. The fact, mentioned on p. 94 sub **19**, that there are about the same number of food vacuoles in green sponges after their culture in aquaria in light as in darkness; in other words: that the want of food would not be larger in sponges in darkness than in those in light.

By these facts the chief argument, that „killing from want of food" could in general be a cause for the dying of the green algae in the amoebocytes (p. 106), would fall.

With a view to this we would do better to exclusively admit as cause for the dying: „poisoning" of the algae by products of metabolism present in the amoebocytes, stronger in the dark and active in all cases when for some reason the power of resistance of the algae has weakened (p. 103, point **I, 2**, p. 106).

On the other hand, however, there are even more and stronger arguments which speak for the fact, that „killing from want of food" (p. 103, point **I, 1**; p. 106) certainly must be a cause for the dying of the algae in the sponge. I therefore refer to the points **II, 7** and **4**. That increase of the mortality of the algae, when the green sponges are transported into water from the conduit (in light) — where the sponges will have to miss many food materials, which they found in nature, so that they will have to make up for it in some way or other (see p. 97) — shows very much in the direction of „killing from want of food". But point **II, 4**, the fact namely, that the mortality in weak concentration of the algae in the sponge in the light is equal to that

in strong concentration (per unit of sponge volume) can even only be explained by this last cause for the dying. From this would follow, that a sponge in light, whether it lodges many or few green algae, wants one and the same number of these for food. It is quite clear, why (**II, 4**) in a sponge in darkness the mortality in weak algal concentration is less than in a strong concentration: in darkness the mortality is much larger than in light; if however the concentration is weak, it is impossible that many algae die. Why (**II, 2**) the mortality in all stages of development of the tissue remains constant, could be explained by „killing from want of food" as well as by „poisoning by products of metabolism".

Finally there are arguments which show, that in sponges in darkness there is certainly reason to speak of an increased want of food — in consequence of lack of O_2 —; which would make a stronger „killing of the algae to digest" from the side of the sponge cells quite intelligible (p. 103, point **I, 1**, p. 106):

α. The argument, mentioned on p. 97, that in colourless Spongillae from darkness, examined when newly caught, the number of food vacuoles appears to be greater than in green ones from light (p. 94, point **21**); which argument formed exactly the starting point for the supposition about the changed katabolic phase in lack of O_2 (in this case = lack of light) (p. 98).

β. When speaking about the growth of the sponges, I have mentioned (p. 53) that one could generally state, that green Spongillae (in light) are larger, so grow more quickly, than colourless ones (in darkness). What can be the reason? Very likely, there is no question of a stronger nutrition of the green sponge from the side of its green algae, while even somewhat more algae are digested in the colourless sponge than in the green one, as mentioned on p. 97. Consequently, there is no question about, that the difference in rapidity of growth is founded on a smaller quantity of food, which the colourless sponge would have at its disposal. But it will have to be founded on a greater want of food, namely chiefly of proteins, in consequence of lack of sufficient O_2 in the tissues of the colourless sponge in darkness (p. 98, 103, **I, 1** and p. 88).

When we look over the results obtained, we might conclude the following causes for the dying of the algae in the sponge tissue: **A.** *In light in fact only „killing from want of food" (point* **I, 1***), and such of the (few) algae, the power of resistance of which is somewhat weakened already for some or other reason;* but not „poisoning" by products of metabolism (point **I**, **2**) nor lack of food, lack of O_2, and accumulation of CO_2 in the algae themselves (point **I**, **3**, **4**). **B.** *In darkness either „killing from want of food" (point* **I, 1***) — perhaps even stronger now — or (and) „poisoning" by products of metabolism (as reaction of defence of the sponge against a foreign intruder) (point* **I**, **2**), *and such again of algae, the power of resistance of which has certainly weakened now much more, in consequence of lack of food or of O_2 and accumulation of CO_2 (point* **I**, **3**, **4**).

For the present a more detailed conclusion cannot be given; more data are required for that, among others about the problem if there is really question about lack of O_2 — with all its consequences, as for instance increased want of food — in the tissues of a sponge in darkness.

Let us now pay attention to the two points (α, β, p. 108), regarding this last problem and which are so important, because they give us an insight into the *significance, which the O_2, secreted by the green algae in light within the sponge tissue, might have for the life of the sponge.* One cannot say, that the conclusion, made in connection with those points α and β, quite satisfies us.

The hypothesis, that in lack of O_2 the katabolic phase of the metabolism will have quite another course (than in abundance of O_2) and consequently will cause an increased want of food — as is given on p. 98 and 103 sub **I**, **1** — may be right in general, it seems a bit far-fetched to consider this hypothesis applicable to our case of a sponge in darkness. Certainly, the sponge in darkness will possess a much smaller quantity of O_2 in its tissues than the green sponge in light. But is there really lack of O_2, while sponges have even an extremely strong circulation of

water? Should one not rather suppose, that by this circulation the necessary quantity of O_2 will already be kept up sufficiently in the tissues of the sponge in darkness, though it will be lower than in the green sponge in light — perhaps the green sponge in light wil not do anything with its larger quantity of O_2 and simply let it slip —? If this were not the case, how would then be the course of that katabolic phase in all higher water organisms not lodging chlorophyll, when lack of light already changed it so enormously in Spongillae (that is to say, made it go on accompanied with freeing of but relatively little energy). One then had to come to the conclusion, that all these higher water organisms, not lodging chlorophyll, can only produce less complete oxidations in their katabolic phase of metabolism (that is to say, less complete than the green Spongillae in light).

On the other side one has to acknowledge that it is difficult to imagine, that this large quantity of O_2, which **is** present in the tissues of a green sponge in light — I just remind that, according to p. 18*a*, green Spongillae in sunlight even develop gasbubbles (very likely of O_2) — would have **no** considerable influence on the katabolic phase of metabolism of the sponge. Besides, how would otherwise the phenomena, mentioned on p. 108 α β, have to be explained? [1])

But let us leave now this question about the lack of O_2 (with all its consequences) in the tissues of a sponge in darkness. It cannot be dissolved before we have stated experimentally, if in fact Spongillae in darkness excrete less complete oxidated products of metabolism than green sponges in light. Certainly this question would be worth such a research! I hope to be able to do this afterwards.

Also the question which was our starting point on p. 106, namely: which in fact are the exact causes of the dying of the algae in the amoebocytes, must, as said before, wait for its decision. The best thing is that, for the present, we content our-

1) And how (see p. 51 note) the perhaps smaller „filtering power" of a (colourless) sponge in dark?

selves with the general formulating of the answer given on p. 109.

The final result of our research into the use of the „symbiotic" association (of sponge and green alga) to the sponge is therefore:

It is either the want of food of the sponge — which might be strongly increased in darkness — or (and) the „poisonous" influence of harmful products of metabolism of the sponge (to be considered as a reaction of defence against a foreign intruder) which continually destroys green symbiotic algae in the amoebocytes; and exactly those algae, the power of resistance of which is already weakened for some or other reason — for instance by having been in darkness — (p. 102—109). All algae killed in this way come to the benefit of the nourishment of the sponge; as this one digests and dissolves them entirely either free in the protoplasm of its amoebocytes or in food vacuoles, keeps the products of the decomposition (p. 96—97) and rebuilds its own cell parts with them, namely for instance the oildroplets and carbohydrate globules (p. 98—102). These oildroplets and carbohydrate globules in their turn are, among others, the source of the great quantity of energy, which the sponge transforms in the flagellar motion of its choanocytes (p. 100).

For the present no decision can be given about the exact significance for the life of the sponge of the O_2, which the living green algae in light secrete within its tissues (p. 93). It may be, that this O_2 is of much significance; even so much, that the katabolic phase of the process of metabolism in a green sponge in light has quite another course by it — namely gives a relatively much larger quantity of energy to the sponge — than in the sponge in darkness (p. 98, 103, 109—110). Some indications were found for this; but this important question requires quite a seperate research, before anything can be said for certain.

As we saw (p. 96, 87, 92) it is very likely, that direct transfer of products of photosynthesis from the living green algae into the sponge tissue does not take place at all.

When next we ask, what in fact the „symbiotic" relation of

sponge and green alga is, considered from the point of view of the use to the sponge, we cannot very well answer that question, before the problem, mentioned above, about the significance for the sponge of the O_2 secreted by the green alga in the light, has come to solution:

a. *If the significance of that O_2 is in fact so important, as was thought possible above, we must conclude — notwithstanding the fact, that the sponge continually destroys and digests numbers of algae, and notwithstanding all other phenomena, which do not seem to go together with a symbiosis — that the relation of sponge and green alga, considered from the point of view of the use to the sponge, is in fact a symbiosis, though this symbiosis is by no means so complete as that of the Lichens.*

b. *If, on the contrary, the significance of the O_2 secreted by the alga is only of little importance, we can conclude — whatever may be the real cause of the dying of the algae in the sponge tissue, wether it be the want of food of the sponge or (and) the „poisoning" of the algae by products of metabolism of the sponge — we* **must** *conclude that, practically spoken, that so called symbiotic relation of sponge and alga is in fact nothing but simply a process of nutrition of the sponge, or, if you like, a very first transition of a process of nutrition into a symbiosis. At any rate this always counts for a sponge in darkness.*

For we could state the following:

The sponge continually imports green algae from the surrounding water into its amoebocytes (p. 50), where those algae then — it should be explicitly mentioned — are killed and digested (p. 111) by the sponge only for a part, when circumstances are favourable; while the rest of the algae can live on, photosynthesise and multiply (and will give their O_2, produced in light, to the sponge tissues (p. 93) — the only argument one can mention in favour of the conception of symbiosis!). This favourable case is only realized in sponges growing in light (p. 70—72) — for in light $i > mo$ [1]) —

---

1) This follows from p. 70 IV, p. 51, and p. 60—61, 59. In darkness $i = mo$; so in light $i > mo$.

and then not even always (p. 41, 75—76). If, however, the circumstances are somewhat less favourable — as is the rule in sponges in darkness (p. 69—70) and as sometimes happens also in those in light (p. 41, 75—76) — then all imported algae (and all that might be present already) are continually and unavoidably destroyed and digested by the sponge (p. 111).

What happens to the number of green algae of a sponge under certain circumstances, entirely depends upon the value, which each quantity takes under those circumstances in this formula:

$$i + r + mu \gtreqless e + g + mo$$

the formula, which we have got to know on p. 68—75 as decisive for the number of green algae of a sponge.

For the „symbiosis" considered from the point of view of the use to the alga, I refer to p. 83—84.

In order to show even more clearly, how much the relation of fresh-water sponge and green alga is still removed from a real symbiosis (in the sense of mutualism), I want to mention what we should require from a relation between two organisms, which are closely connected, to be justified in calling this relation a symbiosis (mutualism): That relation should be one of mutual use; the symbiontes should be interested in each others existence, so spare, if possible, even nourish each other. Both the symbiontes should in fact behave as one, new individual — in extreme cases, for instance, not be able to exist one without the other and die together. The symbiosis should be kept up simply by multiplication of both symbiontes; but should not need a continual supply from outside (import) of one of the symbiontes, to restitute the destroyed ones.

So Noll (56) says about the Lichens: „Die Pilzhyphen umspinnen im Flechtenkörper die Algen, überlassen ihnen den zur Assimilation günstigen Platz ...., treten mit ihnen in innige Berührung und entziehen ihnen einen Teil ihrer Assimilate. Dafür

liefert der Pilz nicht nur das, durch oft kräftige Säure-ausscheidung gehaltreich gewordene Nährwasser, sondern, wie es nach den Untersuchungen ARTARIS wahrscheinlich ist, auch Pepton, so dass die Algen in dem Flechtenkörper nicht nur nicht erschöpft werden, sondern sogar sich kräftiger entwickeln als in freiem Zustande und sich durch Teilung lebhaft vermehren." And SCHENCK (56): „Die Flechten besitzen untereinander so viel übereinstimmendes in Bau und Lebensweise und haben sich als Konsortien" (of fungus and alga) „phylogenetisch weiter entwickelt, so dass sie zweckmässiger als besondere Klasse behandelt werden .... Die Symbiose der flechtenbildenden Pilze mit Algen führt zur Bildung von zusammengesetzten Organismen mit eigenartiger Form des Thallus, welcher entsprechend seiner durch die Algen bedingten selbständigen Ernährungsweise andere Gestalten als bei den nicht flechtenbildenden Fadenpilzen aufweist.... Nur für ganz wenige Flechtengattungen ist festgestellt, dasz ihr Pilz auch ohne Algen in der Natur existenzfähig ist".

I will not finish this chapter, before I have said somewhat more about the often mentioned (p. 111, 103, 109, among others) „poisonous" influence of the products of metabolism of the sponge, which influence would be deadly to the algae. There was no certain proof for its existence, but there were arguments for its probability. For if it might prove that the want of food of the sponge, mentioned on p. 111 and 109, cannot be accepted as a cause of the dying of the algae in darkness, there remains as only possible the one mentioned by the name of „poisoning by products of metabolism". The term is vague, but all the same gives exactly what one would have to understand by it.

Of course, I am not going to take the question once more in consideration, which I said on p. 110 to leave till later on. But I have made some interesting finds among my sponge cultures, which certainly justify the question, if the sponge could exert poisonous influences on other organisms (c. q. the algae), which have come inside its body. In other words, if there would not

be some reason to accept, that here we should have to do with a true reaction of defence of the sponge against the foreign intruder, in the way an unhealthy organism defends itself against the infector.

I have found: 3 different types of algae (2 filamentous and 1 unicellular), which appeared to occur in great number in the tissues of several Ephydatiae (from the Brasemer lake near Leyden and cultivated in my aquaria) (see chapter IX). Two of these, the filamentous algae, proved to destroy entirely the sponge tissue with their growth; the 3rd, the unicellular one, on the contrary, after having penetrated the sponge tissue in the beginning — the original colourless sponge had become light-green by it — was finally conquered and destroyed by the sponge.

In the first place one could imagine this destroying as caused by ferments of defence of the sponge, the above mentioned „poisonous" influence of products of metabolism. But there is still another possibility. It proved to me, that a sponge may eject such unicellular „infecting" algae from its body together with detritus (for instance captured carmine grains). (See lateron, in the description of the defecation-process, chapt. E.)

So here we have seen cases of positive infection of the sponge by filamentous algae, by which the sponge was destroyed. But we also saw a case, in which the sponge finally succeeded in conquering and destroying the intruder — the unicellular alga — which was spreading further and further.

That unicellular alga appeared to be probably a Pleurococcacea, so closely related to the „symbiotic" alga of the Spongillidae (p. 34). Is it not evident then, that one is inclined to see also something in that „symbiosis", which is like an infection? An infection, against which the sponge must also defend itself?

There is still another view in connection with the case of the mentioned unicellular alga. If the „relation" between sponge and „symbiotic" alga is no other than was mentioned (p. 111—113), it might be possible, that the sponge does not want one certain

alga for such a „relation", but that it would content itself with all sorts of other unicellular algae, according to circumstances. So for instance, in the case mentioned above, with a relative of the normal „symbiotic" alga. In this way one might think possible, in different countries, an association of our fresh-water sponge with quite different algae!

IX. Some other algae occurring in the tissues of Ephydatia.

After the theoretical consideration to which these cases gave reason I want to give now a short description with illustrations of the 3 infecting filamentous and unicellular algae which I have found. I must add that I have met with these algae not once, but in several specimina of Ephydatia (never in Spongillae); but only in one spring, and never in sponges immediately from nature, but always in specimina which had been in my aquaria for some time (2—3 months). Generally all 3 of them were to be found together in one sponge, but the unicellular alga also very often alone.

The Ephydatiae infected by filamentous algae were to be recognized at dark-green, almost emerald irregular spots here and there in the colourless or light-green normal sponge tissue. The sponges were also (partly) surrounded at the outside by those algae, which freely spread in the water. Under binocular microscope one could observe very distinctly that those green spots might be continued till within the sponge body, so under the normal tissue; but generally they were to be found in tissue layers more at the surface.

If one examined a piece of such a green tissue-spot under oil-immersion, one found parts, where nothing but the skeleton of the sponge tissue had remained, but the place of the cells was entirely filled in by filamentous algae; and next to that some almost intact sponge tissue parts, in which however among the normal sponge cells filamentous algae began to spread. One could also see how such a growing algal filament quite makes an exterior wall of the sponge protude (tent shaped), when trying to pierce it from within.

On closer examination of the filamentous algae there proved to be two different types present in the tissue at the same time (Fig. 43—45, drawn after living material). The last one (Fig. 45) consisting of long, 5—6 μ thick, unbranched filaments with girdle-shaped chloroplast appeared free in the aquarium as well as in the sponge tissue. I think it to be Ulothrix subtilissima. The other one (Fig. 43, 44), however, was never free in the aquarium but only to be found in the sponge tissue. As one can see from the illustrations (Fig. 43, 44), it consisted of often very irregularly branched filaments, consisting of cells which could have all sorts of shapes from cylinder- to almost ball-shape. The chlorophyll was scattered all over the cell without any regularity — probably in a great number of chromatophores, while very likely each cell contained one nucleus. The filaments were about 8—9 μ thick. As with a view to my other investigations I could not spend much time on studying these infecting algae, I have not been able to discover their mode of reproduction (nor of the other filamentous alga). Nevertheless I want to draw attention to the fact that this alga, given in Fig. 43 and 44, is much like the Trentepohlia spongophila, which professor Weber and Mrs. Weber-Van Bosse (64) found in 1890 also in the tissue of Ephydatia fluviatilis, but of specimina originating from a lake on Sumatra.

Finally I want to speak about the unicellular alga which, as mentioned, appeared either alone or with the two filamentous ones in the tissue of Ephydatiae. Where of course the latter were situated between the amoebocytes, the unicellular one occurred just within the amoebocytes, mostly free in the protoplasm, but sometimes also in different stages of digestion within food-vacuoles. The alga is shown in Fig. 46—52 (drawn after living material). The shape was oval, the diameter 5,5—7 μ. The cell contained: a chloroplast lining the wall, which let the centre of the cell free and seemed to be one time single and another time composed of several parts; next a rather big refractive globule and a number of small refractive points, which, with different adjusting of the microscope, were one time lilac-brown and another time blue-green. The wall was rather thin. I have also found a stage of division

(Fig. 52); from which we see that „freie Zellbildung" does not take place, but simple vegetative division of the whole cell. Though I have not been able to give sufficient time to the research into this alga either, I came to the conclusion that we have probably to do with a Pleurococcacea, so with a relative of the ordinary „symbiotic" alga of the fresh-water sponges.

Besides the 3 „infecting" algae mentioned here, there were of course also the normal „symbiotic" algae in the tissue of the „infected" sponges, but their number was always relatively small. It stands to reason, that there might be in general inside the sponges — in the canal-system for instance — also quite other kinds of algae as well as protozoa and also diatoms, which might of course be captured at their turn by the sponge tissue to serve as food, and so might get into the amoebocytes. But this category does not come into consideration here at all. Here we have only to do with the algae, which appear and keep up either regularly (the symbiotic alga) or accidentally (the 3 infecting algae) in a great number within the sponge tissue.

B. THE CURRENT OF WATER IN THE CANAL-SYSTEM OF THE FRESH-WATER SPONGES.

As mentioned in the Introduction, I have found out a method, which enables us to observe wholly intact, normally living tissue of sponges with an oil-immersion for many hours, on several consecutive days. The way in which the necessary microscopic preparations were obtained is indicated above on pag. 12—13. It was by means of these living preparations that I have been able to state, that the generally acknowledged theory concerning the cause of the current of water through the sponge body is not right, as it is based on a mode of movement of the flagella of the choanocytes, which proved to me abnormal and caused by exhaustion.

Anatomy. — Before proceeding to the examination of the

watercurrent, I want to give a description and a diagrammatic illustration (Fig. 53) of the canal-system in fresh-water sponges. They are taken from DELAGE and HÉROUARD (16) 1899: „..... La surface (de la Spongille) est soulevée par les extrémités saillantes des spicules (Fig. 53 *cnli.*), qui lui donnent un aspect hérissé; çà et là se voient quelques oscules (*os.*), assez larges, irrégulièrement distribués. L'Eponge est partout, sauf naturellement au niveau des oscules, revêtue d'une mince membrane dermique (*ects.*) dans laquelle sont percés les pores (*p.*)" (better: ostia) „et qui forme la voûte d'une vaste cavité hypodermique. Cette voûte, malgré sa minceur, contient des éléments mésodermiques, parmi lesquels des cellules contractiles, et est tapissée sur ses deux faces d'un mince épithélium de pinacocytes. La cavité hypodermique (*cv. hy.*) est continue mais traversée çà et là par des spicules ou des faisceaux de spicules, qui se dressant des parties profondes, soulèvent sans la percer la membrane dermique. Le plancher de la cavité hypodermique formé par la surface du choanosome est criblé de trous, de taille très inégale, qui sont les orifices d'entrée du système inhalant. Les canaux inhalants (*cn. inh.*) plongent dans le choanosome et s'y ramifient largement, mais sans aucune régularité ni dans la forme, ni dans la distribution de leurs branches. — De chaque oscule part un large canal qui plonge directement dans la profondeur, formant une cavité atriale irrégulière (*cv. atr.*) d'où partent en tous sens des canaux exhalants (*cn. exh.*) d'abord à direction tangentielle, puis ramifiés dans toute l'épaisseur du choanosome sans plus de régularité que les canaux inhalants, ni sous le rapport de la forme, ni sous celui de la distribution. — De la sorte, l'Eponge tout entière est réduite à un système caverneux de cavités extrèmement irrégulières, les unes inhalantes plus étroites, plus canaliformes, les autres exhalantes plus spacieuses, plus camériformes, intriquées en tous sens, réduisant la parenchyme (*chs.*) à des cloisons peu épaisses. — Mais, au milieu de cette irrégularité, une règle persiste, absolue: c'est la non-communication directe des systèmes inhalant et exhalant, qui restent séparés. Toutes les lacunes inhalantes communiquent entre elles, toutes les exhalantes

de même; mais pour aller des premières aux secondes, on se heurterait partout à une cloison de choanosome. Dans ces cloisons sont les corbeilles, petites, arrondies, dépourvues de prosodus et d'aphodus, s'ouvrant d'une part dans les lacunes inhalantes par deux à cinq petits orifices prosopylaires et d'autre part dans les lacunes exhalantes par un large orifice apopylaire, ce qui permet de distinguer sur les coupes les deux sortes de lacunes. Dans ces cloisons sont aussi, entre les autres éléments mésodermiques.... les spicules..... formant dans le réseau du choanosome un réseau squelettique..... Ces spicules sont soudés entre eux, soit dans toute leur longeur, soit par leurs extrémités seulement, par la spongine".

Delage and Hérouard's illustration with few alterations (Fig. 53) gives rather a good idea of the canal-system; it is, however, but a diagram. A better illustration of the surroundings of the flagellated chambers in Spongillidae is given by Fig. 54.

Finally a beautiful illustration of a flagellated chamber of Spongilla lacustris (Fig. 55) is taken from Vosmaer and Pekelharing (61). The authors mention that this figure was drawn with great care from a very carefully preserved preparation. The apopyle (*ap.*) and the excurrent canal is shown. The figure represents a section of a chamber; therefore we see but one flagellum at its real length. The thin line uniting the bases of the choanocytes represents the outline of the chamber. So we see that the chamber is lined by the well known choanocytes, whose collar and flagellum can be easily distinguished in the figure as well as their nucleus, a vacuole (at the base of the flagellum) and a certain number of black spots, which are doubtless the above mentioned (p. 90, 9) oildrops. Finally I want to mention that the choanocytes are able to entirely retract their collar and perhaps also their flagellum.

Function. — Proceeding to the description of the water-current in the canal-system, I want to remind in the first place that the water enters the inhalant (incurrent) system through the ostia, it then passes from the incurrent canals trough the proso-

pyles into the flagellated chambers and from there through the apopyles into the exhalant (excurrent) system, in order to finally leave the sponge body by a large osculum (Fig. 53, 54).

This current of water is caused by the movements of the flagella of the choanocytes in the flagellated chambers, as is generally acknowledged. The great question which had been discussed for ever such a long time, and which seemed decided in 1898 by the research of VOSMAER and PEKELHARING (62) — as mentioned in the Introduction — was: what is the exact way in which the flagella move; how is the movement of the water within the flagellated chambers; how is the whole water-current explained?

It is a matter of course that, on account of the difficulty of the research, one has not been able to make many direct observations concerning this question. So LIEBERKÜHN (38) says to have seen the movement of the flagella in Grantia botryoides (a calcareous sponge), viz. „Wimpern" which „äusserst lebhaft schwingen"; he does not give more details. BOWERBANK (7), however, says about the flagella of Grantia compressa: „When in vigorous condition their motions are rapid and cannot readily be followed, but in some in which the action was languid, the upper portion of the cilium was thrown gently backward towards the surface of the sponge, and then lashed briskly forward towards the osculum, and this action was steadily and regularly repeated. Their motions are not synchronous, each evidently acts independently of the others". v. LENDENFELD (37) says — I quote from VOSMAER and PEKELHARING —: „it appears that the cilia in the entodermal collar-cells move, pendulum-like, backward and forward, similarly to the cilia of the polyciliar epithelium-cells in the respiratory-tracts and other parts of vertebrates" — a remark, however, apparently not based on observations. Finally COTTE (13): „on peut voir le mouvement des flagella se produire sous forme d'ondes, avec un rythme particulier au moment où se fait l'observation, mais qui à ce moment est le même pour tous les flagella d'un même territoire" (so in the way of polyciliar epithelium). „Par contre, à côté des cellules à mouvement régulier, on

en trouve d'autres qui ont une allure absolument désordonnée". And: „..... le mouvement des flagella est comparable à celui d'un fouet..... le mouvement de l'eau resultant de l'action des flagella doit être, dans l'ensemble, perpendiculaire à l'axe des choanocytes".

In close relation to these interpretations of the motion of the flagella (as Fig. 56 *d*, 57 *d*) is the way in which the movement of the water within the flagellated chambers was supposed to be. In the theory of BOWERBANK, LENDENFELD (and of COTTE) one should imagine this movement to be regular and rapid, passing from the prosopyles through the chamber to the apopyle. In this way, however, it would not be clear how the sponge is able to capture the food particles from the circulating water; for the greatest deal of it would flow rapidly through the canal-system without ever having been in contact with the cells lining the canals! COTTE resolves this difficulty in the following way: „Il est certain que cette disposition morphologique" (of the prosopyles) „a pour résultat la formation d'un remous ou d'un tourbillon au point où l'eau pénètre dans la corbeille vibratile. Dans cette dernière les flagella, par leurs battements actifs, produisent un brassage énergique de l'eau et par conséquent des particules en suspension dans celui-ci". And: „Il y a en ce point ..... contact plus intime de l'eau et des aliments qu'elle renferme avec les choanocytes qui sont les organes de l'absorption". But this seems to be based on reasoning, not on personal observation.

Therefore it has been the great importance of the theory of VOSMAER and PEKELHARING (62), that, based on experiments and observations of the motion of flagella and the water-current itself, it explained these phenomena in such a way, that it was evident at once that such a movement of the water in the flagellated chambers, as was stated by the investigators, was exceedingly fit for bringing food particles within reach of the sponge-cells.

I am now going to treat VOSMAER and PEKELHARING's theory more at large, as it has also been the starting-point of my own research. Their principal experiment, giving most important observations, was made directly in the neighbourhood of the habitat

of the sponge on a thin-walled Leucosolenia, a tube-like calcareous sponge, the inside of which is entirely covered with choanocytes. I will quote now: „A piece of about 1 c.m. was cut from a tube and then split open and immediately observed. The piece was covered with a cover-glass; but this could hardly harm the choanocytes, as it was carried by the apical rays of the tetrascleres. The preparations were observed with ZEISS's homog. imm. 1.40, 3; Oc. 12. It was evident then that the flagella were beating quite independently from each other, all in different directions" (as Fig. 56 *d*, 57 *d*, 58 *b*). „The movement of each flagellum was not always in the same plane, and one moment it was stronger in one direction, another moment stronger in another. Sometimes a flagellum was stretched for a while almost horizontally. It happened also that one or more flagella were motionless, in order to beat again vividly a few moments afterwards. Every now and then flagella crossed, without ever becoming entangled. Particles suspended in the water were whirling about, never carried forward. In short, the aspect of the motion was absolutely different from what is observed in ciliated membranes of higher animals. There was no trace of a coordination of neighbouring cells".

The authors continue:

„.... this mode of motion cannot be but advantageous for capturing particles by the choanocytes. This is not only the case for choanocytes forming flagellated chambers, but eminently so for those lining the cloacae of Leucosolenia. If all the flagella lashed briskly towards the osculum, the particles, entered through the pores, would be directed chiefly towards the axis of the tube and rapidly removed through the osculum. On the contrary the movement of the flagella has the effect that particles can easily reach the collars and thus come into contact with the protoplasma of the choanocytes".

„Moreover it seems possible to us, to explain by the irregular motion of the flagella, the regular current through the canals of the sponge, as this is so often observed, and so carefully studied by GRANT ..... it seems to us that in the living sponge the water would find more resistance in flowing out from the chamber

through a pore than it finds in streaming in. For we found that in carefully mounted preparations where all the choanocytes remained fixed in their place, these cells surround the pores closely and are placed, not exactly perpendicular on the wall, but somewhat oblique, so as to narrow the cloacal opening of the pore. We found this to be the case in a flat, stretched piece of Leucosolenia. Their position must be all the more oblique in the living state if the wall is of course not flat but concave. If therefore in the cloaca the pressure of the water becomes higher, the collars of the choanocytes will become somewhat inflated and the pore will be narrowed. If, on the contrary in the cloaca the pression of the water in the neighbourhood of a pore is lessened, water can easily flow in through the pores; the choanocytes with their collars thus act as valves. Now by the irregular motion of the flagella the pressure on the wall of the tube, which by the spicules is kept rigid, is continually changing. If the pressure becomes higher, this is of little effect, but if the pressure becomes less, water will flow in through the pores, as long as they are open. The sponge will thus suck in water, which will leave the body again through the osculum".

Thus VOSMAER's and PEKELHARING's theory. Then the investigators call attention to the fact that the arrangement of the canal system of the sponges becomes more and more appropriate according as one examines higher developed forms.

Personal Research. — *How much this theory of* VOSMAER *and* PEKELHARING's *may correspond with their observations and how well it may make us understand that with such a movement of the water a great number of foodparticles are brought within the reach of the choanocytes, it could not quite satisfy me from the beginning. It appeared very unlikely to me that the flagellar motion of the choanocytes should principally be quite different from that of the unicellular Flagellata eg. the Choanoflagellata, which for the rest remind us so much of the choanocytes.* This supposition was the more tempting, because it would make us quite independent of the synchronism (whether existing or not) and of the direction

(whether mutually deviating or not) of the separate flagellar beatings.

The movement of the flagella, which had hitherto been established in the choanocytes, was a rowing-movement (as Fig. 56 *d*, 57 *d*, 58 *b*); so a motion which is not peculiar to the long flagella but to the short cilia of the protozoa; while, on the contrary, the flagella of the unicellulars are moving in spiral-lines (compare Doflein 17). If the latter, now, could be proved to be also the case with the choanocytes, than the solution would be quite easy. For the spiral- or screw-motion of the flagellum of the unicellulars pushes the water on, just as the screw of a steamer, in the direction of and turning round the axis of the flagellar spiral, either towards the cell (Flagellata) or in the opposite way (spermatozoa). — This is simply determined by the fact, whether a spiral wave (optically) moves on from the top to the base of the flagellum or in the opposite way; but I will not enter further into this question here. — If in the choanocytes the motion of the flagella took also place in this manner (as a spiral), it is a matter of fact, that by the action of all choanocytes of a flagellated chamber together there should be a constant flow of water either from the centre of the chamber towards the wall or from the wall towards the centre, provided that the spiral-motions of all choanocytes moved on in the same way. We would however not have anything to do with their synchronism or their direction. Now, according to Doflein (17) the Choanoflagellata push the water off along the axis of the flagellar spiral. Might it not be possible then that the same should count for the choanocytes too?

The question was therefore to observe the movement of their flagella under circumstances that were as normal as possible. On the other hand one had to claim, that the study of the flagellar motions and of the water-current, produced by as many choanocytes together as there are within a flagellated chamber, had to be preceded by an accurate research into the motion of the flagellum of one, isolated, choanocyte and into the water-current it caused in a free and open space.

Now, I can state that I have succeeded in both ways. *The study*

of the movement of the isolated choanocytes, as well as of that in the intact flagellated chambers, has shown me that the flagellar motion, under normal circumstances, is in fact the same as that of the Flagellata, viz. the Choanoflagellata: a spiral- or undulating-motion; but that this movement, after exhaustion, very soon changes into quite a different one, viz. the rowing-motion.

The research into the motion of the flagella of isolated choanocytes was made by me in February 1915. In fact it is best to be done in winter, at a low temperature. For one has to make use of ravel-preparations (p. 12) of living sponge tissue, which one watches as soon as possible with oil immersion in an ENGELMANN case. As in winter Spongilla dies, only Ephydatia was to be used for this research.

I shall now give a description and some illustrations of the flagellar motion, which could be beautifully observed owing to the isolated situation of the choanocyte at the top of a number of other cells. For the observation, namely, one must have the flagellum isolated, but on the other hand the choanocyte itself may not be separated from the others, but it must, just as in the flagellated chamber, be fixed quite fast with its base to have a support against the heavy vibrations of its flagellum. I have noted the description literally during the observations and the figures were drawn immediately from the living material:

5.55 p. m. (immediately after isolation) *Flagellum shows very rapid undulating-motions (probably in spiral-line) of small amplitude. The water with the particles is pushed away strongly, straight through the axis of the flagellar spiral, while at the side it flows on to the base* (Fig. 56 *a*).

5.57 p. m. The amplitude of the flagellar motion becomes much greater, the movement less quick. The water with the particles is still pushed on through the axis of the spiral, while at the side it flows towards the base (Fig. 56 *b*).

6.00 p. m. Just a slight undulating-motion to be seen, very large amplitude, even less rapidity. The water with

the particles is at first stirred to and fro, but finally pushed away in upward slanting direction (Fig. 56 *c*).

6.10 p. m. The last movement (Fig. 56 *c*) was evidently a transition from the spiral- or undulating-motion to this new one: *A slow* [1]) *beating to and fro of the flagellum, without waves. The water with the particles is moved to and fro, but it is not pushed away* (Fig. 56*d*).

6.15 p. m. *The flagellum stops* in a more or less straightened condition (Fig. 56*e*).

To this I must add that after the phase, given in Fig. 56 *a*, the motion of the flagellum became less regular; it was interrupted by resting-periods of unequal, but ever increasing length till 6.15 p. m., when it stopped finally. There was no collar to be seen in this choanocyte, evidently it had been retracted.

One will acknowledge, now, that the first phase (Fig. 56 *a*) of the flagellar motion is in fact quite different from that which the investigators have hitherto observed. It goes without saying that the water current caused should prove quite a different one also. But there is still more to be seen in the figures: the mode of motion of the flagellum, hitherto described (by BOWERBANK, LENDENFELD, COTTE, VOSMAER and PEKELHARING), fully agrees with our phase given in Fig. 56 *d* — even the current of water! Now, the phase of Fig. 56 *d* however is quite abnormal, and caused by exhaustion, as after 5 mnts. it is already followed by final stagnation of the flagellar movements. The explanation, why these last phases (Fig. 56 *c*, *d*), the rowing-movement, have always been found by the investigators and never the first one (Fig. 56 *a*), the screw- or undulating-motion, is quite clear now. Moreover, I will just call attention to what BOWERBANK said, as I quoted already on p. 121: „When in vigorous condition their motions" (of the flagella) „are rapid and cannot readily be followed, but in some in which the action was languid" etc.! *Probably one has always observed the movements of more or less exhausted flagella.*

1) That is to say, compared with the former intense motion; it still goes rather quickly.

I say „probably"; for I can only speak of the motion of the flagella of the Spongillidae; it might be possible, though to me it does not seem very likely, that in other sponges the movement is quite a different one.

It stands to reason that my observations are not confined to the one given here. I have made many, and all of them with the same result. I shall not describe them again, but I will only give a few illustrations; they speak for themselves (Fig. 57 *a*—*f*). The same for a number of choanocytes still joined within a part of a flagellated chamber (Fig. 58 *a*—*c*).

