

OBSERVATIONS ON SOME OUTSTANDING FEATURES
OF PROTOZOAN LIFE

A Thesis

by J.Y. Simpson

(M.A., B.Sc., F.R.S.E.)



INTRODUCTION

The following pages represent for the most part the results of two summers' work under Mr. Sedgwick at Cambridge. In this place I would record my grateful indebtedness to that gentleman, not only for the ample provision - from a room down to the smallest necessary - put at my disposal, but also for the kindly encouragement and stimulus that are now associated in my mind with his name. Nor do I forget the interest and help accorded by Messrs Shipley and Graham Kerr.

Proceeding on the assumption that since the daughter forms produced as the result of binary fission were duplicates, we need not look for any variation there, I endeavoured in the first instance to carry out a suggestion of Mr. Sedgwick's which he ultimately published in his presidential address to the Zoological Section of the British Association in 1899, and laid plans under his direction to find out by observation of the external characters how much variation could be detected in exconjugates as the result of conjugation by comparing them before and after the process. For this purpose it was necessary to choose a Ciliate with well-marked characters, and *Stylonichia pustulata* with its systems of cirri and membranelles appeared to offer the necessary requisite. The only line along which it seemed that I could work was to draw the creatures as accurately as was possible just before conjugation,

repeat this after they had come out of conjugation , and then deduce results from measurements and other comparison of the drawings. The first difficulty was to get the creatures quiet so as to enable one to carry out this programme. A common method of quieting Protozoa is by pressure with a cover-glass , but the distortion and displacement connected with this method rendered it impossible for the work in hand. Another method that has long been recommended is entanglement with cotton wool , but here the success that one obtains is distinctly limited and insufficient for the purpose. Cocaine and other narcotics have been faintly recommended: I have tried the former , but it not only did not produce the amount of quiet that was desirable , but it seemed to disturb the after-life of the creature. After many attempts with various agents , I found that a drop or so of a 4 per cent solution of gelatine had the effect of slowing down these Ciliata: they even had frequent spells of absolute rest , except for the occasional movement of a cirrus. Accordingly eight well-marked specimens , all differing in size , were selected from a culture of *Stylonichia* , of which some members had already entered into conjugation. They were then drawn after being stilled by gelatine , and as they were easily recognizable , they were all put together in a watch-glass containing water from the original medium. Not a single case of conjugation occurred. I then took other specimens and subjected

them to the gelatine medium for the same interval of time as was required to draw the others - each about a quarter of an hour or twenty minutes - and then re-transferred them to a watch-glass also containing water from the original culture.⁺ Again I had no success in conjugation: it would appear that the short sojourn in the gelatine was quite sufficient to change the peculiar bodily equilibrium that must be associated with the stage previous to conjugation. I have little doubt that a similar result would have attended all attempts with narcotics. Accordingly although working all the while at other points I had to acknowledge defeat in this particular instance.

It was, however, while making these more careful examinations of individual forms that I came to doubt the initial assumption upon which one had been proceeding. Observation of a dividing Paramecium with a cleft tail suggested that even though the case was abnormal there was no reason why the products of binary fission should be exactly alike, unless indeed to afford theorists a rationale of conjugation. In several cases I observed that actually it was not the case, but drawings of these, poor in themselves, might unconsciously suggest

⁺An attempt was made to get rid of the gelatine in between by a "washing" with pond water.

that they represented theory rather than fact. Accordingly it seemed worth while to see what could be done by micro-photography, and although I was assured by a young scientific photographer that it was impossible to photograph living Ciliata, an attempt was made. As I am not aware that living Protozoa - Ciliata at any rate - have been photographed before, I have added one or two pictures of forms that otherwise have no definite connection with the point to be illustrated. Ordinary Ilford chromatic plates were used, as also a Leitz 3 lens, which with the associated extension gave a magnification of about 80: the pictures were all taken ^{simultaneously} instaneously by incandescent light. Those that are offered are but a few successes out of about two hundred failures. The chief difficulty was in obtaining a cell small enough to be wholly included within the magnification of the lens. Ultimately a block of soft parafin was employed in which a hole was pierced with a fine needle: it was then sectioned with the microtome, and in this way, by regulating the thinness of the section, a cell was obtained with the minimum of water in which the infusorian could live and yet be in focus all the time. A cover-glass was then super-imposed, and as it was held in position by a generous application of vaseline round the edge, we were enabled to take the photographs in a horizontal position. They were taken at the Laboratory of the Royal College of Physicians with the kindly assistance of Mr. Hume Patterson.

With regard to the remaining methods not much need be said. In pursuing the investigations relating to binary fission I employed damp chambers, constructed very much upon Maupas' system. In killing Protozoa I have pursued all the better known methods and many that are less known, but I have found nothing so satisfactory as the vapour of 2 per cent osmium, although good results were sometimes obtained with a formula given by Hoyer in the Archiv für Mikroskopische Anatomie, Vol.54:Part I - viz. one volume of 5 p.c. corrosive sublimate added to 2 volumes of 3 p.c. bichromate of potash.

For the high-power examination of living Ciliata I often made use of Professor Marshall Ward's culture tubes which can also be converted into very excellent miniature damp chambers. It at least seems a more natural state of affairs to have the living Protozoan in a drop, suspended from a cover-glass attached to one of these culture tubes than to have it prisoned and pressed under a cover-glass, however supported.

Part I of this thesis contains some observations upon the intimate structure of Protozoa, as deduced from sections longitudinal and transverse of Spirostomum ambiguum and Stylo-nichia pustulata. Part II consists of two sections. The first contains confirmation of Scheel's observations upon the sporulation of Amoeba proteus. The second contains an incomplete account of another method of reproduction which I have ventured to call Fragmentation. It may be regarded

as a preliminary account in which the main steps are given: certain of the details have however yet to be ascertained. The main contention of the thesis will be found in Part III. It is only recently that Maupas' classical studies on the rationale of binary fission and conjugation have been called in question. I have attempted to go over his work in part, and while on the whole I find myself in agreement with him as against his critic Joukowsky, I venture to offer a few modifications in some details. To this part is appended some evidence for my belief that binary fission is not duplication. To prove this thoroughly would be the work of many months. At the same time that work would only be multiplication and amplification of the data that I offer. In the second section of this part some notes upon conjugation are inserted. Part IV contains a short account of Encystment as it occurs amongst the Ciliata, together with descriptions of the little known cysts of Paramecium and Spirostomum.

The attempted diagrams and photographs are well-nigh self-explanatory. The former were made with Abbé's drawing-apparatus: the methods adopted in connection with the microphotography have already been described. Much of the latter is sadly lacking in definition, but it is a matter of extreme difficulty to calculate and adapt that amount of water in the cell which is sufficient for the free movement and life of the Protozoan and yet not too great to allow it to get out of

focus during the exposure. We may however hope for still better success in future attempts.

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PART I

INTIMATE STRUCTURE OF PROTOZOA.

There are two methods by which our knowledge of the intimate structure of Protozoa can be best advanced - intra-vital staining and sectioning. Of these the former is the older and has been attended with a certain measure of success. According to Bütschli . Gleichen (1778) was the first to attempt the artificial feeding of Protozoa with carmine , indigo and such like substances , although in his hands the method did not lead to any real results. Ehrenberg , who considered Gleichen's work somewhat in the light of a joke , adopted the method with greater success , not unmixed however with error. In more recent times intra-vital staining has been largely adopted with varying modifications. Prowazek (Zeitschrift für Wissenschaftliche Zoologie:Vol.LXIII:pp.187-194:) cites Brandt's experiments with haematoxylin and bismarckbrown , Certes' success with cyanine and quinoline blue , and Marian Przesmycki's use of methylene blue. To these must be added his own experiments with neutral-red , as a result of which , together with those of his predecessors , great light has been shed upon many problems relating to the internal organisation of infusoria. Thus Brandt shewed that nuclein was not limited to the nucleus , and Prowazek has offered

reason to believe that there are discrete particles in the endoplasm which, inasmuch as they seem to stand in some direct relation to digestion and assimilation, may be held to be the bearers of ferments.

While carrying on other work, I have from time to time kept Infusoria under observation in different coloured media, employing several of the above mentioned stains. There seems to be no doubt that the Infusoria can be accustomed to a greater strength of carmine than of any other stain. The effect however is less than might be imagined. Even at the close of three weeks' confinement in a carmine medium, the strength of which was daily increased, *Paramecium caudatum* shewed no sign of nuclear stain, a fact which has long been known but which makes it increasingly difficult to understand the nature of the metabolic changes that go on between the macronucleus and cytoplasm. And yet the carmine would appear to affect the macronucleus in some way, for the macronuclei of these particular individuals stained relatively quicker than those of ordinary *Paramecia* when both were placed in stain of full strength.

If the stain (in particular dahlia) was added in too great quantity, certain pathological phenomena generally resulted. Of these by far the most common was an out-flowing of the contents of the body into a saclike swelling which formed in front of the mouth, giving the whole ~~the whole~~ the

appearance of some early fish-stage with the corresponding yolksac. This points to extraordinary elasticity of the ectoplasm, for wellnigh a half of the somatic content was often pressed out into the sac. How exactly the process commenced was not easily made out, but inasmuch as there was inhibition of the contractile vacuole, and food vacuoles were still being formed, the internal pressure must have been increased which finally caused rupture at some point near to the formation of the vacuoles where the tension might conceivably be greatest. Paramecia thus affected generally made little progress in a direct forward or backward direction, motion being commonly restricted to a slow rotation round the long axis.⁺ In some instances it was possible, by careful treatment, and especially by immediate change to normal surroundings, to restore the creature to its natural condition, but these were very rare. More commonly the incoming stream continued

⁺ Carl Bruno Schürmayer (Ueber den Einfluss äusserer Agentien auf einzellige Wesen: Jena. Zeits. Naturw: Bd. XXIV: pp. 402-470:) obtained a very similar result after treating Infusoria with such agents as antipyrine, cocaine, chloroform, etc. " Bei Paramecium waren die Folgen einer anhaltenden Diastole sehr auffällig; die zur Vacuolen führenden Canäle nämlich " lösten sich in rasch anwachsende runde Vacuolen auf, " und es entstanden sogar blasige Ausbuchtungen des Ectoplasmas."

to flow till the sac reached enormous proportions and finally burst.

In other cases the stimulus of the colour caused increased ciliary activity. If an ectoplasmic sac was also formed this excessive motion of the cilia sometimes caused disruption of the body, when it was noted that the movements were kept up by the fragments, and sometimes at a rate superior to that of the recent whole. The movements of these fragments usually ceased within sixty seconds, but observation of them forcibly suggested the autonomous character of their movements^{of the cilia}, possibly more evident in these parts than it was in the whole.

In connection with some Paramecia that were kept on a slide in a medium that was daily and increasingly tinged with $\frac{1}{4}$ per cent dahlia solution the following phenomenon was noticed. More especially at either end and round the oral aperture, but also faintly and apparently along lines corresponding to those of the characteristic markings, were formed small transparent blisters of a bluish tinge. They had the appearance of droplets under or in the pellicula. One may suppose that with the inhibition of the contractile vacuoles which ~~were~~^{was} induced at a certain stage they represented the waste of assimilative processes which thus gathered near the points of formation. When the forms were restored to normal conditions these excrescences^c disappeared but whether from absorption from within or by osmosis (partly) to the exterior I cannot definitely state: some-

times it seemed as if one had reason to believe that such osmosis took place. But at any rate it is probable that the impermeability of the infusorian pellicula (where it exists) is not so absolute as is commonly believed. Prowazek had a somewhat similar experience with *Paramecia* fed on neutral-red. (op.cit)

On the first page of the accompanying book of diagrams are given some drawings of sections of *Spirostomum ambiguum* and *Stylonichia pustulata*. These were prepared in very much the ordinary way. After being taken out of absolute alcohol the protozoa were placed in equal parts of ether and absolute alcohol and thence into celloidin where they were left for 48 hours. After that they were put in chloroform for half an hour and then placed in cedar-wood oil until they became perfectly transparent.

In discussing the ectoplasm of the Ciliata , Bütschli distinguishes two main regions , an outer (in most cases) more or less homogeneous pellicula and an inner alveolar layer. The innermost zone of the alveolar layer may however be further differentiated as the cortical plasm. It is the seat of the trichocysts and possibly also of pigments where these occur. All these layers are more or less in connection with one another and the innermost one is not entirely discontinuous with the entoplasm: at the same time the ectoplasm is sharply marked off by the facts that it takes no part in cyclosis and

never shews any trace of a food vacuole. Only rarely are these three zones well developed in single species: most frequently two only are present, sometimes but one, while in the case of *Actinotricha* and *Gonostomum pediculiforme* Maupas denies the existence of ectoplasm altogether. The entoplasm forms the great proportion of the Ciliate body: indeed the only cases that one knows where the conditions are reversed is that of the small *Amöbæ* described in Part II. The sections of *Styloniichia* seem to indicate that here we have but a pellicula, a superficial skin of 1-2 μ in thickness. It is practically hyaline and is of greater thickness and less homogeneous in character on the ventral and lateral aspects. This pellicula is an integral part of the ciliate and in no wise comparable to the cellulose cell membrane of plants which is of the nature of a secretion: the homologue of the latter is rather the various ciliate cysts. In Figs. 8 and 11 it will be noticed that certain thickenings are indicated in the superficial layer at the base of the cirri. The observations bearing on the relations between the cirri and the entoplasm are not numerous. Engelmann (*Zür Anatomie und Physiologie der Flimmerzellen: Pflüger's Arch. f. Physiologie: XXIII: pp. 505 seq.*) noticed that an extremely delicate plasmatic fibril sprang from the base of each of the lateral cirri and could be followed in its course through the cytoplasm to somewhere near the ventral mid-line. This observation was confirmed by

Maupas , and extended to other cirri in other positions and different species. In some of my sections (Fig.12) I was able to connect a very fine fibril running into the entoplasm with these thickenings at the base of the cirri.

The alveolar layer is well developed in Spirostomum and the pellicula on the other hand is hard to differentiate. It however becomes clear from sections of this form that the body striping is due to folds or wrinkles which separate the grooves or furrows in which the cillia lie: the latter seem to have their origin in dimples or depressions rather than in papillae. The alveolar structure is very fine and does not appear to be altogether unconnected with that of the outer entoplasm. Fig.2 , which though apparently transverse (owing to the peculiar striping and unusual nucleus of the form) is really longitudinal , shews a certain concentration in the centre of the ridges which , as Moore suggests , probably indicates the " existence of denser tracts of substance running down the entire length of each ridge , or in reality an apparently resistant structure which may have much to do with the rapid undulations and contractions of the animal when in activity." The received view of the origin of the contractile fibrillae characteristic of so many ciliate species is that they are differentiations of the mesh-work of the alveolar layer: in this case there appears to be some measure of support for the explanation.

All the sections alike shew that the entoplasm is alveolar in structure , only more distinctly so , than the ectoplasm. They disclose a very complicated vacuolation pervading the entire entoplasm. The meshes are of irregular size and shape now rounded , now polygonal: they are , as a rule , larger towards the centre but closer in the neighbourhood of nuclei and , especially in the case of Spirostomum , towards the periphery of the entoplasm. The points and lines of intersection of the different meshes appear somewhat thicker , and the walls whether thin or thick , seem to be composed of delicate fibrillar elements. Within these actual walls , more especially at the points of intersection are different granules mostly circular in outline. In the internal reticulum of Spirostomum Moore observed and has figured numerous small refractive dots that were seen " along the fibres of the net-work and which became more numerous towards the periphery on all sides." He considers that these refractive dots " are the last residuum of the animal's food , and that as such they make their way out along the lines of the enclosing membrane." As distinct from the granules mentioned above I have noticed such refractive dots both in Spirostomum and Stylonichia , but not at all in the same numbers as suggested by Moore's figures.

There appears to be some difference of opinion with regard to the amount of vacuolisation visible in the ordinary entoplasm of the Ciliata. We find all degrees of it from the

very fine honey-comb of the outmost entoplasmic zone , as seen e.g. in Spirostomum to the large alveolar structure , e.g. of Bursaria. All the intermediate stages are traceable and several of them may be found in the one form: it is impossible to absolutely differentiate them. It is also well known that in the encysted state this vacuolisation is much reduced , indeed may be altogether absent. Bütschli however would appear to deny the existence of the markedly alveolar condition in certain cases where it seems to occur. On page 1392 of the 3rd volume of his Protozoa there are words to this effect. " The frequent presence of a coarsely alveolar or vacuolar structure should be distinguished from that of the fine honey-comb structure. Many Ciliata do not appear to develop the former but rather have a compact entoplasm in which , of course, the fine honey-comb formation is not wanting. Most of the Hypotricha fall to be included here , and the same may also be observed even more frequently in the case of smaller forms. Likewise amongst the Vorticellidae and many Holotrichae no proper vacuolisation has been really observed." In smaller type we are told that individuals of the species *Stylonichia mytilus* " welche an Wassermangel litten " as also sometimes normal individuals were found by Sterki to have their cytoplasm " durchaus vacuolar-netzig " : and he concludes " demnach tritt diese Erscheinung auch bei Hypotrichen auf , aber wohl vorzugsweise pathologisch." Now all the sections of Stylo-

nichia pustulata of which drawings are given and indeed all those that I have examined, with the exception of a few in the extreme anterior or posterior regions, shew this vacuolisation fairly well developed. Fig. 13 roughly gives the appearance of a typical section of this nature. A magnification of 1000 shews however that the greatest alveolae are themselves filled with what appears to be a finer alveolar structure - a mesh-work within a mesh-work - but none the less the appearance of vacuolisation is quite distinct and in some instances actual. I have no reason to believe that the individuals thus examined were other than absolutely normal, and am therefore inclined to maintain with Sterki that a vacuolar entoplasm may be found in the case of two species of *Styloni-
chia* and interpreted without any reference to the pathological.

Before adding one or two final notes on the structure of the macronucleus we may refer briefly to its position in the ciliate body. Cyclosis is probably in all cases a movement of the entoplasm as a whole. Within this entoplasm the macronucleus is found and the question that requires to be determined and to which in comparison with its importance relatively little attention has been directed is how far its position is modified by this continuous internal movement. If it lies absolutely free in the entoplasm it is difficult to see how it can avoid sharing in its movements, unless these be too gentle to bear it along. Again, while on the one

hand in view of its function we might suppose that it would be for the benefit of the creature that a compact macronucleus should circulate throughout the cytoplasm, yet if it maintained its position relatively to the mass of the entoplasm all through these movements the amount of benefit might conceivably be less than if it were to remain stationary all the time and let the entoplasm circulate round and past it. Now if the macronucleus is fixed at all it must be moored in some way to the stationary ectoplasm, and in a few instances such definite attachment by fibrils or otherwise has been observed. In other cases, such as that of the *Hypotricha* no change of position has been observed during the ordinary phases of vegetative life, although the means of attachment have not yet come to light. I have noticed in the case of the products of a binary fission of *Stylonichia pustulata* that the paired macronuclei relatively occupy slightly different positions, and if they are absolutely fixed we would have a good point in their position in which to note any variation in daughter forms. In the case of *Paramecium caudatum* the difference in the position of the macronucleus in the two daughters of a divided individual was often considerable, but in this particular species Balbiani observed displacement of the nucleus as far back as 1861. Examination of the living form has shown me that the area within which the macronucleus can circulate is nearly two-thirds of the whole. The entire question

requires re-investigation , for I do not know that there is a single case concerning which it is maintained that the macronucleus retains its position permanently throughout the whole life of the individual especially ^{during} the stages previous to binary fission , and it is probable that the area and amount of local change is greater than is commonly imagined; while on the other hand , as already stated , if it can be definitely shewn that the macronucleus is thus permanently anchored we have an important standard by which to calculate the amount of variation that will be shewn to exist (Part III) in the products of binary fission.