Always — in favourable conditions of course: the choanocytes are often immediately damaged — the motion of the flagellum was at first a rapid succession of (spiral-) waves of small amplitude. The waves were moving — without any exception — from the base to the top of the flagellum; and consequently the water with the particles was pushed on from the cell quickly and in a straight line through the axis of the flagellar spiral, while it flowed towards the base laterally. One can understand that with such a rapid succession of actions it was not easy to make out, whether the flagellum moved exclusively in one plane, as a flat motion, or as a spiral one; one time it showed a flat wave, next decidedly a spiral; however, it does not matter very much. (I will revert to this subject later on.)

After a short time this regular, rapid motion passed always into a slower, less regular one, during which at first the undulating-motion (and the water current) persisted, but this soon ceased, to pass into the still slower and more irregular rowing-motion, by which the water was only stirred a little, and no more pushed away. The end was absolute stopping of the stretched flagellum; but not always for long. Sometimes (Fig. 57) the movement began again after a quarter of an hour's rest, but very seldom the original rapid undulating-motion (and then only for a few moments), but more the later phases, alternatively, now the rowing-motion (Fig. 57 *d*), then the „lash" (Fig. 57 *e*); and not fluent but jerking, interrupted by periods of rest, and very soon followed by entire stagnation, the flagellum (Fig. 57 *f*) stretched.

out. Such a period of weak motion may be repeated several times.

As is shown in the figures, the collars were sometimes visible for a small part; generally they appeared more clearly when the motion had almost ceased (sign of relaxation of the protoplasm-contractility when death is approaching?).

We might now go into a theory about the contractions of the protoplasm, which must take place in a flagellum in order to bring about these (spiral-)waves. And we should be the more justified in doing so, 1st because the spiral- or undulating-motion, as we shall see presently, is in fact the normal way of flagellar movement of the choanocytes, and 2nd because in my opinion it is very likely that in the changing of this movement by exhaustion we could find a good starting-point for such a theory about its mechanism.

As all this, however, would only be more distantly connected with the subject we are treating at present, I prefer to leave it till lateron.

Though we may have shown now, that the normal motion of the flagellum is more likely a spiral- or waving- than a rowing-motion, as the former investigators had found, we have not yet proved, however, that *the normal motion of the flagella within the intact flagellated chambers* is really also a spiral- or undulating one. For up to now I described observations of ravelled sponge tissue.

Here my method, which makes it possible to observe wholly intact normally living sponge tissue with oil-immersion for a long time, has rendered me good service. This method has been described on p. 12—13. Only Spongilla is fit for it (as Ephydatia grows too slowly) and exclusively in summer. If the microscopic preparations have been made with care and if the outward circumstances have been favourable (sufficient warmth!), then we find, within a week's time, the vigorously acting flagellated chambers with in- and ex-current canals everywhere in the sponge-rim — so in the newly formed tissue.

A remarkable sight to watch such a flagellated chamber! At first one sees only a round hole into which, so to say, the water runs in from all sides in incessant current, like a round cascade (the water disappearing in the middle).

Of course, this is only optical delusion. The current of water itself cannot be observed, and what one took for the undulating streamlets are simply the (spiral-)waves of the flagella, that run on from all sides of the chamber to the centre. For the choanocytes are, with their base, attached to the inside of the wall of a globular space (the chamber), so the flagella are all directed towards the centre (Fig. 55). The collars will be treated lateron.

Examining now the flagella very accurately, one immediately recognizes the flagellar motion, that we have got to know above as normal in the isolated choanocytes (*eg. Fig. 56a*); *but this one here is much stronger! The (spiral-)waves are running on very clearly in rapid succession from the base towards the top of the flagellum; their amplitude is very small*, still smaller than Fig. 56*a* shows; sometimes the flagellum is almost stretched; another time it seems as if there are some smaller waves superposed on the larger ones, which is certainly possible.

An accurate illustration of such a strongly acting flagellated chamber is given in Fig. 59, drawn from nature. Beside the (generally occurring) very rapid spiral- or undulating-motion some flagella of a chamber may show a somewhat slower one with a larger amplitude, in the way as shown in Fig. 56 *b*. Probably, this last phase is inserted now and then, as a rest-period, between the normal, quick undulatings; I at least saw it sometimes pass into the rapid one.

For hours one may observe the movements; one never sees any other than those, mentioned here: the quick (spiral-)waves with now and then a slow one between. I have observed them repeatedly in numerous different preparations, in the course of several years (1915—'16—'17); always with the same result. Also, Professor Vosmaer and Professor Pekelharing — I am very glad to mention — have enabled me to demonstrate to them, in my living preparations, the flagellar motion of the in-

tact chambers, described here. Both considered my conception as convincingly proved by the preparations.

As an exception, however, the chambers did not show these forms of the flagellar motion. It occasionally happened, that after hours of observation and experiment (with carmine) the movement became different: a relatively very slow one, and no more the normal but the same abnormal ones, which I have described in Fig. 56 *b-d*, as being caused by fatigue and exhaustion. Accordingly, they were always soon followed by entire stopping.

Now the collars and cell-bodies of the choanocytes need treating. The cell-bodies, in my living tissue preparations, were in general not to be distinguished separately, as one easily understands; one only sees their joined layer as a whole, round the chamber; just as is given in Fig. 59.

The collars are to be seen in great number in the chambers; they are very long and leave their flagellum uncovered only for rather a short part at the top (Fig. 59). (I will return to this subject.) Watching a collar vertically on its longitudinal axis, one never sees its apical edge; evidently, this is so thin that it escapes to the eye. In this way of a collar one only observes two straight lines (the extending wall) at the side of the flagellum. As there are such a great number of cells in a chamber, it is not astonishing that one often has great difficulty in finding out the flagellum with its own two collar-lines (of course, one immediately distinguishes the former from the straight collar-lines by its motion). It is quite an other case with the collars on the top of which one can look; so when our eye is in their longitudinal axis. Then one sees the edge of the collar very distinctly (as a little circle), in which the flagellum (as a tiny spot) (Fig. 60). Of course, I could not give all this in Fig. 59; I, therefore, only for clearness' sake have drawn the apical edge of some of the collars, though in fact they can not be distinguished in this position.

Finally I should mention that I have never observed anything of the so called SOLLAS' membrane; this quite agrees with the results obtained by VOSMAER and PEKELHARING (61).

I now still have to speak of the shape of the flagellar move-

ments. Is this in fact a spiral, or is the whole wave in one flat plane? It could not be made out very well in the isolated choanocytes; but all the better in the intact flagellated chamber. For there we see, as I said before, a number of collars from above on the top (as a circle), the flagellum within (as a spot) (Fig. 60). It then proves that that spot — the section of the flagellum — usually describes a flat ellipse or an almost straight line (Fig. 61, 62); consequently, that the flagellum itself performs its undulating-motion either as a flat spiral or in one flat plane. (As above mentioned, this makes but little difference to the effect on the water).

So we have shown definitively that the normal flagellar motion of the choanocytes is: a very rapid succession of (spiral-)waves of small amplitude, going on from the base to the top of the flagellum (Fig. 56a, 59) and causing a current of water straight through the axis of the flagellar spiral also in the direction from base to top, while the water flows laterally towards the base. Exhaustion causes wholly different flagellar motions with abnormal current of water.

The water-current caused by all the flagella together in a flagellated chamber must of course be the resultant of all the little currents, caused by each of the flagella separately. We have got to know the current of water during the normal motion of the flagellum of the isolated choanocytes (p. 126—128). Within a flagellated chamber the current of water, caused by each flagellum, must, as the mode of moving of each flagellum is the same, necessarily be equal to that which the flagellum would show in isolated condition of the choanocyte; so here there must be again for each flagellum a current of water through the axis of the flagellar spiral away from the cell, so now directed towards the centre of the chamber, while the water flows on towards the base of the flagellum laterally (Fig. 58*a*). *The whole water-current within the flagellated chamber, as the resultant of all little currents caused by each flagellum separately, can be made clear in the best*

way by a diagrammatic figure (*Fig. 63*). This figure does not need special explanation; it is planned after Fig. 55, mentioned above (p. 120). *It shows how the water must flow rapidly and regularly from the prosopyles* (*there is only one indicated here, but there are 2—5 in a chamber*) *between the cell-bodies and the collars of the choanocytes to the base of the flagella* (*here really the opening of the collars*), *to be pushed from there by the flagellar motions to the centre of the chamber, thence to flow away through the apopyle.* It is impossible to prove here (eg. by adding carmine) that the current of water has indeed entirely this course, because — the next chapter will show this — the carmine grains, carried along by the water between the choanocytes, are kept there. This penomenon, however, proves already quite sufficiently that the water-current within a chamber has in fact the course given here (Cnf. Fig. 65, 66).

If one asks about the differences of the water-pressure within the canal-system of the sponge, it is quite clear that, with the diagram given here (Fig. 63), this pressure must be highest in the centre of the flagellated chamber (higher than in the surrounding water) and lowest (lower than in the surrounding water) at the flagellar bases, while it is increased exactly in and by the zone of the flagella. In order that a powerful, steady current may be maintained by the chamber and that, therefore, the water may enter rapidly and exclusively at the prosopyles and flow out by the apopyle, the structure of the flagellated chamber must comply with definite requirements. And these requirements do not only count for the sponges of the type of Spongilla but mutatis mutandis for sponges of any canal-system, either Calcaria or Incalcaria (provided of course that there exists the same flagellar motion). These requirements are: that the incurrent openings (prosopyles, pori) of the chambers (mastichore) are relatively narrow, the excurrent openings (apopyles, osculum) relatively wide and that the former are placed among the choanocytes (all this is generally realized). For the high pressure in the centre of the chamber naturally makes the water flow out through the largest opening, while over and above the flagellar motion in

connection with the placing of the prosopyles among the choanocytes prevents any outflow by this last way. On the other hand the low negative pressure, that rules at the base of the flagella and consequently in the whole zone of the cell-bodies of the choanocytes, must suck up the water from as near as possible, so here through the prosopyles; while moreover all water displacement from the centre of the chamber, therefore also from the apopyle, to the base of the flagella is excluded by the action of the latter (perhaps except on the apopylar edge of a choanocytic layer, so there, where this one passes into the pinacocytic covering; but this water displacement is either of little importance (Homocoela) or measures must have been taken against it — more about this later on).

So in fact we must be able to distinguish always *three sharply separated parts in each acting flagellated chamber* with regard to its contents of water: 1st the zone of the negative pressure, that is the zone of the cell-bodies of the choanocytes with the prosopyles; 2nd the zone of the flagellar function, so the zone in which the water-pressure is increased from negative to positive; 3rd the zone of the positive pressure, that is — let me say so for the present — the centre of the chamber with the apopyle. All passing of water from the 3rd into the 1st zone must be absolutely excluded; of course also immediate passing from the 1st into the 3rd; but passing from the 1st into the 2nd and from the 2nd into the 3rd zone must certainly take place, and that as quickly as possible.

Besides the above mentioned relative width of the in- and excurrent openings and the situation of the former among the collar-cells, also the situation of the choanocytes with respect to the excurrent opening must be considered as an important factor for a good regulation of the water-current within a flagellated chamber. The movements of the flagella together give a certain direction to this current, so if there is an opening in the wall of a chamber exactly in the direction of that current, it is inevitable that the water will flow out by it. The importance of this factor appears very clearly from both these cases: the first,

taken from SCHULZE (52) and already mentioned by VOSMAER and PEKELHARING (62), regards sponges which show the diplodal type of canal-system, where in general there does not seem to be such a great difference in width of the prosodi and aphodi, as both are narrow. It now appears that here (in Chondrosia eg.) the flagellated chambers are pear-shaped and that they only carry choanocytes at the side, opposite to the „stem", while the „stem"-side is covered by pinacocytes. Now the „stem" is formed by the excurrent canal, while the incurrent canal ends among the choanocytes. It is a matter of fact that, with such a structure, the separation of the flagellated chamber into the three pressure-zones wanted is guaranteed.

The importance (to the water-current within a sponge) of well placed choanocytes, with respect to the apopyle of a chamber, appears, however, even more clearly from what I have been able to state on my living microscopic preparations of Spongilla. It proved several times, when I had the chance to observe a flagellated chamber with its excurrent canal exactly from the side, that here too the choanocytes are almost exclusively attached to the wall opposite to the apopyle (see also Fig. 55, after VOSMAER and PEKELHARING) and that, when moving, all flagella are directed towards that opening, that even, sometimes, they are beating with the tops outside the apopyle, so in the excurrent canal (Fig. 64, 73). The latter is of much importance, as will soon appear.

First something else. On p. 131 I mentioned that the collars only left their flagellum uncovered for rather a small part at the top (Fig. 59). The consequence will be, that the flagella only influence the water with a small part (the top outside the collar); for it goes without saying that the water-circulation within the narrow collar can not be important and, besides, will be closed for the greater part in itself. Consequently, the choanocyte possesses in the length of the collar a very good means of regulating the effect of its flagellar motion: the shorter the collar, the stronger the effect. One might make the objection that energy would then be wasted by the flagellar motion within the collars. But con-

sider that the energy which is lost there for the sponge cannot possibly be of much importance, as hardly any water is moved and, besides, the water that *is* moved circulates for the greater part closed in itself, as I mentioned.

Let us return to our question. What does it mean when on the one side we see in a Spongilla that only the tops of the flagella protrude outside the collars, and on the other side that sometimes these tops are beating outside the apopyle (Fig. 64)? That means that in such a case 1st the centre of the flagellated chamber with the apopyle has become zone **2**, the zone of the flagellar function, so of increasing pressure, and 2nd that zone **3**, that of positive pressure, is removed from the chamber into the excurrent canal. Consequently, it is absolutely excluded that any water might flow from zone **3** into zone **1**; and the more so, as we know that the apopyle is always much narrower than the chamber itself (I even think to be justified in supposing that it may be able to change its lumen as a sphincter; which WELTNER too mentions (65)). One can also understand, however, what a powerful effect the motion of so many flagella crowded together in an apopyle, and all of them acting in one direction, must have; what a very strong current of water it must cause!

In the same way as I have described here for the flagellated chambers of the Spongillidae, there will also be accessory arrangements to be discovered in the chambers of the other sponges, helping to perfect the circulation of the water; of course always by improving the system of the 3 zones of pressure, mentioned on p. 134, and the rapidity of the removal of the water from the 1st into the 2nd and into the 3rd zone.

Of course there occur also a number of accessory arrangements outside the flagellated chambers — in other parts of the canal system — for the same purpose. For shortness' sake I will not enter into this question here; and the more so, because it has been treated in extenso by VOSMAER and PEKELHARING (62) and my results do not give anything new.

So we have seen in this chapter that the current of water through

the canal-system of the fresh-water sponges is caused by the flagellar motion of the choanocytes in the flagellated chambers. This motion (in normal condition) takes place in a spiral- or an undulating-line, namely in a very rapid succession of waves of small amplitude, passing along the flagellum from the base to the top (Fig. 56a, 59); by which a current of water arises straight through the axis of the flagellar spiral, and similarly in the direction from base to top, while the water flows on at the side of the base (Fig. 56a). Exhaustion causes quite different motions of the flagellum, with abnormal current of water (Fig. 56 b-d). The whole water-current within a flagellated chamber is of course the resultant of the little currents caused by each flagellum separately; it is rapid and regular (Fig. 63). In order that a powerful and steady current may be maintained by the chamber, and that, therefore, the water will flow in quickly and exclusively at the prosopyles and flow out by the apopyle, the structure of the flagellated chamber must comply with definite requirements. This structure must be such that:

a. the 3 zones, which can be distinguished in a functionating chamber, 1st the zone of negative, 2nd that of increasing, 3rd that of positive water-pressure, remain absolutely separated, so that no water can pass from one zone into the other in any other way than from the 1st into the 2nd and from the 2nd into the 3rd zone (Fig. 63).

and that:

b. this way of passing of the water goes as quickly as possible (Fig. 64).

Finally some separate points:

The motion of the flagella in the chambers does not change when the ostia close, as I repeatedly stated.

Besides the above (p. 135) mentioned function of regulation of the current, we can probably ascribe to the collars that of protection of the flagella against injury and mutual entanglement. A third and much more important function will be treated in the next chapter.

Finally I should mention that Delage and Hérouard (16)

1899 and SOLLAS (53) 1906, either of them in their own way, must have felt something of the theory, described and proved here, of the movement of water in sponges. SOLLAS is nearer the truth than both the other investigators; none of them, however, gives proofs or even mentions experiments.

C. THE INGESTION OF FOOD IN THE FRESH-WATER SPONGES.

Preceding Researches. — The question of the ingestion of food is closely related to that of the water-current; therefore the investigators have usually studied them together.

When studying this problem one should discern the ingestion of solid food from the feeding upon substances in solution in the water.

According to PÜTTER (48, 49) 1909 and 1914, it would be absolutely impossible that a sponge feeds on solid food only; on the contrary, it would be more likely that it feeds on organic substances in solution, which would be present in the water in (relatively) large quantities and would diffuse through its surface into its tissues. I shall not speak now about the — to my opinion exact — critic on PÜTTER's theory by BIEDERMANN (6) and LIPSCHÜTZ (40). I myself have never found a proof of its exactness during my investigations; on the contrary, the argument on which this theory its based (the deficit of solid food) seems rather not binding for the (green) fresh-water sponges — see pag. 96. But, of course, I do not think the possibility entirely excluded, that a sponge absorbs also feeding substances in solution. Already HAECKEL (25) 1872 thought this probable. On the other hand, however, one certainly has no right to consider the research of LOISEL (41) 1898, who saw vital staining solutions taken up by sponge tissue, as a proof that sponges may really take up feeding substances in solution, as MINCHIN (45) and SOLLAS (53) do, and also TOPSENT (57) to a certain extent. For we know from the researches of OVERTON (1902) that, although

a certain number of substances may enter a cell by diffusing (eg. the vital stains), a large quantity of other substances may not, and especially those, that might be food to the cell. This entering or not-entering would in this case be a physical phenomenon, independent of the activity of the cell (lipoid-theory of Overton). Although we are obliged, as Höber (30) 1911 points out rightly, to admit beside this physical permeability still a physiological permeability of the cells, in order to make the absorption of nutriment (in solution) conceivable, we may never consider the absorption of vital stains by cells as a proof to the possibility of also absorbing nutriment in solution.

The capturing of solid food is generally stated for sponges. As to this, the view of Vosmaer and Pekelharing (62) 1898 is almost generally acknowledged in literature, as I mentioned already in the Introduction. These investigators showed once more and by better proofs than the others, that the flagellated chambers would be the chief „eating-organs" of the sponge.

These experiments were made in the following way: A Spongilla or a Sycon, having been for some time in water with carmine or milk, was either immediately killed in 1 % osmic acid or placed back into pure water and killed afterwards. The sponges were examined in sections or in maceration preparations. I will quote here the description given by Vosmaer and Pekelharing: „In sponges which had been for half an hour to two hours in water with carmine or milk we found ..... a considerable quantity of carmine in the choanocytes, while in the pinacocytes and in the cells of the parenchyma particles were seen here and there, but in a considerably smaller quantity than in the choanocytes ..... If the sponge had remained for hours (to 24 hours) in the carmine, there was more carmine in the cells of the parenchyma than in the choanocytes. If, after a stay of many hours in carmine, the sponge was placed back into pure water for some hours, the carmine was abundantly found in the cells of the parenchyma, and hardly at all in the choanocytes. Feeding with milk had about the same results ..... we believe we are entitled to

say that the choanocytes really are the organs by which particles suspended in the water, passing the canals, are captured and thus brought into the tissue of the body".

It is a matter of course that VOSMAER and PEKELHARING conceived the mode of capturing food by the choanocytes in perfect agreement with their theory of the water-current, which theory I mentioned at large on page 123—124. So the investigators say: „The particles (suspended in the water) are .... transported to the flagellated chambers ..... here the regular current at once changes into a very irregular movement" (as in Fig. 58*b*). „The particles are moved to and fro in the chamber, and though they partly leave the chamber through the apopyle, a number will, however, arrive *within* [1]) the collars of the choanocytes. The protoplasm of the cells then seizes the particles in order to give them off again to the cells of the parenchyma. This does not prevent that now and then particles can be seized by cells lining the canals; but this will always be of less importance. METSCHNIKOFF's opinion that the flagellated chambers were not the real „eating-organs" ..... is not sufficiently supported by his observations".

Thus runs the theory of VOSMAER and PEKELHARING. MINCHIN (45) 1900 holds a somewhat different view, namely that of METSCHNIKOFF (44) 1892. Although this theory of METSCHNIKOFF has been contested by several investigators — eg. by VOSMAER and PEKELHARING — and BIEDERMANN (6) declares: „diese letztere Behauptung" (the METSCHNIKOFF theory) „erfuhr ..... keine Stütze, indem sich herausstellte, dass die Kragenzellen wirklich die einzigen d i r e c t nahrungsaufnehmenden Elemente sind", I shall quote MINCHIN's words, as they become of importance by the results of my research. MINCHIN says: „Although the problem might seem a simple one, there is no question which has been so much discussed as the nutrition of sponges ..... With regard to the ingestion of food two opposite opinions have prevailed, one set of investigators attributing an ingestive function to the

1) Italics from me, v. T.

collar cells, another set regarding the „mesoderm cells" as the true phagocytes. Those who hold the former view explain the presence of ingested particles in mesoderm cells as having passed on to them by the collar cells. The true explanation seems to lie, as METSCHNIKOFF has pointed out, between these two opinions. The „mesoderm" shows a great difference as regards its degree of evolution in different types. While in some, eg. Ascons, the parenchyma is scarcely developed, in others it reaches a high grade of complication. In accordance with these differences the part played by the parenchyma in capturing food may, in some cases, be very slight, in others very great. There can be no doubt whatever, from numerous experiments that have been performed by various investigators from CARTER and LIEBERKÜHN in the fifties up to VOSMAER and PEKELHARING at the present time, that in many sponges at least the collar cells are very active in capturing food. On the other hand, these cells are from their nature and size incapable of ingesting large bodies such as Infusoria or Diatoms. Food of the latter kind could only be absorbed by becoming entangled in the webs of tissue in the incurrent canal system, there to be absorbed by the phagocytic wandering cells, or, it may be, by porocytes".

„Considered generally, sponges present a gradual evolution as regards the power of ingesting food materials, corresponding to the evolution of the canal system. In the simplest forms, such as Ascons, microscopic food particles are ingested by the collar cells; larger bodies, such as diatoms may be captured by the porocytes, which close upon them like a trap when they enter the intracellular lumen of the pore. The collar cells represent however the chief „eating organ" of the sponge".

„In other sponges the complications of the incurrent system represent a progressive elaboration and perfection of an apparatus for assimilation, doubtless, in the first instance, of bodies too large to be absorbed by the collar cells. As the water passes through the inhalant canals and spaces, food in it is captured by cells in the parenchyma, either by phagocytic amoebocytes or, perhaps, also by porocytes. The function of ingestion may

finally be usurped almost entirely by cells in the parenchyma; the collar cells then become concerned only with the production of the current, their ingestive activities being in abeyance (METSCHNIKOFF)."

Thus MINCHIN. Finally I will just mention what COTTE (12) 1902 says about the mode of ingestion of food by the choanocytes: „je suis disposé à croire que .... l'ingestion peut se faire par toute la surface de la cellule active". But further: „L'ingestion paraît se faire généralement dans une espace annulaire situé entre le flagellum et la collerette". And „Le seul rôle que nous puissions actuellement prêter (aux collerettes) en dehors d'une intervention active dans les faits de phagocytose, est celui de guider les particules alimentaires vers la base du flagellum, point où la phagocytose paraît se faire avec le plus d'énergie".

Personal Research. — *The 4 principal questions, which I shall have to treat, are therefore: 1st Are the food particles captured from the water by means of the choanocytes of the flagellated chambers? 2nd In what way does this capture by the choanocytes take place? 3rd What happens to the particles captured? 4th Does the sponge dispose of still other means of capturing floating particles from the water?*

I have been able to answer these 4 questions, among others by observing my normally living microscopic preparations of sponge tissue (p. 12—13). I therefore placed these preparations in water from the conduit, to which I added some carmine, or in a very diluted suspension of green symbiotic algae isolated from another sponge. To make the observation succeed, it is necessary to transport the preparations already some hours in advance into the glass vessel (with the suspension) finally used for microscopising, otherwise one never sees the capturing of the particles; for, probably, the ostia remain closed after the transport of the sponges, to be opened only after some time, so that only then the normal water-circulation starts. The phenomena can never be observed so beautifully with a suspension of symbiotic algae as with a carmine suspension.

It goes without saying, after the results obtained by VOSMAER and PEKELHARING with carmine-feeding to Spongillae, that I too found *the first question* affirmatively answered: *The particles floating in the water are captured in a mass by the choanocytes of the flagellated chambers;* very often their layer is dyed quite red by it (in case of carmine nutrition). It also stands to reason that, since I had stated a mode of motion of the flagella and the water within the flagellated chambers quite different from that described by both the investigators mentioned, also *the way in which the choanocytes capture the food particles* was bound to prove wholly different: *those particles are not captured inside the collars at all, as* VOSMAER *and* PEKELHARING *thought, but on the contrary outside-between the collars (especially at their base) or between the bodies of the choanocytes themselves.* That this must necessarily be the case, immediately appears from Fig. 55, 59 and from Fig. 63, the diagrammatic representation of the water current in a flagellated chamber as the resultant of the streamlets produced by each flagellum separately. *For the bodies and collars of the choanocytes must, so to say, filter the water, circulating between them, free from floating particles.*

I shall now give a description of the capturing of carmine, as I have been able to observe so many times in my living sponge preparations. So the little sponge is in carmine suspension under oil immersion in an Engelmann case; one has selected a favourably situated flagellated chamber.

It is beautifully to be seen how the carmine is captured! Continually grains run on rather rapidly to the flagellated chamber, carried along by the water in the incurrent canal; they slip into the prosopyle, but then they are either immediately kept or first they move quickly a little aside into the choanocytic layer, and stick there. On more accurate observation, however, the grains, after entering the prosopyles, prove in most cases to slip through the choanocytic layer, but, when having got to the base of the collars, to suddenly deviate aside and to be soon captured — still at the bases of the collars. Only very seldom a grain penetrates any farther, in the zone of the collars themselves; as most of

them have been captured in advance, either between the bodies of the choanocytes or between the bases of the collars. The movement of the flagella is always the rapid spiral- or undulating-motion, which was mentioned above as the normal one; the collars are normal, far extended.

These different ways of capturing carmine grains are represented in Fig. 65, 66. In both figures the choanocytic layer is drawn for convenience's sake as a broad circle; and the way taken by the grains is indicated by dots; while in the last figure — drawn from nature — the choanocytic layer has been represented as loaded with carmine.

After all I have said and drawn about the structure and the water-current of the flagellated chambers (p. 132—134, Fig. 55, 59, 63), the here described way of capturing carmine between the choanocytes — by which the water circulating in a chamber is so to say filtered — is quite intelligible. I only want to point out that the phenomenon, that the carmine grains generally immediately pass the choanocytic layer, but then suddenly deviate aside along the base of the collars to be kept there, can be explained by the fact that in a living chamber on the one side the bodies of the choanocytes are shorter and bigger — which makes the open spaces between the separate cell bodies much smaller — than has been represented in Fig. 55 and in the diagrammatic Fig. 63, while on the other side the collars are approaching each other more and more in the direction of the centre of the chamber. In other words: the flowing water will find the widest passage in a chamber exactly at the bases of the collars (compare Fig. 59). Therefore, however, one should not think that zone of the bases to be extremely unfit for capturing floating particles; that only depends upon the relative size of the latter. The carmine grains now are $\frac{1}{2}$—1 μ, the symbiotic algae 2—3 μ; thus so small that they can just pass the prosopyles, while they will stick between the bases of the collars. The more so, as we may suppose that the collar cells, for the advancement of that purpose, will be provided with a sticky, mucous surface, as is also accepted for protozoa.

Many times I have observed this phenomenon of carmine capturing, in different preparations during several years (1915, '16 and '17); it always took place in the way described here. I had also the opportunity of demonstrating it to Professor VOSMAER and Professor PEKELHARING; both held my conception convincingly proved by the living preparations.

In exactly the same way as described now for carmine, I have also observed several times green symbiotic algae being captured by the choanocytes in a chamber.

As mentioned before, only very seldom a carmine grain gets into the zone of the collars, as most of them have generally been captured already in advance. If it does take place, however, it is the collars which prevent their escaping. For these appear to be very active enlargements of the capturing-surface of the choanocytes, as the particles which might have escaped from their cell-bodies or collar-bases are, in most cases, held between the long collars, as I have been able to observe. A representation of such a case is to be seen in Fig. 67, a carmine grain which I saw captured between 3 collars (seen from above). Afterwards one can see the grain slowly descending along a collar to the base. (Fig. 68, *1–2*). I saw the same thing happen to green symbiotic algae.

So here we have stated the remarkable fact, that the choanocytes capture the food particles in exactly the same way as the Choanoflagellata.

Only very rarely a carmine grain quite succeeds in escaping from the collar cells; then one sees it slip through the chamber. Sometimes also, the way, gone by a grain in the chamber before being captured, seems different from the normal one; an explanation might be given for it, but cannot be proved.

Now the prosopyles still need treating. They are generally not to be distinguished, even if one sees the carmine grains enter the flagellated chamber at a certain point; and no wonder. For one does not see the separate collar-cells either, but only their joined layer as a whole. So it may count as a peculiarity, that there were even two prosopyles to be distinguished in the chamber of Fig. 66. The left one was varying in width; I measured it as

7—8 μ on an average. The right one, on the contrary, was more normal viz. narrower, with the ordinary width of 3—4 μ, and constant as to size. Further, I think to have observed once a great change of a prosopyle of another chamber, viz. that it narrowed from an at first long, rather wide fissure to an opening of 4 μ. Taken for itself this seems a queer observation, rather to be explained by optical delusion. But it deserves our attention, when one considers it in connection with the preceding and with what DELAGE (15) says about the prosopyles of Ephydatia: „méats intercellulaires de grandeur et de forme extrêmement variables, produits par écartement peut-être temporaire des cellules flagellées pour donner accès à l'eau", while also WELTNER (65) writes that they can arise and disappear again. In fact, this would be logical; as in that way a flagellated chamber, after the choanocytes near the existing prosopyles had been overloaded with particles from the water, could open quite new prosopyles simply by separating some other choanocytes.

Finally I want to remind, that all these experiments with the normally living microscopic preparations could only be made with tissue of Spongilla (as Ephydatia grows so very slowly).

I now must answer the 3rd question, namely what happens to the particles from the water, after they have been captured by the choanocytes at the base of the collars.

Those particles — carmine grains or symbiotic algae — *are then taken up by the protoplasm of the choanocytes and carried along into the cell (Fig. 66).* How, I have never been able to observe; but one sees them enter the choanocytic layer; while one also sees them very distinctly, either in intact flagellated chambers or in isolated choanocytes, within the separate cells and always free in the protoplasm, never enclosed in a vacuole.

Next those particles are rather soon ejected by the collar cells again into the surrounding tissue — let me say here, *into the „intercellular plasmic groundsubstance"*, in which also the chlorophyll carrying amoebocytes are to be found (chap. F.) — *from whence those amoebocytes take them later on.* This fully corresponds

to the results of VOSMAER and PEKELHARING (p. 139—140). I have been able to observe it several times in my living preparations, regarding symbiotic algae as well as carmine.

A description may follow here. Fig. 69 represents a flagellated chamber with its normal surroundings of „intercellular" substance and amoebocytes (the figure has been drawn true from nature). The shuttle-shaped amoebocytes with the green algae continually slide in various processions — often directed oppositely (see arrows) — past the chamber; no canals are to be seen. The flagella show the normal rapid spiral- or undulating-motion. In the choanocytic layer a number of green symbiotic algae are lying together at the base, in groups of 5—15; sometimes such a group is to be found in a protrusion of this layer (Fig.: *1*). There all at once, in less than no time, the algae of that group are lying outside the layer, free in the „intercellular" space, but still on their original place (Fig.: *2*). So the protrusion of the choanocytes must have been withdrawn and must have „left behind" the algae. At least in this way one should explain the phenomenon, that has such an exceedingly rapid course. Next the algae, which got free in this manner, are slowly spread all over the „intercellular" substance (Fig.: *3*), from where the amoebocytes will be able to take them up at their desire. That, in fact, the amoebocytes do so, will appear from a following observation.

But first I will describe another flagellated chamber in a preparation, in carmine suspension. Situation almost as in the preceding figure, though here canals are to be seen. Here is to be observed very distinctly how the carmine is ejected into the parenchyma (Fig. 70, I—III; from nature); namely two small grains (*a* and *b*) ejected one after the other from a protrusion of the choanocytic layer. The figures speak for themselves; in I the original condition is given; in II the successive removals of grain *a*, after it has been expelled, are indicated by *1—5*; and in III the same is given for grain *b*. Here the ejecting takes place into a very plastic tissue-bridge.

Then a flagellated chamber overloaded with carmine in the same preparation (Fig. 71, true from nature), while the carmine

from the chamber as centre begins to spread through the „intercellular" substance by little streamlets moving to and fro. The flagella are in rapid spiral- or undulating-motion. In the choanocytic layer the small carmine grains are united at the base into conglomerates, just as we saw already for the algae; while also outside the chamber in the tissue the conglomerates are more numerous than the separate grains (Fig.). Already some amoebocytes in the neighbourhood have taken up carmine. This must be originating from the choanocytes and have been taken from the „intercellular" substance, as there is nowhere any carmine to be seen in the whole preparation, except in 6 flagellated chambers with their nearest surrounding. Now, after the preparation has been in pure water all night, the next morning the above mentioned chambers are almost without carmine; but in the surrounding there are many carmine conglomerates, sometimes still free in the „intercellular" substance, most often however situated within the amoebocytes with algae (and then by times within a vacuole).

The here described phenomena of taking up carmine or algae within the choanocytic layer, followed by ejecting into the „intercellular" plasmic substance and being taken up again by the amoebocytes with green algae, have been studied by me several times, although not in all their minor subdivisions; and this not only by observing normally living preparations of sponge-tissue, but also with the aid of ravel preparations.

One might now ask what happens to the particles captured, after they have got within the amoebocytes with symbiotic algae. I shall postpone this question to the next chapter, to first answer the last question of p. 142.

Does the sponge dispose of still other means of capturing floating particles from the water? I have been able to answer this question affirmatively, again by observation of my normally living microscopic preparations. The phenomenon, however, is very difficult to observe, as a number of favourable conditions must be realized together — which only happens very seldom. It was not

until I had obtained in another way the proofs, that there should still exist in the sponge an entirely different method of capturing food, that I succeeded in observing it in my living preparations.

Those proofs were in short:

1. If one makes a ravel preparation of a little sponge (Spongilla or Ephydatia) that has been in a suspension of carmine or symbiotic algae for some hours — and thereby has become light-red or light-green —, then under the microscope the carmine or the algae prove to be present:

a. in a great quantity of course in the choanocytes of the flagellated chambers.

b. little or not yet in the amoebocytes with symbiotic algae — for the transport from the choanocytes to these amoebocytes takes time.

c. in a relatively great quantity in a not very numerous kind of amoeboid cells, which distinguish themselves from the ordinary amoebocytes with symbiotic algae by their generally almost entire lack of such algae, while sometimes they hold all sorts of detritus (by times situated in vacuole). Their nucleus is, as that of the amoebocytes, vesicular.

2. While now in the choanocytes the carmine generally occurs as small grains or as conglomerates of small grains, it appears to be present in the cells, mentioned under *c*, principally in big grains or their conglomerates. (Perhaps one will doubt, if carmine grains and conglomerates are so easy to be distinguished. In fact this is the case: the grains are generally simple in outline, straight and angular, as pieces of a crystal, and internally homogeneous, refractive red; while the conglomerates are apparently more rounded off, but in reality more irregular by numbers of re-entering angles, and internally not homogeneous but red, everywhere interrupted by black; which is conceivable by their structure.)

3. Very often one can clearly see in a normally living microscopic preparation, which has been in carmine suspension for some hours already, that the carmine, except in the choanocytic layer of the flagellated chambers, is also to be found in mass in

an apparently undifferentiated plasmic substance (in which few or no symbiotic algae) lining the canals. A lively transport of carmine takes place there. By itself this does not say anything; that carmine could be proceeding from the flagellated chambers, though then it would be rather peculiar that it should exclusively extend along the canal walls. Sometimes, however, it also appears that there is a certain difference in size between the carmine grains (not conglomerates!) within the choanocytes and those in the canal walls. The former are almost exclusively small (0.5—0.7 μ), those in the walls often much larger (1.5—4 μ). This fact now is supported and completed in a very desirable manner by what we could state above under **2** in ravel preparations.