It is only in a limited sense that the shape and size of the macronucleus can be said to be constant for each species. Possibly the variation is not so great as one has observed , e.g. in the nuclei of the individuals of various Amoeban species , but still it exists. Unfortunately this nuclear variation was noticed too late to include statistics concerning it amongst those relating to other points given in Part III. It was perhaps more noticeable in *Paramecium caudatum* than in *Stylonichia pustulata* , but is present in both.

The sections that I have examined suggest that the structure of the macronucleus is comparable to that of the outer layer of the entoplasm , viz. finely honey-combed: it is however more close and dense. The slightly thickened lines , representing as we may suppose the lines of intersection of

the cell-walls , and the certain presence of numerous deeply-staining microsomes within them prevent one from applying the term homogeneous to the concentrated structure. Bütschli (Arch.f.mikr.Anat.Vol.IX:p.657:) has given a fuller description of certain " Binnenkörper " first noticed by Stein which he found in both parts of the macronucleus of this and other forms. Fig.9 , (Plate I) represents these " Binnenkörper " as they appeared in some of my sections , more regularly spherical ^(although sometimes polygonal) and possibly larger than those figured by Bütschli. Commonly in the interior there was a refractive body surrounded by a lighter area which was distinctly marked off from the lighter area of the neighbouring " Binnenkörper." Of their real character nothing at present seems to be known.

PART II

REPRODUCTION OF RHIZOPODA: (a) Sporulation of Amoeba.

That the Rhizopoda encyst has long been known, but mainly in reference to protection against an unfavourable environment, as is so common in the case of the Ciliata.⁺ In connection with Reproduction Bütschli only refers to two series of observations - those of Cien^kowsky on the encysted spindle-cells of *Labyrinthula*, and those of Haeckel on *Protomyxa aurantiaca*. The latter may be briefly noticed for reasons that will appear in the sequel. The *Protomyxa* formed round cysts with a simple though somewhat thick gelatinous wall within which the cell contents divided up into numerous circular bodies. These after a while escaped through fracture of the cyst wall, developed a flagellum, and after swimming about for a time settled down, withdrew the flagellum and became amoeboid in character. Either by gradual growth or by the fusion of several forms in a plasmodium, the adult stage was reached. Certain considerations however suggest that we are here dealing with a form that should be included among the Mycetozoa.

Schaudinn's observations also bear on a form (*Paramoeba*

⁺ They are also known to encyst for purposes of undisturbed food assimilation.

Eilhardi) by no means closely related to the Amoebae proper. This form likewise secretes a cyst in which division of the contents takes place. In this process the way is shown by a paranucleus (nebenkern) and the nucleus responds sympathetically , dividing into as many portions as does the paranucleus. Daughter nuclei and daughter paranuclei then approach one another and the cytoplasm finally divides into the same number of portions. A slow movement of the daughter forms is set up in the interior and the cell wall bursts , so that the contents escape as small colourless biflagellata. These divide longitudinally , and the paranuclei during division play the part of centrosomes to the dividing nucleus.⁺ It is these grandchildren which after losing their flagella develop pseudopodia , and eventually reach the adult form.

The following observations do not pretend to be other than a general confirmation of the observations communicated by Scheel for the first time in his Beiträge zur Fortpflanzung der Amöben , and hitherto unconfirmed. The few points in which there is any difference have been carefully noted.

The species under observation was Amoeba proteus. It may be kept in various ways , but apart from attempts to make the conditions of its life as natural as possible by supplying

⁺The paranucleus of course , in each case , itself divides so as to function as centrosome.

fresh food material from the locality from which the specimens were originally obtained, I have found that minute fragments of albumen, preferably boiled, when added to the water serve as well as the more complicated "nährboden" that are possibly better known. The forms were originally kept under observation for the purpose of seeing if there was any process comparable to conjugation in their life-history. Of this no trace was seen, but it was remarked that in the course of 24 hours all the forms had suddenly encysted. For this I am unable to suggest any cause unless it be that possibly the Amoebae were overfed. The form of the cysts was approximately circular; the diameter as measured from the drawings was 120μ on an average. The wall of the cyst was moderately transparent and one could see especially round the circumference the nature of the process that had taken place within. Towards the centre the contents - and by this term may be understood the individual units into which the cytoplasm had divided - were not so discernible as distinct units. The colour of the cysts was whitish-yellow. Unfortunately their number was not great - some four or five - but they were equivalent to all the forms that had been isolated at that particular time for observation. The process took place in a summer month - June; Scheel's cysts were formed during the winter. He speaks of them as "unstalked, and without any organs of prehension, whilst to their under-surface adhered stony granules,

plant rootlets , and various detritus." The first two of these characters hold good for those cysts that I had under observation , but the conditions under which they were kept did not offer any demonstration of the gluey nature of the cyst wall. +

No free space was apparent between the cyst contents and wall: the one lay close upon the other. In regard to the actual formation of the cyst in the case of the individual Amoebae , one noticed the gradual assumption of a circular form , the retraction of pseudopodia , the slow diminishing of internal activity (as evidenced especially in the slackening plasma-streamings) , and the disappearance of the fatty circular inclusions. Eventually all movement ceased , and with this seemed to vanish the differentiation into hyaline ectoplasm and darker entoplasm: no change was remarked in the nucleus. The formation of what is the basis of the cyst-wall seemed to be associated with the slackening movements - not merely those that were internal but those of the creature as a whole (commonly a slow rotation about its middle point.)

+ A ~~strong~~^{very} note , discovered since the above was written , distinctly states that one of the cysts had adhering to it in some quantity ejecta which had evidently been excreted before encystment and had remained in the vicinity.

Scheel's encystment was epidemic , and numerous instances gave him the opportunity of subjecting several of the cysts to the ordinary methods of preservation , by which he was able to make out the intimate structure of the cyst-wall. This so far as one could observe was double , and evidently a secreted product of the enclosed creature; as such it would probably be formed in concentric lamellae as is commonly the case in these circumstances. Scheel indeed made out three distinct zones in the cyst-wall; of these the two inner ones were perfectly homogeneous and structureless , differing only in their thickness and in the degree to which they took up stain. In no one of the zones was he able to demonstrate the existence of pores , although they must necessarily be permeable to a certain degree in some such way as e.g. a Pellicula. (It must be confessed however , that , on examination , Scheel's figures suggest that there are but two zones. If figure 8 be compared with figure 5 , it would indeed seem as if a in figure 5 , the innermost zone , represented the contour of the protoplasm and could hardly be regarded as a distinct lamella). The outermost zone of the cyst-wall was , according to Scheel , neither regular nor homogeneous. He speaks of it as clear , and of considerably greater breadth than either of the inner zones. In places it is actually shriven , contains various organic inclusions , and shews other characteristics of cuticular secretions of some standing. In the case of the living

cysts that I had under observation none of these peculiarities were obvious; on the contrary the external appearance of the cyst-wall was fairly regular but certainly transparent.

As already stated , scarcity of material prevented me from making examination of stained and mounted specimens: observation was alone made on the living form. Accordingly many of the following data regarding the cytoplasm and its contents are transcribed from Scheel's completer examination. In the case of young cysts of five days' standing the cytoplasm consisted of a hyaline matrix in which lay numbers of small sharply-outlined granulations: the latter did not stain in the preserved state. In fact the appearance was much like that of a free amoeba except for the absence of differentiation between ectoplasm and endoplasm and the lack of the fatty spheres. In older cysts there were apparent in addition larger strongly refractive bodies. They possessed an opaque appearance and recalled the fatty spheres of the free amoeba. Their shape was variable - oval to circular. Scheel found that they resisted the action of dilute acid , were not blackened by osmium , and did not dissolve in a mixture of absolute alcohol and ether: accordingly he declares against their fatty nature and offers the suggestion that they may be regarded as by-products of assimilation stored up as reserve material. He is also uncertain about their origin , noticing them first in stages characterised by a multiplicity of nuclei.

With regard to the nucleus , Scheel noticed in one or two cases of early stages that it was rudely horse-shoe-shaped , and from the fact that in no single latter stage did he find a hint of mitotic division , he concluded that the nuclear division was direct. He notes also a form of increase through budding; certain nuclei are described as having lateral growths , which give them a starred appearance. This observation of the budding nucleus is of special interest in relation to what will be said later about the fragmentation of amoeba. (He also finds additional proof of the budding in the varying sizes of the nuclei; the products of ordinary division are usually of approximately equal size). All the nuclei however do not thus divide , but maintain a temporary inactivity.

The nuclei stain with great ease , but on the other hand they contain a variable number of clear circular bodies which look like vacuoles , but are proved not to be so by sections. They resist all nucleolar stains and seem to be generally achromatic. Their origin and function are unknown. Scheel found cysts with numbers of nuclei varying from 1 to 600. In the cysts with 20-30 , the nuclei already begin to assume a peripheral position. A ripe cyst will contain 500-600 nuclei.

When some 200-300 nuclei have been formed , it will be remarked that at the circumference the cytoplasm is becoming

separated off into sharply defined regions each of which contains a single nucleus. This movement of separation is centripetal and indicates the last stage in the development of the cyst. Scheel's stained preparations shew no nuclei in the centre of the cyst. In appearance this region is quite unlike the peripheral plasma and shews a highly vacuolar structure enclosing numerous very fine granulations with sundry delicate fibrillar structures. Towards the end of their existence the cysts under observation gradually lost their fairly circular form, mainly because they lost their firmness owing to the weakening and thinning of the walls. One day on the application of a drop of water to make up for what had been lost by evaporation, the walls gave way and the contents of the cysts were set free. It is satisfactory to note however that Scheel was not able to make out any regular exit in his more numerous empty cysts.

As the result of Haeckel's study of the life-history of *Protomyxa aurentiaca*, as also of Schaudinn's observations on *Paramoeba Eilh.*, one was prepared to expect a flagellate stage in the development of the young freed Amoebae. Of this however I found no trace. Scheel's experience was also identical - "ich habe mich aber trotz vielfacher Beobachtungen nie überzeugen können, dass eine solche flagellaten Generation hier auftritt."

The young amoebae are difficult to observe owing to their

great transparency. They contain very few granules in the first instance and are excessively slow in their protoplasmic movements. Scheel describes and figures those he had under observation as possessing numerous pointed pseudopodia.

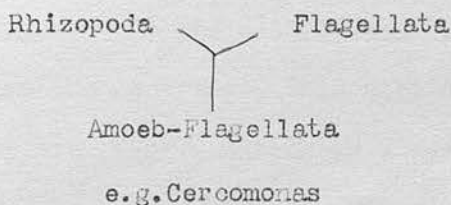
But this character of marked pointedness was not manifest in the youngest forms that I examined, nor was it present in the still smaller Amoebae produced by the process of fragmentation.⁺ When measured in an approximately spherical condition, they shewed a diameter of from 9 to 13 μ . The nucleus was very distinct. They developed into the adult form in about three weeks.

Accordingly we reach the conclusions to which later will be added others, that *Amoeba proteus* has at least one other method of reproduction than that of ordinary fission, - that is to say, it forms reproductive cysts. This brings its life-history further into line with that of the Ciliata, many of which also form cysts that have usually been described as of two kinds - reproductive and protective, although I believe that this distinction is not so absolute as is commonly supposed. It has been long known that *Amoeba* formed protective cysts, which I unfortunately have had no opportunity of comparing with these newer reproductive cysts. Why exactly or

⁺ Later. This is not strictly the case, as in the fragmentation of *Amoeba princeps* I have found very occasionally the young amoebae with such pointed pseudopodia.

under what particular conditions the latter are formed we do not really know, but the shell is a secretion of the animal and may apparently be formed at any season of the year.

Scheel has further demonstrated that within this protective covering the nucleus divides up directly into a great number of daughter-nuclei which may reach a maximum of 500-600. At a certain stage commences a corresponding dividing-up of the cytoplasm into daughter fragments, each of which is supplied with one of the daughter-nuclei. On the bursting of the cyst-wall these become free and develop into adult Amoebae without the interpolation of a flagellate phase. This last point is of especial importance in view of the fact that the Rhizopoda and Flagellata are commonly supposed to be very closely related. It certainly is the case that several Flagellata (e.g. *Cercomonas*) have an amoeboid phase in their life-history, and we have already referred to other forms that seem to combine both phases likewise in their life-history. But it would also appear that if certain lower forms such as those mentioned do combine both phases, the extreme forms which may be supposed to have evolved from them are specialised and constant in their structure and life-history alike.



PART III

REPRODUCTION OF CILIATA: (a) Binary Fission.

The simplest and most common form of reproduction amongst the Ciliata is by binary fission. In this ordinary, possibly vegetative, method of reproduction the plane of division is generally perpendicular to the long axis of the creature. To this generalisation the Vorticellidae form an apparent exception, but on the view that their evident long axis really corresponds to the dorso-ventral axis of other Ciliates, their case falls into line with that of the rest of the sub-class. Formerly many instances of fission in the direction of the long axis were described; they may safely be considered to have been mere instances of conjugation.

Binary fission most commonly takes place while the creature moves about; i.e. it is an activity, in most cases at least, temporarily added to all the other activities of Ciliate existence. As such it may be considered to be the original method of reproduction. Under other conditions fission may take place when the creature is at rest, or, in other words, in certain cases binary fission is not associated with free movement: on the contrary, this stationary fission is usually associated with the formation of a cyst. Under these circumstances the operation may take place more than once

in succession. Such stationary fission together with budding - which is simply a form of fission where the products are so unlike in size as to be distinguishable as parent and offspring - are best considered as modifications of ordinary binary fission.

By ordinary binary fission, then, we understand the division of a ciliate during its active free existence into two daughters by a constriction more or less transverse to its long axis. It is confessedly difficult to arrive at a rationale of binary fission. One might suppose that it was associated with a certain limit of size so that, as is more evident in the case of globular Rhizopoda, while the bulk increases as the cube of the diameter, the surface increases only as the square, ~~and accordingly~~ relief is obtained by the process. But it has been shown more than once - indeed is matter of common observation - that binary fission takes place at all stages in the development of certain Infusoria and is not merely postponed till they reach a definite size. That is to say, binary fission is not necessarily connected with growth beyond the specific mass of the species.

For example, in encysted forms, there is no possibility of growth previous to division, and in other cases, as the result of continuous division, there may be an actual decrease in size. To put the matter briefly actual increase in size is neither a constant precursor or result of binary fission per se

in the case of the Infusoria. All that can be said is that while in certain cases e.g. *Stylonichia*, *Euplotes*, a distinct lengthening is noticeable at the commencement of the process, in others, e.g. *Stentor*, *Spirostomum*, no such phenomenon is observable.

Another question has interest in this connection - where are the first signs of the process noticeable, in the nucleus or in the cytoplasm? In view of the fact that both answers have been given by first-class workers, Bütschli contents himself with stating for the majority that there are undoubtedly many instances where there are hints of new formations in the plasma, e.g. the "Anlage" of the new ciliary apparatus, mouth, or contractile vacuoles, before any change in either macronucleus or micronucleus is observable.

Simple binary fission, apart from nuclear considerations, is not a very complicated process. As already stated, the plane of division lies more or less at right angles to the long axis of the body, and usually approximately near the middle. It is necessary to make the qualification "more or less" because in the case of *Spirostomum teres* at any rate, the plane of division is somewhat oblique, as Stein observed so long ago as 1867. (*Die Organismus der Infusionsthierchen*: Bd. II). In the more highly organised Ciliata the special organs have to be duplicated, and this is achieved either by division of the already existing organ or by fresh formation in one of the

offspring. The former method is comparatively rare , and only occurs where the organ or system in question as e.g. the canals connected with the contractile vacuoles , runs practically the entire length of the creature. When the twin organ is entirely formed anew , the position of the original one usually decides upon which of the daughters this work devolves. Be it in the anterior portion of the parent , it is generally found that the daughter that develops from the posterior half will have to form the new organ. Of Paramecium , however , it may be said that the mouth lies as a rule slightly in the posterior half of the body , and in this particular instance the new mouth is always formed behind the old one , and this involves considerable consequent displacement. One or two abnormal Paramecia in which the mouth lay unusually close to the tail gave one the opportunity of verifying the correctness of this exception even in such extreme cases. Still it is however the anterior contractile vacuole that is formed anew in either daughter.

In the case of the Hypotricha there is a renewal of the whole ventral ciliary apparatus of both daughters as a consequence of division: the same phenomenon occurs after conjugation. The process has been studied by Stein , Balbiani , Engelmann , Sterki⁺ and Prowazek , and even yet we cannot claim to know all the details. I have attempted to follow the process in the case of Stylonichia , the favourite object of

⁺ Reference given on next page.

examination , but it is a work of exceptional difficulty.

Previous to division new frontal cirri are formed in the anterior half of the dividing creature, practically under the old: they are remarkably transparent and clear. Then follow in succession , according to Prowazek , the new adperistomal cirri and finally the still insignificant anal cirri. Behind the marginal cirri the rudiments of their successors are formed at the same time; they have a very crowded appearance. " The new adoral membranellae arise close behind the old so that at a later stage the latter seems ruptured." This sentence settles the one point most in dispute among the older workers. as to whether the adoral zone was renewed or not. Personally I have no doubt of the fact. Much the same can be also observed in what will be the posterior daughter. The new cirri appear first as cilia and are very irregular and often violent in their motions , contrasting with the old ones which are generally lifeless. Their area of origin is very much less than that which they eventually occupy. The new anal cirri in the case of the posterior daughter are formed above the old. The area occupied by the latter is marked off by a sort of furrow and undergoes degeneration although there is no actual separation from the body of the creature - rather absorption.

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In binary fission the nucleus is naturally the seat of the most complicated processes. Even when changes are first noticeable in the cytoplasm, it can be sufficiently well maintained on a priori grounds that there are previous changes in the nucleus, which though invisible are yet the inciting cause of those that are visible in the plasma. Macronucleus and micronucleus alike divide, the latter usually in advance of the former. It is not yet possible to state authoritatively whether in the case of two micronuclei, the two halves of the same micronucleus go to one daughter, or whether it is a half of each of the micronuclei that go to form the daughter micronucleus in anyone of the offspring.

With regard to the duration of the process, no definite rule can be laid down. I have noted the following periods for the forms named when under observation:

Paramecium caudatum: $1\frac{1}{2}$ -2 hours.

Stylonichia pustulata: 1-2 hours.

Lachrymaria Olor: $1-1\frac{1}{2}$ hours.

Spirostomum ambiguum: 2-3 hours.

Spirostomum teres: 2-3 hours.

As regards the rate of fission it may be noted in the first place that it is by no means constant throughout the Ciliata but varies with the species. Each species has its own normal rate of division depending upon its specific qualities. The following list of rates of division is based upon Maupas:

I have ventured to modify it slightly in accordance with my own results. The range of temperature on which I base my modifications is 16° - 22° C.

Stylonichia pustulata: every 12-16 hours.

Euplotes patella: every 24 hours.

Onychodromus grandis: every 12 hours.

Oxytricha pellionella: every 8 hours.

Spirostomum ambiguum: every second day.

Spirostomum teres: every two to three days.

Paramecium aurelia: every 24 hours.

Paramecium caudatum: every 24 hours.

Colpidium colpoda: every 8 hours.