The 3 points mentioned sufficiently indicated, that *in a sponge had to be still quite a different method of capturing food-particles — and especially coarse particles —; and such in the canals themselves, outside the flagellated chambers*; *for wich then, of course, only the incurrent canals had to be considered.* By chance I discovered that method; though, after all, one must say that it had to be functioning in a sponge.

Just think of the structure of the canalsystem: incurrent canals — flagellated chamber — excurrent canals. One always used to say that the narrow ostia (dermal-pores), placed at the entrance of the incurrent canals, prevent the too large particles floating in the water from entering, and so from blocking up the canal system. But these ostia measure in living Spongillae, as I have been able to state several times, even to $63 \times 84\ \mu$, while DELAGE (14, 15) could fix their width in killed Ephydatiae on 6—30 μ. Now, generally the prosopyles only measure, as we saw, 3—4 μ. So it goes without saying that numbers of particles will enter by the ostia, which are too large to pass the prosopyles. What must happen to these particles, what must the sponge do with them, when they have come with the water-current to a flagellated chamber and remain sticking in a prosopyle, so stop it up? They must be removed, otherwise — in nature there are so many particles in the water — the sponge would unavoidably die within short,

by all its prosopyles being stopped up. Of course the sponge cannot do it in any other way than by constantly making itself master of those particles, by taking them up within its cells, within its tissues, with the help of protoplasm current; in order to push them out again afterwards (about this in another chapter (E)).

Now, in fact I have observed this phenomenon several times in my normally living microscopic preparations.

It is known, that a choanocytic layer of a flagellated chamber is covered with a thin tissue layer at the side of the incurrent canal. I refer to the figures of the different authors: eg. DELAGE (14, 16), MINCHIN (45), VOSMAER (59, 62) a. s. o.

I myself observed in my living preparations, that outside and against the flagellated chamber at the side of the incurrent canal, against the base of the choanocytes, there is a thin layer of apparently undifferentiated protoplasm, which one time is relatively thick (1—3 μ) and thus easily to be recognized as being separated from the choanocytes (and of course from the lumen of the canal), but next time is so thin, that it appears as a whole with the choanocytic layer. Symbiotic algae occur but few in it, or not at all. That layer, which one must imagine to be covering more or less the whole prosopylar side of the chamber (except of course the prosopyles), appears to be simply a continuation of the lining of the incurrent canal extending over the flagellated chamber, and, when visible, distinguishes itself from the choanocytic layer by a lighter tint (and of course by a darker one from the lumen of the canal). In that plasmic layer, now, very often all sorts of particles — eg. oildroplets [1]), or carmine grains if the preparation is in a carmine suspension — are carried on slowly by protoplasm current and are so removed over considerable distances (eg. $^1/_4$ of the outer surface of a flagellated chamber) [2]). Thus it is seen, for instance, that by this current carmine particles are carried off aside of the chamber into the parenchyma. All this is given in Fig. 72—74, which have been drawn from life. (In

1) One remembers that, as mentioned on p. 100, those oildroplets would be the source of the energy in the flagellated chambers.

2) By which a plasmic layer, which is not visible by itself, may be recognized.

Fig. 74 one also sees the transport of carmine along a little „bridge" bent through a canal; cnf. Fig. 70).

That this layer of flowing plasm must exist on the incurrent-canal-side of a chamber, is again very logical. For what would the choanocytes, eg. in Fig. 73, do with their captured carmine, if that layer was not there?

Many times I have observed this layer on a chamber. I have also had the opportunity to demonstrate it to Professor VOSMAER and Professor PEKELHARING.

The rest of my observation concerned: A flagellated chamber with much carmine in the choanocytes already; carmine grains run up through the incurrent canal and enter by a prosopyle. There approaches a large carmine ball ($7 \times 8\ \mu$), also runs towards the prosopyle, but remains sticking in it. During 10 minutes nothing is seen to happen; but then the ball is going to move and it is, along the outside of the chamber — so between chamber and incurrent canal —, very slowly — as by protoplasm current — carried off aside into the tissue over a distance of more than $18\ \mu$. A moment later a similar phenomenon is to be observed on a somewhat smaller carmine ball, at another prosopyle of the same chamber. I also observed exactly the same thing happen to an alga and to a protozoon in other similar preparations.

So it is the layer of apparently undifferentiated flowing plasma, situated outside and against the flagellated chamber at the side of the incurrent canal, that takes up these big particles — which, carried along by the current of water, got into or against the prosopyles and threaten to stop them up permanently — and that carries them off into the tissue, so that the prosopyles again become accessible (Fig. 75). If these particles might be of any use to the sponge as food, this will undoubtedly keep them and carry them to the amoebocytes (and digest them); which, as we saw already, also happens to the particles captured by the choanocytes. If they are of no value to the sponge, this will try to get rid of them as soon as possible. More about this later on.

If one now inquires after the morphological meaning of this layer of apparently undifferentiated flowing plasma, I must declare that for this moment I don't dispose of sufficient data to answer this question (but see Appendix). Considering the above mentioned (p. 149 sub **1** *c*), one is inclined to look upon it as consisting of amoeboid cells (one or more for each flagellated chamber). But one should not forget that that state mentioned of the sponge, tested as ravel preparation, answers to what we got to know as the state of the normal intact sponge on p. 149 sub **3.** There it proved that the canal walls in general, not especially the exterior covering of the flagellated chambers, were loaded with carmine. So it is quite possible that the amoeboid cells, treated on p. 149 sub **1** *c*, have simply been canal-wall-cells, and have not had anything to do with that covering. The same counts for what follows.

I killed a living sponge preparation, that, according to observation, had reached in a carmine suspension a stage as given on p. 149 sub **3,** in osmic acid and afterwards had the tissue macerated in water, to finally study the separate cells under the microscope. The carmine now proved to be present: 1st in a great number and in f i n e grains within the choanocytes 2nd in a great number and in b i g grains or conglomerates within very irregularly branched amoeboid cells. These amoeboid cells can take the most simple up to the most fantastic shapes; one sees isolated cells with long and broad protrusions, even extended to membranes or stretched as bands (of course all pseudopodial processes), exactly as was observed in living preparations as the apparently undifferentiated plasmic substance; one sees cells with the appearance of a hollow tube, which evidently lined a canal, with plasmic „bridges" and even membranes extended in the lumen. All of them contain carmine. These amoeboid cells now carry an often clearly visible nucleus, little or no symbiotic algae and sometimes detritus; they are rather numerous. But carmine is hardly ever to be found in the ordinary amoebocytes with a great number of symbiotic algae.

One sees the striking conformity with what was found on p. 149 sub **1, 2, 3.** So the apparently undifferentiated plasma lining

the canals, which lodges the big carmine grains, does belong to amoeboid cells. Perhaps one is now inclined to look upon these as being pinacocytes, because in general the canals are supposed to be lined by this sort of cells — see eg. DELAGE 14. But, probably, the latter is not right for Spongillidae; a fact which WELTNER (67) pointed out already, and which I too could state. I namely found the canals lined: here by flat pinacocytes, there by amoebocytes with symbiotic algae, yonder again by apparently undifferentiated plasmic substance (Fig. 71). (Or must one, in both last cases, imagine the pinacocytes to be present, but extended so thin that they escape to our sight?) As the pinacocytes, moreover, generally do not show such an irregular shape (as the carmine-carrying cells in the above mentioned macerated material) and as they usually are also easily to be recognized as cells in a living preparation (while here we are speaking of apparently undifferentiated plasma), one had better explain this plasma, these amoeboid cells, as belonging to the parenchyma, which then is not lined here by pinacocytes — or by very thin, not separately visible pinacocytes? —. I will return to this subject in the Appendix.

At any rate the mentioned apparently undifferentiated plasma belongs to amoeboid cells. But, as said, one may not yet conclude from this, that the flowing plasmic layer at the exterior of the flagellated chambers also belongs to amoeboid cells. If, however, this was in fact the case, we could ascribe several morphological meanings to those cells. In the first place one would be inclined to look upon this layer as being pinacocytes again or better parenchyma lined with thin pinacocytes — as DELAGE (14) gives — or parenchyma without pinacocytes. On the other side, however, one might say that here we had amoeboid cells, which probably entirely surround the prosopyles of the chambers, in other words, something as the well known porocytes of calcareous sponges. I will also return to this question in the Appendix.

For the rest, the whole question of the morphological meaning of the apparently undifferentiated plasma of the canal walls only

stands in a distant relation to the problem, we are treating just now, the ingestion of food.

Which of the two methods of capturing food, the one with the choanocytes or the one with the plasmic layer, is the most important one for the sponge, will depend, in my opinion, simply on the size of the food-particles present. If the size is small the 1st, if it is larger, then the 2nd method preponderates. With carmine nutrition the capturing by choanocytes was the chief one.

Finally one might ask, if the sponge can capture food in still more ways. I must answer that I think it quite possible. In the first place I think of capturing particles in the incurrent canals themselves, which show all sorts of irregular lumina, while fine plasmic bridges, sieve-like membranes and what not, are extended in them, so that they have plenty of opportunity of capturing. Also ingestion of food at the outer-surface of the sponge seems possible. I have, however, never observed these ways of capturing. One thing would prove against them, viz. that with a carmine nutrition of short duration there is hardly ever carmine to be found anywhere else in the sponge than just in and at a short distance from flagellated chambers, as I often stated. But I have also made observations which are in favour of them.

In a living preparation there were numerous Flagellata moving quickly within the canals and at the outside of the sponge. Such organisms, now, some together and sometimes with carmine grains, were also moving within small vacuoles in the canal-walls or in the tissue at the outer-surface. It is quite impossible that these living organisms have been captured by the choanocytes or the plasmic layer; for then they would have been killed, at least they would be motionless. They are more likely to have been captured after having arrived in a blind ending part of the canal, the latter partly narrowing — for the tissue is very plastic, the canals arise and disappear while one observes them — and closing, when its size had been reduced to that of a vacuole; while finally

a cell took up the remaining vacuole with the Flagellata in it. At least, one can explain the phenomenon in this way.

Sometimes one also finds diatoms in the sponge tissue, which are too large to have been ingested by choanocytes and perhaps also by the plasmic layer. These are more likely to have been captured by the tissue bridges and sieve-like membranes of the canals.

Finally there must necessarily exist a system at the ostia to remove the too large particles kept there, so those which may not enter the incurrent canals; this need not be accompanied with taking up within the cells. On the other hand, however, it is difficult to think, that food captured in that way should be of no use at all. So here might be still another means of capturing nourishment. I have never seen anything of it.

So we saw in this chapter that in the fresh-water sponges:

1st the small (food-) particles are captured from the circulating water within the flagellated chambers, viz. outside-between the collars (especially at their base) or between the bodies of the choanocytes, while thus, so to say, the water is filtered clear (Fig. 63, 65—68). Next these particles are taken up within the choanocytes, united to conglomerates and ejected again into the „intercellular" plasmic groundsubstance (Fig. 66, 69—71), from whence the amoebocytes with symbiotic algae take them up in their turn (Fig. 71).

2nd the coarse (food-) particles are captured from the circulating water outside and against the flagellated chambers at the side of the incurrent canal, and such, because they remain sticking in or against the prosopyles. The thin layer of apparently undifferentiated flowing protoplasm, which covers that side of every flagellated chamber with the exception of the prosopyles (Fig. 72—74), then takes up each particle and carries it off aside into the tissue, so that the prosopyles again become accessible (Fig. 75). If these particles may be of any use to the sponge as food, it is very likely that they are carried on to the amoebocytes with symbiotic algae, just as those captured by the choanocytes.

So we see that it is not right, when Biedermann (6) declares

„dass die Kragenzellen wirklich die einzigen direct nahrungsaufnehmende Elemente sind".

While on the other hand MINCHIN (45) wrote rightly: „There can be no doubt whatever, ... that in many sponges at least the collar cells are very active in capturing food. On the other hand, these cells are from their nature and size incapable of ingesting large bodies such as Infusoria or Diatoms. Food of the latter kind could only be absorbed by becoming entangled in the webs of tissue in the incurrent canal system, there to be absorbed by phagocytic wandering cells, or, it may be, by porocytes". Especially this last supposition, the ingestion by porocytes (here = plasmic layer, p. 154), has proved to be exact.

D. THE DIGESTION OF FOOD IN THE FRESH-WATER SPONGES.

I shall be quite short about this subject, as I only possess very few other data concerning it, except all I told above in extenso about the digestion of the green symbiotic algae in the sponge tissue, under the head „Chlorophyll" (p. 16—17, 42—45, 94—116).

As we have seen, however, (p. 96) that exactly the symbiotic algae are a very important, perhaps even the chief source of nourishment for the fresh-water sponges, we certainly may consider the results, regarding their digestion, to be decisive with regard to the problem of the digestion in the fresh-water sponges in general.

We saw that the symbiotic algae, which the sponge has captured from the water in the ways described in the preceding chapter and carried on to the amoebocytes, die within those amoebocytes, either for a part, or all of them, and are digested there and dissolved, while the decomposition-products come to the benefit of the sponge (p. 111—113). This digesting and dissolving mostly took place free in the protoplasm of the lodging cells, sometimes however within a vacuole (p. 97). As I remarked

already before (p. 97), both these methods of digesting are probably not different in principle, but only in quantity of secerned enzyme (in other words, in rapidity).

I made no investigations into the enzymes acting (p. 96), except that the presence of a lipase was made probable (p. 89, 98—99). Perhaps I will have the opportunity later on to extend my investigation in this direction.

As for the carmine captured by the sponge, I can mention that, after it had got into the amoebocytes with symbiotic algae, it was generally soon ejected by these again and removed out of the sponge body afterwards — about which in the next chapter. Only very seldom I have been able to observe a beginning of digestion, a solution of the carmine; but then also, very remarkable indeed, a solution was to be distinguished within a vacuole (this became entirely light-red) from a solution quite free in the protoplasm of an amoebocyte, in which case from the carmine grain as centre the red colour slowly spread through the plasma. In fact a nice illustration of what I have so often mentioned for the symbiotic algae as plasma and vacuole digestion. A proof now, that indeed the difference is a matter of rapidity (of digestion), is given in these observations of carmine-solution in the fact, that carmine grains were never to be found any more in a light-red vacuole, on the contrary there were, when the solution took place free in the protoplasm.

I obtained no data concerning a possible digestion of food elsewhere, eg. in the „intercellular" spaces of the parenchyma.

This about the digestion of food. It had already been acknowledged in literature [see among others Biedermann (6) and also Cotte (13)] that especially the amoebocytes took part in it.

E. THE DEFECATION AND EXCRETION IN THE FRESH-WATER SPONGES.

Also these phenomena I have observed in my living preparations of sponge tissue.

On the whole, there is but little known for certain in litera-

ture about these processes. In the first place one has of course always thought probable the excretion of dissolved matter by diffusion (see Burian (10) 1910) all over the inner or outer surface of the sponge, though in fact no one has ever mentioned any strong proof for it. On the other hand Haeckel (25) 1872, Lendenfeld (36) 1883, Weltner (65) 1891 and Delage and Hérouard (16) 1899 supposed defecation and (or) excretion as being performed by the choanocytes; while Minchin (45) 1900 ascribes this function for a considerable part to the amoebocytes, Bidder (5) 1892, on the contrary, to the porocytes, and Loisel (41) 1898 thinks the excretion being performed by the contraction of the mesogloea (ground-substance).

None of these investigators, however, mentions a definite observation of the phenomena. On the contary the following do:

Masterman (42) 1894 found, if a sponge (Grantia compressa), after having been for some minutes in a carmine suspension and afterwards in pure water, had been killed with osmic acid, that amoeboid cells filled with carmine protruded from the external surface, as if they were just going to be ejected. While something the like seemed to have happened also at the internal surface, as there were cells, ejected and laden with carmine, to be found inside the canals too. Masterman says: „We have here an example of a process of intracellular excretion for the removal of waste solids".

Also the opinion of Cotte (13) 1904 is partly founded on observation; as far as I know it is the last extensive publication about excretion in sponges. As summary he gives: „Les cellules mésogléiques (amibocytes, spongoblastes, etc.) rejettent leurs produits de désassimilation, sous forme de sphérules, dans la substance interstitielle qui les expulse graduellemement. Les sphérules usées des cellules sphéruleuses clasmatosées sont expulsées par la substance fondamentale; un certain nombre de sphéruleuses vont s'éliminer d'elles-mêmes an niveau des canaux. Les choanocytes excrètent directement dans les chambres". This about the excretion, about defecation: „Après l'ingestion de produits inertes les chaonocytes rejettent dans les chambres une grande

quantité de ceux-ci. Les cellules sphéruleuses entraînent dans leur élémination quelques-unes des particules qui ont été déversées dans la substance fondamentale. La plus grande quantité de celles-ci, après avoir été transportée dans tout l'organisme par les amibocytes, est directement expulsée par la substance interstitielle; quelques-unes sont transportées jusqu'aux canaux par les amibocytes qui les y rejettent". As far, however, as I can gather from the description, COTTE has never observed the often mentioned ejection of particles by the „substance interstitielle"; but he did observe the ejection of (or by) „cellules sphéruleuses" into the canals, as well as defecation by choanocytes (within the collar, just as in the Choanoflagellata!). COTTE experimented on Reniera simulans and Sycandra raphanus.

As one sees, the whole problem of excretion and defecation is still a long way from being solved; particularly because so often hypothesis and observation have been intermingled.

Now, I myself have come to the conclusion, by observing the phenomena in my normally living microscopic preparations of sponge tissue, that defecation — and very likely excretion at the same time — takes place on a large scale by means of vacuoles, which occur along the walls of the (excurrent) canals in an apparently undifferentiated plasmic substance, that in reality consists of amoeboid cells.

I now pass on to my experiments.

A proof, that defecation — so a process, by which solid particles captured from the water and food-rests, which are of no use to the sponge, are removed from its tissues — is really acting in the sponge-body, follows already from what I said in the first part of this paper (p. 15—16). There we saw that a sponge, newly caught from nature, has a dirty (green or brown) colour, as its tissue is loaden with particles from the (also brownish) water of the lake; this dirty tint, however, entirely disappears and the bright (green or creamy white) colour comes in its place, when the sponges have been cultivated for some days in pure

water from the conduit. *Proof that defecation must take place even on a large scale.* For with a microscopic observation the brown particles, which orginally were in a great number all over the tissue, proved then to have almost disappeared. The same counts for colourless sponges which have captured such a great number of carmine grains from a suspension, that they have become quite red; here too the carmine is removed in pure water, so that the sponges become colourless again; while one finds the ejected carmine conglomerates at the bottom of the culture-vessel.

Next I will mention that, just as I discovered the phenomenon of the capturing of coarse (food-) particles by (cells of) the canal-walls for the first time in ravel preparations of sponge tissue (p. 148—152), I obtained the first indications of the way in which defecation takes place by means of the same preparations:

As I mentioned on p. 149, one finds in a sponge (Spongilla or Ephydatia), which has been in a carmine suspension for some hours, a great number of carmine grains 1st in the choanocytes and 2nd in amoeboid cells (without symbiotic algae but often with all sorts of detritus), while on the contrary carmine is not or rarely to be found in the amoebocytes with symbiotic algae. Has the sponge been in pure water for some time after it got out of the suspension, one finds, as was partly mentioned on p. 146—148, only little carmine in the choanocytes, but now much (as conglomerates, upto 4 μ large) in the amoebocytes with symbiotic algae, while it is also to be found rather much, and then in big conglomerates (upto 14 μ), in amoeboid cells (present in a small number) without symbiotic algae but with (often) all sorts of detritus. The sponge itself then appears to be less red than it was immediately after it came out of the carmine, while now on the contrary the water, in which it has been, is coloured slightly red. If the sponge remains in pure water for some days more, one does not only find but little carmine in the choanocytes but also in the amoebocytes with symbiotic algae. The carmine, however, is still present in a great quantity and in large conglomerates in the often mentioned amoeboid cells with-

out, or with few, symbiotic algae, and then sometimes within a vacuole. These cells, as said before, are not manifold; they lodge besides a vesicular nucleus (which is very much like that of the ordinary amoebocytes) and the carmine conglomerates, also often all sorts of detritus and sometimes some unicellular, green algae as described on p. 117, while a single time a vacuole has been formed round all these foreign parts together. I believe, however, to have a reason for supposing that such vacuoles do occur more often in these cells round those parts than it seems, but that then they are only temporarily invisible by the accidental grouping of the particles. For I have noticed, that such a vacuole entirely disappeared by a movement of the cell (so that its contents seemed to be quite free in the protoplasm), to become visible again a few moments later. Very often those foreign particles are united to a more or less compact mass. Only once I stated such a detritus mass being ejected by a vacuole of an isolated cell.

In the mean time the sponge itself has lost very much of its red colour in the pure water, while this now in its turn has taken a red tint. As it proves that the sponge does not show any destruction of tissue, we may explain the red tint in the water as caused entirely by the carmine the sponge has (by way of defecation) removed from its body. Now we examine the culture water; then it appears that the following parts have sunk to the bottom: carmine conglomerates (eg. 7×10, 7×13 μ.) together with all sorts of detritus and sometimes a big unicellular, green alga, which every time are united to more or less compact masses, just as we found them above within the amoeboid sponge cells (without symbiotic algae). But there never was an enveloping cell to be seen round these detritus-masses in the culture water.

So here we have stated in ravel preparations, that in the freshwater sponge the function of defecation is performed by amoeboid cells (with few or no symbiotic algae), which by means of vacuoles eject the detritus masses outside the sponge tissue, but which themselves remain within the tissue.

If one examines a normally living preparation of sponge tissue that remains in carmine suspension for some hours, one will find carmine, as has been mentioned on p. 146 and 149—150, on the one side in a great quantity in the choanocytic layer of the flagellated chambers (Fig. 66, 71) and on the other side also in a great quantity in an apparently undifferentiated plasmic substance (in which few or no symbiotic algae) in the walls of the canals — to which it will have been transported, for instance, after having been captured in the plasmic layer at the outside of the flagellated chambers (p. 152, Fig. 75). 2nd. If then the preparation is in pure water for some time, we find, as we saw on p. 146—148, hardly any carmine in the choanocytes; but it now appears to be in small conglomerates within the amoebocytes with symbiotic algae. Besides we also find it in large conglomerates (upto 17 μ), and sometimes together with detritus, within vacuoles in the apparently undifferentiated plasmic substance (in which few or no symbiotic algae) situated along the canal walls. 3rd. *The sponge having been in pure water for some time longer, the carmine also disappears from the amoebocytes with symbiotic algae, to be found almost only as large conglomerates in an apparently undifferentiated plasmic substance (in which few or no symbiotic algae) and mostly situated along the canal walls and often together with detritus within a vacuole (Fig. 76a, 77).*

One will have to acknowledge that these observations on living tissue correspond very well with the results obtained in ravel preparations. *The matter now was to observe the phenomenon of the ejecting of feces itself.*

In this too I succeeded in my normally living preparations. But also here I had to exert much patience.

I shall now give a description of such a phenomenon of defecation, as I observed it on a preparation which, after having been in carmine suspension, was in pure water for some time: A carmine conglomerate lies, together with some detritus and some green symbiotic algae, along the wall of a canal in the often mentioned apparently undifferentiated plasmic substance. At first no vacuole is to be seen round these parts, but shortly

after there is; so then the carmine, the green algae and the detritus are together within it. *Now the vacuole is going to protrude far into the canal (Fig. 76a); it is even pushed forward, so to say, on a broad stem. All at once — one does not see how — the very thin vacuole wall disappears at the side of the canal, some small carmine grains begin to loosen from the conglomerate, move to and fro, and all of a sudden they run off; next some green algae do the same (Fig. 76b); and at last the large conglomerate, moves a little to and fro, as if it were still kept back — then suddenly it runs off through the canal!*

So one sees that here there is no question of the feces being ejected together with the lodging cell, as MASTERMAN *says; the cell, or whatever this apparently undifferentiated plasma may be, simply stays behind in the wall.* I have observed this phenomenon of defecation several times in the same way.

Let us now attentively examine such a feces conglomerate before it is ejected. So it is in the apparently undifferentiated plasmic substance within the tissue. Later on I shall speak about this plasmic substance. Often no vacuole is to be seen around the conglomerate at first; this only appears later on and then increases rapidly. One can also observe how, from all sides, small feces-particles (being within vacuoles or not) are carried on to the large conglomerate (also being within a vacuole or not) and are united with it. In the mean time the conglomerate is continually carried along in the tissue by the plasmic substance over considerable distances, so that for instance it happens but too often, that it escapes to our sight by disappearing into deeper tissue-layers. In the same way one can see a conglomerate — then always within a vacuole — moving repeatedly from one canal to the other; while the vacuole often protrudes so far into the canal, that one thinks it will burst; but it withdraws again entirely into the tissue and goes to another canal, to repeat the same. A remarkable sight, which I want to describe somewhat more at large.

A normally living microscopic preparation of sponge tissue, which has first been in carmine suspension and afterwards in pure water. A large carmine conglomerate lies again in an ap-

parently undifferentiated plasma, between three canals (Fig. 77, *1*); there are some green symbiotic algae to be found in the plasma, but by no means so many as in the ordinary amoebocytes; no vacuole present. A few moments later a vacuole arises around the conglomerate, while at the same time also some green symbiotic algae are enclosed. The vacuole rapidly increases and protrudes more than half way into the lumen of a canal (Fig. 77, *2*). But it does not burst! On the contrary, the vacuole withdraws into the tissue, to protrude then into another canal (Fig. 77, *3*). But here it does not burst either, but withdraws again. Now it lasts for some time. At last it protrudes into the 3rd canal (Fig. 77, *4*). All at once the thin vacuole wall disappears at the outside; the conglomerate still remains in its place, it only moves a little to and fro, as if it were still kept back, then it slightly moves on and again to and fro, a pull — and it runs off through the canal.

A remarkable process, that protruding into a canal, to withdraw again after some time! I have repeatedly observed it, as said before. Why dit not the vacuole burst immediately?

The only answer I can give, based on my observations, makes us acquainted — if it is right — with a remarkable phenomenon of sponge-life, which, however, is very logical, even indispensable. Just consider: *The fecal conglomerates must necessarily be ejected exclusively into the excurrent canals of the sponge*, otherwise the whole defecation-system would unavoidably fail. As we saw, however, the vacuoles with the feces are not bound to certain permanent places, but they are, just as the whole sponge tissue is continually moving, always carried along. But how does a vacuole „know" then if a canal is an incurrent one, in which it may not eject its contents at all, or an excurrent one? The vacuole — or better the protoplasm, to which the vacuole belongs — must examine this on the spot and then it will show the phenomenon which we saw it perform above: it will protude into the canal. Thus the thin protoplasmic vacuole-wall will get to know in some way or other the state of the canal, either, for instance, by the rapidity of the current or by the pressure of

the water. If the right (excurrent) canal has been found, the plasma contracts and the vacuole bursts; if, however, it proves to be an incurrent canal, the vacuole will withdraw to try the same somewhere else.

Now, my observation relative to this was in fact, that the vacuoles withdrew from canals which, if it was possible to distinguish, proved to be incurrent canals. (One can distinguish it from the situation of the flagellated chambers).

We now got to know the process of defecation, which, as we saw, must very often take place in nature, where the water contains so many particles, that the sponges have quite a dirty tint, which however they soon lose in clean water (p. 160—161). This defecation proved to take place by means of large vacuoles. These vacuoles are partly filled with liquid — undoubtedly originating from the sponge tissue —; this liquid is ejected with the feces. *Have not we found here, then, besides a powerful defecation process also a strongly acting excretion process?* I think we have.

But we have not yet quite finished the process of defecation. *There exists, besides the here described way of defecation everywhere at arbitrary points of the excurrent canal walls, still another method* which, as I believe, we shall have to distinguish from the first one. And this, because it is apparently *bound to a more or less fixed place:*

In sponges „fed" on carmine one repeatedly meets with one large accumulation of this matter near each flagellated chamber, and again in an apparently undifferentiated plasmic substance, in the wall of the excurrent canal (Fig. 73). While, now, on the one side new carmine grains are constantly added to the large heap by the flowing plasmic layer, situated at the incurrent-canal-side of the chamber (p. 151, 156), one sees on the other side now and then a big conglomerate being ejected from the heap into the excurrent canal; both of which is represented in Fig. 73 (drawn from life). I observed this, of course, again in my living tissue preparations.

Of course it cannot be said, if this accumulation of carmine —

which, apparently, has been deposited gradually by the flowing plasmic layer, so in small quantities at the time — is exclusively formed by fine carmine grains, captured by the choanocytes and then passed on to this layer, or by coarser particles, which remained sticking when entering the prosopyles and then have been carried off by this plasmic layer (p. 156). Probably both is the case.

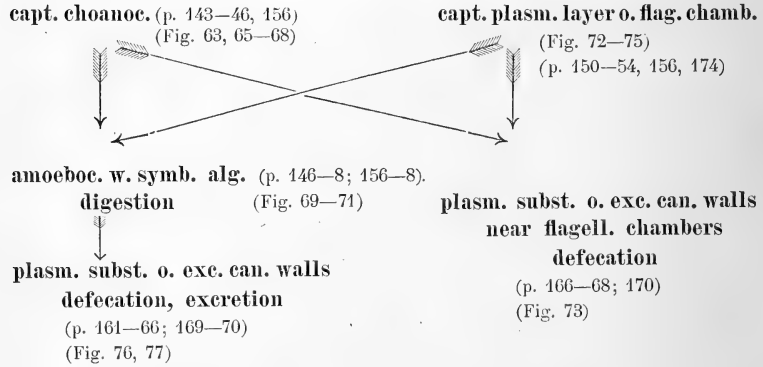
That in this way particles, which block up the prosopyles and are of no food value, are removed as soon as possible from the canal system, is very well conceivable and of much importance to the sponge. So here we get to know *a very powerful system of cleansing the sponge: coarse particles, which, having passed the ostia, remain sticking in the prosopyles and threaten to block them up permanently, are taken up by the plasmic layer and are carried off to a point in the tissue somewhere in the neighbourhood, from where they are soon ejected into the excurrent canals, to be removed with the water current (Fig. 73).*

On the other hand it is hardly to be accepted that coarse particles from the water, which might be of use as food, should be ejected in this way without any reason. But then one has to suppose, that the sponge knows to decide whether a captured particle contains nutrition or not, in the short distance between taking up in the plasmic layer and delivery at the deposit of feces. This seems very unlikely to me; the more so, because above we have stated several times (p. 146—148, 157—158) that carmine, most certainly, is carried into the sponge tissue and is moved on for digestion to the amoebocytes with green symbiotic algae, to be expelled only later on (p. 161—164). So it proves, that only the amoebocytes with symbiotic algae — which are performing the function of digestion (p. 157) — decide about food or no food with regard to the particles captured. Consequently, one may consider it as excluded that the same could also happen already near the flagellated chamber.

The explanation of the phenomenon, that on the one hand the sponge carries the carmine even into its amoebocytes, while on the other hand it disposes of a very rapid method of getting rid of it immediately, seems to me the following: With a relatively

small number of particles in suspension in the surrounding water, the sponge will carry all particles captured into its amoebocytes with symbiotic algae, so into its digestive organs (Fig. 71); but, when the particles in suspension are very numerous, it will also carry a number of them within its amoebocytes, but soon „satisfied" will leave off, to simply eject them, directly after capturing (in or near the flagellated chamber), along the shortest way at the excurrent side of the chamber, of no consideration whether the particles are food or not (Fig. 73).

We now can make the following diagram of the course of the (food-) particles captured by a fresh-water sponge (imagine the often mentioned „intercellular plasmic groundsubstance" (chapt. F) in the places of the arrows). The pages of the text, in which the process was treated, and the figures referring to it are put in parenthesis. So we begin with the capturing in the choanocytes and the capturing in the plasmic layer at the outside of the flagellated chamber:



I now want to point out some more accidental peculiarities:

1st. Just remember that we could stade a few times (p. 162—165) that vacuoles together with feces also ejected unicellular, green algae, even green symbiotic algae. I want to mention this emphatically with a view to what I said, in the part about the chlorophyll of the fresh-water sponges, regarding a possible

export of symbiotic algae (p. 52) and a contest against infecting algae by ejecting them (p. 115). Especially the question about the export is important. I do not venture to decide whether it can be large or not; in both cases here it concerned a green sponge, so a sponge that came into consideration for possessing an excess of green symbiotic algae (p. 70—72).

2nd. Sometimes it appears in a living sponge preparation, which has first been in carmine and afterwards in pure water, that at last there is still some carmine present in large conglomerates in amoebocytes with numerous symbiotic algae, and such, in the rim of the little sponge. This makes us suppose that, besides the above mentioned defecation inside the sponge at its inner surface, also defecation could take place at the outer surface, for instance by the ordinary amoebocytes. I have not observed any thing more certain, however.

3rd. I have never discovered pulsating vacuoles in the choanocytes, nor ever anything of defecation by these cells.

4th. Finally a question, as I put also on p. 153: What is in fact the morphological meaning of that often mentioned apparently undifferentiated plasmic substance, which, situated in the wall of the excurrent canals, proved to perform the defecation. Up till now I have omitted to enter into this question purposely, in order to evade unnecessarily complicating the problem of defecation, we wanted to know in the first place.

From the conformity of the results obtained from living- and from ravel preparations (p. 161—164) one would immediately decide, that the apparently undifferentiated substance consists of amoeboid cells. Also the following supports this view: Just as I described on p. 153, that I killed and macerated a living sponge preparation, which had been in carmine and the contents of carmine of which I had stated, I did the same with a preparation, which, according to microscopic research, was in the stage of carmine defecation given on p. 163 sub 3. All cells were to be seen isolated. Carmine now proved present: not or little in choanocytes and amoebocytes with symbiotic algae, but numerous and mostly in large conglomerates — and sometimes together with

detritus within a vacuole — in simple to very irregularly branched amoeboid cells, which lodged a nucleus but few or no symbiotic algae and for which, in general, the same description counts, which I gave on p. 153 for the there isolated amoeboid cells. So these cells have, in short, the appearance of, what I called in my living preparations, the apparently undifferentiated plasmic substance. One also sees the striking conformity with what was found on p. 161—164. Consequently this apparently undifferentiated plasma, which, all over the wall of the (excurrent) canals proved to bring about the defecation and excretion, belongs to amoeboid cells. That this also counts for the plasma, which also performs the function of defecation but then near an apopyle (p. 166—167), was not yet quite decided by this, but I have been able to prove it in the same way in another preparation.

Perhaps one is inclined — just as on p. 154 — to look upon these amoeboid cells in the canal walls as pinacocytes. But for the same reason as I mentioned there, I believe to be justified also here in taking these cells rather as belonging to the parenchyma. I will return to this subject afterwards.

Next one could put the questions: 1st. If, in fact, there is in principle any difference between these two kinds of amoeboid cells — situated in the living sponge as apparently undifferentiated plasma in the walls of the excurrent canals, the one however spread all over, the other only near to the flagellated chambers —, both of which bring about the carmine defecation. 2nd. If, in fact, there exists in principle any difference between these two kinds of amoeboid cells (those of defecation) and the amoeboid cells, mentioned on p. 153—154, which — situated in the living sponge also as apparently undifferentiated plasma in the walls of the incurrent canals — lodge the large grains of carmine (p. 149—150). I suppose we shall have to answer these questions negatively; we shall rather have to consider all these amoeboid cells as identical, each sort only temporarily performing another function (cnf. p. 149, **1** *c*; 161—163). It would even be quite possible that a cell, which has first taken a number of carmine grains in an

incurrent canal, transports them immediately to an excurrent one, to eject them. I specially think of the case of Fig. 73 (supposed that the plasmic layer outside and against the flagellated chamber also belongs to an amoeboid cell). Even, with the existing dimensions it would be possible, that one single amoeboid cell extends as well at the outside (incurrent canal side) of the flagellated chamber as near the apopyle; this cell, in other words, would be able (without getting out of its place) to take the carmine from the incurrent canal and eject it into the excurrent one, by simple protoplasm current within its own body. A very simple and rapid process!

F. APPENDIX.

Some separate observations [1]).