In the second place we may note that the rate of fission depends intimately upon the food-conditions to which the creatures are subjected. In conducting these experiments I have been in the habit of employing two distinct foods - either a hay infusion of a light straw colour into which one put a piece of meat to hasten the production of bacteria, or else the forms produced by making a very dilute paste with ordinary flour and water, ^{of letting it stand.} *Paramecium* and *Stylonichia* take kindly to either of these media of which a drop was added daily to the slide on which they were isolated. The conditions were kept as constant as possible by the withdrawal of a couple of drops of the medium (some ^{five} ~~four~~ or ^{six} ~~five~~ drops in all) in which they had passed the night, which were replaced by one of food and

another of distilled water. Pond water was also sometimes employed but greater constancy was secured by the other method. I could not find that either of these two food media made any appreciable difference on the rate of division. But after a certain amount of manipulation one learned that there was a minimum of food that kept e.g. *Paramecia* as they were, that there was also a definite amount, usually one drop, which caused one division in 24 hours, and that there was also a maximum which seemed to have an inhibitory effect upon the forms in question. In this case the body of e.g. *Stylonichia* became positively black with unassimilated food matter, a condition of affairs which is reproduced in the photograph on Plate X. ^{Fig. 2}. Change to a less rich medium soon resulted in a return to the normal state of affairs.

We may note in the third place that the rate of division bears a direct relation to temperature. To Maupas belongs the credit of having established this fact upon a comparatively sound basis. As far back as 1776 Spallanzani had observed that the multiplication of ciliata was accelerated by increased temperature. But it is Maupas' chief count against the defective work of his predecessors that they had not properly attended - in some cases not at all - to the temperature and food conditions. The following rates of division under different temperatures are taken direct from Maupas:

Species	5°-10° C.	10°-15° C.	15°-20° C.	20°-25° C.
<i>Stylonichia Myt.</i>	48 hrs.	24 h.	12 h.	8 h.
<i>Euplotes pat.</i>			24 h.	12 h.
<i>Onychodromus gr.</i>	48 h.	24 h.	12 h.	6 h.
<i>Oxytricha fallax</i>		212 h.	8 h.	
<i>Colpidium colpoda</i>		12 h.	8 h.	
<i>Glaucoma scintillans</i>	8 h.	6 h.	5 h.	

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 Balbiani has also suggested that the volume of water in which the Infusoria are kept has a direct influence upon their power of increase. Thus he says that *Paramecium aurelia* requires to be kept in a cubic centimeter of water in order to realise its full power of multiplication. In view of the interesting results that E. Warren has obtained with different bulks of water in the case of *Daphnia* (Q.J.M.S.1900) it would seem as if similar treatment of the infusoria offered a field in which good results could be obtained.

It has long been known that the comparative regularity with which binary fission was carried out in favourable circumstances decreased as the period lengthened since the last conjugation. I have observed this phenomenon several times in the case of *Paramecium caudatum* and *Stylonichia pust.* , but have been unable to express the gradually decreasing energy in the terms of any formula. On the other hand it has been maintained that immediately after coming out of conjugation

+ Reference on next page.

these two forms shew a marked increase in the rate of fission - the expression of a surplusage of energy; of this phenomenon, however, I have never seen any trace. So far as I am aware, conjugation results in no difference in the after rate of ordinary multiplication, and this also would appear to be true of light and darkness.

Before proceeding to examine Maupas' theory of binary fission and the recent attack upon it, I should like to refer to his specification of two distinct species of *Paramecium* - *aurelia* and *caudatum*.⁺⁺ He gives a definite account of these two forms in which the latter is described as possessing an elongated body, as being fusiform, obtuse in front and thinner behind. This species is also credited with one micronucleus and the zygote nucleus was said to give rise to eight corpuscles. The other species had a broader body, was almost oval, and obtuse at both extremities. It further possessed two micronuclei, and the zygote nucleus gave rise to only four corpuscles. Since this account no special notice

⁺ Observ. et expér. s. les phénom. de la reprod. fissipare chez les infus. ciliées: Compt. rend. Ac. Sc. Paris: T. 50: P. 1191.

⁺⁺ No reference is made for the present to other species, e.g. *putrinum*, *bursaria*.

seems to have been taken of the two species except to cast doubt upon their existence. Thus in the Zoologie Descriptive Fabre-Domergue states that neither he nor Balbiani have ever come across this Paramecium with the double micronucleus, and he makes the remark in such a way as to suggest that Maupas was drawing on his imagination in his description of it.

Accordingly we find but one species - caudatum - with the single micronucleus - recognised generally in the text-books and other literature. There is no doubt however that these two distinct species do exist. On Plate X will be found photographs of the two species which give a very good idea of their relative sizes, while on Plate IV, fig.1 are given drawings of stained specimens which bring out the nuclear characteristics. The latter figures give *P. aurelia* a length of 80μ and a breadth of 40μ , while on the same scale *P. caudatum* has a length of 130μ and a breadth of 50μ .

These figures though hardly exact for the living form, bring out the peculiar feature of *aurelia* as compared with *caudatum*, viz. the high proportion that its breadth bears to its length.

The magnification of the photographs is about 80. I may also mention here that I made frequent endeavours, through isolation of pairs, to get the two species to conjugate.

The disproportion in size offered no a priori objection, as one often sees equal disproportion in the case of conjugating *Stylonichia*, and even in the case of *P. caudatum* the inequality

is often marked. The representatives of *P. caudatum* were selected from a culture in which an epidemic of conjugation had set in, while the aurelia were taken from another culture which was far advanced in the number of its divisions. I never had the chance of contemporaneous epidemics amongst the two species, and accordingly always selected *P. caudatum* as the form that one certainly knew was ready for conjugation, inasmuch as it is the larger and probably more forceful species. Out of 21 attempts I ~~made~~^{had} but two partial successes. Conjugation took place on two slides: the period was normal. After separation each of the ex-conjugates divided once: on the third day they died off. In anticipation of something of this sort from analogy in higher forms, I intended to let the two pairs run their natural course, foregoing the desire to examine their nuclear condition. In view, therefore, of the incompleteness of the experiment it is perhaps unwarrantable to draw any results regarding hybridisation and the "fixity" of species so far down in the animal scale.

As has been previously mentioned, numbers of observers have remarked that the comparative regularity with which binary fission proceeds under favourable circumstances decreases as the time increases since the last conjugation, and one has often wondered if it would not be possible to express this decrease by means of a mathematical curve or formula. In this connection it is Maupas' chief distinction to have established

that in the case of each species this power of binary fission comes to an end after a definite number of divisions , and that were there no other method of restoring this potentiality to the individual the species would come to an end. With the latter stages of this gradual loss of fission-energy he found distinct degeneration of the creature associated. In this degeneration he found two well-marked stages. The first stage is not accentuated by any particular external change in the infusorian , unless possibly a slight reduction in size.⁺ It continues to feed and to multiply in the normal manner but all the while it is giving rise to successors that are entering the second stage of degeneration. Moreover when preserved in the ordinary method it is found to have undergone a certain atrophy of its nuclear apparatus. The macronucleus fragments (Styl. pust.) or may disappear altogether (Styl. Myt.). The micronuclei are reduced to one , or even none (Styl.pust. and Oxytricha sp.) : on the other hand after such reduction they may later increase to numbers in excess of the norm. (Styl. myt and Onychodromus). In the second stage of this senile degeneration the Infusorian loses its power of multiplication. It no longer takes in food , and its body in consequence becomes quite clear. There is now a marked decrease

⁺ In the case of *Stylonichia pust.* this reduction varied from 25 to 50 μ .

in size⁺, and atrophy of external organs and appendages sets in. Finally this degeneration further expresses itself in a sort of sexual hyperaesthesia, causing sterile conjugations that inevitably end in the death of the partners. Now Maupas determined that senile degeneration began in the case of *Styl. pust.* about the 170th generation or division, and that death ensued at the 316th. Similarly cultures of *Onychodromus* became extinct after 330 generations, and so on. It is also an integral part of his theory that it is impossible to induce conjugation during the earlier bipartitions which cover a definite period of immaturity: in the case of *Styl. pust.* this extended to the 130th division. At the end of these earlier divisions - at the 31st in the case of *Stylonichia* - puberty is attained, and conjugation can be induced. This period of eugamy lasts over a definite number of divisions - until the 170th, as we know in the case of *Stylonichia* - when senile degeneration sets in, ending in death.

The first thing that strikes one when examining Maupas' tables of binary fission is their mechanical regularity. The following represents the first fortnight of the well-known *Stylonichia pustulata* table:

⁺ *Stylonichia pustulata* had measured 70-90 μ in place of the normal 160 μ .

Date	Temperature	Individuals	Number of Bipartitions in 24 hours	Total
February 27	16	1		
28	16	2	1	1
March 1	16	4	1	2
2	16	32	3	5
3	17	147	2	7
4	18	483	2	9
5	118	935	1	10
He isolated one of the 935				
6	19	2	1	11
7	19	8	2	13
8	18	64	3	16
9	17	230	2	18
He isolated one of the 230				
10	17	4	2	20
11	17	16	2	22
12	16	126	3	25

I have taken the figures for the first fortnight, but greater regularity could have been shewn if one had taken a fortnight at a later date. Fabre-Domergue confesses that he never succeeded in obtaining such regularity in any cultures that he undertook, and it seems to me ^{that} in so saying he intim-

ates that he obtained a series of divisions that is much more natural than anything represented in Maupas' mathematical tables. When cultures of *Stylonichia* or *Paramecium* are kept in glass vessels where they may have some small bulk of water in which to live they do not multiply at this rate, or with such regularity. It is not my intention however, to impeach Maupas' tables for with his results I find myself largely in agreement as against his latest adversary Joukowsky. Nevertheless, apart altogether from venturing to enquire how such exactness was acquired as is expressed in 935 *Stylonichia*, I would maintain that the ends and results which Maupas has taught us are reached by a process of division that is far from regular and depends to a great extent upon the individuality of the infusorians. Even under the happiest possible conditions (so far as one can judge), artificial or natural, binary fission does not proceed with that constant regularity that the French savant would ascribe to it. The following table representing a few weeks of a short series expresses, I believe, a more natural rate of progress than one would gather to be the case from Maupas' table. The form experimented with was *Paramecium caudatum*, and in every case the series was commenced with two exconjugates. I have reckoned that case as 1 bipartition in 24 hours where half or more of the creatures on the slide divided.