I.

First I want to speak about *the often mentioned* (p. 17, 146—148, 150—154, 156, 163, 166, 168—170) *undifferentiated intercellular plasmic substance* (*ground-substance*, *mesogloea*).

As is generally accepted, the tissue mass, which occurs in the sponges between the pinacocytic epithelium of outer and inner surface and the choanocytic layers of the flagellated chambers, the so called parenchyma, consists of ground-substance with cells. Minchin (45) says about it: „The skeletogenous stratum (parenchyma) is developed to a very variable extent in different sponges. Scarcely recognisable in some, in others it attains great proportions, making up all but a relatively insignificant portion of the total bulk of the sponge body. It consists of a gelatinous ground-substance or mesogloea, which contains cells of various kinds. The mesogloea is the first portion to appear as a structureless layer between the dermal and gastral epithelia, and is

1) Here should have followed a description of a remarkable phenomenon in living gemmule cells of Spongilla; however, I have meanwhile published this elsewhere (van Trigt, 57 c).

probably a secretion of the former. Cells from the dermal epithelium next migrate into the mesogloea, forming a parenchyma which is concerned primarily with the task of furnishing skeletal structures for the support of the sponge body".

However, the opinion that the mesogloea should be a „structureless" (undifferentiated) intercellular plasmic substance, seems to be not right, at least not for Spongillidae.

In 1870 already Lieberkühn (39) came to this conclusion; and such in consequence of warming the living Spongillae; for it always appared then that this groundsubstance split up into a number of separate cells (with nucleus). „In der homogenen durchsichtigen Grundsubstanz tritt durchweg eine Zerklüftung auf, welche sie in gleichmässige Stücke zertheilt, die je einen Kern mit Kernkörper besitzen". „Diese Beobachtungen lehren, dass die contractile Substanz" (groundsubstance) „durch Erwärmung wieder in dieselben Zellen zerfällt werden kann, aus welchen sie ihren Ursprung nahm, und das die durchsichtige contractile Substanz nichts ausserhalb der Zellen Existirendes ist, sondern nur ein Bestandtheil derselben bildet". „Man kann sich überzeugen, dass die Zellen die einzigen Bestandtheile der contractilen Substanz darstellen und nicht etwa noch eine homogene Sarkode daneben existirt, wie von einigen Forschern angenommen wird". Lieberkühn obtained the same result by simply pressing living Spongillae to pieces.

Also Weltner (67) comes to such a conclusion in 1900: „Was nun die Grundsubstanz, in welcher die verschiedenen Zellen eingebettet sind, anbetrifft, so möchte ich das Folgende bemerken. Bei einem in Alcohol conservirten Süsswasserschwamme stellt die Intercellularsubstanz eine hyaline Masse dar, in der die Zellen deutlich hervortreten. Untersucht man aber eine lebende ausgewachsene Spongille, so bietet die mittlere Gewebsschicht ein wesentlich anderes Aussehen dar. Man sieht allerdings hier und da deutlich abgegrenzte Zellen zwischen hyalinen Streifen (die Grundsubstanz) frei bleiben, daneben bemerkt man Körnerhaufen, in denen man gelegentlich in Folge ihrer amoeboiden Bewegung einen Zellkern zu Gesicht bekommt. Der übrige Theil des Ge-

webes lässt aber keine Zellen mehr erkennen, sonder das Ganze stellt eine mit Körnchen von verschiedener Grösse erfüllte Masse dar, in der man am lebenden Object weder Zellen noch Kerne unterscheiden kann. Man erhält an solchen Stellen den Eindruck, als ob sämmtliche Zellen mit einander verschmolzen seien, ohne dass man Zellgrenzen wahrzunehmen vermag. Wir haben also hier an einer Stelle ein Syncytium vor uns, an einer anderen ein gallertiges Bindegewebe. Um die geschilderten Verhältnisse zu demonstriren habe ich in Figur..... von einer lebenden Ephydatia fluviatilis ein Stückchen des Parenchyms abgebildet, welches sich zwischen zwei Nadeln befand. Es liessen sich hier mit Deutlichkeit 4 Zellen erkennen, 2 davon mit einem Inhalt von ungleich grossen Körnern" (the amoebocytes with symbiotic algae) „und 2 andere die mit gleich grossen Körnchen erfüllt sind. Von einer zwischen den Zellen liegenden hyalinen Substanz war nichts zu sehen. An zwei Stellen liessen sich Flüssigkeitsvacuolen erkennen. An der freien Aussenfläche war nur in dem dickeren Theile eine Epithelzelle wahrzunehmen, der übrige Theil, sowie die grossen Lacunen im Inneren, durch die sich ein dünner Gewebsbalken hindurchzieht, sind ohne Epithel. Solche epithelfreie Gewebsbalken sind bei Süsswasserspongien eine ganz gewöhnliche Erscheinung, so lange die Balken noch von geringer Dicke sind; sie bestehen sogar oft nur aus einer einzigen lang ausgezogenen Zelle. Ausser den genannten 4 Zellen liessen sich in dem abgebildeten Gewebestück keine weiteren zelligen Elemente erkennen, vielmehr bestand die ganze Masse aus einer dickflüssigen Substanz, in der zahllose gröbere und feinere Körnchen eingelagert waren, wie man sie sonst in den Spongillenzellen findet. Es ist für mich kein Zweifel, dass eine solche Masse aus zusammengeflossenen Zellen besteht, welche man, wie das Lieberkühn zuerst gethan hat, durch Anwendung von Wärme sichtbar machen kann".

With the help of my living preparations I have now been able to state exactly the same thing as what Lieberkühn and Weltner found, before I knew anything of the observations of both these investigators. So my observations have been made

quite independent of the former. I too found in living tissue the amoebocytes with symbiotic algae and the cells with equally large grains imbedded in an apparently undifferentiated intercellular plasmic ground-substance (p. 17, 146—148, 156, 168, Fig. 69, 71), in which numbers of enclosures (eg. oildroplets and sometimes symbiotic algae) and also vacuoles (p. 163—165), and which, at the side of the canals, was one time lined by cells (eg. pinacocytes) and not so another time (p. 154). When rubbed to pieces as well as when warmed, or after killing and macerating, *the whole tissue, so also that undifferentiated plasmic substance, proved to consist of amoeboid cells with nucleus* (see also p. 153—154, 169—170).

As to the observation, that that plasmic substance (i. e. the ground-substance of the parenchyma) was not entirely lined with pinacocytes, I mentioned already on p. 154 that the possibility is not excluded that pinacocytes were present in fact, but not to be discovered by their fine extension. With regard to the above described phenomena of ingestion of food in the flowing plasmic layer outside and against the flagellated chamber (p. 152) and of defecation by vacuoles in the excurrent canal walls (p. 163—166), it seems more likely that, at least there where those processes take place, the pinacocytic covering of the canals will be missing. For that would certainly advance the rapidity. On the other hand my observation made during the capturing of coarse food particles outside and against the flagellated chamber, viz. that first the particle remains quiet for about 10 minutes before being carried off by the flowing plasmic layer (p. 152), might possibly show that thin pinacocytes, which then must first be passed by the food particle, are present.

As we have now seen that all apparently undifferentiated plasmic ground-substance consists of amoeboid cells, we may conclude that the layer of flowing plasm at the outside of the flagellated chambers (p. 152, 156) is also formed by such cells. And we may also conclude that the (food-) transport (from the choanocytes to the amoebocytes with symbiotic algae, for instance) which, as we saw above (p. 146—148, 168), takes place through the so

called intercellular ground-substance, is in fact performed by amoeboid cells.

In connection with what was said on p. 170 we can put the question again, if in fact there is in principle any difference between all those various kinds of amoeboid cells, which — appearing in the living sponge as undifferentiated plasmic substance (p. 168) — on the one side, as we saw, capture coarse (food-) particles from the water (p. 152, 156, 174) or lodge them (p. 149—150, 153—154), on the other side perform the function of defecation and excretion (p. 163—166, 169—170), and in the third place form the „intercellular" groundsubstance of the parenchyma (p. 171—174). I suppose to have to answer also this question negatively. They are more likely to be all the same cells, each kind only temporarily performing another function. See also p. 170—171.

And at last the question, if these amoeboid cells — which, as one knows, lodge but few or no symbiotic algae — are perhaps identical to the amoebocytes with numbers of symbiotic algae, again differing from them only by a temporarily other function. Also this would be possible (cf. p. 149, 162). But I do not venture to answer this question; I only want to ask. See also Weltner (68).

II.

One must not confound the often mentioned food-(digestion-) vacuoles of the amoebocytes with symbiotic algae (p. 94—97, 111) and the defecation and excretion vacuoles of the amoeboid cells without symbiotic algae (p. 162—166) with a peculiar vacuolising of the tissue of the outer, and sometimes also the inner sponge surface, which gets something of a foamy structure by it. I mentioned it already on p. 17, and such with regard to a similar structure in isolated amoebocytes (Fig. 4). I think that this last kind of vacuoles will properly not belong to the cells, but are rather parts of the surrounding water, which were temporarily enclosed by the pseudopodia during the amoeboid motion

of the cells. Therefore one should sharply distinguish them from the food- and the defecation- and excretion vacuoles, the contents of which of course belong to the cells themselves. LIEBERKÜHN (39) already drew attention to this.

III.

We have a very easy criterion to make out whether a fresh-water sponge, in which there are neither gemmules nor finger-shaped branches, is a Spongilla lacustris or an Ephydatia fluviatilis, viz. in its smell. Spongillae, namely, have a pungent smell (pungent for instance as formol); while Ephydatiae entirely lack it. Several persons have, on my request, stated this difference; but it also proved to me that not everybody is able to notice it.

SUMMARY.

This summary gives the chief results of my research, and the pages of the text, the tables and illustrations concerning them. See also VAN TRIGT 57b.

A.

1. The two chief forms of Spongilla lacustris and Ephydatia fluviatilis are a grass-green and a colourless (creamy-white) one (p. 15—16, Fig. 1, 2).

2. The sponge owes its green colour to numerous little green corpuscles present in its tissues, especially in the amoebocytes (p. 16—17, Fig. 4, 69).

3. These green corpuscles produce O_2 (p. 18—20, Table 1, 2) and photosynthesise (oil) in light (p. 20—21, Table 3), but not so in darkness. Consequently — in connection with the points of similitude already known (p. 17) — we are justified in declaring that the green colouring-matter of the Spongillidae is identical with vegetable chlorophyll (p. 21).

4. The green chlorophyll corpuscles of the fresh-water sponges are round or oval, 1.7—3.8 μ in diameter, surrounded by a cell-wall and consisting of protoplasm and a chloroplast; while perhaps a nucleus is present, but a pyrenoide is absent. They enclose oildrops, but carbohydrates were never to be found within them (p. 21—27, Fig. 5, 40, 41, 6—11).

5. These green chlorophyll corpuscles, isolated from the sponge tissues, remain normal and alive for 6 months and even longer, and multiply (p. 27, Table 4). They also occur free in nature, in the waters in which the sponges are living, where they also multiply (p. 27—28).

It proved possible to me to durably transmute colourless Spongillidae into the green form, by infecting them with isolated green chlorophyll corpuscles (p. 28, Table 7, 8).

So the green chlorophyll corpuscles prove to be organisms, on the one hand capable of living by themselves without the sponge body, on the other hand of accommodating themselves to the life within the sponge tissues, when taken from the surrounding water by the sponge. This result together with that of **4,** mentioned above, justifies the conclusion, that the green chlorophyll corpuscles are algae, associated to the sponge in „symbiosis" (p. 21—24, 29).

6. This green „symbiotic" alga of the Spongillidae multiplies by simple, vegetative division of the whole mother cell, not by „freie Zellbildung" (p. 30—33, Fig. 5, 12—34). Thus the alga does not at all answer the definition of a Chlorella (p. 29—30, 34), but — in connection also with its structure (p. 24—27) — that of a Pleurococcus (p. 34).

7. Generally speaking, green Spongillidae grow in light, colourless ones in darkness or in twilight (p. 35); while green sponges grow colourless in darkness, and colourless ones grow green in light (p. 35, Table 8).

8. In the green sponges in light as well as in the colourless ones in darkness green as well as colourless „symbiotic" algae occur (p. 35, 46, 48, Table 6).

9. Several of those colourless algae have exactly the same structure as the green ones (p. 36, Fig. 35).

10. The isolated green „symbiotic" algae of the Spongillidae, in water, can produce chlorophyll in darkness (p. 37, Table 4 B). Those green algae also, whether cultivated in light or in darkness in poor or in rich organic feeding media, remain normal (green, for instance) and alive for months, and multiply; while the isolated colourless algae under similar conditions disappear from the culture and never pass into the green form (p. 38—41, Table 4). It proves, therefore, quite impossible that the green algae pass into the colourless ones and that the colourless algae pass into the green ones, by the combined influence of darkness or light and a certain feeding milieu (p. 41).

11. The green „symbiotic" algae, whether isolated or in the tissues of the Spongillidae, become colourless exclusively by dying, in order to gradually pass from colourless algae with clear structure into the successive stages of „solution", colourless algae with shade of structure, colourless ones without structure, vague shades of colourless ones, and to finally disappear (p. 42—45, Table 5, Fig. 35—37).

12. Analyses were made of the intrinsic amount of the various green and colourless stages of the „symbiotic" algae in the tissues of a large number of green Spongillidae from light and of colourless ones from darkness, and such in tissues in different stages of development (Table 6). The results are too numerous to be repeated here; they are mentioned on p. 46—48, point 1—11. In short we can say, that a green sponge in light contains an excess of green living algae and a smaller number of colourless dead ones; a colourless sponge in darkness, on the contrary, an excess of colourless dead algae and a smaller number of green living ones (p. 48).

13. The factors, ruling the number of the „symbiotic" algae in the sponge tissues, were studied separately (per unit of time and per unit of sponge-volume). These factors are 6 in number:

a. The import (*i*) of the algae from the surrounding water into the sponge tissue, a powerful and continually acting factor in nature and equally active in light as in darkness (p. 49—51, Table 7).

b. The export (*e*) of the algae from the sponge into the surrounding water, an uncertain but probably not important factor (p. 52, Fig. 76).

c. The reduction (*r*) of the sponge tissue to a smaller volume, a factor which in nature only occurs in autumn and then might cause increase of the concentration of the algae in the tissue (p. 52—53).

d. The growth (*g*) of the sponge tissue, which in a long space of time must strongly lower the concentration of the algae in the tissue and which is more active in green sponges in light than in colourless ones in darkness (p. 53).

e. The intensity of multiplication (*mu*) of the algae, which in sponge tissue in equal concentration of the algae is much larger in light than in darkness, in light in a strong concentration larger than in a weak one, but in darkness in both cases $\pm$ 0 (p. 54—56, Table 9, 10).

f. The mortality (*mo*) of the algae, which in sponge tissue in equal concentration of the algae is much larger in darkness than in light, in darkness in a weak concentration somewhat smaller than in a strong one, but in light in both cases almost just as large (p. 56—62, Table 6, 8).

14. By cultivating the sponges under such circumstances, that the factors of import, export, reduction and growth were almost entirely excluded, we could show that the phenomena — that green sponges grow colourless in darkness and colourless sponges grow green in light — must be explained as being caused, under these circumstances, by the combined action of the multiplication and the mortality of the „symbiotic" algae (p. 62—66, Table 8). And we were able to prove, by means of the above mentioned data concerning those two factors, why they must cause those phenomena (p. 66—67).

15. Next we have proved with the help of the above given data concerning all 6 factors (import, export, reduction, growth, multiplication and mortality): I. Why in nature the Spongillidae must contain such an amount of the various green and colourless stages of the „symbiotic" algae in light and in dark-

ness, as we have experimentally stated, in short above sub **12** and in extenso on p. 46—48 sub 1—11. II. How in nature these sponges keep up their „colour" (green or colourless), and how both „colour"-types arise from each other. For, what happens eg. to the number of green algae of a sponge under certain circumstances, in other words how the colour of the sponge is affected, entirely depends upon the value which each of those 6 factors takes under these circumstances in the formula

$$i + r + mu \begin{matrix} > \\ = \\ < \end{matrix} e + g + mo$$

the formula, which we have got to know as decisive for the number of green algae of a sponge (p. 68—75).

16. By a comparison of the behaviour of the „symbiotic" algae when cultivated in sponge tissue and isolated in water, we got to the following conclusion: In darkness the „symbiotic" association of sponge and alga offers much less advantage to the alga than a life free in the water, as in the sponge all algae are destroyed (p. 77, 83, Table 10). In light, on the contrary, that „symbiotic" association offers more advantage to the alga than a life free in the water; that advantage, however, only consists in the fact, that the sponge protects the alga against destruction, eg. by enemies (p. 76—80, Table 10). The milieu — the feeding milieu —, on the contrary, is in the sponge not at all more favourable to the alga than in the water, neither in light nor in darkness, but about just as favourable or even less favourable (p. 77, 80—83, Table 9, 10). When further we know that also in light algae are constantly destroyed in the sponge — though less than in the water —, we must conclude, that from the point of view of the use to the alga that association with the sponge cannot be called at all a symbiosis in the meaning of that of the Lichens (p. 84, 113—114).

17. We could establish in 26 points the facts which bear upon the question about the use of the „symbiotic" association (with the alga) to the sponge (p. 84—95, Table 11—15).

With the help of these data we got to the following conclusion concerning this question (p. 111—114):

It is either the want of food of the sponge or (and) the „poisonous" influence of harmful products of metabolism of the sponge (to be considered as a reaction of defence against a foreign intruder), which continually destroys green „symbiotic" algae in the amoebocytes; and exactly those algae, the power of resistance of which is already weakened for some or other reason (p. 102—109). All algae killed in this way come to the benefit of the nourishment of the sponge; as this one digests and dissolves them entirely either free in the protoplasm of its amoebocytes or in food vacuoles, keeps the products of the decomposition (p. 96—97) and rebuilds its own cell parts with them, for instance the oildroplets and carbohydrate globules (p. 98—102). These oildroplets and carbohydrate globules in their turn are, among others, the source of the great quantity of energy, which the sponge transforms in the flagellar motion of its choanocytes (p. 100).

For the present no decision can be given about the exact significance for the life of the sponge of the O_2, which the living green algae in light secrete within its tissues (p. 93). It may be, that this O_2 is of much significance; even so much, that the katabolic phase of the process of metabolism in a green sponge in light has quite another course by it — namely gives a relatively much larger quantity of energy to the sponge — than in the sponge in darkness (p. 98, 103, 109—110). Some indications were found for this possibility.

Direct transfer of products of photosynthesis from the living green algae into the sponge tissue does, most probably, not take place at all (p. 96, 87, 92).

When next we ask, what in fact the „symbiotic" relation of sponge and green alga is, considered from the point of view of the use to the sponge, we cannot very well answer that question, before the problem, mentioned above, about the significance for the sponge of the O_2 secreted by the green alga in the light, has come to solution:

a. If the significance of that O_2 is in fact so important, as was thought possible above, we must conclude — notwithstanding the fact, that the sponge continually destroys and digests numbers of algae, and notwithstanding all other phenomena, which do not seem to go together with a symbiosis — that the relation of sponge and green alga, considered from the point of view of the use to the sponge, is in fact a symbiosis, though this symbiosis is by no means so complete as that of the Lichens.

b. If, on the contrary, the significance of the O_2 secreted by the alga is only of little importance, we can conclude — whatever may be the real cause of the dying of the algae in the sponge tissue, whether it be the want of food of the sponge or (and) the „poisoning" of the algae by products of metabolism of the sponge — we must conclude that, practically spoken, that so called symbiotic relation of sponge and alga is in fact nothing but simply a process of nutrition of the sponge, or, if you like, a very first transition of a process of nutrition into a symbiosis. At any rate this always counts for a sponge in darkness.

For we could state the following:

The sponge continually imports green algae from the surrounding water into its amoebocytes (p. 50), where those algae then — it should be explicitly mentioned — are killed and digested (p. 111) by the sponge only for a part, when circumstances are favourable, while the rest of the algae can live on, photosynthesise and multiply (and will give their O_2, produced in light, to the sponge tissues (p. 93) — the only argument one can mention in favour of the conception of symbiosis!). This favourable case is only realized in sponges growing in light (p. 70—72), and then not even always (p. 41, 75—76). If, however, the circumstances are somewhat less favourable — as is the rule in sponges in darkness (p. 69—70) and as sometimes happens also in those in light (p. 41, 75—76) —, then all imported algae (and all that might be present already) are continually and unavoidably destroyed and digested by the sponge (p. 111).

18. Also some other views were given concerning the association of sponge and green alga (p. 114—116).

19. Some cases were mentioned, in which quite other algae (than the normal ones) occurred in a great number in the tissues of Ephydatiae, viz. two filamentous algae and an unicellular one. The two former proved to destroy the sponge tissue; the latter, on the contrary, was finally conquered by the sponge (p. 115—118, Fig. 43—52).

B.

20. The current of water through the canalsystem of the fresh-water sponges is caused by the flagellar motion of the choanocytes in the flagellated chambers. By studying isolated choanocytes as well as wholly intact flagellated chambers we could state, that this motion (in normal condition) takes place in a spiral- or an undulating-line, namely in a very rapid succession of waves of small amplitude passing along the flagellum from the base to the top (p. 124—132, Fig. 56*a*, 59); by which a current of water arises straight through the axis of the flagellar spiral and similarly in the direction from base to top, while the water flows on at the side of the base (p. 126—128, Fig. 56*a*). Exhaustion causes quite different motions of the flagellum, with abnormal current of water (p. 127—128, 131, Fig. 56*b-d*). The whole water-current within a flagellated chamber is of course the resultant of the little currents caused by each flagellum separately; it is rapid and regular (p. 132—133, Fig. 63). In order that a powerful and steady current may be maintained by the chamber, and that, therefore, the water will flow in quickly and exclusively at the prosopyles and flow out by the apopyle, the structure of the flagellated chamber must comply with definite requirements; these requirements were studied (p. 137, 133—136).

C.

21. By studying the phenomena of ingestion of food in normally living microscopic preparations of sponge tissue, I could state that in fresh-water sponges:

The small (food-) particles are captured from the circulating water within the flagellated chambers, viz. outside-between the collars or between the bodies of the choanocytes, while thus, so to say, the water is filtered clear (p. 142—146, Fig. 63, 65—68). Next these particles are taken up within the choanocytes, united to conglomerates and ejected again into the „intercellular plasmic ground-substance" (p. 146—148, Fig. 66, 69—71), from whence the amoebocytes with symbiotic algae take them up in their turn (p. 146—148, Fig. 71).

The coarse (food-) particles are captured from the circulating water outside and against the flagellated chambers at the side of the incurrent canal, and such, because they remain sticking in or against the prosopyles. The thin layer of apparently undifferentiated flowing protoplasm, which covers that side of every flagellated chamber with the exception of the prosopyles (p. 151—154, Fig. 72—74), then takes up each particle and carries it off aside into the tissue, so that the prosopyles again become accessible (p. 148—152, 155, Fig. 75).

Finally the question was discussed, whether food can be captured by the sponge in still more ways (p. 155—156).

22. No observations were made which could fortify the theory, that sponges feed especially on organic substances in solution (p. 138).

D.

23. My principal results regarding the digestion of food have already been mentioned in the discussion of the symbiotic relation of sponge and alga (see point **17**). I only added some new observations (p. 157—158).

E.

24. By observing the phenomena in my normally living microscopic preparations, I have come to the conclusion, that in fresh-water sponges defecation — and very likely excretion at the same time (p. 166) — takes place on a large scale by means of vacuoles, which occur along the walls of the excurrent

canals in an apparently undifferentiated plasmic substance (p. 160—168, Fig. 76, 77, 73), that in reality consists of amoeboid cells (p. 169—171).

25. Defecation perhaps also can take place at the outer surface of the sponge (p. 169), but I have never observed choanocytes performing the function of excretion or defecation (p. 169).

F.

26. The common opinion, that the ground-substance of the parenchyma, the mesogloea, should be an undifferentiated intercellular plasmic substance, proved to me not right for Spongillidae. The mesogloea entirely consists of amoeboid cells, just as LIEBERKÜHN and WELTNER stated (p. 171—175).

Leyden, Zoological Laboratory,
August 1918

BIBLIOGRAPHY.

1. Artari, A., Untersuchungen über Entwicklung und Systematik einiger Protococcoideen. Dissertation, Basel 1892.
2. —— Chlorophyllbildung durch grüne Algen. Ber. Dtsch. Botan. Ges., 1902.
3. Babak, E., Die Mechanik und Innervation der Atmung. II Porifera. In: Winterstein, Handb. d. vergl. Physiologie, Bd. I II, 1912.
4. Beijerinck, M. W., Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. Botan. Zeit., 1890.
5. Bidder, G., Note on Excretion in Sponges. Proc. R. Soc. London, Vol. 51, 1892.
6. Biedermann, W., Die Aufnahme, Verarbeitung und Assimilation der Nahrung. In: Winterstein, Handb. der Vergl. Physiologie, Bd. II I, 1911.
7. Bowerbank, J. S., A Monograph of British Spongidae. London, 1864. Vol. I.
8. Brandt, K., Ueber die morphologische und physiologische Bedeutung des Chlorophylls bei Thieren. I. Arch. f. Anat. u. Physiol., Physiol. Abth., 1882.
9. —— Idem II. Mitth. a. d. Zoolog. Stat. z. Neapel, Bd. IV, 1883.
10. Burian, R., Die Exkretion. B. Coelenteraten. In: Winterstein, Handb. der vergl. Physiologie, Bd. II II, 1910.
11. Chodat, R., l'Histoire des Protococcoidées. Bull. d. l'Herbier Boissier, T. 2, 1894.
12. Cotte, J. Comment les choanocytes de Sycandra absorbent-ils les particules alimentaires? Compt. rend. Soc. d. Biologie, T. 54, 1902.
13. —— Contribution à l'étude de la nutrition chez les Spongiaires. Bull. scient. d. l. France et d. l. Belgique, T. 38, 1904.
14. Delage, Y., Embryogénie des Eponges. Arch. d. Zoolog. expérim. et Génér., 2me Sér. T. 10, 1892.
15. —— Ephydatia fluviatilis. In: Zoologie Déscriptive, Vol. I, 1900.
16. —— et Hérouard. Traité de Zoologie concrète, Vol. II I, 1899.
17. Doflein, F., Lehrbuch der Protozoenkunde, 1911.
18. Engelmann, Th. W., Die Erscheinungsweise der Sauerstofausscheidung chromophyllhaltiger Zellen in Licht, etc. Pflüger's Archiv, Bd. 57, 1894.
19. Etard et Bouilhac, Présence des chlorophylles dans un Nostoc cultivé à l'abri de la lumière. Compt. Rend. Acad. d. Sc. Paris, T. 127, 1898.
20. Gay, F., Recherches s. l. développ. et l. classif. de quelques Algues Vertes. Dissertation. Paris, 1891.

21. Geddes, P., On nature and functions of the »Yellow cells" of Radiolarians and Coelenterates. Proc. R. Soc. Edinburgh, Jan. 1882.
22. —— Further researches on animals containing chlorophyll. Nature, Vol. 25, 1882.
23. Gerneck, Beihefte Botan. Zentralbl., 21 [2], 1907.
24. Grintzesco, J., Contribution à l'étude des Protococcacées. Rev. Génér. d. Botanique, T. 15, 1903.
25. Haeckel, E., Die Kalkschwämme, 1872.
26. Hammarsten, O., Lehrbuch der physiologischen Chemie, 1910.
27. Heinricher, E., Zur Kenntniss der Algengattung Sphaeroplea. Ber. Botan. Ges., I, 1883.
28. Hermann, L., Lehrbuch der Physiologie, 1905.
29. Hertwig, R., Lehrbuch der Zoologie, 1903.
30. Höber, R., Physikalische Chemie der Zelle und der Gewebe, 1911.
31. Hogg, J., On the action of light upon the colour of the River-sponge. Mag. Nat. History, N. S. 4, 1840.
32. Jordan, H., Vergleichende Physiologie Wirbelloser Tiere, Bd. I, 1913.
33. Jost, L., Pflanzenphysiologie, 1908.
34. Kny, L., Die Abhängigkeit der Chlorophyllfunction von den Chromatophoren und vom Cytoplasma. Ber. Botan. Ges., Bd. 15, 1897.
34*a* Keeble, F., Plant-Animals, A study in Symbiosis. Cambridge, 1912.
35. Lankester, E. Ray, On chlorophyll-corpuscles and amyloid deposits of Spongilla and Hydra. Quart. Journ. of Microsc. Science, 1882.
36. Lendenfeld, R. v., Ueber Coelenteraten der Südsee. Ztschr. wissensch. Zool., Bd. 38, 1883.
37. —— A Monograph of the Horney Sponges. London, 1889.
38. Lieberkühn, N., Beiträge zur Anatomie der Kalkspongien. Arch. f. Anat. Physiol. u. wiss. Medic., 1865.
39. —— Ueber Bewegungserscheinungen der Zellen. Schrift der Ges. z. Bef. d. ges. Naturwiss. zu Marburg, Bd. 9, 1870.
40. Lipschütz, A., Die Ernährung der Wassertiere durch die gelösten organischen Verbindungen der Gewässer (Eine Kritik). Ergebn. d. Physiologie, Asher u. Spiro. 1913.
41. Loisel, G., Contribution à l'histo-physiologie des éponges. Journ. de l'Anat. et Physiol. d. l'homme et d. animaux, 1898.
42. Masterman, A. T., On the nutritive and excretory processes in Porifera. Ann. a. Mag. of Nat. History, Ser. 6, Vol. 13, 1894.
43. Matruchot et Molliard, Variations de structure d'une Algue Verte, Stichococcus bac., sous l'influence du milieu. Compt. Rend. Acad. d. Sc. Paris, T. 131, 1900.
44. Metschnikoff, E., Leçons s. l. pathologie comparée d. l'inflammation, 1892.
45. Minchin, E. A., Sponges. In: Lankester, Treatise on Zoology, 1900.
46. Müller. K., Regenerationsvermögen der Süsswasserschämme, Arch. f. Entwickl. mechanik., Bd. 32, 1911.
47. Oltmanns, F., Morphologie und Biologie der Algen, 1904—5.
47*a* Pfeffer, W., Pflanzenphysiologie.
48. Pütter, A., Die Ernährung der Wassertiere etc., 1909.

49. Pütter, A. Der Stoffwechsel der Kieselschwämme. Ztschr. f. Allg. Physiologie, Bd. 16, 1914.
50. Radais, Sur la culture pure d'un algue verte etc. Compt. Rend. Acad. d. Sc. Paris, T. 130, 1900.
51. Schimper, A. F. W., Untersuchungen über die Chlorophyllkörper etc. Pringsheim's Jahrbücher d. wiss. Botanik, Bd. 16, 1885.
52. Schulze, F. E., Untersuchungen über den Bau und Entwicklung der Spongien. Ztschr. f. wiss. Zoologie, Bd. 29, 1877.
53. Sollas, I., Porifera. In: Cambridge Nat. History, 1906.
54. Sorby, Quart. Journ. of Microsc. Sc., Vol. 15.
55. Stahl, E., Zur Biologie des Chlorophylls, 1909.
56. Strasburger, Noll, Schenck, Karsten, Lehrbuch der Botanik, 1908.
57. Topsent, E., De la digestion chez les éponges. Arch. d. Zoologie ex-. périm. et génér., 1898.
57*a* Treub, M., Quelques recherches s. l. rôle du noyau dans la division des cellules végét. Verh. Kon. Akad. v. Wetensch., Amsterdam, Vol. 19, 1879.
57*b* van Trigt, H., A contribution to the physiology of the fresh-water sponges. Proceed. Kon. Akad. v. Wetensch. Amsterdam, Vol. 20, 24 Nov. 1917.
57*c* —— Un phénomène remarquable des cellules vivantes des gemmules etc. Archiv. Néerland. d. Physiologie, T. 2, 1918.
58. Verworn, M., Allgemeine Physiologie, 1909.
59. Vosmaer, G. C. J., Spongien. In: Bronn's Klassen und Ordnungen des Tierreichs, 1887.
60. —— Leerboek der Dierkunde, 1911.
61. —— and Pekelharing, C. A., On Sollas's membrane in Sponges. Tijdschr. Ned. Dierk. Ver., S. 2, Bd. 4., 1893.
62. —— —— Over het opnemen van voedsel bij sponsen, Proc. Kon. Akad. v. Wet. Amsterdam, Maart 1898; Observations on sponges, Verh. Kon. Akad. v. Wet. Amsterdam, 2e Sect., Dl. 6, Nº. 3, 1898; Arch. f. Anat. u. Physiol., Physiol. Abth., 1898; Onderzoek. Physiol. Lab. Utrecht, 1899.
63. de Vries, H. Plantenphysiologie, 1906.
64. Weber, M., et A. Weber—van Bosse, Quelques nouveaux cas de symbiose. Zoolog. Ergebn. e. Reise in Niederl. Ost-Indien, 1890.
65. Weltner, W., Süsswasserschwämme. In: Zacharias, Tier- und Pflanzenwelt des Süsswassers, 1891.
66. —— Der Bau des Süsswasserschwammes. Blätter für Aquar. u. Terrar. Freunde, Bd. 7, 1896.
67. —— Süsswasserschwämme. In: Zoolog. Forschungsreisen in Australien etc. R. Semon. Bd. 5. Denkschr. d. Med. Natw. Ges. Jena, Bd. 8, 1900.
68. —— Spongillidenstudien V. Arch. f. Naturgeschichte, 73. Jahrg., 1 Bd., 1907.
69. Wille, N., Chlorophyceae. In: Engler und Prantl, Die Natürlichen Pflanzen-familien. Nachträge z. I², 1911.
70. Wilson, H. V., Development of Sponges from dissociated tissue cells. Bull. of Bureau of Fisheries, V. 30, 1910.

TABLES.