Date	Temperature	Slide	Number of		Total
			Individuals	Bipartitions in 24 hours	
June 13	16	a	2	0	0
		c	2	0	0
		f	2	0	0
		g	pair still in conjugation		
		h	pair still in conjugation		
		q			
June 14	16	a	4	1	1
		c	3	1	1
		f	4	1	1
		g	2	0	0
		h	2	0	0
		q	pair in conjugation		
June 15	17	a	4	0	1
		c	4	0	1
		f	4	0	1
		g	2	0	0
		h	4	1	1
		q	2	0	0
An increase in size was apparent in the case of a and g					
June 16	20	a	5	0	1
		c	4	0	1

Date	Temperature	Slide	Individuals	Number of	
				Bipartitions	Total
		f	7	1	2
		g	2	0	0
		h	4	0	1
		q	2	0	0
June 17	21	a	8	1	2
		c	6	1	2
		f	11	1	3
		g	2	0	0
		h	8	1	2
		q	3	1	1
18	23	a	10	0	2
		c	8	0	2
		f	22	1	4
		g	3	1	1
		h	11	0	2
		q	3	0	1
19	21	a	13	0	2
		c	10	0	2
19	21	f	31	0	4
		g	5	1	2
		h	21	1	3
		q	5	1	2
20	20	a	16	0	2

Date	Temperature	Slide	Number of Bipartitions		
			Individuals	in 24 hours	Total
		c	15	1	3
		f	32	0	4
		g	8	1	3
		h	27	0	3
		q	7	0	2
June 21	23	a	20	0	2
		c	27	1	4
		f	52	1	5
		g	8	0	3
		h	42	1	4
		q	7	0	2

With the exception of g and q 5 Paramecia were removed from each slide

22	20	a	30	1	33
		c	36	1	5
		f	70	1	6
		g	9	0	3
		h	51	0	4
		q	14	1	3
23	19	a	45	1	4
		c	36	0	5
		f	72	0	6
		g	18	1	4

Number of
Bipartitions

Date	Temperature	Slide	Individuals	in 24 hours	Total
		h	70	0	4
		q	14	0	3
June 24	20	a	45	0	4
		c	45	0	5
		f	73	0	6
		g	18	0	4
		h	70	0	4
		q	18	0	3
25	23	a	63	0	4
		c	51	0	5
		f	76	0	6
		g	36	1	5
		h	70	0	4
		q	20	0	3
26	24	a	81	0	4
		c	55	0	5
		f	77	0	6
		g	44	0	5
		h	86	0	4
		q	25	0	3
26	24	a	81	0	4
		c	56	0	5
		f	88	0	6

date	Temperature	Slide	Individuals	Number of	
				Bipartitions	Total

		g	70	1	6
		h	104	0	4
		q	37	1	4

The numbers on these slides were reduced to 13 , 7 , 14 , 14 , 10 and 7 respectively.

June	28	23	a	26	1	5
			c	7	0	5
			f	20	0	6
			g	27	1	7
			h	18	1	5
			q	11	1	5
29	23	a	45	1	6	
		c	9	0	5	
		f	27	0	6	
		g	42	1	8	
		h	25	0	5	
		q	16	1	6	
30	23	a	53	0	6	
		c	14	1	6	
		f	34	0	6	
		g	46	0	8	
		h	38	1	6	
		q	23	0	5	

Number of
Bipartitions

Date	Temperature	Slide	Individuals	in 24 hours	Total
July 1	22	a	90	1	7
		c	28	1	7
		f	60	1	7
		g	77	1	9
		h	54	1	7
		q	23	0	5

The numbers on the first five slides were reduced to 43 , 9 , 13 , 14 , and 17 respectively.

2	20	a	64	1	8
		c	18	1	8
		f	16	0	7
		g	28	1	11
		h	12	1	8
		q	27	0	5
3	18	a	99	1	9
		c	23	0	8
		f	20	0	7
		g	51	1	12
		h	15	0	8
		q	34	0	5
4	18	a	99	0	9
		c	25	0	8
		f	30	1	8

Date	Temperature	Slide	Number of Bipartitions		
			Individuals	in 24 hours	Total
4	18	g	51	0	12
		h	15	0	8
		q	34	0	5

and so on.

Here then in a period of three weeks with a temperature ranging from 16° to 24° C. there is, over six slides, an average of eight divisions. This series was by no means the first that I inaugurated, and the slides were numbered from a to r. The majority were treated daily in a constant manner - given the same definite amount of food (calculated from previous experiments) and provided with a certain amount of fresh water at definite intervals. With the others I experimented in the amount of food given, in the period of time that they were left without any change of water, in the amount of water on the slide, and so on. In no single instance did I obtain such clock-work regularity as Maupas' tables shew. The slides whose history is given were amongst these that were treated with regularity so far as I was able, and consequently they were all treated alike. Hence a day like June 25 when but one of the slides shews a complete Maupasian division, appears to me to represent the more natural state of affairs, and for no reason more than this. Maupas' table of

Stylonichia pustulata admittedly deals with a form that multiplies more quickly than *Paramecium caudatum* - possibly about twice as an average over all temperatures. But at the close of the first three weeks his *Stylonichia* has divided no fewer than 39 times with a temperature that ranged from 15° to 19°. Now no one has laid more stress upon the influence of temperature in raising the rate of division than Maupas, and yet I do not find from the table that his high rates of division bear any relation to the temperature. Quite the contrary is the case, for out of the four occasions on which the *Stylonichia* divided three times in 24 hours within the first three weeks, on two of them the temperature was actually a degree lower than the previous day when it divided a less number of times. Accordingly, though I believe that ultimately, continued binary fission involves a certain degeneration, and that Maupas' theory of the matter is largely correct, still it is altogether false to imagine that under natural conditions a *Stylonichia* will rush through 316 divisions in $4\frac{1}{2}$ months: that is to say, the validity of Maupas' method is open to question, and where this is so the results are always in jeopardy, more or less.

I may refer here to two peculiar cases of division that came under my notice. On May 13th, 1900, two *Paramecian* exconjugates were isolated on a slide and subjected to ordinary culture treatment. On the 14th they were as before; on the 15th there were three; they remained at this number on

the 16th , and presented no abnormality.⁺ On the 17th there were four Paramecia on the slide , but one of them had developed a cleft tail. The cleft was in a plane perpendicular to the dorso-ventral axis. From the first it extended to a depth of about 25μ and did not grow deeper. Otherwise the creature appeared to be perfectly normal: the two contractile vacuoles functioned and the internal circulation swept round , clear of the divided tail. On the 18th it remained as it was. On the 19th it had divided , and the anterior half , though distinctly undersized , and resembling rather the species aurelia in configuration , was yet normal in every other respect and continued afterwards to divide by itself. On the 20th the original form again divided , but not on the 21st. However on the 22nd it resumed operations , and while the anterior one still retained its aurelian characters , I noticed that the internal circulation of the posterior half no longer swept clear of the tail but had partially entered into one (the dorsal) lobe , which now contained excretory granules and one or two small food vacuoles. At the same time this lobe had slightly increased in size , while the other had correspondingly decreased. On the 23rd it divided again , but not on the 24th. The anterior parts still retained the same characteristics as formerly and gave rise themselves to

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No temperatures are given as in this case they probably had no influence on the sequel.

otherwise normal Paramecia. In the posterior cleft-tail Paramecium the dorsal lobe continued to grow, while the other was more and more absorbed. The mouth also was driven unusually far back. On the 25th and 26th it again divided, but not on the 27th. The ventral lobe had now been completely absorbed while the other had increased in size till it now measured some 60μ : there was, however, no disproportionate increase in breadth, and there seemed to be a tendency for it to get blocked. At any rate the number of excretory granules increased, and the circulation slowed down. On the 28th and 29th it again divided, but the dorsal tail seemed thoroughly congested and by the 30th it was dead. None of the daughters reproduced the peculiarity in themselves or their descendants. Illustrations are given on Plate IV.

The other case is still more peculiar. About the same time as the preceding exconjugates were isolated - one of several series - on another slide two had been set aside which on the following day were found to be still in a sense the same number. Yet evidently one of the creatures had begun to divide but stopped in the process, so that while on the slide there was one normal form, the other was a monster, composed of two full-grown Paramecia with organic union between the anterior part of what should have been the posterior daughter, and the posterior part of the anterior one. There was no constriction between the two or other hint of their origin.

The two bodies formed one continuous whole with one circulation and was so flexible throughout that the two extremities could touch. On the 17th May it appeared , and on the 18th was in no way changed. On the 19th the slide on which it was isolated contained three forms: the monster had given off a daughter from either end. These daughters were ordinary *P. caudatum* of good average size; they continued to divide by themselves and in every way appeared to be normal. On the 20th the monster remained as it was , but again on the 21st it repeated the operation of giving off a daughter from either end. On the 22nd it had not multiplied , but on the 23rd for the third and last time it had given off a daughter from either end. These last , however , were markedly smaller in size , and otherwise , like the " aurelian " daughters that had been given off by the cleft-tail individual. Previous to this the posterior creature had gradually been becoming inclined at an angle to the anterior one. Up to this point the combined activity of the monster had been as great as that of any normal *Paramecium*. The anterior half , perhaps naturally , was the more active , and in a sense the guiding part. Its cilia were feverishly active: they were also longer and better developed especially in the anterior regions , than those of the posterior creature. This greater anterior activity may also have found expression in a process that began to come off from it a little above the angle made with the pos-

terior form. Further, the two contractile vacuoles of the anterior creature were close together and contracted simultaneously. From the 23rd, i.e. about a week after its appearance, growth ceased to show itself, as we have seen, in the regular separation from either end of two daughters on every second day, and began rather to express itself in the growth of the aforesaid process and in remarkable lateral expansion of the anterior half. This and an earlier stage are figured on Plate IV. In this peculiar condition it remained with slight modifications about another week, but was dead by the 28th.