TABLE 1. *The production of gasbubbles (O_2?) by green Spongillidae in bright day- or sun-light.* Sponges newly captured; placed separately into cylindrical glass vessels — capacity 5 L. (*a. b.*) or 3 L. (*c.*) — filled with water from the conduit, in the open air; sponge-pieces of equal volume in all series of experiments; an inverted funnel and tube placed under water over each piece. The sponges in darkness under a light-tight case. Column A indicates the presence (+) or absence (—) of gasbubbles within or at the outside of the sponge tissue; column B the same for the inside of funnel and tube; column C the same for all outside the funnel and tube. The sign = indicates the number of gasbubbles to be unchanged. All series of experiments are mentioned. As for the discussion, see pag. 18. In *c.* the green sponge-pieces are taken from one specimen.

a. Spongillae; in light; vol. 10 cM³.

colour of sponge		green			colourless		
n⁰ of culture		297			296		
date	hour	A.	B.	C.	A.	B.	C.
25. VII. '15	10 a.m.						
„	1 p.m.	++			—		
„	6 „	++	+++		—	+	
26. VII.	6 „	=	=		=	=	

b. Spongillae; in light; vol. 3 cM³.

colour of sponge		green			colourless		
n⁰ of culture		302			303		
date	hour	A.	B.	C.	A.	B.	C.
28. VII. '15	11 a.m.						
„	1 p.m.	++		+⁻	—		+⁻
„	4 „	++		+⁻	—		+⁻
„	7 „	++	+++	+⁻	—	+⁻	+⁻
29. VII.	7 „	=	=	=	=	=	=

c. Spongillae; vol. 1½ cM³.

colour of sponge		green			green			colourless		
n⁰ of culture		401			400			402		
in light or in darkness		darkness			light			light		
date	hour	A.	B.	C.	A.	B.	C.	A.	B.	C.
2. IX. 16	11 a.m.	—	—	—	—	—	—	—	—	—
„	11½ „	—	—	—	+	—	—	—	—	—
„	6 p.m.	—	+⁻	+⁻	++	+++	+⁻	—	+⁻	+⁻

TABLE 2. *The production of O_2 by the isolated green chlorophyll corpuscles of the Spongillidae in light*; proved by means of the ENGELMANN bacteria-method. Ravel preparations of living sponge tissue, to which some material from a bacterial-culture was added and also some small airbubbles, under coverglass surrounded with vaseline (pag. 12, *1*). Column A indicates after how long a time in light or in darkness the preparations were observed; column B indicates the distribution and the intensity of motion of the bacteria then stated in the preparation. The chief experimental series has been mentioned; in all others the result was the same. As for the discussion, see pag. 19—20.

	green sponge tissue	green sponge tissue	green sponge tissue		colourless sponge tissue	colourless sponge tissue
A.	B.	B.	B.	A.	B.	B.
immediately	equal distribution rather violent motion	equal distribution rather violent motion	equal distribution rather violent motion	immediately	equal distribution rather violont motion	equal distribution rather violent motion
after $\frac{1}{4}$ of an hour in darkness	accumulation } round violent motion } airbubbles very weak motion elsewhere	accumulation } round violent motion } airbubbles very weak motion elsewhere	accumulation } round violent motion } airbubbles very weak motion elsewhere	after $\frac{1}{4}$ of an hour in darkness	accumulation } round violent motion } airbubbles very weak motion elsewhere	accumulation } round violent motion } airbubbles very weak motion elsewhere
aft. $\frac{3}{4}$ o. a. h. in darkness	very weak motion all over	very weak motion all over				
after $1\frac{1}{2}$ h. in darkness			very weak motion all over			
after $\frac{1}{2}$ h. in light	violent motion all over	violent motion all over	violent motion all over	after $\frac{3}{4}$ o. a. h. in light	very weak motion all over	very weak motion all over
after ? h. in darkness			very weak motion all over			
after ? h. in light			violent motion all over			
after ? h. in darkness			very weak motion all over			
after ? h. in light			violent motion all over			

TABLE 3. *The production of oildrops by the isolated green chlorophyll corpuscles of the Spongillidae in light.* Ravel preparations of living sponge tissue (pag. 12, *1*). Column A indicates wether the chlorophyll corpuscles were in light (l.) or in darkness (d.) during the period preceding the examination; column B indicates the number stated of green chlorophyll corpuscles containing an oildrop per 100 green corpuscles. Some less exact experimental series are left out. As for the discussion, see pag. 20.

a. (Spongilla)

n⁰ of culture	161		162		163		164		165	
date	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.
25. II. '15	l.	68	l.	71	d.	47	d.	71	d.	41
1. III.	„	77	„	68	„	38	l.	70	l.	42
16. III.	„	93	„	100	„	40	„	93	„	100
9. IV.					l.	90				

b. Spongilla

n⁰ of culture	208		209		210		211	
date	A.	B.	A.	B.	A.	B.	A.	B.
16. III. '15	d.	38	d.	18	d.	42	d.	16
9. IV.	l.	85	l.	87	„	38	„	
„½ h. later					l.	70	l.	68
11 V.	„	90	„	97	d.	88	d.	92

c. (Spongilla)

n⁰ of culture	396 a		396 b		396 c		396 d		average %	396 e		396 f		396 g		396 h		average %
date	A.	B.	A.	B.	A.	B.	A.	B.		A.	B.	A.	B.	A.	B.	A.	B.	
16. VIII. '16	d.	42	d.	30	d.	34	d.	60	42	d.		d.		d.		d.		
18. VIII.	l.	82	l.	74	l.	82	l.	80	79	„	44	„	40	„	22	„	36	35
20. VIII.	„	96	„	92	„	94	„	94	94	l.	78	l.	74	l.	86	l.	76	78
22. VIII.										„	88	„	78	„	92	„	90	87

TABLE 4. *Cultures of the isolated green and colourless chlorophyll corpuscles of the Spongillidae in various feeding media in light and in darkness; their composition examined by means of microscopic preparations* (pag. 12, *1*). The manner in which these cultures were obtained and kept, as well as the composition of the media, is mentioned on pag. 9—12.

In the tables A—D is indicated: *a*. The n° of each culture. *b*. The species (sp. = Spongilla; ep. = Ephydatia) the culture has been taken from. *c*. The date of the beginning. *d*. The nature of the culture-medium (last column). *e*. The composition of the culture from the first day till as long as it was kept; so column 1 indicates by +, 0, or — wether in the culture the green substance (n.b. exclusively that of the green chlorophyll corpuscles, not that of other algae which might the present) is increased, remained the same or decreased; column 2 wether in the culture occurs a strong (+), a small (—) or no (n) [1]) infection of algae (a.), bacteria (b.), diatoms (d.) or of mould (m.); column 3 indicates the number of the green chlorophyll corpuscles present in the whole (microscopic) preparation; column 4 the number of the green stages of division of those corpuscles; column 5 the number of the „colourless chlorophyll corpuscles with structure" (p. 42); column 6 the number of the „colourless chlorophyll corpuscles without structure" („vague shades" excluded) — again always the number present in the whole microscopic preparation, consequently, present in an almost equal volume of each culture. As for the mode of stating these numbers as well as for the meaning of the symbols I—XII, see pag. 13—15. All data concerning one culture are united in one horizontal line. Next indicates: an asterisk to a n° (eg. 69*) that the culture orginated in a colourless sponge, a zero to a n° (eg. 84°) the culture originated in a green one; in all other cases the cultures of green corpuscles were taken from green sponges, that of colourless corpuscles from colourless ones; dr. means that the chorophyll corpuscles are degenerate, p. that they have grown pale; while c. denotes that there occur centra of growth of green corpuscles.

All cultures are mentioned. To one series belong the following numbers (put together in parenthesis) — therefore these may be compared directly —: (37, 43), (65, 66, 72), (68, 69), (80, 81), (84—87), (91, 92), (113—120), (124—129), (141—142), (145—148), (188—198), (199—204), (240—242), (309—316), (319—321). The cultures n° 72 and 86 were in the inorganic solution I of pag. 10; n° 114, 118, 125, 128 in the inorganic solution II; n° 87, 141, 142, 146—148 in the diluted organic solution III; n° 115, 119, 126, 129 in the concentrated organic solution II; n° 188—195 and 199—204 in the concentrated organic solution I; all mentioned on pag. 10—11. The feeding media of the other cultures are indicated in the tables (conf. pag. 10—11).

As these cultures should inform us, if and under which circum-

1) mentioned in special cases only.

stances green and colourless chlorophyll corpuscles pass into each other (pag. 37—41) — for we will try to explain in this way, why green sponges occur in light and colourless ones in darkness, and why green sponges grow colourless in darkness and colourless ones grow green in light (pag. 35) —; and as we can see from Table 8, that in general a green sponge must be in darkness for at least a month in order to grow almost colourless — during which period its number of green corpuscles strongly decreases, that of the colourless ones strongly increases — and that a colourless sponge must be in light for at least a month in order to grow somewhat green — during which period its number of green corpuscles strongly increases and that of the colourless ones increases a little —; we should observe our cultures of the corpuscles for more than a month.

From the tables 4 A—D results (see also p. 37—44):

1. The isolated green chlorophyll corpuscles, when cultivated in light or darkness in all kinds of feeding media, remain normal (green for instance) and alive for months, and multiply by normally green descendants.
2. The green corpuscles multiply more rapidly in light than in darkness (pag. 55).
3. In general the number of isolated colourless chlorophyll corpuscles in the cultures does not increase, but even decreases under all circumstances mentioned sub *1*; the corpuscles disappear from the culture and never pass into the green form.
4. When the green corpuscles decrease in number, the colourless ones increase; so the first ones probably pass into the latter (while these disappear after some time).
5. Bacteria do not seem to harm the green corpuscles; but mould, diatoms and algae make them grow colourless (n° 84!) (which colourless corpuscles disappear).

ophyll corpuscles in li

after $1^2/_3$ month						months			after 9 months						
1	2	3	4	5	6	4	5	6	1	2	3	4	5	6	
—	+ d.a.	XII	VII	I	.	.	.	.	.	.	.	.	.	.	cultures in water
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	XII	IV	I	I	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	cultures in inorgan. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	cultures in diluted organ. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	cultures in concentr. organ. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	cultures in diluted sponge-liquid.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	cultures in concentr. sponge-liquid.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

TABLE 4 A.

Green chlorophyll corpuscles in light.

n°	species.	date	immediately	after 1/4 month	after 1/2 month	after 1/2 month	after 3/4 month	after 1 month	after 1 1/4 month	after 1 1/2 month	after 2 months	after 2 1/2 months	after 3 1/4 months	after 4 1/2 months	after 5 1/4 months	after 6 1/2 months	after 8 months	after 9 months	
			1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	
37		10. VII	. . X . . .	+ XII . I .	— d. X . X .	. . . . .	. . . . .	. . X VII . .	+? d.a.	— d.a. XII VII I .	d.a. IV . I .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
65	sp.	11. VIII	. . . . .	+ b. XII . . .	. . . . .	— . XII . . .	. . . . .	. . . . .	+ d. X VI V .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
68*	»	»	. . V . VII X	b. VI . I .	. . . . .	+ . VIII . I .	+	. . . . .	+ . XII X I .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
84	»	27. VIII	. . XII VII I I	+ . XII VII I .	. . . . .	. . . . .	— d. IX . X I	— d. II . X I	+ d. V . XII .	. . . . .	d. IV . XII X .	. . IV . VII I	d.a. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
113	»	6. XI	. b. X I II VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ b. XII VII I II	. . . . .	. . . . .	b.m. IX VII II	. . . . .	— m.a. VII IV XII VII	. . . . .	. . . . .	. . . . .	. . . . .	
147*	ep.	19. I	. . II . V VIII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ a. c. VII IV V .	. . . . .	. . . . .	a. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	cultures in water
290	sp.	6. VII	. . X V VI IX	. . X VI V VII	+ . X V I II	. . . . .	. . XII VII IV III	. . . . .	. . . . .	. XII IV I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
309	»	9. VIII	. . X I III VI	. . . . .	. . . . .	. . . . .	. . XII VII IV IV	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . XII III I VIII	. . . . .	. . . . .	
310	»	»	. . X I III VI	. . . . .	. . . . .	. . . . .	. . XII VIII V VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . XII I I III	. . . . .	. . . . .	
311	»	»	. . X I III VI	. . . . .	. . . . .	. . . . .	. . XII VIII IV IV	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . I I I I	. . . . .	. . . . .	
312	»	»	. . X I III VI	. . . . .	. . . . .	. . . . .	. . XII V VII VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . I I I VI	. . . . .	. . . . .	
72	sp.	11. VIII	. . . . .	+	. . . . .	+ . c. . . .	. . XII VII I .	. . . . .	. . XII . I .	. . . . .	— . XII VII I .	. . . . .	— d.a. X VI III .	. . . . .	d.a. I . I I	. . . . .	. . . . .	. . . . .	cultures in morgan. feed. sol.
144	»	6. XI	. . X I II VI	. . . . .	0	. . . . .	. . . . .	. . . . .	. . VII . VI	. . . . .	. . . . .	+ X VII VIII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
141	ep.	19. I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . X III I II	. . . . .	. . . . .	d.a. IV . VII X	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted organ. feed. sol.
148	»	»	. . II . V VIII	. . . . .	. . II . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . III . V VII	. . . . .	. . . . .	a I . II II	. . . . .	. . . . .	. . . . .	. . . . .	
115	sp.	6. XI	. . X I II VI	. . . . .	m. X IV I VII	. . . . .	. . . . .	. . . . .	m. IV . VII	. . . . .	. . . . .	m. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. organ. feed. sol.
188	»	10. III	. . X III III VI	. . . . .	. . . . .	m. VIII . VII .	— . I . IV X	. . . . .	. . . . .	. . . . .	. . . . .	m. I . VII IX	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
189	»	»	. . X III III VI	. . . . .	. . . . .	m.	— m. III . V IX	. . . . .	. . . . .	. . . . .	. . . . .	m. I . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
190	»	»	. . X III III VI	. . . . .	. . . . .	m. X . IV VII	— m. IX III IV IX	. . . . .	. . . . .	. . . . .	. . . . .	m. I . II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
146	sp.	6. XI	. . X I II VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . X . I I	. . . . .	. . . . .	m. IV . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted sponge-liquid.
86*	»	27. VIII	. . I . VII IX	. . IV . I	. . . . .	. . . . .	. . a. V . VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
319	sp.	7. VIII	. . X II IV VIII	. . . . .	. . . . .	. . . . .	0 b. X I I II	. . . . .	. . . . .	. . . . .	0 . X I I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. sponge-liquid.
320	»	»	. . X II IV VIII	. . . . .	. . . . .	. . . . .	0 b. X I I I	. . . . .	. . . . .	. . . . .	0 . X I I V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	

ll corpuscles in darl

after $1^2/_3$ months						after 9 months						
1	2	3	4	5	6	1	2	3	4	5	6	
.	.	X	.	I	.	.	.	.	.	.	.	cultures in water
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	c. X	V	V	V	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	cultures in inorgan. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	c. X	VII IV	V	V	cultures in diluted organ. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	cultures in concentr. organ. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	cultures in diluted sponge-liquid
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	cultures in concentr. sponge-liquid
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	

TABLE 4B.

Green chlorophyll corpuscles in darkness.

n°	species	date	immediately	after 1/4 month	after 1/2 month	after 1/2 month	after 2/3 month	after 3/4 month	after 1 month	after 1 1/4 month	after 1 1/3 month	after 2 months	after 2 1/2 months	after 3 1/2 months	after 4 1/2 months	after 5 1/4 months	after 6 1/2 months	after 8 months	after 9 months	
43	.	22. VII	. . . . .	. . . . .	. . . . .	. . . . .	+ . . VII . .	. . . . .	. . . . .	+ d.a. XII VII I .	. X . I	. . . . .	. . IX VI . IV	d.a. VII V V V	— . VI IV I I	. . . . .	+ m. VII II I I	. . . . .	. . . . .	
66	sp.	11. VIII	. . . . .	. . X . . .	. . . . .	+ . . X III .	. . . . .	. . . . .	. . . . .	J. X . I .	. . . . .	— . VII . III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
69	"	"	. . . V . VII X	. IV . I V	. . . . .	+ . . VII . I	+ IX V III	. . . . .	. . . . .	. IV . I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
85	"	27. VIII	. . XII VII I .	0 . XII VI I .	. . . . .	. . . . .	+ . XII V I .	. . . . .	. . . . .	. . XII V I .	. . . . .	. . XII . I .	. . . . .	. XII III I I	. . . . .	. XII V I I	+ m. X V II III	. . . . .	. . . . .	cultures in water
117	"	6. XI	. + b. X I II VI	. . . . .	+ . . XII VII II IV	. . . . .	. . . . .	. . . . .	. . . . .	. XII III I I	. . . . .	. . . . .	. . XII V I I	. . . . .	— m. XII VII IV V	. . . . .	. c X IV III V	. . . . .	. . . . .	
145a	sp.	19. I	. . II . V VIII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. III . I I	. . . . .	. . . . .	. IV . II III	. . . . .	. . . . .	. . . . .	. . . . .	
313	sp.	9. VIII	. . X I III VI +	. . . . .	. . . . .	. . . . .	+ . XII III I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . VI . III VI	. . . . .	. . . . .	
314	"	"	. . X I III VI 0	. . . . .	. . . . .	. . . . .	+ . XII III I III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . VII . III VIII	. . . . .	. . . . .	
315	"	"	. . X I III VI +	. . . . .	. . . . .	. . . . .	+ . XII III I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. XII I I V	. . . . .	. . . . .	
316	"	"	. . X I III VI +	. . . . .	. . . . .	. . . . .	! ? . XII I I IV	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— . X I III VIII	. . . . .	. . . . .	
86	sp.	27. VIII	. . XII VII I .	0	. . . . .	. . . . .	. . . . .	+	. . . . .	+ . XII V I .	. . . . .	— . XII . I .	. . . . .	. . IV . I I	. . . . .	. IV . I I	. . I . I I	. . . . .	. . . . .	cultures in inorgan. feed. sol.
118	"	6. XI	. . b. X I II VI	. . . . .	0	. . . . .	. . . . .	. . . . .	. . . . .	+ . XII VI I I	. . . . .	. . . . .	. . XII VII I I	. . . . .	— . IX V I V	. . . . .	— . VII IV III III	. . . . .	. . . . .	
125	"	"	. . VII IV II X	. . . . .	. . IV . II IX	. . . . .	. . . . .	. . . . .	. . . . .	+ . IX I I I	. . . . .	. . . . .	. . V III I I	. . . . .	. III I I III	. . . . .	. V I III V	. . . . .	. . . . .	
87	sp.	27. VIII	. . XII VII I .	. . XII VI I .	. . . . .	. . . . .	. . IX . I .	. . . . .	. . . . .	+? . XII . II .	. . . . .	+? . c IX IV I II	. . . . .	. . XII VI I I	. . . . .	. . X V I I	. c VII X V I I	. . . . .	. c VII X IV V V	cultures in diluted organ. feed. sol.
142	ep.	19. I	. . . . .	. . . . .	0 . X . IV IV	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . XII V II II	. . . . .	. . . . .	+ . c XII . II IV IV	. . . . .	. . . . .	. . . . .	. . . . .	
146	"	"	. . II V VIII	. . . . .	. . II V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . II V VII	. . . . .	. . . . .	. . II I II	. . . . .	. . . . .	. . . . .	. . . . .	
119	sp.	6. XI	. + b. X I II VI	. . . . .	. . — m. IX ! II X	. . . . .	. . . . .	. . . . .	. . . . .	+ m. dr V . IV	. . . . .	. . . . .	. . . . .	. + m. dr II . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
191	"	10. III	. . X III III VI	. . . . .	. . . . .	. + m.	. . IX I III V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. + b.m. VI ? I III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. organ. feed. sol.
192	"	"	. . X III III VI	. . . . .	. . . . .	. + m.	. . IX I III V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. + m. VII IV I II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
193	"	"	. . X III III VI	. . . . .	. . . . .	. + m.	. . IX I III V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. + m. VII I I II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
194	"	"	. . X III III VI	. . . . .	. . . . .	. . n. X . I I	. + m. X I I VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. + b.m. VII IV I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
195	"	"	. . X III III VI	. . . . .	. . . . .	. . m.	. + m. IX I I VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. + b.m. VIII IV II I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
120	sp.	6. XI	. . + b. X I II VI	. . . . .	. . . IX IV III VII	. . . . .	. . . . .	. . . . .	. . . . .	. . VII III I I	. . . . .	. . . . .	. . . . .	. . VII III II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted sponge-liquid
196	"	10. III	. . X III III VI	. . . . .	. . . . .	. . n.	. . VII I III III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ c VIII III I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
197	"	"	. . X III III VI	. . . . .	. . . . .	. . n.	. . c VII I IV V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ c VIII II II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
198	"	"	. . X III III VI	. . . . .	. . . . .	. . n.	. . VII III IV V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . VII II I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
240	ep.	3. VI	. . VII IV V VIII	. . . . .	. . . . .	. . . . .	+? — c VII a. VI II III — IV —	. . . . .	. . . . .	. . VII I II — VII	. . . . .	. . VI I II — IV —	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. sponge-liquid
241	"	"	. . VII IV V VIII	. . . . .	. . . . .	. . . . .	. + a. VI II V — III —	. . . . .	. . . . .	. . VII I IV — VII —	. . . . .	. . VI II I III —	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
242	"	"	. . VII IV V VIII	. . . . .	. . . . .	. . . . .	+? — c a. V II II — IV —	. . . . .	. . . . .	. . VI II II — V —	. . . . .	. . V I II — V —	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
243	sp.	"	. . X V VII IX	. . . . .	. . . . .	. . . . .	+ c VI ? n. IX III I I	. . . . .	. . . . .	. . IX V III — III —	. . . . .	. . IX III III — III —	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
321	"	7. VIII	. . X II IV VIII	. . . . .	. . . . .	. . . . .	. . . . .	0 + b. IX I I II	. . . . .	. . . . .	. . . . .	+? . XI I II — II —	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	

chlorophyll corp

after $1^2/_3$ mon					nths	after 9 months						
1	2	3	4	5	6	1	2	3	4	5	6	
												cultures in water
												cultures in inorgan. feed. sol.
												cultures in diluted organ. feed. sol.
												cultures in concentr. organ. feed. sol.
												cultures in diluted sponge-liquid.
												cultures in concentr. sponge-liquid.

TABLE 4 C.

Colourless chlorophyll corpuscles in light.

n°	species	date	immediately	after 1/4 month	after 1/3 month	after 1/2 month	after 2/3 month	after 3/4 month	after 1 month	after 1 1/4 month	after 1 2/3 month	after 2 months	after 2 1/2 months	after 3 1/3 months	after 4 1/3 months	after 5 1/4 months	after 6 2/3 months	after 8 months	after 9 months	
			1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	
68	sp.	11. VIII	. . V . VII X	. b. VI . I	. . . . .	+ . VIII . I	— . IX VII V	. . . . .	. . . . .	+ . XII X I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in water
80	»	20. VIII	. . VI . VII X	. . VII . II VI	+ ? . VI IV II	. . . . .	. . . . .	. . . . .	. . . . .	b VII . IV	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
92	»	27. VIII	. b. II . VII IX	. . V . I VI	. . . . .	. . . . .	. . . . .	. . II . II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
127	»	6. XI	. . VII IV . II X	. . . . .	. . . . .	. . VII . III V	. . . . .	. . . . .	. . . . .	. . V . II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
147	sp.	19. I	. . II . V VIII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	— a. c. VII IV V	. . . . .	. . . . .	+ a. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	
84*	sp.	27. VIII	. . XII VII I I	+ . XII VII I	. . . . .	. . . . .	. . . . .	— d. IX . X I	— d. II . X I	. d. V . XII	. . . . .	. d. IV . XII X	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
113	»	6. XI	. b. X I II VI	. . . . .	+ . XII VII I III	. . . . .	. . . . .	. . . . .	. . . . .	+ ← b. XII VII I I	. . . . .	. . . . .	. . . . .	. . . . .	— m.a. VII IV XII VII	. . . . .	. . . . .	. . . . .	. . . . .	
128	sp.	6. XI	. . VII IV II X	. . . . .	. . . . .	. . IV . II II	. . . . .	. . . . .	. . . . .	. a. VII . I VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in inorgan. feed. sol.
114*	»	6. XI	. . X I II VI	0	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . VII . VI	. . . . .	. . . . .	. . . . .	. . . . .	+ . X VII VII	. . . . .	. . . . .	. . . . .	. . . . .	
148	sp.	19. I	. . II . V VIII	. . . . .	. . II . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . III . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted organ. feed. sol.
129	sp.	6. XI	. . VII IV II X	. . . . .	. . . . .	m. IV . IV X	. . . . .	. . . . .	. . . . .	m. I . I VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
130	sp.	10. III	. . II . VI VIII	. . . . .	. . . . .	m	. . I . V VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . b.m. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
200	»	»	. . II . VI VIII	. . . . .	. . . . .	m	. . I . V VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . b. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. organ. feed. sol.
201	»	»	. . II . VI VIII	. . . . .	. . . . .	m	. . I . V VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . m. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
188*	sp.	»	. . X III III VI	. . . . .	. . . . .	m. VIII . VII	— . I . IV X	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . m. I . VII IX	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
189	»	»	. . X III III VI	. . . . .	. . . . .	m	— m. III . V IX	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . m. I . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
190	»	»	. . X III III VI	. . . . .	. . . . .	m. X . IV VII	— m dr. IX III IV IX	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ . m. I . II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
89	sp.	27. VIII	. . I . VII IX	. . IV . I	. . . . .	. . . . .	. . . . .	+ . a. V . VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted sponge-liquid.
146*	»	6. XI	. . X I II VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . X . I I	. . . . .	. . . . .	. . . . .	. . . . .	— m. IV . I I	. . . . .	. . . . .	. . . . .	. . . . .	
319	sp.	7. VIII	. . X II IV VIII	. . . . .	. . . . .	. . . . .	. . . . .	0 — b. X I I II	. . . . .	. . . . .	. . . . .	0 . X I I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. sponge-liquid.
320	»	»	. . X II IV VIII	. . . . .	. . . . .	. . . . .	. . . . .	0 + b. X I I I	. . . . .	. . . . .	. . . . .	0 X I I V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	

ss chlorophyll cor

onth		after		nths		after 9 months						
5	6	1	2	5	6	1	2	3	4	5	6	
I	.	.	.	.	.	.	.	.	.	.	.	cultures in water
.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
I	VI	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	
-	.	.	.	I	III	.	.	.	.	.	.	
I	I	.	.	.	.	.	.	.	.	.	.	cultures in inorgan. feed. sol.
.	.	.	.	.	.	.	.	.	.	.	.	cultures in diluted organ. feed. sol.
V	VI	.	.	.	.	.	.	.	.	.	.	cultures in concentr. organ. feed. sol.
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	cultures in diluted sponge-liquid
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	
V=	VII=	.	.	.	.	.	.	.	.	.	.	cultures in concentr. sponge-liquid
=	IV=	.	.	.	.	.	.	.	.	.	.	
=	VI=	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	.	

Table 41b.

Colourless chlorophyll corpuscles in darkness.

n°	species	date	immediately	after 1/4 month	after 1/2 month	after 1/2 month	after 3/4 month	after 1 month	after 1 1/4 month	after 1 1/2 month	after 2 months	after 2 1/2 months	after 3 1/2 months	after 4 1/2 months	after 5 1/4 months	after 6 1/2 months	after 8 months	after 9 months	
			1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	
69	sp.	14. VIII	. . V . VII X	. . IV . I V	. . . . .	+ VII I . .	+ . IX V III .	. . . . .	. . . V . I .	. . IV . . I .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
81	„	20. VIII	. . V . VII X	. . IV . II .	. . . V I . I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
92	„	27. VIII	. . II . VII IX	. . V . I VI	. . . . .	. . . . .	. . II . I .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in water
124	„	6. XI	. . VII IV II X	. . . . .	. . . . .	. . IV . II IV	. . . . .	. . . . .	. . V . I VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
145	ep.	19. I	. . II . V VIII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . III . I I	. . . . .	. . . . .	. . IV . II III	. . . . .	. . . . .	. . . . .	. . . . .	
291	sp.	6. VII	. . IV . VII X	. . VI . IV VI	. . . . .	. . VI . III V	. . VII . II VI	. . . . .	. . . . .	. . VI I II V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . I . I III	. . . . .	
125	sp.	6. XI	. . VII IV II X	. . . . .	. . . . .	. . IV . II IX	. . . . .	. . . . .	+ . IX I I I	. . . . .	. . . . .	. . V III I I	. . . . .	. . III I I VI	. . . . .	. . V I III V	. . . . .	. . . . .	cultures in inorgan. feed. sol.
146	ep.	19. I	. . II . V VIII	. . . . .	. . II . V VII	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . II . V VII	. . . . .	. . . . .	. . II . I II	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted organ. feed. sol.
126	sp.	6. XI	. . VII IV II X	. . . . .	. . . . .	+ m. IV . III X	. . . . .	. . . . .	+ m. I . IV VI	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
262	ep.	10. III	. . II . VI VIII	. . . . .	. . . . .	m	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	+ m. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. organ. feed. sol.
203	„	„	. . II . VI VIII	. . . . .	. . . . .	m	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
204	„	„	. . II . VI VIII	. . . . .	. . . . .	m	. . I . I VI	. . . . .	. . . . .	. . . . .	. . . . .	+ m. I . I I	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
194	sp.	„	. . X III III VI	. . . . .	. . . . .	n . X . I I	m . X I I VII	. . . . .	. . . . .	. . . . .	. . . . .	+ m.b VII IV I I	. . . . .	b. VII I I I	. . . . .	. . . . .	. . . . .	. . . . .	
88	sp.	27. VIII	. . II . VII IX	. . II . I .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
196	„	10. III	. . X III III VI	. . . . .	. . . . .	n	. . VII I III III	. . . . .	. . . . .	. . . . .	. . . . .	c VIII III I I	. . . . .	. . VII I I I	. . . . .	. . . . .	. . . . .	. . . . .	cultures in diluted sponge-liquid
197	„	„	. . IX III III VI	. . . . .	. . . . .	n	. . c VII I IV V	. . . . .	. . . . .	. . . . .	. . . . .	+ c VIII II II II	. . . . .	. . I I I I	. . . . .	. . . . .	. . . . .	. . . . .	
198	„	„	. . X III III VI	. . . . .	. . . . .	n	. . VII III IV V	. . . . .	. . . . .	. . . . .	. . . . .	. . VII II I I	. . . . .	. . IX II I I	. . . . .	. . . . .	. . . . .	. . . . .	
241	ep.	3. VI	. . VII IV V VIII	. . . . .	. . . . .	. . . . .	a. VI II V III	. . . . .	. . VII I IV VII	. . . . .	. . VI II I III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	cultures in concentr. sponge-liquid
242	„	„	. . VII IV V VIII	. . . . .	. . . . .	? a. c V II II IV	. . . . .	. . . . .	. . VI e II III IV	. . . . .	. . V I II V	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
243	sp.	„	. . X V VII IX	. . . . .	. . . . .	? n. IX III I I	. . . . .	. . . . .	. . IX V III VI	. . . . .	. . IX III III III	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	
221	„	7. VIII	. . X II IV VIII	. . . . .	. . . . .	. . . . .	0 b. IX I I II	. . . . .	. . . . .	. . . . .	+? XI I II II	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	

TABLE 5. *The transition of isolated green chlorophyll corpuscles of the Spongillidae into the successive colourless stages*, brought about under various circumstances either in cultures (conf. Table 4) or in vaseline preparations (pag. 12, *1*), and studied by stating the number of the various chlorophyll corpuscles present in a microscopic preparation (pag. 13–14).

In the table is indicated: *a*. The n⁰ of each culture or of each preparation. *b*. The species (s. = Spongilla; e. = Ephydatia) from which the corpuscles were taken. *c*. The date of the examination. *d*. Wether the corpuscles were kept in light (l.) or in darkness (d.). *e*. The number of the various corpuscles stated: sub 1 that of the green ones, sub 2 that of the „colourless ones with clearly marked out structure" (p. 42), sub 3 that of the „colourless ones with shade of structure", sub 4 that of the „colourless ones without structure", and sub 5 that of the „vague shades of colourless ones" without structure. In the last column is mentioned, wether these data concern a culture or a vaseline preparation and under what special circumstances it was. As for the meaning of I—XII, see pag. 14; and for the discussion, see pag. 43, II; p. indicates that the green corpuscles have grown pale.

n⁰	species	date	l. or d.	1	2	3	4	5	
37		10. VII	l.	X					culture
		17. VII		XII	I	I			
		21. VII		X	X				diatom-infect.
		31. VIII		XII	I	I			
68	s.	19. IX	l.	XII	I	I			vas. prep.
p.		6. X		XII p.	I	I	I		in bright
		30. X		II p.	X	X	XII		daylight.
		15. XII			VI		X		
		25. I		I	III	IX	XII		
		14. IV		I	I	III	XII		
		3. VI		I	I	I	XII		
		25. VIII		I	I	I	X		
		3. XII		I	I	I	IX		
		20. II		I	I	I	VIII		
84	s.	2. IX	l.	XII	I	I	I		culture,
		21. IX		IX	X		I		diatom-
		6. X		V p.	XII				infection.
		28. X		IV	XII		X		
		10. XI		IV	VII		I		
		11. XII		I	I	1	I		
85 l.	s.	28. V	l.	X	V		V		culture, alga
		1. VII		I	VIII	IV	VIII		and diatom-
		4. VIII		I	VII	I	VII		infect.
94	s.	9. IX	l.	XII	I	I			prep. 3h in
		21. IX		VI p.	XII				damp at 83°.
		26. IX			destr.	destr.			

n°	species	date	l. or d.	1	2	3	4	5	
95	s.	11. IX	l.	XII	I	I	I		vas. prep.
		17. IX		XII p.	I	I	I		in bright
		19. IX		VI p.	XII				daylight.
		6. X		I	XII	I	I		
		2. XI		I	XII		I		
		15. XII			XII				
		25. I		I	VII	IX	X		
		14. IV		I	VII		IX		
		3. VI		I	I	I	X		
113	s.	11. XII	l.	XII	I	I	I		culture,
		21. I		IX	II				transitory
		17. III		VII	XII		VII		alga and
		25. V		XII	IX				mould infect.
		21. II		I	I	I	I		
114	s.	21. I	l.	X	VII				culture, alga
		18. III		IX	VIII		VIII		infection.
		25. V		V		V			
		21. II		I		I			
141	e.	24. III	l.	X	I	I	II		culture, alga
		27. V		IV	VII		X		and diatom
		21. II		I	I	I	I		infect.
146	e.	19. I	d.	II	V		VIII		culture,
		24. III		II	V		VII		normal.
		28. V		II	I	I	II		
149	e.	1. III	l.	XII	I	I	I		vas. prep.
		3. VI		I	X		X		
		3. XII		I	II		XII		
		20. II		I	I	I	XII		
188	s.	10. III	l.	X	III		VI		culture,
		26. III		VIII	VII				large inf.
		30. III		I	IV		X		of mould.
		15. VII		II	IV		VII		
189	s.	10. III	l.	X	III		VI		culture,
		30. III		III	V		IX		large inf.
		15. VII		I	III		VI		of mould.

n⁰	species	date	l. or d.	1	2	3	4	5	
190	s.	10. III	l.	X		III	VI		cult., smal-
		26. III		X		IV	VII		ler inf. of
		30. III		IX		IV	IX		mould, later-
		1. VI		I		II	II		on larger.
194	s.	10. III	d.	X		III	VI		cult., very
		26. III		X	I	I	I		small inf. of
		31. III		X	I	I	VII		mould, later-
		16. VII		VII p.	I	I	I		on large.
269	s.	24. VI	l.	X		V	X		vas. prep.
		6. VII		X	I	V	IX		
		21. VII		X	I	IV	VIII		
		2. IX		X	I	V	I	VIII	
		3. XII		II p.	II	VII	X	VIII	
272	s.	24. VI	l.	X		V	X		vas. prep.
		6. VII		X	I	I	VII		
		21. VII		X	I	II	VII		
		2. IX		X	I	I	I	VII	
		3. XII		X p.	II	III	VI	V	
275	s.	24. VI	d.	X		V	X		vas. prep.
		21. VII		X	I	IV	VII		
		2. IX		X	I	V	I	VIII	
		3. XII		X p.	l	III	V	VII	
		20. II		X p.	I	III	III	VIII	
277	s.	24. VI	d.	X		V	X		vas. prep.
		2. IX		X	I	V	I	VIII	
		3. XII		X p.	I	III	V	VI	
281	s.	24. VI	l.	V		V	XI		vas. prep.
		2. IX		II	I	I	I	VIII	
287	s.	24. VI	d.	V		V	XI		vas. prep.
		2. IX		II		VII	I	VII	
290	s.	9. VII	l.	X	V	VII	IX		culture,
		12. VII		X	III	V	VII		normal.
		17. VII		X	I	I	II		
		31. VII		XII	IV	I	III		
		24. VIII		XII	I	I	I		

n°	species	date	l. or d.	1	2	3	4	5	
290	s.	7. VII	l.	X	IV	VI	IX		vas. prep.
a.		31. VII		X	II	I	V		
		2. IX		X	I	I	I	VII	
290	s.	7. VII	l.	X	IV	VI	IX		vas. prep.
b.		31. VII		X	II	I	VI		
		2. IX		X	II	II	I	VI	
290	s.	7. VII	l.	X	IV	VI	IX		vas. prep.
c.		31. VII		X	I	IV	IV		
		2. IX		X	I	I	I	V	
290	s.	7. VII	d.	X	IV	VI	IX		vas. prep.
e.		2. IX		X	I	I	I		
290	s.	7. VII	d.	X	IV	VI	IX		vas. prep.
f.		2. IX		X	I	I	VI		
290	s.	17. VII	l.	X	I	I	II		vas. prep.
k.		2. IX		X p.					in bright
		4. XII		I	I	I	X		daylight.
290	s.	4. VIII	l.	XII	I	I	III		vas. prep.
n.		3. IX		X	VII	VII	X		in sunlight.
		5. X		IX p.	VII	VII	XI		
		4. XII		I	I	VII	XII		
		20. II		I	I	V	XII	VIII	
290	s.	4. VIII	l.	XII	I	I	III		vas. prep.
p.		3. IX		X	VII	VII	X		in sunlight.
		5. X		X p.	VII	VII	XI		
		4. XII		X p.	III	VII	VII	VIII	
		20. II		X p.	I	IV	VII	III	
291	s.	7. VII	d.	IV	V	VII	X		culture,
		9. VII		IV	V	VII	VIII		normal.
		12. VII		VI	I	IV	VI		
		21. VII		VI	II	III	V		
		21. II		I	I	I	III		
307	s.	3. IX	l.	XII	I	I	V		vas. prep.
l.		5. X		I	III	VII	XII		in sunlight.
		3. XII		I	I	VII	XII		
		20. II		I	I	II	XII	VII	
		28. VIII		I	I	II	XII	VII	

n°	species	date	l. or d.	1	2	3	4	5	
308	s.	3. IX	l.	XII	I	I	I		vas. prep.
l.		5. X		I	I	III	XII		in sunlight.
		3. XII		I	I	VI	I	XII	
		20. II		I	I	I	IV	VIII	
318	s.	7. VIII	l.	X	III	III	VI		vas. prep. 3h
		5. X		I	I	I	X		at 83°.