Up till quite recently Maupas' classical work has been permitted to go comparatively unchallenged. In the *Verhandlungen des Natur-historisch-Medizinischer Vereins zu Heidelberg*, however, D. Joukowsky publishes certain Beiträge zur Frage nach den Bedingungen der Vermehrung and des Eintritts der Konjugation bei den Ciliaten which go contrary somewhat to the received views. Although part of what follows would come more naturally under the latter half of this section, yet the subject matter of the greater portion of it justifies its consideration here.

Joukowsky's observations were made upon *Pleurotricha lanceolata* - a form allied to *Stylonichia* - *Paramecium caudatum* and *Paramecium putrinum*. He says that he got irregular divisions at first: only after a month did the forms divide regularly.

After the numbers on a slide had reached one hundred , division was slower. I have already referred to this question of regular and irregular division. My own experiments were more than once carried on considerably more than a month , and I did not find any greater regularity after the first four weeks than I did during that time. Nor is it easy to see why this should be so. To imagine that these infusorians will " settle down " after a month into regular methods of division simply means failure to appreciate the conditions of the experiment. Joulowsky indeed says that the abnormalities in the division rate were due to the abnormal relations in which the creatures live. Bacteria generate and hinder ordinary division , and one may well suppose that the secretions and excretions of the creatures themselves may be ultimately dangerous in such a circumscribed area. But then this investigator deliberately states that after a month the divisions became regular , and yet we are not led to believe that he had found any means of overcoming the difficulties in which he sees the cause of the earlier irregular divisions. Obviously therefore , they cannot have played the part that he imagines. I may also mention here that Maupas , while making these largely statistical experiments in binary fission , employed cover-glasses on his slides in the damp chamber. This appears to me to have been the introduction of an altogether unnecessary artificial condition: so far as regards the obser-

vation of the mere rate and other simple aspects of binary fission I never employed cover-glasses: any infusorian requiring high power examination was easily isolated. Maupas also states that in his damp chamber there was very slight evaporation, and that "when it was necessary" he made compensation for the loss with rain water. If he added food daily it is difficult to see how it was ever necessary in addition to make up for evaporation. If the latter had to be done at all, it were surely better to change the water in greater or in less quantity with regularity and so give less occasion to bacteria to generate. I cannot say that I found the latter method unsuccessful when I tried it.

Joukowsky kept *Pleurotricha lanceolata* for a period of eight months, in the course of which 458 divisions occurred, and during that time he got neither conjugation - not even when he starved the creatures and set them in pure water - nor evidence of degeneration. In a certain degree there is correspondence here with Maupas' experiments on *Stylonichia mytilus* where senile degeneration (which however Joukowsky did not find) did not seem to stimulate this species to conjugation as it did in the case of *Stylonichia pustulata*. Joukowsky nevertheless observed a certain shrinkage in size which he found depended on the quantity and quality of food. The following is his temperature table:

	30° C.	23° C.	15° C.
13 XII 1894:6 p.m.	1 individual	1 individual	1 individual
14 XII 1894:6 p.m.	18 individuals	8 individuals	2 individuals

The question of degeneration is probably the most important that he raises. As we have already seen, Maupas distinctly states that at the end of the period of eugamy which covers a definite number of divisions of the creature, senile degeneration sets in which ends in death if conjugation does not intervene: we have also seen the method in which this degeneration expresses itself. On this subject he had already been challenged by Bütschli, who maintained that the fission-capacity of the Ciliata was specially great and much in evidence after conjugation, but that thereafter it gradually ebbed away. If by this Bütschli meant that immediately after conjugation the rate of fission is above the normal, I can only say that I have never observed anything of this nature in the several forms that have come under my observation. But if, as seems more evident (Protozoa. Bd.1:Heft III:p.1592:) he is simply entering a protest against Maupas' action in limiting the process of degeneration to one special late period in the infusorian's life - thus in the case of *Stylonichia pustulata* it is not reached until from the 170th to the 200th generation - he is surely to be commended. If there is degeneration at all, it is most improbable on all other analogy that it should set in at a certain fairly definite point - so late

as the last third of the creature's life. If there is degeneration, it has commenced invisibly long before these outward manifestations in the loss of frontal cirri and other appendages: it is ever so with decay. And in referring to Maupas' *Stylonichia* series with its increasing temperature from the middle of the period onwards, and bearing in mind the effect that temperature has on the rate of fission, Bütschli is only asking a common-sense question when he demands how, under these conditions it could have been possible to recognise a gradual ebb in the fission-energy such as we may suppose to constitute the initial stages of degeneration. Joukowsky then, found no degeneration in his eight months cultured *Pleurotricha*. He never saw the disappearance of the frontal membranellae: he found no abnormal relations in the condition of the nuclei, unless in two cases when a certain change in the relative positions of the two parts of the macronucleus was noted. He examines Maupas' *Stylonichia* table, and finds that the creature multiplied much more quickly in the later weeks⁺, and to this he in large part attributes the degeneration. "It is very possible that the cause of the degeneration which Maupas observed is not the mere number of generations

⁺ Some four or five times every 24 hours in place of the normal twice or thrice.

alone but the number of generations in association with the rapidity of multiplication." For my own part I have looked for evidence of degeneration throughout 3-4 month slide cultures⁺ of both the *Paramecia* and *Stylonichia pustulata*, as also in the case of other odd forms that I happened to find in quantity previous to an epidemic of conjugation, but have not recognised it in such specific manner as nuclear degeneration or loss of external appendages. Still, none the less am I convinced of a gradual ebbing of vital energy as the series proceeds, which expresses itself in slowed motion, in a tendency to inactivity and general listlessness (if the word be admissible in this connection) as also in a certain diminution in size that was not remedied by any amount of food.

On the other hand I have examined specimens of *Paramecium* and *Stylonichia* taken out of cultures in larger vessels about which I knew nothing in respect of their age, and have found individuals much under the ordinary size shewing a peculiar vacuolation, not only of the plasma but of the macronucleus: I cannot however positively state that this is the later stage of degeneration due to continued binary fission; although it is at least probable. In this connection I may state that Prowazek (Protozoen Studien I) found a sort of senile degeneration in old cultures of *Stylonichia*. The creatures were small and compressed, the plasma was rich in granules, and often large fatty globules were present. He also found

⁺ In some cases these covered the period of eugamy as calculated by generations.

change in the structure of the macronucleus , in the contents of which were large holes and alveolae while the chromatin lay about in irregular heaps. Joukowsky also notes the presence of vacuoles in the nucleus of *Pleurotricha lanceolata* and *Paramecium caudatum* - " helle Kugeln durch die ganze gefärbte Masse des Nucleus zerstreut , und ihre flüssige Natur scheint unzweifelhaft zu sein." He believes that they are normal: may they not be early hints of the degeneration which otherwise he is in straits to find ?

Joukowsky also made observations on a culture of *Paramecium caudatum*. In a temperature of 19°-23° C. he got them to divide one or two times. By the seventh month he noticed that they divided badly. Some of the individuals seemed dead , but on examination they were found to be still alive. The cilia on the upper surface had almost completely disappeared; indeed it was only at either end and in the region round about the mouth that he found ciliation at all. He made out , however , no hint of nuclear degeneration.

Maupas laid great stress on the period of immaturity in the infusorian's life - that definite number of divisions previous to puberty that had to be gone through before it was in a fit state to conjugate. We saw , e.g. that this period was reached by *Stylonichia pustulata* at the 130th division. Joukowsky , experimenting with *Paramecium putrinum* , found that this period of puberty was attained after some 7 or 8 divisions,

that is to say , it is practically always present. In this particular species he succeeded in getting exconjugates to conjugate within that small number of divisions , and maintains in consequence that Maupas' rule does not have universal validity. Now it is well known that by means of starvation not only can Ciliata be prevented from multiplying by binary fission, but after they have reached the period of puberty they can be hurried into conjugation by a similar method. ⁺ I therefore made deliberate attempts by means of starvation and other unfavourable means in another series similar to that of which details have already been given , to induce conjugation with ⁱⁿ the period of puberty , but never succeeded. The forms experimented with were the two *Paramecia* (*aurelia* and *caudatum*) *Stylonichia pustulata* and *Oxytricha pellionella*. It is of course true that ever so many negative results do not contradict one positive result , but I must confess that I am extremely doubtful concerning this phase of Joukowsky's work , and am entirely in agreement with Maupas' view. ⁺⁺

The French biologist also maintained that conjugations

⁺ With regard to the former point we may note that those Ciliata that have been hindered in this way from reproducing themselves by binary fission require some little time to recover the power to do so when food is again supplied to them.

⁺⁺ Reference on next page.

between near relatives were sooner or later sterile. As I have already shewn, this is certainly true in the case of conjugation between members of the two species of *Paramecium*. But in the case of *Paramecium putrinum* Joukowsky observed effective conjugation between the descendants of one individual: at the same time he admits that this probably has its limits. I also am inclined to believe that this peculiar process has its limits - but in the Maupassian sense: for although I have observed conjugation in the case of *P. caudatum* between the descendants of an ex-conjugate, in the four or five instances in which I kept them they all died off within 4 to 8 divisions.

The last point to which I wish to refer in connection with binary fission is its effects upon the products of the process. It is a long-standing generalisation that binary fission is mere duplication, that the products of the process are exactly alike. This generalisation was probably in the first instance botanical: then it spread to zoology. Its use in theory is obvious. In binary fission we do not look for variation: accordingly we are left with an excellent rationale

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Joukowsky fails to observe that in *Leucophrys patula* and *Paramecium putrinum* Maupas recognised possible exceptions to his puberty theory (*Le Rajkaryog. chez les Ciliés*: p.410:) while he also admits that the period of immaturity may be greatly shortened under certain unknown conditions. Of these conditions I have been able to find out nothing.

of conjugation , and so , finally , of sexual reproduction , viz. a means to produce variation in the interests of evolution. In the presidential address to the Zoological Section of the British Association at the Dover Meeting , 1899 , Adam Sedgwick spoke as follows: " I say that the constitution of the exconjugates , as we may call them after they are separated, must be different from what it was before conjugation , but so far as I know no difference in structure corresponding with this difference in constitution has been recorded. I feel no sort of doubt , however , that structural differences , i.e. variations , will be detected when the exconjugates are closely scrutinised and compared with the animals before conjugation , and I would suggest that definite observations ^{be} ~~are~~ made with a view to testing this point." I have already stated the circumstances under which an attempt was made to carry out these observations , and how in the course of it a conclusion was reached opposed to the assumption concerning binary fission. It is then , my firm belief that binary fission is not mere duplication , and that the products of such division are more or less ^{un} alike. So far as I am aware no other similar statement has been made before except by Ernest Warren in Volume LXV of the Proceedings of the Royal Society of London , where in a paper entitled An observation on Inheritance in Parthenogenesis (p.155) he says " From twenty-three Daphnia (themselves originating from parthenogenesis) broods were produced

consisting of three to six individuals. The parents were measured, and the offspring were allowed to grow up. On measuring the offspring it was at once obvious that the children of the same brood exhibited very considerable variability."