TABLE 6. *The number of the various green and colourless chlorophyll corpuscles present in different stages of development of the tissue of living green and colourless Spongillidae grown in light or in darkness*, examined microscopically by means of ravel preparations of the tissues (pag. 12—14).

It was of much importance to know the intrinsic amount of chlorophyll corpuscles in the sponge tissue during its different stages of development, viz. in its prime youth — when growing vigorously —, in full-grown state — growing no more —, and in its stage of rest — as gemmulae in winter. Young, growing tissue we find 1st in the small (2—5 m.M.) sponge-disks, attached for instance to stones, and being probably newly germinated gemmules; 2nd in the tops of the branches of Spongilla (p. 16); probably, however, the disks will appear younger than the tops. Full-grown tissue we generally find all over the sponge body except in the tops, so for instance at the base of the branches or in the crust (p. 16). This only concerns Spongilla; as mentioned (p. 16), Ephydatia does not form long branches but cushions; so in this sponge we can not distinguish a certain limited region of growth. There the growth is more general, but then locally not so vigorous as it is in the tops of Spongilla.

Consequently, for Spongilla I shall indicate in the table the amount of chlorophyll corpuscles in 4 groups, viz. for the tissue of 1st sponge-disks, 2nd branch-tops, 3rd branch-bases or crusts, 4th gemmules. In this last group one may distinguish again the gemmules in different stages of development; their last stage then joins the group of the sponge-disks. In this way the circle is accomplished. For Ephydatia we can not give groups of old and young tissue — as mentioned —; of course it could be done for disks and gemmules, but this has been left out here.

In the tables is indicated: *a*. The month of the analysis of each

sponge. *b.* Wether the sponge, at the moment of its analysis, was brought from its habitat in nature to the aquaria for more (n.) or less (i.) than 2 weeks ago; in the first case we may say, that probably a good deal of the influence of the import of chlorophyll corpuscles (p. 50) on the amount of these corpuscles in the sponge will have disappeared; but not, however, in the last case. *c.* The number of the various chlorophyll corpuscles present in the tissue: in column 1 that of the green ones, in col. 2 that of the green stages of division, in col. 3 that of the „colourless corpuscles with structure" (p. 42), in col. 4 that of the „colourless ones without structure" („vague shades" excluded); always the number present in the whole microscopic preparation, present, therefore, in an almost equal volume of each sponge.

The data concerning one sponge piece are given on one horizontal line. Sub 5 the analyses of different pieces of a same sponge are indicated by the same letters. All analyses are mentioned. As for the meaning of I—XII, see pag. 14. At last for each tissue-group of the green and colourless sponges the amount of the various chlorophyll corpuscles, present in all analyzed sponges together, is composed. This composition simplifies of course the mutual comparison of the results of the different groups.

As for the discussion, see pag. 46—48 and 76.

TABLE 6 A.

month	i. or n.	green ones from light 1	2	3	4	5		month	i. or n.	colourless ones from darkness 1	2	3	4	5	
IX	i.	XII	VI	I	III	.	tissue of very young sponge-**disks** (2—5 m.M. diameter); vigorously growing.	IX	i.	VII	I	VI	IX	.	tissue of very young sponge-**disks** (2—5 m.M. diameter); vigorously growing.
IX	„	X	VI	I	I	.		IX	„	II	.	V	.	.	
VII	„	XII	IV	II	VII	.		IX	„	V	I	VII	.	.	
VII	„	XII	VI	V	VII	.		IX	„	III	I	VII	.	.	
VII	„	XII	IV	V	VII	.		II	„	II	.	V	.	.	
VII	„	XII	II	IV	VII	.		VII	„	II	I	III	VIII	.	
VII	„	X	IV	III	VI	.		VII	„	II	I	VI	VIII	.	
VII	„	XII	IV	V	VII	.		VII	„	III	I	V	VIII	.	
VII	„	XII	V	I	VI	.		VII	„	VI	I	V	VII	.	
								VII	„	VI	I	VI	IX	.	
								VII	„	VI	I	V	VIII	.	
							the 9 together: col. 1. : 2 X + 7 XII col. 2. : 1 II + 4 IV + 1. V + 3. VI col. 3. : 3. I + 1 II + 4 III + 1 IV : 3 V col. 4. : 1. I + 1 III : 2. VI + 5. VII								**the 11 together:** col. 1. : 4 II + 2 III + 4. V + 3. VI + 1. VII col. 2. : 9. I col. 3. : 1 III + 5. V + 3. VI + 2 VII col. 4. : 1. VII + 4. VIII + 2. IX
VII	i.	X	V	IV	VIII	.	tissue of branch-**tops**; vigorously growing.	VII	i.	VII	II	V	IX	.	tissue of branch-**tops**; vigorously growing.
VII	„	XII	VII	IV	VII	a		VII	„	VII	II	VI	VIII	k	
VII	„	XII	III	IV	VIII	a		VII	„	VII	I	VI	XII	k	
VII	„	XII	V	IV	VII	a		VII	„	VI	I	VI	IX	l	
VII	„	XII	V	III	VII	b		VII	„	VII	I	VI	IX	l	
VII	„	XII	V	III	VII	b		VII	„	VIII	IV	V	IX	m	
VII	„	XII	VII	III	VII	c		VII	„	VI	I	V	IX	n	
VII	„	XII	VI	III	VII	c		VII	„	VI	I	VII	IX	o	
IX	„	XII	IX	V	IX	.		VII	„	VII	I	IV	IX	p	
IX	„	XII	VII	I	III	d		VII	„	IV	I	V	IX	q	
VI	„	X	VI	IV	VI	e		VII	„	III	I	IV	IX	r	
VI	„	X	VI	IV	VII	f		VII	„	V	I	VI	IX	s	
VI	„	X	VI	III	VII	f		VII	„	VII	I	III	IX	t	
VI	„	XII	VII	III	VII	g		VII	„	VI	I	IV	VIII	u	
VI	„	XII	VII	III	VII	g		VII	„	V	I	V	IX	v	
VII	„	XII	III	IX	IX	.									
VII	„	XII	III	VI	VIII	.									
VII	„	XII	IV	IV	VIII	.									
VII	„	XII	III	V	VIII	h	tissue of branch-**bases** and of **crusts**; full-grown.								
VII	„	X	I	IV	VIII	i									
VII	„	XII	I	IV	VII	j	**the 30 together:** col. 1. : 1 IX + 13. X + 2. XI + 14. XII col. 2. : 5. I + 3. II + 5. III + 3. V + 3. VI + 3. VII + 3. VIII col. 3. : 1. I + 5. II + 8. V + 4. VI + 1. VII col. 4. : 1. VI + 6. VII + 9. VIII + 5. IX + 8. X								**the 15 together:** col. 1. : 1. III + 1. IV + 2. V + 4. VI + 6. VII + 1. VIII col. 2. : 2. I + 2. II + 1. IV col. 3. : 1 III + 3. IV + 5. V + 5. VI + 1. VII col. 4. : 2. VIII + 12. IX + 1. XII
VI	i.	XII	II	III	VII	.									
VI	„	XII	I	V	VIII	.									
VI	„	XII	II	IV	VII	.									
VI	„	XII	II	IV	VIII	.									
IX	„	XII	V	VI	VII	.									
VI	„	X	V	IV	VII	e		VII	i.	IV	I	VI	IX	l	
VI	„	X	VII	IV	VIII	.		VII	„	VI	I	VI	XII	l	
VI	„	X	III	IV	VIII	f		VII	„	VI	I	VI	XII	k	
VI	„	X	VII	IV	X	g		VII	„	V	II	VIII	XII	k	
VI	„	XII	III	III	VIII	.		VII	„	II	I	VI	XII	q	
VI	„	XII	.	III	VII	.		VII	„	VI	I	VII	XI	m	
VI	„	XII	III	III	VIII	.		VII	„	II	I	VIII	XII	n	
VI	n.	X	IV	V	X	.		VII	„	II	I	VI	XII	o	
VI	„	X	IV	IV	X	.		VII	„	II	I	VI	XI	p	
VII	i.	XII	VII	IV	VIII	c		VII	„	II	I	VI	XI	r	
VII	„	X	VI	V	IX	b		VII	„	V	I	V	XI	s	
VII	„	XII	III	V	VIII	b		VII	„	VI	I	VI	XII	t	
VII	„	X	VI	IV	X	b		VII	„	IV	I	VII	XI	u	
VII	„	X	V	V	IX	a		VII	„	III	I	VI	X	v	
VII	„	IX	III	VI	IX	a									
VIII	n.	X	II	IV	VIII	.									
VIII	„	X	II	IV	VII	.									
VIII	„	X	I	III	VI	.									
X	.	XII	.	V	.	.									
VI	i.	X	I	VII	X	.									
VI	„	XI	IV	V	X	.									
VII	„	XII	.	VI	IX	h									
VII	„	XII	I	VI	X	i									
VII	„	XI	I	V	IX	j									
XI	n.	X	.	II	.	.	young gemmulae	XI	n.	V	.	IX	XII	.	young gemmulae
XI	„	X	.	II	.	.		XI	„	III	.	IX	XII	.	
XI	„	IX	I	V	IX	.	older gemmulae in Nov.	.	.	.	.	.	.	.	
XI	„	IX	I	V	X	.		.	.	.	.	.	.	.	
I	„	IX	I	I	.	.	old gemmulae in Jan.	I	n.	I	I	II	.	.	old gemmulae in Jan.
I	„	IX	I	I	.	.		I	„	I	I	II	.	.	
I	i.	X	IV	I	.	.	newly germinated gemmulae	II	i.	V	II	III	IV	.	newly germinated gemmulae
I	„	X	III	.	.	.		II	„	VI	I	II	V	.	
I	„	.	IX	I	.	.		II	„	VI	I	II	VI	.	
I	„	X	III	I	.	.	skeleton wholly filled up by light-green germinated gemmules	II	„	IV	I	V	VIII	.	skeleton wholly filled up by germinated gemmules.
I	„	X	X	I	.	.		II	„	II	.	VII	IX	.	
II	n.	X	V	II	VI	.	idem, but tissue now green.								
II	„	XII	VII	IV	VII	.									

TABLE 6 B. Spongillae.

green ones from darkness							colourless ones from light						
month	i. or n.	1	2	3	4		month	i. or n.	1	2	3	4	
VII	i.	X	I	IV	IX	tissue of **bases** and **crusts.**	VII	i.	VI	I	V	XI	tissue of **bases** and **crusts.**
VII	„	X	I	IV	X		VII	„	VI	I	V	X	
VII	„	IX	II	IV	IX		VII	„	VII	I	IV	IX	
VII	„	X	I	V	X		VII	„	VII	II	IV	IX	
VII	„	VIII	II	III	VIII		VII	„	VI	I	IV	IX	
VII	„	VIII	I	V	VIII		VII	„	V	I	IV	IX	

TABLE 6 C. Ephydatiae.

green ones							colourless ones						
month	i. or n.	1	2	3	4		month	i. or n.	1	2	3	4	
II	i.	IX	V	IV	IX	**the 20 together:** col. 1. : 1. VIII + 4. IX + 8. X + 2. XI + 5. XII col. 2. : 1. II + 1. III + 4. V + 7. VI + 7. VII col. 3. : 2. I + 5. III + 2. IV + 6. V + 4. VI + 1. VII col. 4. : 8. VII + 8. VIII + 2. IX	II	i.	III	I	VI	X	**the 13 together:** col. 1. : 3. I + 4. II + 2. III + 1. IV + 2. V + 1. VI col. 2. : 11. I + 1. II + 1. IV col. 3. : 1. V + 9. VI + 3. VII col. 4. : 1. VII + 2. VIII + 7. IX + 3. X
II	„	X	VI	III	VIII		II	„	V	I	VI	IX	
II	„	X	VII	III	VIII		II	„	IV	I	VI	X	
II	„	X	VI	III	VII		XI	„	V	I	VII	IX	
II	„	X	VI	IV	VIII		XI	„	VI	IV	VI	VII	
II	„	X	VI	III	VII		XI	„	II	I	VII	IX	
XII	n.	XII	V	I	.		II	„	III	II	VI	IX	
XII	„	XII	V	I	.		II	„	II	I	V	VIII	
II	i.	VIII	VI	III	VII		II	„	II	I	VI	VIII	
II	„	IX	VI	VI	VIII		II	„	II	I	VII	X	
II	„	XII	VII	V	VII		II	„	I	I	VI	IX	
II	„	X	VII	V	VIII		VII	„	I	I	VI	IX	
II	„	IX	VII	V	VIII		VII	„	I	I	VI	IX	
II	„	XII	VII	VI	VII								
II	„	X	VII	V	VII								
II	„	XI	VII	V	VII								
II	„	X	V	V	VII								
II	„	IX	VI	VI	VIII								
VI	„	XII	II	VI	VIII								
VI	„	XI	III	VII	IX								

TABLE 7. *The capture of green chlorophyll corpuscles by colourless Spongillae from a diluted suspension (in water) of such corpuscles isolated from a green sponge.*

Some equal cylindrical glass vessels were filled with 3 L. of water from the conduit; then a same quantity of the material, pressed out from a green Spongilla, was added to each vessel of one series; so all vessels looked equally grayish. This suspension always proved to contain green chlorophyll corpuscles only, no other algae. Next an equally large piece of colourless Spongilla was placed in each vessel of the series, except in one, and all vessels exposed to light or kept in darkness. Sometimes also an equally large piece of colourless Spongilla was put into a vessel filled with water from the conduit only, and exposed to the same conditions.

In the tables is indicated: *a*. The degree of troubling of the culture suspension (++++ = very much troubled; 0 = clear). *b*. The colour of the sponge. *c*. The presence (+) or absence (0) of oscular-tubes. These data are given for several days. The chief series have been mentioned. As for the discussion, see pag. 28, 49; for the continuation of the experiments, see Table 8.

TABLE 7 A. in light; volume sponge 10 cM³; 9—VI—'15.

n°	244	245	246	247	248	251	
contents of vessel	suspens. + sponge	suspens. + sponge	suspens. + sponge	suspens. + sponge	suspens. + sponge	suspens.	
degr. of troub.	++++	++++	++++	++++	++++	++++	1st day
col. of spong.	colourless	colourless	colourless	colourless	colourless		
osc. tub.							
degr. of troub.	++++	+++	+	+++	0	++++	2d day
col. of spong.	somewhat greenish	greenish	the greater part light-green	greenish	entirely light-green		
osc. tub.	+	0	+++	0	++++		
degr. of troub.	++++	+++	0	+++	0	++++	3d day
col. of spong.	somewhat greenish	greenish	the greater part light-green	greenish	entirely light-green		
osc. tub.	0	0	+++	0	+++		

TABLE 7 B. in light; volume sponge 7 cM³; 11—VI—'15.

n⁰	253	254	255	256	257	258	259	
contents of vessel	suspens.	suspens. + sponge	suspens. + sponge	suspens. + sponge	suspens. + sponge	suspens. + sponge	conduit-water + sponge	
degr. of troub.	++++	++++	++++	++++	++++	++++	0	1st day
col. of spong.		colourless	colourless	colourless	colourless	colourless	colourless	
osc. tub.								
degr. of troub.	++++	++	++	+++	+	++	0	2d day
col. of spong.		2/5 light-green	1/2 light-green	somewhat greenish	3/5 light-green	1/2 light-green	colourless	
osc. tub.		0	0	0	++	0	++	
degr. of troub.	++++	+	+	++	0	+	0	4th day
col. of spong.		1/2 light-green	3/4 light-green	1/2 light-green	2/4 light-green	1/2 light-green	colourless	
osc. tub.		0	0	0	0	0	+	

TABLE 7 C. in darkness; volume sponge 3 cM³; 24—VIII—'15.

n⁰	325	326	327	328	
contents of vessel	suspens.	suspens. + sponge	suspens. + sponge	conduit-water + sponge	
degr. of troub.	++++	++++	++++	0	1st day
col. of spong.		colourless	colourless	colourless	
osc. tub.		+++	+		
degr. of troub.	++++	++	+	0	2d day
col. of spong.		1/1 light-green	1/1 light-green	colourless	
osc. tub.		++	+++	+	
degr. of troub.	++++	0	+	0	4th day
col. of spong.		1/1 light-green	1/1 light-green	colourless	
osc. tub.		++++	+++	+	

TABLE 8. *Cultures of green and colourless Spongillidae in light or in darkness in aquaria filled with flowing water from the conduit; while the colour of the sponges and the number of the various green and colourless chlorophyll corpuscles in the tissues changes or remains constant.*

Generally of each sponge some pieces were cultivated in light and some other ones under equal circumstances in darkness; such pieces of one sponge are indicated in the tables with one n°, adding l. or d., event. I and II, to it. These cultures, of course, may be compared directly. The colour, and generally also the number of the various chlorophyll corpuscles in the tissues of each sponge (= piece) was exactly examined (pag. 13—15) at the beginning as well as at the end of the experiment. Mostly the material of the branch-tops was well discerned from that of the branch-bases (see Table 6 A).

The cultures took place, as mentioned, in aquaria with flowing water from the conduit or sometimes in glass vessels with 3 L. of the same (but not flowing) water. As for the arrangement, see pag. 8—9. The cultures were kept till the sponges began to die or to reduce their tissue; Spongillae usually after about 1 month, Ephydatiae after about 2 months. In this way the factors of import, (export), reduction and growth (p. 49—53) were excluded as much as possible in these experiments; while as only active factors remained multiplication and mortality (pag. 54—62).

That import was excluded follows from the fact that the sponges were cultivated in water from the conduit. The export is, as stated above, an uncertain but probably not important factor. The reduction, as mentioned, was excluded by putting a stop to the experiments, as soon as it appeared; while, moreover, a continual vigorous circulation of fresh water through the aquaria tried to limit its troubling consequences as much as possible (p. 53). Had these measures been not entirely sufficient (which cannot be decided), one may consider, however, safely that the reduction will have equally influenced all experiments of one series. The factor of growth, of course, can never be entirely excluded; but in these experiments it was generally but very weak only (p. 53).

In the table is indicated: *a.* The n° of each experiment. *b.* The species of the sponge piece (s. = Spongilla; e. = Ephydatia). *c.* Whether the piece was cultivated in an aquarium (a.) or in a glass vessel (v.). *d.* The date of the examination. *e.* Whether the examined material was taken from a branch-top (t.) or from a branch-base (b.). *f.* Wheter the sponge, at the moment of examination, was in good condition (g.) — showing no or but few reduction —, or that more

TABLE 8.

cultures in light													cultures in darkness												
n°	s. e.	o. v.	date	t. b.	g. ½ r.	— + +	colour	1	2	3	I II III		n°	s. e.	o. v.	date	t. b.	g. ½ r.	— + +	colour	1	2	3	I II III	
7	?	a	2 IV	.	g.		colourless																		
			5 V	.	.	.	partly green	.	.	.	I														
7 3 eq.	?	a	2 IV	.	g.		green																		
			27. V	.	.		"	.	.	.	II														
7 3 eq.	s.	a.	20. VIII	.	g.		green																		
			16. IX	.	½		"	.	.	.	II														
7 3 eq	s.	a	22. VII	.	g.		green																		
			21. VIII				"	.	.	.	II														
10.	s.	a.	4. VIII	.	g.	.	green																		
			11 VIII	.	.	.	partly yellow-green																		
			18. VIII	.	.	.	partly light yell-green				III														
			26. VIII	.	g.	-																			
33. l.	s.	v.	4. VII	.	g.	.	colourless						33. d.	s.	v.	10. VII	.	g.	.	light-green					
			10. VII	.	g.	.	greenish									20. VII	b.	r.	—	yellow-green	.	.	.	III	
			16 VII	b.	g.	.	light yellow-green																		
			20 VII	b.	r.	.	light-green			.	I														
35. l.	?	v.	10. VII	.	g.	.	green						35. d.	?	v.	10. VII	.	g.	.	green					
			20 VII	.	r.	.	"									20. VII	.	.	.	"					
			23 VII	.	.	.	"	.	.	.	II					23. VII	.	.	.	light-green	.	.	.	III	
39.	s.	a.	4. VII	.	g.	.	green																		
			27. VII	.	.	.	"	.	.	.	II														
49.	s.	v.	3. VIII	.	g.	.	green						48.	s.	v.	3. VIII	.	g.	.	green					
			17. VIII	.	.	.	"									17. VIII	.	.	+	light-green					
			24. VIII	.	.	.	"									24. VIII	.	.	.	"					
			31. VIII	.	g.	—	"									31. VIII	.	g.	.	yellow-green					
			8 IX	t.	.	.	"	X	IV							8. IX	b	½	.	greenish	IX	I	X	III	
			"	b.	.	.	light-green		.		III														
51.	s.	v.	3 VIII	.	g.	.	colourless						50.	s.	v.	3. VIII	.	g.	.	colourless					
			17 VIII				"									17. VIII	.	.	.	"					
			24. VIII	.	e.	.	"									24. VIII	.	r.	.	"					
			31. VIII	.	.	.	"									31. VIII	.	.	.	"					
			8 IX	b.	.	.	"	V	I	VI	II					8. IX	b.	.	.	"	II	I	VI	II	
57.	s.	a.	3 VIII	.	g.	.	green																		
			14. IX	.	v.	e.	"	.	.	.	I														
99.	s.	v.	24. IX	.	g.	.	green																		
			21. X	.	r.	.	"	.	.	.	II														
100. l.	s.	v.	24. IX	.	g.	.	green						100. d.	s.	v.	24. IX	.	g.	.	green					
			3. X	.	.	.	"									3. X	.	.	.	"					
			21. X	.	g.	.	"									21. X	.	g.	.	light yellow-green					
			2. XI	.	.	.	somewhat light-green	X	V	VII	III					2. XI	b	½	.	greenish	VII	VII	X	III	
102. l.	?	v.	24. IX	.	g.	.	colourless						102. d.	s.	v.	24. IX	.	g.	.	colourless					
			3. X	.	r.	.	"									3. X	.	r.	.	"					
			21. X	.	r.	.	greenish									21. X	.	r.	.	"					
			2. XI	b	½	.	"	VII	X	X	I					2. XI	b	.	.	"	II	X	XII	II	
102. l. suc	s.	v	24. IX	.	g.	.	clrl. i. susp. o. gr. ch. c.																		
			3 X	.	.	.	colourless																		
			21 X	.	g.	.	greenish																		
			2 XI	b	½	.	light-green	X	IX	IX	I														
134. 3 eq.	e.	a.	26. XI	.	g.	.	colourless						138. 4 eq.	e.	a.	25. XI	.	g.	.	colourless					
			15 XII	.	.	.	yellow-green				I					15. XII	.	.	.	yellow-green					
			22. II	.	.	.	2 colrless, 1 yellow-gr.				III					15. I	.	.	.	light-green				I	
																22. II	.	.	.	light yellow-green					
																3. IV	.	.	.	colourless				III	
135.	e.	a.	29. XI	.	g.	.	colourless						/ 3 eq.	e.	a.	26. XI	.	g.	.	colourless					
			15 XII				light yellow-green									15 XII	.	.	.	"					
			22 II	.	.	.	yellow-green				I					22 II	.	.	.	"					
136.	e.	a.	26. XI	.	g.	.	colourless									3. IV	.	r.	.	"				II	
			15 XII				light yellow-green																		
			22 II	.	.	.	yellow-green						/ 3 eq.	e.	a.	27. II	.	g.	.	colourless					
			2 IV	.	.	.	light-green				I					3 IV	.	.	.	"					
137. 3 eq.	e.	a.	26. XI	.	g.	.	partly green									15. V	.	.	.	"				II	
			15 XII	.	.	.	" "																		
			22 II	.	.	.	entirely green				I		/ 3 eq.	e.	a.	13 IV	.	g.	.	colourless					
																15. V	.	.	.	"				II	
/ 3 eq.	e.	a.	29 IX	.	g.	.	colourless																		
			13 X	.	.	.	yellow-green				I														
/ 3 eq.	e.	a.	26. XI	.	g.	.	partly green																		
			15 XII	.	.	.	" "																		
			22 II	.	.	.	entirely green																		
			2 IV	.	.	.	" "				I														
/ 3 eq.	e.	a.	26. XI	.	g.	.	partly green																		
			15 XII	.	.	.	" "																		
			22 II	.	.	.	entirely green																		
			2 IV	.	.	.	" "				I														
139. l.	e.	a.	19. I	.	g.	.	green						139. d.	e.	a.	19. I	.	g.	.	green					
			22. II	.	.	.	"									22. II	.	.	.	"					
			2. IV	.	.	.	"									2. IV	.	.	.	"					
			15. V	.	.	.	"	.	.	.	II					15. V	.	.	.	"				II	
144. l.	e.	a.	19. I	.	g.	.	colourless						144. d.	e.	a.	19. I	.	g.	.	colourless					
			22. II	.	.	.	"									22. II	.	.	.	"				II	
			2 IV	.	.	.	"				II														
206.	e.	v.	10. III	.	g.	.	colourless						205.	e.	v.	10. III	.	g.	.	colourless					
			5 V	b.	g.	.	"	III	IV	IX	II					30. III	.	.	.	"					
			22. V	.	r.	.	"									22. IV	.	.	.	"					
																5. V	b.	g.	.	"	II	IV	IX		
207.	e.	v.	10. III	.	g.	.	clrl. i. susp. o. gr. ch. c.									22. V	.	g.	.	"				II	
			10. III	.	.	.	greenish																		
			30. III	.	.	.	light yellow-green	.	.	.	I														
			22. IV	.	g.	.	" " "																		
			5. V	b.	.	.	greenish	IX	V	VII															
			22. V	.	r.	.	colourless	.	.	.	III														

TABLE 8 (continued).

cultures in light													cultures in darkness											
n°	s. e.	a. v.	date	t. b.	g.	colour	1	2	3	I II III	n°	s. e.	a. v.	date	t. b.	g.	colour	1	2	3	I II III
216.	s.	v.	9 IV		g.	green					217.	s.	v.	9 IV		g.	green				
			22 IV		g.	"								22 IV		g.	light-green				
			5 V	b.	y.	"	XII	II	VIII	II				5 V	b.	g.	yellow-green	IX	II	IV	III
											218.	s.	v.	9 IV		g.	green				
														22 IV		g.	"				
														5 V	b.	g.	light yellow-green	X	III	IV	III
246.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.					256.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.				
			14. VI			light-green								14. VI			light-green				
			22. VI			greener								22. VI			"				
			2. VII		g.	almost normally green								2. VII			almost colourless				
			10. VII	t.		normally green	X	V	VIII	I				10. VII	t.	g.	" "	VIII	V	X	
248.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.								21. VII		$\frac{1}{2}$	" "				III
			14. VI			light-green					255.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.				
			22. VI			greener								14. VI			light-green				
			2. VII	b.	g.	almost normally green	X	V	VIII	I				22. VI			"				
			10. VII			normally green								2. VII			yellow-green				
254.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.								10. VII	t.	g.	almost colourless	VII	IX	IX	
			14. VI			light-green								21. VII		$\frac{1}{2}$	colourless				III
			22. VI			greener															
			2. VII		g.	still greener															
			10. VII			almost normally green				I											
257.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.															
			14. VI			light-green															
			22. VI			greener															
			2. VII		g.	still greener															
			10. VII	t.		almost normally green	X	IV	VIII	I											
258.	s.	a.	14. VI		g.	chl. i. susp. o. gr. ch. e.															
			14. VI			light-green															
			22. VI			greener															
			2. VII		g.	still greener															
			10. VII			almost normally green				I											
259.	s.	a.	14. VI		g.	colourless															
			22. VI			"															
			2. VII		g.	greenish															
			10. VII			"															
			21. VII	t.	g.	light-green	VIII	IV	VII	I											
260. l.	s.	a.	11. VI		g.	green	XII	III	VIII		260. d.	s.	a.	11. VI		g.	green	XII	III	VIII	
			3. VII			"								3. VII		g.	light-green				
			10. VII	t.	g.	more light-green	X	III	VI	III				10. VII	t.	g.	yellow-green	IX	VII	XII	III
261. l.	s.	a.	11. VI		g.	green	XII	III	VIII		261. d.	s.	a.	11. VI		g.	green	XII	III	VIII	
			3. VII			"								3. VII							
			10. VII	t.	g.	"	X	III	VII	III				10. VII	t.	g.	" "	IX	V	VIII	
			22. VI		g.	"								22. VI		g.	light green				III
262.	s.	a.	11. VI		g.	green	XII	III	VII												
			3. VII			"															
			9. VII	t.		more light-green	XII	VII	XII												
			9. VII	b.		" " "	IX	IV	XII	III											
263. l.	s.	a.	12. VI		g.	colourless	II	III	IX		263. d.	s.	a.	12. VI		g.	colourless	II	III	IX	
			3. VII	t.		light yellow-green	VIII	III	X	I				3. VII	t.		"	VI	III	X	III
264. l.	s.	a.	15. VI		g.	colourless	III	IV	X		264. d.	s.	a.	15. VI		g.	colourless	III	IV	X	
			3. VII	t.		light yellow-green	VIII	IV	X	I				3. VII			"	VII	III	X	III
242. l.	s.	a.	23. VII	b.	g.	green	XII	IV	VIII		252. d.	s.	a.	23. VII	b.	g.	green	X	V	IX	
			17. VIII	b.		"	XII	IV	IX	I				17. VIII	b.	g.	gray-green	IX	V	XII	III
243. l.	s.	a.	23. VII	b.	g.	green	XII	V	VIII		253. d.	s.	a.	23. VII	b.	g.	green	X	IV	X	
			18. VIII	b.		more light-green	X	V	X	II[10]				18. VIII	b.	g.	gray-green	X	VII	XII	II[10]
244.	s.	a.	24. VII	b.	g.	green	X	V	IX		254. d.	s.	a.	24. VII	b.	g.	green	IX	VI	IX	
			19. VIII	b.	g.	"	X	V	X	II				19. VIII	b.	g.	gray-green	VII	VII	XI	III
245. l.	s.	a.	23. VII		g.	light-green					255. d.	s.	a.	23. VII		g.	light-green				
			16. VIII	b.	g.	almost normally green	X	IV	X	I				16. VIII	b.	g.	colourless	VI	III	XII	III
"	s.	a.	23. VII		g.	light-green					"	s.	a.	23. VII		g.	light-green				
			16. VIII		g.	almost normally green				I				16. VIII		g.	light yellow-green				III
"	s.	a.	23. VII		g.	light-green					"	s.	a.	23. VII		g.	light-green				
			16. VIII		g.	almost normally green				I				16. VIII		g.	"				II
258. l.	s.	a.	25. VII	b.	g.	colourless	IV	VI	IX		258. d.	s.	a.	25. VII	b.	g.	colourless	VI	VI	XI	
			16. VIII	b.	g.	light-green	VIII	V	XII	I				16. VIII	b.	g.	"	VII	III	XII	III
259. l.	s.	a.	26. VII	b.	g.	colourless	VI	VI	XII		259. d.	s.	a.	26. VII	b.	g.	colourless	V	VIII	XII	
			16. VIII	b.	g.	light-green	VIII	VII	XII	I				16. VIII	b.	g.	"	VI	VI	XII	III
232. l.	s.	a.	21. VIII		g.	green					232. d.	s.	a.	21. VIII		g.	green				
			16. IX			"				II				16. IX			light-green				III
233. l.	s.	a.	21. VIII		g.	green					233. d.	s.	a.	21. VIII		g.	green				
			16. IX			"				II				16. IX			light-green				III
234.	s.	a.	21. VIII		g.	green					234. d.	s.	a.	21. VIII		g.	green				
			16. IX			"				II				16. IX			light-green				III
236.	s.	a.	27. VIII		g.	chl. i. susp. o. gr. ch. e.															
			27. VIII			light-green															
			16. IX			somewhat greener?				II											
237.	s.	a.	27. VIII		g.	chl. i. susp. o. gr. ch. e.															
			27. VIII			light-green															
			16. IX			somewhat greener?				III											
238.	s.	a.	27. VIII		g.	colourless															
			16. IX			"				II											
239. [sq]	s.	a.	24. VIII		g.	light-green															
			16. IX			normally green				I											

TABLE 8 (continued)**.

cultures in light

n°	s. a. e. v.	date	t. b. r.	g. ± +	colour	1	2	3	I II III
333. l.	e. a.	22. II	b.	g. .	dark green	X	III	VIII	
		23. IV	.	g. .	" "				
		1. V	b.	g. —	" "	XII	VI	IX	II[1]
334. l.	e. a	23. II	b.	g. .	green	X	IV	VIII	
		23. IV	.	g. .	dark green				
		30. IV	b.	g. —	" "	X	VII	X	II[1]
336. l.	e. a.	24. II	b.	g. .	colourless	V	VI	IX	
		23. IV	.	g. .	"				
		1 V	b.	g. —	"	III	VI	IX	II[10]
337. l.	e. a	24. II	b.	g. .	colourless	V	VI	IX	
		9. IV	.	g. .	greenish				
		7. V	b.	g. —	yellow-green	VIII	V	XI	I
338. l.	e. a.	24. II	b.	g. .	colourless	VI	V	VIII	
		9. IV	.	g. .	greenish				
		8. V	b.	g.	yellow-green	IX	IV	XII	I
339. l.	e. a.	25. II	b.	g. .	green	IX	IV	IX	
		2. IV	.	g. .	light-green				
		24. IV	b.	g. —	light yellow-green	VI	VI	X	III
340. l.	e. a.	25. II	b.	g. .	light-green	VIII	III	VII	
		24. IV	b.	g. .	dark green	XII	VII	VIII	I
341.	e. a.	25. II	b.	g. .	colourless	III	VI	X	
		9. IV	.	g. .	greenish				
		30. IV	b.	r. .	"	VI	VI	XII	I
343.	e. a	26. II	b.	g. .	dark green	XII	V	VII	
		30. IV	b	g. .	" "	X	VI	IX	II[10]
345.	e. a.	26. II	b.	g. .	colourless	II	V	VIII	
		9 IV	.	g. .	greenish				I
		7. V	b.	g. .	colourless	II	VI	IX	III
347.	e. a.	26. II	b.	g. .	colourless	II	VII	X	
		9. IV	.	g. .	greenish				
		7. V	b.	r. —	yellow-green	VIII	III	XII	I
355.	e. a.	27. II	b.	g. .	dark green	XII	VI	VII	
		7. V	b.	r. —	green	X	V	XII	III
357.	e. a.	27. II	b.	g. .	green	XI	V	VII	
		7. V	b.	g. —	dark green	XI	IV	XI	II[1]
359.	e. a.	27. II	b.	g. .	light-green	IX	VI	VIII	
		7. V	b.	r. —	green	XI	V	XI	I
365. l.	e. a	20. VI	b.	g. .	colourless	V	VII	XII	
		10. VII	b	g —	"	I	VII	XI	II[10]
365. l.	e. a	20 VI	b	g. .	colourless	III	VI	XI	
		10. VII	b	g.	"	II	VII	XII	II[10]
366. l.	e. a	21. VI	b.	g. .	dark green	XII	VI	VIII	
		12. VII	b.	g.	light-green	X	VI	X	III
366. l.	e. a.	21 VI	b.	g. .	dark green	XI	V	IX	
		13. VII	b.	g.	light-green	X	VII	X	III
367. l.	s. a	22 VI	b.	g.	light-green	X	VII	X	
		14. VII	b.	g. —	green	XII	VI	X	I
368. l.	s. a.	23. VI	b.	g. .	yellow-green	VIII	III	IX	
		17. VII	b.	g. .	green	X	IV	X	I
369. l.	s. a.	24. VI	b.	g. .	light yellow-green	VIII	III	IX	
		15. VII	b.	g.	light-green	X	IV	X	I
370. l.	s. a.	24. VI	b.	g. .	colourless	VII	IV	IX	
		13. VII	b.	g. —	yellow-green	VIII	V	X	I
371. l.	s. a.	24 VI	b.	g.	dark green	XII	V	VIII	
		17. VII	b.	g —	" "	XII	V	XII	II
372. l.	s. a	24. VI	b.	g. .	dark green	XII	IV	VIII	
		17 VII	b.	g. —	" "	XII	V	IX	II
374. l.	s. a.	25. VI	b.	g	colourless	III	IV	IX	
		14 VII	b.	g. .	light-green	IX	V	XI	I
375. l.	e. a	25. VI	b.	g. .	colourless	I	IV	X	
		14. VII	b	g —	"	I	V	XI	II
376. l.	e. a.	25. VI	b.	g.	colourless	I	VII	X	
		17. VII	b	g	"	II	II	XII	II[1]
377.	s. a.	26 VI	b.	g.	light-green	VIII	IV	IX	
		14. VII	b.	g.	green	X	V	X	I

cultures in darkness

n°	s. a. e. v.	date	t. b. r.	g. ± +	colour	1	2	3	I II III
333. d.	e. a.	22. II	b.	g. .	dark green	X	III	VII	
		23 IV	.	g. .	yellow-green				
		1. V	b.	r. —	colourless	VI	VII	XII	III
334. d.	e. a.	23 II	b.	g. .	green	X	III	VII	
		23. IV	.	g. .	yellow-green				
		30. IV	b	r. —	greenish	VI	VII	XII	III
336. d.	e. a.	24. II	b.	g. .	colourless	IV	VI	X	
		23. IV	.	g. .	"				
		1. V	b	g. —	"	II	VI	X	II[10]
337. d.	e. a.	24. II	b.	g. .	colourless	III	VI	IX	
		23. IV	.	g. .	"				
		7. V	b.	g.	"	VI	IV	X	II[1]
338. d.	e. a	24. II	b.	g. .	colourless	VI	IV	VIII	
		23. IV	.	g.	"				
		8. V	b.	g. —	"	III	IV	X	II[10]
339. d.	e. a.	25. II	b	g. .	green	X	III	VIII	
		9. IV	.	g. .	almost colourless				
		24. IV	b	g	colourless	II	VII	XII	III
340. d.	e. a.	25. II	b.	g. .	light-green	IX	VI	VIII	
		9. IV	.	g.	yellow-green				
		24. IV	b.	r.	" "	VI	VI	XII	II[10]
342.	e. a.	25. II	b.	g.	colourless	III	VI	IX	
		8 V	b.	g.	"	II	IV	VIII	II[10]
344.	e. a.	26. II	b.	g. .	dark green	X	V	VIII	
		9. IV	.	g. .	yellow-green				
		8. V	b.	g.	light yellow-green	VII	III	XII	III
346.	e. a.	26. II	b.	g. .	colourless	II	VI	VIII	
		30. IV	b.	g. —	"	II	VI	X	II
348.	e. a.	26. II	b.	g.	green	IX	V	VIII	
		9. IV	.	g.	yellow-green				
		8. V	b.	g. —	colourless	V	IV	XII	III
356.	e. a.	27. II	b.	g.	green	X	V	VII	
		9. IV	.	g. .	yellow-green				
		30. IV	b.	g. —	colourless	VII	VII	XII	III
358.	e. a.	27. II	b.	g.	green	X	V	VIII	
		9. IV	.	g. .	yellow-green				
		8 V	b.	g. .	colourless	I	V	XII	III
362.	e. a.	27. II	b.	g. .	colourless	II	VII	X	
		8. V	b.	g.	"	I	IV	XI	II[10]
364.	e. a	27. II	b.	g.	light yellow-green	II	VII	IX	
		1. V	b	g. —	colourless	II	VI	X	II[10]
365. l d.	e. a.	20. VI	b.	g. .	colourless	III	VI	XII	
		10 VII	b.	g. —	"	II	VII	XI	II[10]
365. l d	e. a	20 VI	b.	g.	colourless	II	V	XI	
		10 VII	b.	g.	"	I	VI	VII	II[10]
366. l d	e. a.	21 VI	b.	g.	dark green	XI	VII	IX	
		12 VII	b.	g.	greenish	VI	VII	XII	III
366. l d	e. a.	21. VI	b.	g.	dark green	XI	VII	IX	
		13 VII	b.	g.	greenish	VII	VI	VII	III
367. d.	s. a.	22 VI	b.	g.	green	XI	X	X	
		11. VII	b.	g.	light-green	IX	VII	XII	III
368. d.	s. a.	23. VI	b.	g. .	green	X	IV	X	
		17. VII	b.	g. .	light-green	VIII	VI	XII	III
369. d.	s. a.	24. VI	b.	g.	green	IX	IV	IX	
		14 VII	b.	g.	yellow-green	VII	VI	XII	III
370. d.	s. a.	24 VI	b.	g.	light-green	IX	V	X	
		13. VII	b.	g.	yellow-green	IX	V	XII	II[10]
371. d.	s. a.	24 VI	b.	g.	dark green	XII	III	VII	
		17. VII	b.	g. —	green	X	VI	XII	III
372. d.	s. a	24. VI	b.	g.	dark green	XII	IV	VII	
		17. VII	b.	g. —	green	X	VI	XI	III
374. d.	s. a.	25. VI	b.	g	light-green	IX	IV	X	
		14. VII	b.	g.	light yellow-green	VIII	VI	XII	III
375. d.	e. a	25. VI	b.	g. .	colourless	II	V	X	
		13. VII	b.	g.	"	I	VI	XI	II[10]
376. d.	e. a.	25. VI	b.	g. .	colourless	I	VII	IX	
		17. VII	b.	g.	"	II	V	XII	II[1]
378.	s. a	26. VI	b.	g.	green	X	V	VIII	
		14. VII	b.	g. —	light-green	IX	VI	XII	III

TABLE 8 (continued)***.

cultures in light														cultures in darkness													
n°	s. a. e. v.	date	t. b.	g. — ‡ + r. ‡ +	colour	1	2	3	I II III	n°	s. a. e. v.	date	t. b.	g. — ‡ + r. ‡ +	colour	1	2	3	I II III
410.	s. a.	28. VII	.	g. .	dark green														
		15. VIII	.	g. —	" "	.	.	.	II										
411.	s. a.	28. VII	.	g. .	dark green														
		15. VIII	.	g. —	" "	.	.	.	II										
412.	s. a	28. VII	.	g. .	dark green														
		15. VIII	.	g. —	" "	.	.	.	II										
413.	s. a.	28. VII	.	g. .	dark green														
		15. VIII	.	g.	" "	.	.	.	II										
414.	s. a.	28. VII	.	g. .	dark green														
		15. VIII	.	g.	" "	.	.	.	II										
415.	s. a.	28. VII	.	g. .	dark green														
		15. VIII	.	g.	" "	.	.	.	II										
416.	s. a.	28. VII	.	g. .	colourless														
		15. VIII	.	g. —	light yellow-green	.	.	.	I										
417.	s. a.	28. VII	.	g. .	colourless														
		15. VIII	.	g. —	light-green		.		I										
418.	s. a.	28. VII	.	g. .	colourless														
		15. VIII		g. —	light-green		.		I										
418. 2 eq.	s. a.	28. VII	.	g. .	colourless														
		15. VIII	.	g. —	light yellow-green	.	.	.	I										
418.	s. a.	28. VII	.	g. .	colourless														
		15. VIII	.	g. —	clrl. to light yell.-green			.	I										
419.	s. a.	28. VII		g. .	colourless														
		15. VIII	.	g. —	greenish	.			I										
420.	s. a.	28. VII	.	g.	colourless														
		15. VIII	.	g. —	yellow-green	.	.	.	I										
421.	s. a.	28. VII		g. .	light yellow-green														
		15. VIII	.	g. —	light-green	.	.	.	I										
422. 2 eq.	s. a.	28. VII	.	g. .	light yellow-green														
		15. VIII	.	g. —	light-green	.	.	.	I										
423.	s. a.	28. VII	.	g.	colourless														
		15. VIII	.	g. —	yellow-green		.	.	I										
424.	s. a.	28. VII	.	g. .	yellow-green														
		15. VIII		g. —	green	.	.	.	I										
425.	s. a.	28. VII	.	g.	light yellow-green														
		15. VIII	.	g. —	light-green	.	.	.	I										
426.	s. a.	28. VII	.	g. .	greenish														
		15. VIII	.	g. —	yellow-green		.		I										
427.	s. a.	28. VII	.	g.	yellow-green														
		15. VIII		g. —	green	.	.	.	I										
428.	s. a.	28. VII	.	g. .	light-green														
		15. VIII		g. —	green	.	.	.	I										
429.	s. a.	28. VII	.	g. .	light-green														
		15. VIII	.	g. —	green	.	.	.	I										
430.	s. a.	28. VII	.	g.	light-green														
		15. VIII	.	g. —	green	.	.		I										
431.	s. a.	28. VII	.	g.	light-green														
		15. VIII		g. —	dark green				I										
432.	s. a.	28. VII		g. .	light-green														
		15. VIII	.	g. —	dark green				I										
433.	s. a.	28. VII	.	g. .	yellow-green														
		15. VIII		g. —	light-green				I										
434. 3 eq.	s. a.	28. VII		g.	light-green														
		15. VIII	.	g. —	green				I										
435. 2 eq.	s. a.	28. VII	.	g.	greenish														
		15. VIII	.	g. .	light-green			.	I										
435. 2 eq.	s. a.	28. VII		g. .	greenish														
		15. VIII	.	g. —	yellow-green				I										
436.	s. a.	28. VII	.	g.	light yellow-green														
		15. VIII		g. —	green	.	.		I										
436. 2 eq.	s. a.	28. VII	.	g. .	light yellow-green														
		15. VIII	.	g. —	light-green	.	.		I										

reduction (r.), or some dying (†) could be observed. *g*. Whether the sponge had grown very weakly (—), rather well (+) or vigorously (++). *h*. The colour of the sponge; for which I shall make use of the following expressions, indicating the green colour with decreasing intensity: dark green > green > light-green > yellow-green > light yellow-green > greenish > colourless. *i*. The number of the various chlorophyll corpuscles present in the tissues: sub 1 that of the green ones, sub 2 that of the „colourless ones with structure" (p. 42), sub 3 that of the „colourless ones without structure" („vague shades" excluded); always the number present in the whole microscopic preparation, so in an almost equal volume of each sponge. As for the meaning of I—XII, see pag. 14. *j*. The type to which the sponge proved to belong during the experiment; in type I the green colour, therefore also the number of green chlorophyll corpuscles, increases; in type III the green colour and the number of green corpuscles decrease; in type II the green colour remains constant as well as the number of green corpuscles, or the green colour or the number of green corpuscles changes a little by increase or decrease; in the last case but one we may speak of a type II^{I}; in the last one of a type II^{III}.

All experiments are mentioned; those belonging to one series are placed together in one space. As for the discussion, see pag. 58—66.

In this table has also been registered the behaviour of some colourless sponges, which had first been for some time in a suspension of green chlorophyll corpuscles, which, therefore, had been purposely infected with those corpuscles (see Table 7); viz. n° 246, 248, 254—256 etc. For the discussion of these results, see p. 28, 50.

Table 9. *The intensity of multiplication of the green chlorophyll corpuscles of Spongilla in light, when cultivated in sponge-tissue or in water in a weak or in a strong concentration of the corpuscles.*

Of a green Spongilla a piece was cultivated in an aquarium (p. 8—9), while two cultures of green chlorophyll corpuscles in water were made of the remaining parts (p. 9), one containing the corpluscles in a weak concentration the other in a strong one. The sponge and the cultures were kept at about the same temperature. In all of them the number of the stages of division per 100 green corpuscles was daily examined — sometimes even several times a day —. A darker green rim was soon formed in the cultures to the membrane on the bottom (p. 10) — the usual way in which the multiplication of the material is first recognized. Of course the chlorophyll corpuscles were in a stronger concentration in this rim than somewhere else in the membrane.

In the tables is indicated: *a*. The date and the hour of the examination. *b*. The then stated number of the stages of division per 100 green corpuscles in the green sponge as well as in the cultures in water, in the membrane and in the rim, in strong and in weak concentration of the corpuscles (— means no rim; + rim present).

One day the concentration of the green corpuscles in the sponge and in the cultures was closely examined and compared. As for the method used, see p. 81. We shall call this concentration in a dark green sponge or in a dark green membrane: strong (s.); that in a yellow-green to light-green sponge or membrane: moderate (m.); and that in a greenish sponge or membrane: weak (w.).

From the tables results: *1* Periodicity in the multiplication does not occur (p. 54). *2* The weaker the concentration of the corpuscles present in a culture the higher is their intensity of multiplication (p. 55). *3* In strong concentration the intensity of multiplication in water and in sponge tissue are equal (p. 82). *4* The culture may be destroyed quickly when infected by protozoa (p. 79).

TABLE 9 A. Nº 373.

date	hour	green sponge	water from conduit				remarks.
			strong conc.		weak conc.		
			rim	memb.	rim	memb.	
24. VI	.	0	.	.	.	.	
26.	2. p. m.	4	.	0	.	0	
27.	2. „	4	.	0	.	0	
28.	4. „	3	.	0	.	2	
29.	11. a. m.	3	.	0	—	2	
30.	.	.	.	.	.	.	
1. VII	11. „	3	.	0	$\overset{+}{8.5}$	6.5	
2.	12. m.	5	.	0	16	.	
3.	6. p. m.	9	.	2	6.5	.	
4.	.	.	.	.	.	.	
5.	5. „	2	—	8.5	15	20	
6.	2. „	4	$\overset{+}{0}$	6.5	13	15	
7.	10. a. m.	2	1	9	16	35	
8.	11. „	2	2	8.5	16.5	31	
„	6. p. m.	.	.	.	.	32	
9.	10. a. m.	1	0	5	22	30	
10.	10. „	1	3	12.5	29	32	
11.	2. p. m.	4	6.5	16	24	31	
12.	6. „	0	1	13	5.5	39	
13.	6. „	.	.	.	.	39	
14.	6. „	2	9	6.5	0	30	
15.	12. n.	.	.	.	.	39	
„	4. a. m.	.	.	.	.	33	
„	6. „	.	.	.	.	45	
„	8. „	.	.	.	.	30	
16.	1. p. m.	2	0	14.5	16	29	
17.	6. „	.	.	.	.	39	
23.	11. a. m.	.	2	28	25	30	
26.	.	s.	s.	m.	$m.^{+}$	w.	← concentr.
30.	10. a. m.	.	1	20	13	29	
		2	2.3	13	17	33	average since 6. VII

TABLE 9 B. N° 389.

date	hour	green sponge	water from lake				remarks.
			strong conc.		weak conc.		
			rim	memb.	rim	memb.	
26. VII	.	1	—	2	.	.	
30.	11. a. m.	1	.	1	—	0	
31.	.	.	.	.	.	.	
1. VIII	10. „	0	.	0	6.5	10.5	
2.	10. „	0	.	1	2	7.5	
3.	10. „	1	.	1	7.5	18	
4.	9. „	3	.	0	12.5	19	
5.	9. „	1	.	1	20	18	
6.	.	.	.	.	.	.	
7.	3. p. m.	2	.	1	4	12.5	
„	.	s.		m.+	m.	w.	← concentr.
8.	.	.	.	.	.	.	
9.	7. p. m.	0	.	0	—	10.5*)	*) infect. of protoz., membr. disappring.
10.	12. n.	0	.	0	.	17	
„	4. a. m.	0	.	0	.	10	
„	8. „	0	.	1	.	14	
„	12. m.	0	.	0	.	11.5	
11.	9. p. m.	2	.	1	.	4	
18.	10. a. m.	1	.	7.5	.	5†)	†) membr. ± disappred.
		0.8		1	9	12	average since 1. VIII

TABLE 10. *The intensity of multiplication of the green chlorophyll corpuscles of the Spongillidae and the total increase or decrease of the whole number of green corpuscles, when cultivated in sponge-tissue or in water, in light or in darkness.*

Generally of each sponge a piece was cultivated in an aquarium in light and another one under equal circumstances in darkness (p. 8); such pieces of one sponge are indicated in the tables with the same n°. All these sponges have been mentioned already in Table 8 on account of the changing of their colour and their amount of various chlorophyll corpuscles. As stated there, the factors of import, (export), reduc-

TABLE 10.

n°		concentr. at beginning	date	sponge light 1	2	3	water from conduit light 1	2	3	water from lake light 1	2	3	sponge darkness 1	2	3	water from conduit darkness 1	2	3	water from lake darkness 1	2	3	sp. or c.	remarks
49	48	-	IX	most	m								0	w.	—							sp	after 5 weeks' culture.
246	255	m.	VII	2·5	»	+							0	w.								sp	
248	256	»	»	7	»								0	»	—							»	
254		»	»																			»	after 3 weeks' culture.
257		»	»	11	»	+																»	
258		m.	»	9	»	+																»	
259		w.	»			0+																»	
260		»	VII	5	»	0							0·5	m.								sp	
261		»	»	6	»	0							0	»								»	
262		»	»	9	»	0																»	after 3 weeks' culture.
263		w.	»			0+										0·						»	
264		»	»			0+										0·						»	
282		»	VIII	2	»	+	25	w.	+	18	w.		0·3	m.	—	3	w.	+	2	w.	0	sp	
280		»	»	0·7	»	0	26	»	+	25	»		0	»	—	0	»	+	0·7	»		»	after 3 weeks' culture.
284		»	»	0·4	»	0	32	»	+	16	»		0	»	—	3	»		2·7	»	—	»	in the sponges n° 284,
285		m.	»			+							0	w.								»	285, 288, 289 in dark-
287		»	»			+																»	ness, stages of division
295		»	»			+										0						»	are wholly absent
288		w.	»			+							0	w.	0+							»	
289		»	»			+							0	»	0+							»	
309	313	m.	VIII				VII	»	0+							III	»	0				sp	after 3 weeks' culture;
310	314	»	»				VIII	»	0+							III	m.	0+				»	sub 1 is given the num-
311	315	»	»				VIII	»	+							III	»	0+				»	ber of stages of division
312	316	»	»				V	»	0+							I	»	0+				»	per preparation (p. 14).
322		»	IX	1	»	0	18	w.					0	m.	-	1	w.					sp	after 3 weeks' culture.
323		»	»	4	»	0	35	w.	+				0	»		2	»					»	
324		»	»	3	»	0	28	w.	0				0	»		0	»	0				»	
329	330	m.	X	2	m.	0				24	w.	0+										sp	after 6 weeks' culture;
329	331	»	»	7	»	0				18	»	0										»	in sponge at first increase
329	332	»	»	6	»	0				25	»	0+										»	of concentr. till 8.
333 I, 335 I,	333 d, 335 d.	»	V	1	»	0+	24	w.+	0+				0·9	w.	—	0	w.	0				c.	
334 I, 335 II,	334 d, 335 dd.	»	»	2	»	0+	10	»	0+				0	»	—	0	w.+	0				»	
336 I, 352,	336 d, 349	w.	»	0	w	0	19	m.	+				0	»	0	0	m.	0+				»	
337 I, 353,	337 d, 350	»	»	17	m.	+	23	w.	0				5	»	0+	0	»	0+				»	
338 I, 354,	338 d, 351	»	»	10	»	+	28	»	0				0	»	—	2	»	0+				»	
	339	»	»	3	w.	—							0	»	—							»	
	340	m.	»	6	»	+							0	m.	0							»	
341	342	w.	»	0	w.	0+							0	w.	0							»	after 10 weeks' culture.
343	344	»	»	0	»	0							0	»	—							»	
	346	w.	»			+							0	»	0							»	
347	363	»	»	13	m.	+							0	»	—							»	
355	356	»	»	0	»	0							0	»	—							»	
357	358	»	»	7	»	0+							0	»	—							»	
359	348	m.	»	7	»	+							0	»	—							»	
	362	w.	»										0	»	0							»	
365 I		w.	VII	0	w.	0								w.	0							c.	
365 II		»	»		»	0–							0	»	0–							»	
366 I		»	»	3	m.	—							0	»	—							»	
366 II		»	»	7	»	—							0	»	—							»	
367		»	»	1	s.	0+							0	m.	—							sp	
368		w.	»	7	»	+				14	w.++	0++	1	»	—				0	w.	0+	»	
369		»	»	0	m.	+				7	»	0++	0	m.–	—				0	»	0+	»	after 3 weeks' culture.
370		»	»	11	»	+							1	m.	—							»	
371		»	»	0	s.	0							0	s.–	0							»	
372		»	»	1	»	0							0	»	0							»	
374		w.	m.	2	m.	+							0	w.	—							»	
375		w.	»	0	w.	0							0	»	0							»	
376		»	»	6	»	0+							0	m.	—				0	m.	0+	»	
377, 379 I,	378, 379 d.	m.	»	6	»	0+				7	m.++	0++	0	m.	—				0	m.	0+	sp	
		5·	III							9	w.	280											water from lake; sub 3
		21·	VI							6	»	650											is given the number of
		19·	VI							9	»	850											the green chlorph. corp.
		28·	VII							7	»	3700											present in 1 Litre.
410		15·	VIII	1	»	0	1	s.	—													sp.	
411		»	»	7	»	0	1	»	—													»	
412		»	»	3	»	0			—													»	
413		»	»	0	»	0			—													»	
414		»	»	6	»	0	1	s.	—													»	
415		»	»	0	»	+			—													»	
416		w.	»	20	w.	+	28	w.	0–													»	
417		»	»	11	»	+	22	»	0													»	
418		»	»	16	»	+	17	»	0–													»	
»		»	»			+			-													»	after 3 weeks' culture.
»		»	»			-																»	
»		»	»			0+																»	in this series the column
419		»	»	15	w.	+	20	w.	—													»	»concentr. at beginning«
420		»	»	13	m.	+·	27	»	+													»	gives the concentration
421		»	»	17	»	+	27	»	+													»	in the sponge as well
422		»	»	16	»	+	37	»	+													»	as in the water culture
»		»	»			+			+													»	(they were equal)
423		w.·	»	11	m.	+	22	w.	0–													»	
424		w.	»	7	»	+	25	»	0													»	in this series the algae
425		w.·	»	9	»	+	30	»	+													»	in the water cultures
426		m.	»	7	»	+	11	m.	+													»	were never originating
427		w.	»	15	»	+	28	»	+													»	from the same sponge
428		m.	»	17	w.·	+	23	»	+													»	as their partner sponge
429		»	»	6	»	+	21	w.	0–													»	piece, but those in 2 cul-
430		»	»	8	»	+	15	m.–	0–													»	tures from other (dark)
431		m.	»	5	»	+			—													»	green sponges, and those
432		m.	»	3	»	+			—													»	in m. and w. cultures
433		m.	»	3	m.	+			—													»	from other yellow-green
434		w.	»	9	»	+	13	m.	0													»	to light-green sponges.
»		»	»			+			-													»	
»		»	»			+			-													»	
435		w.	»	9	m	+			-													»	
»		»	»			+			-													»	
»		»	»			+			-													»	
»		»	»			+			-													»	
436		»	»	10	m.·	+			-													»	
»		»	»			+			-													»	
»		»	»			+			-													»	

tion and growth were excluded as much as possible in these experiments. At the same time also cultures of green chlorophyll corpuscles isolated from the same or from other sponges (p. 9) were made in water from the lake or (and) in that from the conduit, in light or in darkness. Also these cultures were placed (in bottles) in the aquaria; consequently, all experiments of one series were exposed to the same temperature. If the cultures (in bottles) arose from the same sponge, which was also used for the sponge-experiment, they have no special n° in the table; but they have, when arising from another sponge (except in n° 410—436). For each sponge piece and for each culture the intensity of multiplication, the concentration and the total increase or decrease in number of the green corpuscles was examined after some weeks.

In the table is indicated: *a*. The n° of the experiment. *b*. The concentration of the green corpuscles in the sponge tissue at the beginning of the experiment; that in the cultures in water was then always weak (w. or w.+), only in n° 309—316 and n° 379 it was moderate (m.), while that in the water-cultures of n° 410—436 is given in the table. *c*. The date the experiment was brought to an end. *d*. The results then stated: sub 1 the number of the stages of division per 100 green chlorophyll corpuscles; sub 2 the concentration of these corpuscles in the part which had been examined; sub 3 the total increase (+) or decrease (—) or the equalness (0) of the whole mass (here = the whole number [1]) of these corpuscles, calculated (per unit of volume) from the colour-changing [2]); all this, concerning the corpuscles present in the sponges or in the water from the lake or in that from the conduit, in light as well as in darkness. *e*. Whether the sponge material had been taken from a Spongilla (sp.) or from an Ephydatia (e.). *f*. Some remarks.

As for the method of examining and comparing the concentration of the chlorophyll corpuscles in the sponge tissue and in cultures, see p. 81. We shall call this concentration in a green to dark green sponge or membrane: strong (s.); that in a yellow-green to light-green (gray-green) sponge or membrane: moderate (m.); that in a colourless to greenish to light yellow-green sponge or membrane: weak (w.) [2]).

All experiments are mentioned; those belonging to one series are placed together in one space.

As for the discussion, see p. 54—56 and p. 77—83.

---

1) As their size in sponge tissue and water remains almost equal.

2) In the table + or — to an indication (eg. m.+, 0−, +−) means, that the quantity is somewhat greater or somewhat smaller, than the indication denotes by itself.

TABLE 11. *Cultures of isolated green chlorophyll corpuscles of Spongilla in water, in light or in darkness. Comparison of the number of oildrops occurring free in the cultures to that present in the green corpuscles, with respect to the number of green stages of division and the number of colourless corpuscles without structure present in the cultures.*

In the chief experiment (n° 290) several other cultures have been made of one original culture, on two different moments. These new cultures are indicated in the tables by braces proceeding from the original culture. All these cultures were from time to time microscopically examined as to oildrops etc. (pag. 13—15). The observations concerning a same culture are united in the table by short lines. The numbers joined to the braces and lines indicate the time (in months) between the two observations; l. means culture in light, d. culture in darkness. For each observation is mentioned: sub 1 the number of the green chlorophyll corpuscles, sub 2 that of the green stages of division, sub 3 that of the colourless corpuscles without structure, sub 4 that of the green ones containing an oildrop, sub 5 that of the free oildrops; always the number present in the whole microscopic preparation. As for the meaning of I—XII, see pag. 14—15; as for the discussion, pag. 86—87.

TABLE 12. *The number of oildrops per amoebocyte in the tissues in different stages of development* (conf. Table 6) *of green Spongillidae from light and in colourless ones from darkness* (may be also from light); microscopically examined by means of ravel preparations from newly captured living sponges (p. 12—15).

For Spongilla these observations are given in 4 groups (conf. Table 6), viz. for the tissue of *1.* branch-tops *2.* branch-bases *3.* different gemmule stages *4.* sponge-disks; for Ephydatia only for full-grown tissue (Table 6).

In the tables is indicated: *a.* the month of the examination. *b.* The number then stated: in column 1 the (average) number of oildrops per amoebocyte, in col. 2 the number of the green chlorophyll corpuscles, in col. 3 that of the green corpuscles containing an oildrop, in col. 4 that of the colourless ones without structure; in the last 3 cases the number present in the whole microscopic preparation. On each horizontal line the data concerning one sponge piece are given; sub 5 the analyses of different pieces of a same sponge are indicated by the same letters. Finally for each tissue group the data, concerning all analyzed sponges together, are composed; this composition simplifies of course the mutual comparison of the results of the different groups. As for the meaning of I—XII, see pag. 14; as for the discussion, pag. 85, 87—89.

TABLE 11.

nº	1	2	3	
290	X	V	IX	V
I.			$\frac{1}{3}$	
	X	V	II	I
			$\frac{1}{2}$	
	XII	VII	III	I
			1	
	XII	IV	I	V
309	X	I	VI	V
310	X	I	VI	V
311	X	I	VI	V
312	X	I	VI	V
313	X	I	VI	V
314	X	I	VI	V
315	X	I	VI	V
316	X	I	VI	V

TABLE 11.

nº	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
290 l.	X	V	IX	VII	II	l. $\frac{1}{3}$	X	IV	VIII	X	VII	$-\frac{1}{2}$	X	III	V	X	VII	-1	X	III	VII	X	VII	-3	XII	I	VII	X	X	$-2\frac{1}{2}$	XII	I	VII	X	V
						l. $\frac{1}{3}$	X	VI	VIII	X	III	$-\frac{1}{2}$	X	III	VI	X	III	-1	X	III	VI	IX	III	-3	X	I	VII	IX	IV	$-2\frac{1}{2}$	X	I	VII	X	IV
						l. $\frac{1}{3}$	X	VII	VIII	X	II	$-\frac{1}{2}$	X	III	IV	X	III	-1	X	I	V	X	III	-3	X	I	VII	X	VII	$-2\frac{1}{2}$	X	I	VII	X	III
						d. $\frac{1}{3}$				X	III	$-\frac{1}{2}$			IV	X	IV	-1	X	I	I	IX	III	-3	X	I	III	X	III	$-2\frac{1}{2}$	X	I	III	IX	III
						d. $\frac{1}{3}$				X	III	$-\frac{1}{2}$			V	X	IV	-1	X	III	VI	IX	VI	-3	X	I	III	X	VII	$-2\frac{1}{2}$	X	I	III	IX	IV
			$\frac{1}{3}$			l. $\frac{1}{2}$	XII	VIII	I	IV	IV	-1	XII	III	I	XII	IV	-3	X	I	III	X	IX	$-2\frac{1}{2}$	X			X	IV						
						l. $\frac{1}{2}$	XI	VI	I	X	IX	-1	X	I	I	X	IX	-3	X	I		IX	IX	$-2\frac{1}{2}$	IV			IV	IV						
	X	V	II	X	IX	l. $\frac{1}{2}$	X	I	I	X	IX	-1				IX	IX	-3	X	I		X	IX	$-2\frac{1}{2}$											
						l. $\frac{1}{2}$	X	I	I	X	IX	-1				VIII	IX	-3	X			IX	IX	$-2\frac{1}{2}$											
			$\frac{1}{2}$			l. $\frac{1}{2}$	X		IV	X	IX	-1				VIII	VI	-3	I	I	X		IX	$-2\frac{1}{2}$	I	I	X		X						
						l. $\frac{1}{2}$	X		V	X	IX	-1				VII	VII	-3	X			VII	IX												
	XII	VII	III	IV	IV																													
			1																															
	XII	IV	I	VIII	III																													
309	X	I	VI	VII	IX	l. $-\frac{3}{4}$	XII	VII	IV	VII	IV	-6	XII	III	VIII	XI	VI																		
310	X	I	VI	VII	IX	l. $-\frac{3}{4}$	XII	VIII	VI	VII	IV	-6	XII	I	III	XI	X																		
311	X	I	VI	VII	IX	l. $-\frac{3}{4}$	XII	VIII	IV	VII	V	-6	I	I	I	I	III																		
312	X	I	VI	VII	IX	l. $-\frac{3}{4}$	XII	V	VI	X	IV	-6	I	I	VI	I	V																		
313	X	I	VI	VII	IX	d. $-\frac{3}{4}$	XII	III	I	XI	IV	-6	VI		VI		VII																		
314	X	I	VI	VII	IX	d. $-\frac{3}{4}$	XII	III	III	XI	III	-6	VII		VIII		III																		
315	X	I	VI	VII	IX	d. $-\frac{3}{4}$	XII	III	I	XI	V	-6	XII	I	V	XI	X																		
316	X	I	VI	VII	IX	d. $-\frac{3}{4}$	XII	I	IV	XI	IV	-6	X	I	VIII	VII	VII																		

green ones from light							colourless ones from darkness						
month	1	2	3	4	5		month	1	2	3	4	5	
VII	VIII	XII	X	VII	a	**tops. the 12 together:** col. 1. : 4. IV + 4. VI + 2. VII + 1. VIII + 1. IX col. 2. : 2. X + 10. XII col. 3. : 2. IX + 7. X col. 4. : 7. VII + 4. VIII + 1. IX	VII	IX	VI	VI	IX	d	**tops. the 12 together:** col. 1. : 5. IV + 2. VI + 1. VII + 1. VIII + 1. IX + 2. X col. 2. : 1. III + 1. IV + 3. VI + 5. VII + 1. VIII col. 3. : 2. VI + 1. VII col. 4. : 1. VIII + 8. IX + 1. X + 1. XII
„	VI	XII	X	VII	a		„	IV	VII	VI	IX	d	
„	IX	XII	X	VII	b		„	IV	VII	VII	VIII	e	
„	IV	XII	X	VII	b		„	IV	VII	.	XII	e	
„	IV	XII	X	VII	c		„	IV	VII	.	IX		
„	IV	XII	X	VIII	c		„	IV	VIII	.	IX	f	
„	IV	XII	.	VII	g		„	VI					
„	VI	X	.	VIII	h		„	X	VI	.	IX	i	
„	VII	XII	.	VIII	k		„	VIII	VI	.	IX	j	
„	VI	XII	X	VIII	l		„	VI	VII	.	X	o	
„	VII	X	IX	IX	m		„	VII	IV	.	IX	p	
„	VI	XII	IX	VII	n		„	X	III	.	IX	q	
VII	VI	XII	X	VIII	a	**bases. the 35 together:** col. 1. : 9. IV + 11. VI + 8. VII + 5. VIII + 2. IX col. 2. : 1. IX + 6. X + 2. XI + 5. XII col. 3. : 1. V + 2. VII + 7. VIII + 4. IX + 19. X col. 4. : 2. VIII + 5. IX + 4. X	VII	IV	IV	.	IX	d	**bases. the 19 together:** col. 1. : 1. III + 4. IV + 1. VI + 4. VII + 2. VIII + 4. IX + 3. X col. 2. : 5. II + 5. III + 1. IV + 1. V + 3. VI col. 3. : 1. III + 1. IV + 1. V + 2. VI + 1. VIII col. 4. : 1. IX + 4. XI + 5. XII
„	IV	X	X	IX	a		„	IX	VI	IV	XI	d	
„	IV	XII	X	VIII	b		„	III	VI	V	XII	e	
„	IV	X	VII	X	b		„	IV	V	.	XII	e	
„	IV	X	X	IX	c		„	VII	VI	.	XI	f	
„	IV	IX	IX	IX	c		„	VII	II	.	XII	i	
VI	VII	X	X	X	r		„	VIII	II	.	XII	j	
„	VII	XI	X	X	r		„	VII	II	.	XI	o	
VII	VII	.	.	.	g		„	VII	II	.	XII	p	
„	VI	.	.	.	h		„	IX	II	.	XI	q	
„	VI	X	IX				„	VIII	X	III			
„	IV	X	VIII				„	VI	.	VIII			
„	VI	XII	X	.	k		„	VIII	III				
„	VIII	XII	VIII	IX	l		„	X	III				
„	VI	XII	X	X	m		„	IX	III				
„	VIII	XI	X	IX	n		„	X	.	VI			
VI	VII	.	X				„	IX	III				
„	VII	.	V				„	IV	.	VI			
„	VI	.	X				„	IV		III			
„	IV	.	X										
„	VII	.	X										
„	VIII	.	X										
„	VI	.	X										
VIII	VI	.	X										
„	IX	.	IX										
„	IX	.	X										
„	VI	.	IX										
„	VI	.	X										
„	VIII	.	VII										
„	VIII	.	VIII										
IX	IV	.	VIII										
„	VII	.	VIII										
„	VII	.	VIII										

Spongillae.

green ones from light							colourless ones from darkness						
month	1	2	3	4	5		month	1	2	3	4	5	
XI	XII	X	.	.	.	young gemm.	XI	X	V	.	XII	.	young gemm.
„	XII	X	.	.	.		„	X	III	.	XII	.	
„	X	IX	.	X	.	older gemm.							
„	X	IX	.	IX	.								
I	X	IX	IX	.	.	old gemm.	I	XI	I	.	.	.	old gemm.
„	X	IX	IX	.	.		„	XI	I	.	.	.	
I	X	X	VII	.	.	newly germin. gemm.	II	X	VI	II	V	.	newly germin. gemm.
„	XI	X	VII	.	.		„	X	V	II	IV	.	
„	X	X	.	.	.	later	„	X	IV	II	VIII	.	later
II	VII	X	X	VI	.	later							
„	VII	XII	X	VII	.								
IV	III	.	.	.	.	still later	IV	VIII	.	.	.	.	still later
„	VII	.	.	.	.		„	VI	.	.	.	.	
„	X	.	.	.	.		„	VII	.	.	.	.	
VII	VIII	XII	.	VII	.	sponge-disks	VII	XII	II	.	VIII	.	sponge-disks
„	IV	XII	III	VII	.		„	IV	II	.	VIII	.	
„	IV	XII	IX	VII	.		„	IV	II	.	VIII	.	
„	IV	XII	IX	VII	.		„	VII	VI	.	VII	.	
„	IV	X	IX	VI	.		„	IV	VI	.	IX	.	

Ephydatiae.

green ones							colourless ones						
month	1	2	3	4	5		month	1	2	3	4	5	
II	VIII	X	X	VIII		**the 16 together:** col. 1. : 5. IV + 1. V + 5. VI + 3. VII + 2. VIII col. 2. : 1. VIII + 3. IX + 7. X + 2. XI + 2. XII col. 3. : 1. VII + 1. VIII + 6. IX + 7. X + 1. XII col. 4. : 4. VII + 8. VIII + 3. IX	II	IV	V	.	IX		**the 18 together:** col. 1. : 10. IV + 4. VI + 1. VII + 1. VIII + 1. IX + 1. X col. 2. : 4. II + 5. III + 1. IV + 4. V + 1. VI + 1. VII col. 3. : 1. V col. 4. : 4. VIII + 4. IX + 3. X + 2. XI + 2. XII
„	VII	X	X	VII			„	IV	IV	.	X		
„	IV	X	X	VIII			„	IV	V	.	IX		
„	VII	X	X	VII			„	IV	III	.	IX		
„	VIII	IX	IX	IX			„	IV	VI	.	VIII		
„	VII	X	X	VIII			„	VI	VII	.	VIII		
„	IV	VIII	VIII	VII			„	IV	III	.	X		
„	IV	IX	IX	VIII			„	VI	III	.	IX		
„	VI	XII	XII	VII			„	IV	II	.	VIII		
„	IV	X	X	VIII			„	VI	II	.	VIII		
„	IV	IX	IX	VIII			„	IV	II	.	X		
IV	V	XII	X	VIII			„	IX	V	.	XII		
„	VI	XI	IX	IX			VI	VIII	III	.	XII		
„	VI	XI	IX	IX			„	VI	III	.	XI		
„	VI	X	IX	VIII			„	VII	II	.	XI		
„	VI	.	VII				„	IV	V	V			
							„	X					
							„	IV					

TABLE 13. *The presence of a lipase in the tissue of Spongilla.*

In order to prove this presence I first made a sufficient quantity of pure emulsion of fat (oildrops) from the sponge tissue, in the following way:

A living Spongilla (green or colourless; or gemmulae) is rubbed and pressed, the parts of the skeleton removed; then the pressed out liquid is centrifuged for about 5 minutes, by which a thick mass (of sponge cells and chlorophyll corpuscles) sinks to the bottom and the liquid remains. The latter contains numerous oildrops (and, may be, chlorophyll corpuscles). Next this liquid is evaporated at 60°; the residue extracted by ether; the ether then filtered and evaporated too. Then remains a substance of vaseline-like consistence, sticky, with a strong smell, melting when warm and then forming a lasting greasy spot on paper, indissoluble in water, but dissoluble in ether and xylol, stained red with sudan III and black with osmic acid. Consequently this substance is fat. By boiling in water it becomes an emulsion again, containing the same oildrops we originally proceeded from. (Besides, this boiling is necessary to destroy all traces of enzymes, that might still be present.) I shall call this boiled liquid „emulsion".

Next another living sponge is rubbed and pressed, etc., etc. (see above); while the liquid, remaining after centrifuging, is kept. This will contain the lipase, at least when it is present in sponge tissue. This liquid I shall call „enzyme".

As one knows, the lipase splits the fat by hydrolysis into its component parts: the glycerine and the acids. It is the arising of the latter we have to show in our experiments; in the following way: A certain quantity of „enzyme" and „emulsion" are mixed; to this we add one drop of the indicator phenolphthaleine — being red in alkaline milieu, but colourless in an acid one — and such a small quantity of an (alkaline) Na_2CO_3 solution that the whole mixture becomes light-red. The acids, then, set free from the „emulsion" by the lipase will make the red colour disappear. In order to get a pure result it is necessary, however, that in this mixture no acids arise in another way than by the hydrolysis due to the lipase; or at least that we reckon with it, if it proves to be the case.

3 Series of experiments (I, II and III) were made at the same time, in which the following substances were mixed:

I. 15 drops of „enzyme" + 2.5 cM^3 of „emulsion" + 1 drop of phenolph. + Na_2CO_3 sol.
I. 15 drops of „enzyme" + 2.5 cM^3 of water + 1 drop of phenolph. + Na_2CO_3 sol.
I. 15 drops of water + 2.5 cM^3 of „emulsion" + 1 drop of phenolph. + Na_2CO_3 sol.

The experiments were kept for hours at the same temperature as the room. Meanwhile the colour was accurately examined; while I used the following indications for the red tint, arranged according to decreasing intensity: light-red > somewhat red > reddish > somewhat reddish > colourless. In the table we find the colour-changings registered; the data concerning one experiment, obtained at the times indicated, are given on one horizontal line.

exper.	I_2	4. p.m.	light-red	9. p.m.	colourless	11. a.m.	colourless
,,	I_3	,,	,,	,,	,,	,,	,,
,,	I_6	,,	somewhat red	,,	,,	,,	,,
,,	I_7	,,	,, reddish	,,	,,	,,	,,
,,	II_1	,,	light-red	,,	somewhat red	,,	somewh. reddish
,,	III_1	,,	,,	,,	,, ,,	,,	colourless
exper.	I_4	11. a.m.	somewh. reddish	2. p.m.	colourless	6. p.m.	colourless
,,	I_5	,,	,, red	,,	somewh. reddish	,,	,,
,,	II_2	,,	,, ,,	,,	reddish	,,	somewh. reddish
,,	III_2	,,	,, ,,	,,	somewh. red	,,	reddish
exper.	I_8	11. a.m.	light-red	$12\frac{1}{2}$ p.m.	reddish		
,,	I_9	,,	,,	,,	,,		
,,	I_{10}	,,	,,	,,	,,		
,,	I_{11}	,,	,,	,,	,,		
,,	II_3	,,	,,	,,	somewh. red		
,,	II_4	,,	,,	,,	,, ,,		
,,	III_3	,,	,,	,,	,, ,,		
,,	III_4	,,	,,	,,	,, ,,		
exper.	I_{12}	5. p.m.	light-red	6. p.m.	somewh. reddish	$6\frac{1}{2}$ p.m.	colourless
,,	I_{13}	,,	,,	,,	,, ,,	,,	,,
,,	I_{14}	,,	,,	,,	,, ,,	,,	,,
,,	I_{15}	,,	,,	,,	,, light-red	,,	light-red
,,	II_5	,,	,,	,,	,, ,,	,,	,,
,,	II_6	,,	,,	,,	somewh. red	,,	somewh. reddish
,,	III_5	,,	,,	,,	,, ,,	,,	,,
,,	III_6	,,	,,	,,	,, ,,	,,	,,


s
n aq.
t.
in aq.
t.
1 aq.
t.
in aq.
t.
n aq.

I should mention that the colours of I_5, II_2 and III_2 were absolutely equal at the beginning of the experiment; this was also the case in I_8—I_{11}, II_3, II_4, III_3 and III_4 as well as in I_{12}—I_{15}, II_5, II_6, III_5 and III_6. The experiments I_8 etc., II_3 etc. and III_3 etc. contained other „enzyme" and „emulsion" than the preceding ones.

In the first place we see from the table, that the „blind" experimental series II and III lose indeed more or less their red colour. Consequently, acids are set free in these. In II one might ascribe this to hydrolysis due to the lipase, for this mixture must have also contained some (few) oildrops. But in III no lipase can have been present at all; nevertheless the fat is hydrolyzed — probably by means of the alkali — (or the red colour diminishes by the entrance of CO_2 from the air).

The experiments of series I, however, prove to lose their red colour sooner than those of II and III. One might be inclined to explain this by the combined influences, which were acting separately in II and III. But that would not be exact, as is shown in the second and in the last group of experiments. Still another factor must have been acting. That must be the hydrolysis of the fat by the lipase. But this lipase does not seem to be very active here; although it is difficult to give a decision.

Table 14. *The number of oildrops and of globules, which can be stained (brown) by I, present in choanocytes in comparison to that in amoebocytes, in green and colourless Spongillidae taken from water of the lake, from water of the canal and from that of the conduit;* microscopically examined by means of ravel preparations (p. 12—15) of sponge tissue.

I examined green Spongillae (s.) and Ephydatiae (e.) from light and colourless ones from darkness. The (average) number of oildrops (oildr.) and of globules which can be stained by I (glob. I) was always stated in the same volume of amoebocytes (sub 1) and of choanocytes (sub 2). As for the meaning of I—XII, see pag. 14. In the column „remarks" is mentioned: the species of the sponge; the month of the examination; whether the sponges, after their capture from the lake or the canal, had been in water from the conduit (aq.) before they were examined, and for how many time; and whether sponge-reduction had occurred.

All experiments are mentioned. As for the discussion, see pag. 90—93.

TABLE 14.

water of canal									water of lake									water from conduit								
green sponge				colourl. sponge				remarks	green sponge				colourl. sponge				remarks	green sponge				colourl. sponge				remarks
oildr.		glob. I		oildr.		glob. I			oildr.		glob. I		oildr.		glob. I			oildr.		glob. I		oildr.		glob. I		
1	2	1	2	1	2	1	2		1	2	1	2	1	2	1	2		1	2	1	2	1	2	1	2	
IX	IX			IX IX	IX IX			s. IX. 1 day in aq.	IX X IX	IX X IX							s. IX. 1 day in aq.	X	X							s. IX. reduct. ? 1¼ month in aq.
VII VII VIII	VIII IX VIII	IV XII	VII XII					s. VI. 1 day in aq.	VI IV VII VI VI	VI VI VIII VII VII	X IX VII IX	X X X IX					s. VI. 1 day in aq.									
VI	VII	X	X	IV	VI	XII	XII	e. VI. 1 day in aq.	IV	VII	VIII	VIII	X	IX	X	X	e. VI. 1 day in aq.									
VI IX	VII X	XII XII	XII XII	X	XII	XII	XII	s. VIII. not in aq.	IX VI	X VIII	XII XII	XII XII	VI	X	XII	XII	s. VIII. not in aq.	VI VIII	X XII	X XII	X XII	VIII X	XI XII	X XII	X XI	s. VIII. no reduct. ¼ month in aq.
																		VIII IV	XII VIII	X X	X X	IX X	XII XII	X X	X XII	s. VIII. no reduct. 5 days in aq.
																						IX IV IV	XII VIII VII	XII X X	XII XII XII	s. IX. no reduct. ½ month in aq.
																		IV VII VII	VI IX VIII	X XII XII	X XII XII					s. IX. no reduct. ¼ month in aq.

TABLE 15. *The number of amoebocytes, containing food-vacuoles, present in the (ravel) preparation (p. 12—15) of green and colourless Spongillidae (before, and after they have been cultivated in aquaria) from light and from darkness, in tissues in different stages of development (conf. Table 6).*

Immediately after the capture of green Spongillae from light and colourless ones from darkness their branch-tops and branch-bases were examined as for their amount of amoebocytes containing food-vacuoles; of Ephydatiae only the full-grown tissue was studied (conf. Table 6). After some weeks' culture in water from the conduit in light or in darkness this examination was repeated, then, only for full-grown tissue. Generally of each sponge a piece was cultivated in light and another one under equal circumstances in darkness. As for the meaning of I—XII, see p. 14. The total number of amoebocytes present in the preparation was always very numerous (XI).

In the table is indicated: *a*. The n° of the experiment. *b*. The date of the examination. *c*. The then stated number of amoebocytes containing vacuoles present in the preparation of a branch-top (t.) or a branch-base (b.), for cultures in light or in darkness; viz. on the 1st line the number immediately after the capture (so at the beginning of the experiment) and on the 2nd the number at the end. Finally for each tissue group the data, concerning all analyzed sponges together, are composed.

All observations are mentioned. The green Ephydatiae came from the light, the colourless ones from darkness or from the light. As for the discussion, see pag. 94.

TABLE 15 A. SPONGILLAE.

green ones from light						colourless ones from darkness					
		cultures in light		cultures in darkness				cultures in light		cultures in darkness	
nº	date	t.	b.	t.	b.	nº	date	t.	b.	t.	b.
292	23. VII	VI	I	I	IV	298	25. VII	VII	IX	IV	IV
	17. VIII		VII		IX		16 VIII		IX		IX
293	23. VII	I	VII	I	VII	299	26. VII	III	III	IV	IV
	17. VIII		VII		VII		16. VIII		IX		VII
294	24. VII	I	VI	III	IX						
	19. VIII		X		IX						
367	22. VI		VI		VIII						
	11. VII		VII		VII						
a	23. VII	III	VI			i	23. VII			II	
b	„	III	III			j	„			III	VII
c	„		VI			k	„			VI	
d	„		VII			l	„			IV	IX
e	„	IV	VI			m	„			IV	VII
f	„	III	VII			n	„			VI	IV
g	„	III	VI			o	„			VIII	VIII
h	„	IV	III			p	„			VI	VIII

together; results of newly captured sponges

tops: 4. I + 5. III + 2. IV + 1. VI	*tops:* 1. II + 2. III + 4. IV + 3. VI + + 1. VII + 1. VIII
bases: 1. I + 2. III + 1. IV + 6. VI + + 4. VII + 1. VIII + 1. IX	*bases:* 1. III + 3. IV + 2. VII + 2. VIII + 2. IX

TABLE 15 B. EPHYDATIAE.

green ones						colourless ones					
		cultures in light		cultures in darkness				cultures in light		cultures in darkness	
n⁰	date	t.	b.	t.	b.	n⁰	date	t.	b.	t.	b.
333	23. II		III		III	336	24. II		IV		IV
	1. V		VII		IV		1. V		V		IV
334	23. II		III		V	337	24. II		IV		IV
	30. IV		VII		IV		7. V		IV		IV
339	25. II		III		IV	338	24. II		IV		IV
	24. IV		VII		X		8. V		IV		IV
340	25. II		IV		III	341–2	25. II		IV		IV
	23. IV		III		III		30. IV		VII		IV
343–4	26. II		IV		V	345–6	26. II		IV		V
	30. IV		IV		VII		30. IV		IV		IV
348	26. II				IV	347	26. II		IV		
	8. V				X		7. V		IV		
366 I	21. VI		IV		IV	365 I	20. VI		III		III
	12. VII		IV		IV		10. VII		IV		VII
366 II	21. VI		III		IV	365 II	20. VI		III		IV
	13. VII		VII		IV		10. VII		VI		VII
together; results of newly captured sponges											
6. III + 7. IV + 2. V						3. III + 11. IV + 1. V					

ILLUSTRATIONS.

EXPLANATION OF PLATES nº I—VI.

For clearness' sake many figures have been drawn more or less diagrammatically from nature. For each object („symbiotic" alga, flagellated chamber, etc.), however, at least one illustration true to nature has always been given.

Fig. 1. Living green Spongillae lacustr. in water (natural size). The sponge-crust cannot be seen. As for the discussion, see p. 15—16.

Fig. 2. Living colourless Spongillae lacustr. (natural size) in water, showing oscular tubes. The sponge-crust cannot be seen. See p. 15—16.

Fig. 3. Living Spongilla growing on coverglass (magnif. 4 times). *1.* = old centre; 2. = newly formed membrane. See p. 12—13.

Fig. 4. Isolated living amoebocyte of Spongilla grown on coverglass (magnif. ± 1000 times). *nu.* = nucleus; *gr. alg.* = green chlorophyll corpuscles. See p. 17, 175.

Fig. 5. Living green chlorophyll corpuscle of Spongillides (= „symbiotic" alga). Magnif. ± 6600 times. *ch.p.* = chloroplast; *odr.* = oildrop. See p. 24—25.

Figs. 6—11. Plasmolysis in the „symbiotic" algae of Spongillidae. The heavily dotted portion represents the chloroplast; the slightly dotted one the protoplasm. See p. 26.

Figs. 12—31. Different stages of the living green „symbiotic" algae of Spongillidae. Figs. 16—22, 25—29, 31 stages of division. The dotted portion represents the chloroplast. See p. 24, 30—33.

Figs. 32—34. Plural stages of division of the green „symbiotic" algae of Spongillidae. The dotted portion represents the chloroplast. See p. 33.

Figs. 35—37. Colourless stages of the „symbiotic" algae of Spongillidae. Fig. 35 represents a „colourless alga with clear structure"; Fig. 36 a „colourless one with shade of structure"; Fig. 37 a „colourless one without structure". See p. 36, 42.

Figs. 38—42. Green „symbiotic" algae of Spongillidae killed, and stained by methylene-blue or haematoxyline. In Fig. 38 and 39 the oildrops have been stained. See pag. 25—26.

Figs. 43—45. Filamentous algae infecting Ephydatia (magnif. ± 350 times). See p. 117.

Figs. 46—52. Unicellular alga infecting Ephydatia (magnif. ± 800 times). The dotted portion represents the chloroplast. See p. 117.

Fig. 53. Diagrammatic representation of a section of the body wall of Spongilla (with few alterations after DELAGE and HÉROUARD). *cnli.* = conulus; *p.* = ostia; *os.* = osculum; *os. t.* = oscular tube; *ects.* = ectosome; *cv. hy.* = subdermal cavity; *cn. inh.* = incurrent canal; *cn. exh.* = excurrent canal; *chs.* = parenchyma; the arrows indicate the direction of the water current. See p. 119—120.

Fig. 54. Flagellated chambers and surroundings in Spongilla. *fl. ch.* = flagellated chamber; *inc. can.* = incurrent canal; *exc. can.* = excurrent canal. Magnif. ± 130 times. The arrows indicate the direction of the water current. See p. 120.

Fig. 55. Section of a flagellated chamber of Spongilla (magnif. ± 1600 times), after VOSMAER and PEKELHARING. *ap.* = apopyle. See p. 120.

Fig. 56. Successive stages of the flagellar movement of an isolated choanocyte. The cell body is still connected with several other choanocytes, the collar is entirely retracted. The arrows indicate the direction of the water current, the dots floating particles; the moment of observation is given in each case; *a.* immediately after isolation; in *e.* the flagellum has finally come to rest. Magnif. ± 1770 times. See p. 126—127.

Fig. 57. As Fig. 56. The collar is partly retracted. *a.* immedately after isolation; in *c.* the flagellum has come to rest; in *d.*—*e.* a (new) period of weak motion began again, which is finished in *f.* Magnif. ± 1770 times. See p. 128.

Fig. 58. Successive stages of the flagellar movement of a number of choanocytes still joined within a part of a flagellated chamber, observed in a ravel preparation. The collars are entirely retracted. *a.* immediately after isolation, in *c.* the flagella have finally come to rest. Cnf. Figs. 56—57. See p. 128, 132.

Fig. 59. Intact flagellated chamber of living Spongilla grown on coverglass; the flagella in the normal spiral- or undulating-motion. The collars are fully expanded. *ch. l.* = choanocytic layer; *odr.* = oildrops. Magnif. ± 1430 times. See p. 130—131.

Figs. 60—62. Representation of flagellum and collar seen on top; in Fig. 60 the flagellum stops, in Fig. 61 it is in spiral-motion, in Fig. 62 in flat undulating-motion. See p. 131—132.

Fig. 63. Diagrammatic representation of the water current inside a flagellated chamber of Spongilla. *pr.p.* = prosopyle; *ap.p.* = apopyle; the arrows indicate the direction of the current; + and — refer to the water pressure. See p. 132—134.

Fig. 64. Semi-diagrammatic representation of a chamber, the flagella of which are beating with the tops outside the apopyle. *ch. l.* = choanocytic layer. See p. 135—136.

Fig. 65. The different ways of capturing (food-)particles within a flagellated chamber of Spongilla (diagrammatic). The prosopyles have not been drawn, nor the separate cells of the choanocytic layer (*ch. l.*). The way taken by the particles is indicated by dots. *a.* and *b.* show the capturing between the bodies of the choanocytes; *c.* the capturing between the collars; *d.* the capturing at the bases of the collars. See p. 143—145.

Fig. 66. The capture of (food-)particles within a flagellated chamber with 2 prosopyles, viz. between the bases of the collars of the choanocytes (semi-diagrammatic). The way taken by the particles is indicated by dots. The separate cells of the choanacytic layer (*ch. l.*) have not been drawn; the layer contains numerous particles which have been captured. Magnif. ± 1000 times. See p. 143—146.

Figs. 67—68. The capture of a carmine grain between 3 collars (Fig. 67), and the descending of the grain along a collar to the base (Fig. 68, *1—2*) (semi-diagrammatic). See p. 145.

Fig. 69. A flagellated chamber and its surroundings in a living green Spongilla grown on coverglass (magnif. ± 800 times). *gr. alg.* = green „symbiotic" algae; *cl. alg.* = colourless „symbiotic" algae; *ch. l.* = choanocytic layer; *mesgl.* = mesogloea; *am.* = amoebocyte; *nu.* = nucleus; *vac.* = food vacuole; *odr.* = oildrops. By *1—2—3* is represented the ejection of „symbiotic" algae by the choanocytic layer into the mesogloea. See p. 16, 147, 174.

Fig. 70. Two carmine grains (*a.* and *b.*) ejected from a choanocytic layer (*ch. l.*) into a parenchymal tissue-bridge (semi-diagrammatic). In *I* the original condition is given; in *II* grain *a.* was ejected and moved on (*1—2—3—4—5*), in *III* grain *b.* ejected and moved on (*1—2—3—4—5—6—7*). See p. 147.

Fig. 71. The spreading of carmine from a flagellated chamber through the mesogloea into the amoebocytes. *ca.* = carmine grains and conglomerates; the other indications as in Fig. 69. Magnif. ± 800 times. See p. 147—148, 174.

Fig. 72. The layer of apparently undifferentiated flowing plasma (*pl. l.*) situated outside and against the base of the choanocytes (*ch. l.*); an oildrop is moved on slowly (*1—2—3*). See p. 151—154.

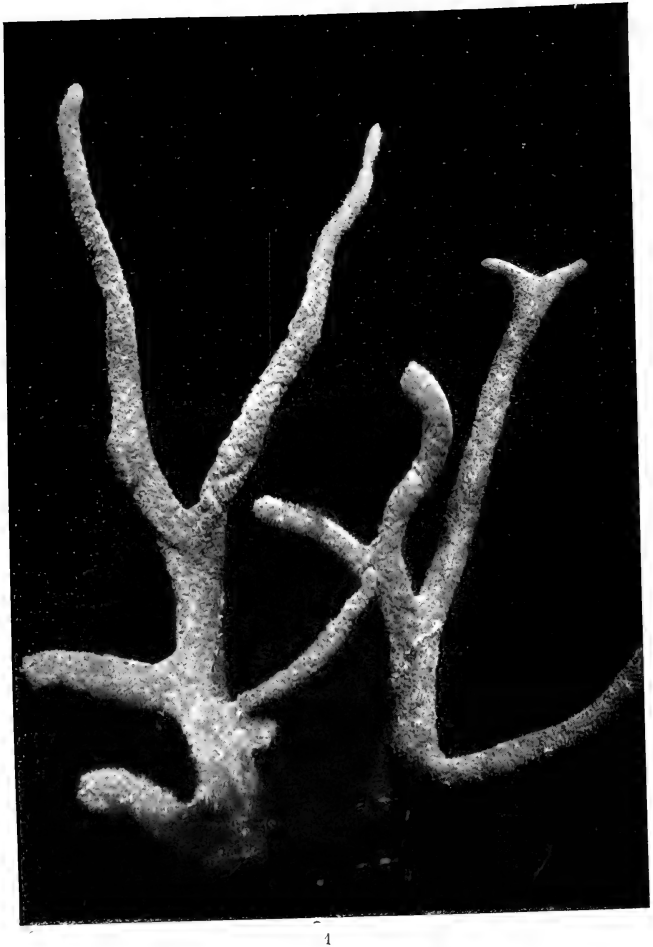
Fig. 73. Semi-diagrammatic representation of a flagellated chamber with the layer of flowing protoplasm (*pl.l.*) lying against the choanocytes at the side of the incurrent canal. The choanocytic layer (*ch. l.*) has been drawn as one whole; it lodges numerous captured carmine particles. Similar particles are carried along in the plasmic layer (*1—2—3*) to a deposit place, from where now and then a large (fecal) conglomerate is ejected. Magnif. ± 1000 times. See p. 151—154, 166—168.

Fig. 74. Three flagellated chambers and incurrent canal (*can.*); layer of flowing plasma against the base of the choanocytes at the side of the canal; tissue „bridge" bent through the canal (semi-diagrammatic). The figure represents the transport of carmine in the plasmic layer (*a—b—c*) and in the „bridge" (*1—2—3—4—5*). *ch. l.* = choanocytic layer; *ca.* = carmine (grains and conglomerates) which has been captured. See p. 151—154.

Fig. 75. The capture (*2*) and the carrying aside (*3—4*) of a coarse (food-) particle, that remained sticking in the prosopyle (*1—2*), by means of the layer of flowing protoplasm (*pl. l.*). The figure is semi-diagrammatic. The separate cells of the choanocytic layer (*ch. l.*) have not been drawn; the layer contains numerous particles which have been captured. Magnif. ± 1000 times. See p. 152.

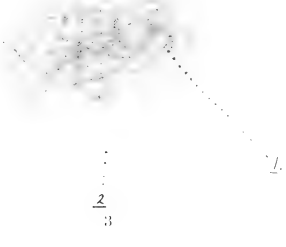
Fig. 76. The defecation and excretion by a vacuole situated in the canal wall. The small circles represent green »symbiotic" algae. Magnif. ± 400 times. See p. 163—164.

Fig. 77. The protruding and withdrawing of a defecation (and excretion) vacuole at several canals; in *1.* a vacuole has not yet been formed; in *4.* it finally protrudes into the 3rd canal and bursts. The small circles represent green „symbiotic" algae. Magnif. ± 400 times. See p. 163—166.



1

2



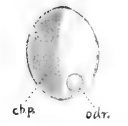
1

2

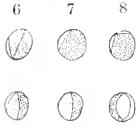
3

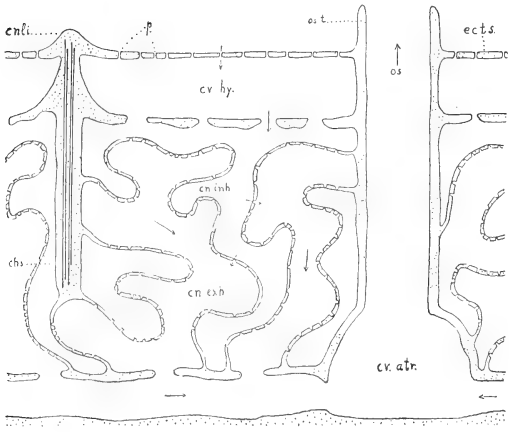
Van Trigt photogr. et del.

4

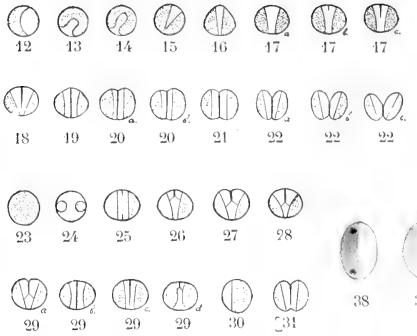


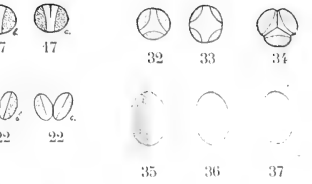
5

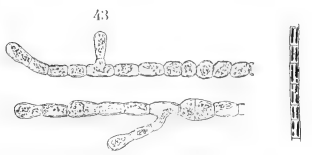


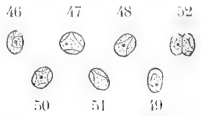


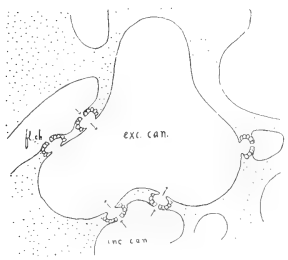
53







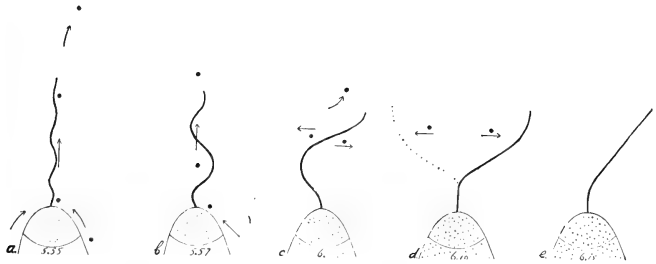




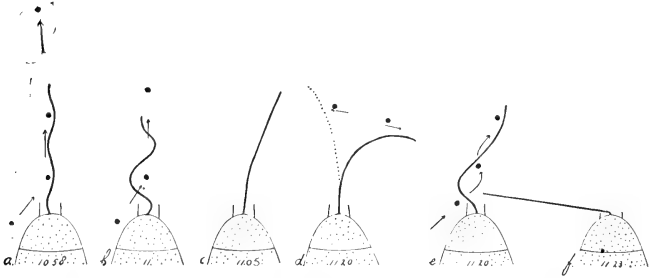
54

55

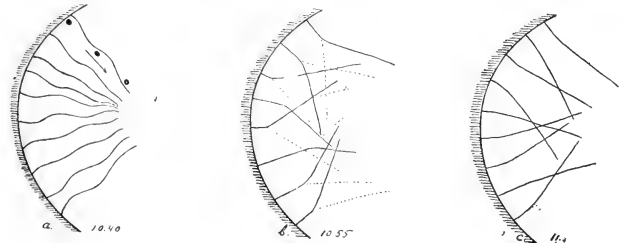
Van Trigt fig. 4—54 del.



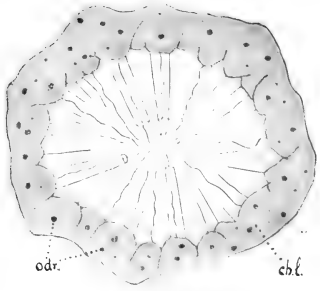
56



57

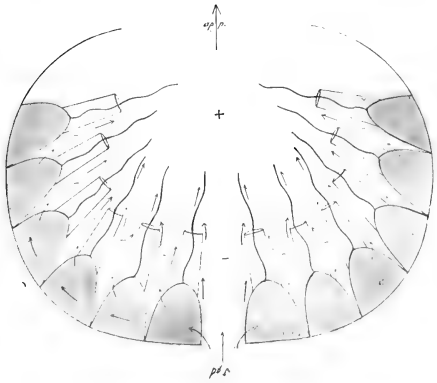


58



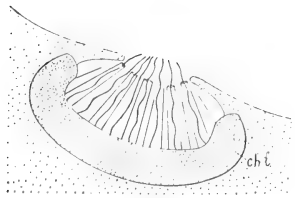
59

60 61 62

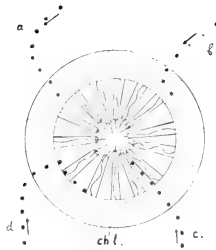


63

Van Trigt del.



64

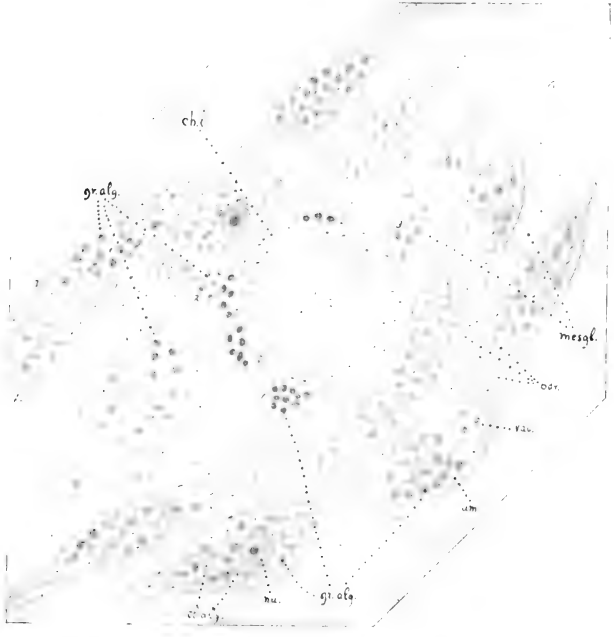


65

66

67

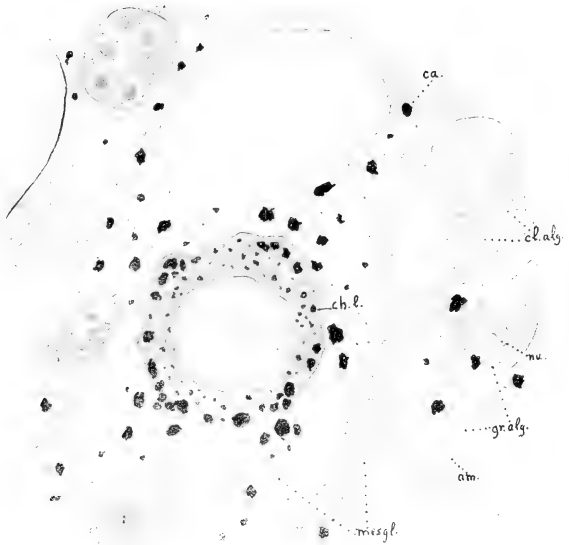
68



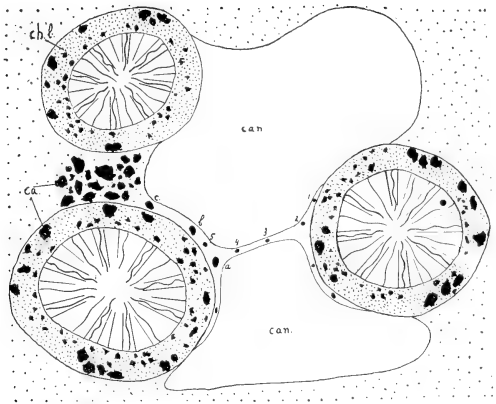
69

70

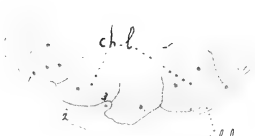
Van Trigt del.



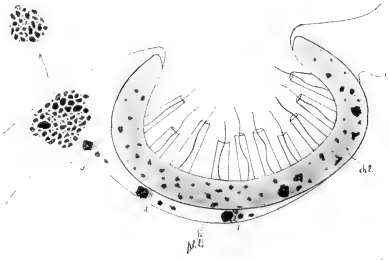
71



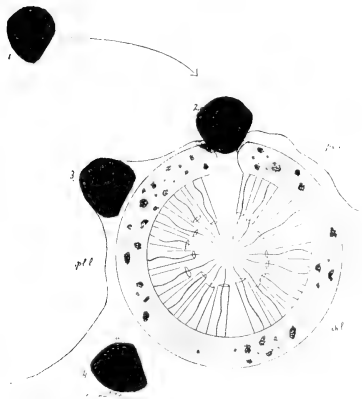
74



72

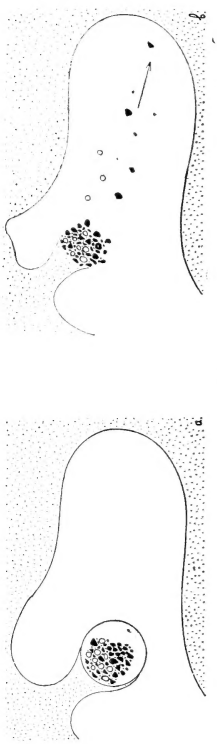


73

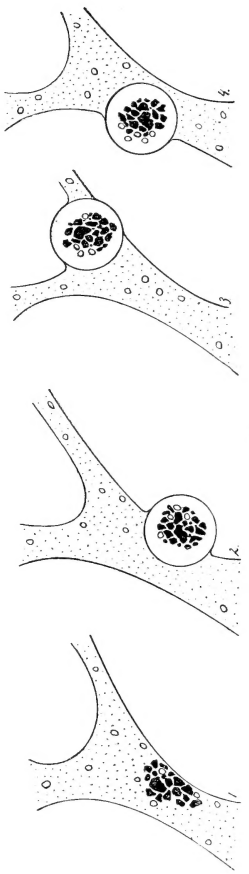


75

Van Trigt del.



76



77

VAN TRIGT del.