The first objection to the belief that binary fission is duplication is of an a priori character. Zoology knows little of origins, but what is known tends to suggest that conjugation was not there from the first. It would still appear to be unknown amongst the Gymnomyxa.⁺ Of this class Sedgwick states generally: "Reproduction is commonly effected by simple division, but in some cases encystment occurs, and the protoplasm breaks up into a number of minute portions called spores." Now if, in harmony with evolutionary conceptions, conjugation was not there from the first and the earliest method of reproduction was by binary fission, it is difficult to see how there could have been any progress - how there could have been evolution - had the products of binary fission been exactly alike. Environment must have affected creatures or hypothetical alike in much the same way. If there was no variation through binary fission then there could not have been any upward advance. We have given some account of the sporulation and fragmentation of

⁺ Schaudinn however has published an account of the conjugation of *Actinophrys sol.* Sitzungsber. d. k. pr. Akad. d. Wiss. zu Berlin: 1896:V.

Amoeba. It is peculiarly difficult to reach any accuracy in measurement of these spores because of their inherent protean character. But even here within 12 hours of their production either by sporulation or fragmentation it was possible to make out diametrical variation in size of from 1 to 2 μ , and by the third day it had reached 1 to 4 μ . The measurements were made on the approximately spherical forms. Accordingly then we find variation in the youngest forms produced under these processes.

Perhaps it should be stated here that the contention is not that there is always variation in binary fission. Of this indeed I have very little doubt - but it would be exceedingly difficult, perhaps impossible, to prove: for where the variation was not quantitative it might be qualitative. Indeed one is bound to confess that there were many instances where the products of binary fission did appear to be singularly alike, nay might well have been called duplicates so far as their qualities were revealed by a magnification of 625. But these instances were by no means the majority: on a rough estimate of all the cases that I examined with any care, I should say that they were less than 50%. On the other hand there were also numerous instances where even with a magnification of 105 it was evident on careful examination that there was considerable dissimilarity between the daughter forms.

The species that I specially examined in this connection were *Paramecium caudatum* and *Stylonichia pustulata*.

The points to which I have for the present restricted examination are:

- a): the general outline.
- b): the total length.
- c): the extremest breadth.
- d): The distance between the two contractile vacuoles (Paramecium).
- e): The ~~total~~ length of the ^{middle caudal bristle} ~~total~~ (Stylonichia).

In all these five points I have found variation varying in b) from 1 to 20μ , in c) from 1 to 20μ , in d) from 1 to 20μ for Paramecium; and for Stylonichia in b) from 1 to 60μ , in c) from 1 to 20μ , and in e) from 1 to 10μ . These figures for Stylonichia are however very much above the average, being those of the second pair, a very extreme case. (see list). I did not find that a greater length in one was always associated with a less breadth - that perchance the area as so calculated was the same. The following statistics ($m\mu$) relate to ten pairs of Paramecia, some of which are figured in the book of illustrations.

	b	c	d
1st pair:	165, 150	45, 40	100, 95
2nd pair:	180, 190	60, 55	105, 100
3rd pair:	200, 190	45, 50	110, 110
4th pair:	140, 150	40, 45	80, 85

the case of every pair.

†† As we have seen, however, (Part I) change in the position of the macronucleus might be expected for other reasons.

	b	c	d
5th pair:	180, 190	50, 55	105, 110
6th pair:	230, 220	60, 50	110, 105
7th pair:	260, 240	85, 90	125, 115
8th pair:	280, 260	80, 65	145, 125
9th pair:	260, 240	80, 70	115, 105
10th pair:	250, 230	75, 95	120, 110

Of these the first pair is figured in the last illustration on Plate IV. Here however the most distinctive feature is the peculiar bend or twist in one of the daughters which was noticeable in whatever position the pair were drawn.⁺ I have also frequently noted variation in the position of the macronucleus, which could be measured as from either extremity.⁺⁺

On Plate V, fig. 3 are drawn two *Stylonichiae* that were the products of a transverse division. It is an ^{slightly} extreme case, in which the measurements for b, c, and e were: 230, 220; 90, 110; 35, 30. I subjoin here measurements of four other pairs:

	b	c	d
1st pair:	240, 230	120, 110	35, 30
2nd pair:	240, 180	110, 90	40, 30
3rd pair:	230, 240	110, 100	40, 40
4th pair:	230, 245	115, 110	35, 40

⁺ The drawings are of course attempted from the same position in the case of every pair.

⁺⁺ As we have seen, however, (Part I) change in the position of the macronucleus might be expected for other reasons.

An interesting question is raised as to at which stage these measurements should be taken so as to represent this variation in its truest and yet greatest degree. It is very natural to suppose that for some little time after division the two daughter Paramecia will be imperfectly formed, e.g. across the line of fission: we may imagine that the posterior end of the anterior daughter and the anterior end of the posterior one are not fully developed. Accordingly one should perhaps wait till the daughters are full grown: on the other hand it may then be objected that the differences that are noticeable may be due to feeding or some modification of the environment. I have the authority of a recognised gynecologist (J.W. Ballantyne) for stating that in the human subject twins are most like at the eighth month after birth: there is usually a difference e.g. in weight of as much as three pounds at birth. The analogy is, I admit, somewhat distant - to be more helpful one would require to go back to the earliest divisions of the fertilised ovum from the two halves of which on ^{one} the received theory twins are formed: but it seems to me that on the whole the fairest time at which to make the comparison is just shortly after division. I have attempted comparison at both stages. The measurements are from full-grown forms. In one set of photographs at least the period is very early. The last figure in the series of the dividing Paramecium was taken several minutes after the

preceding picture , and I do not know whether the differences between the two forms there portrayed are greater or less than those between the full-grown forms (not however of the same individuals) given on the preceding plate.

I consider that *Urostyla grandis* would be an exceedingly good subject on which to make measurements and observations in this variation. I have given a drawing shewing the marked inequality even in the later stages of actual division. These were only slightly lessened after separation and full growth. The separation seemed ultimately to be effected by the gradually increasing activity of the frontal cirri and undulating membranes of the posterior creature. During the **very last moments of the process** , however , they remained at rest. After separation the two daughters stayed beside one another for a few moments , and then swam off. The anterior daughter carried away a little piece out of the posterior one, the loss being represented by a notch in the latter. On this account alone they were recognisable as anterior and posterior daughters for fully 48 hours after separation , when they accidentally met with an untimely death.

(b) Conjugation.

We have already seen on Maupas' shewing that if after a definite number of divisions has taken place , the Ciliate in question does not enter into conjugation with another of its

kind , the gradual loss of energy implied in this continued binary fission will ultimately result in death. We may also take it as established that such conjugation where it takes place between any two descendants of a single exconjugate is sooner or later ineffective, and that a fruitful union is alone obtained when the partners are unrelated in this manner.

The subject of the conditions of conjugation is complicated. They certainly are , in the first instance and mainly , internal. After a certain number of divisions a peculiar state of fitness - a tendency towards conjugation - sets in. It is the period of eugamy. Within that period however , there is little doubt that such an external cause as starvation will help to induce conjugation:[†] it may also be hurried by other methods to which reference will be made later , provided always that the creature has attained to this state of eugamy. Meanwhile we emphasize that the immediate cause of conjugation is to be looked for in the creature itself: it is of an inward character. We may even suppose that the connection between a decrease in the food supply and conjugation that is often observable in large cultures is accidental so far as con-

[†] Maupas states specifically that it is the want of food that excites the conjugal appetite in the mature infusorian. (*Le Rajeunissement karyogamique chez les Ciliés*.pp.168-176.

jugation is concerned: it is possibly a mere coincidence. The facts that we remark are a lively series of divisions, a scarcity of food, and combined with that, conjugation. We are therefore forced to connect conjugation with the preceding series of divisions and the internal disturbances that are induced thereby. The latter are rendered innocuous by conjugation, inasmuch as the effect of that process is to provide the creatures with new nuclear apparatus. The run-down clock is wound up again: the creature responds to the command of Nature - "As you were."

I had hoped to include here some account of the conjugation of *Spirostomum* and of *Lacrymaria*: the investigations are as yet however, too incomplete. I have also worked through the conjugation of *Paramecium caudatum* and of *Stylonichia pustulata*. In the case of the former there is nothing to add to the very complete investigations of Maupas and Hertwig: in the case of the latter an exceedingly minute account of the process by Prowazek that appeared last year (*Protozoen-studien I*) can hardly be amended. The following paragraphs are therefore greatly curtailed.

To obtain conjugation with a permanent fruitful issue it is therefore advisable to mix the cultures if they are the descendants of a single exconjugate: at any time during the period of eugamy conjugation may then set in. Everts (1873) believed that he could induce it in the case of *Vorticella nebulifera* by gradual evaporation of the water. Bütschli's experi-

ments along similar lines had a negative result. Prowazek maintains that he accelerated the advent of the process by gradual evaporation off slides. I have not repeated the experiment with slides, but I have seen the process hastened in partially exposed watch-glasses to which I had also refrained from adding food: I prefer to see the ^{more immediate} primary cause however in the starvation. Conjugation can also be hastened by adding distilled water to the culture, as Prowazek notes: but this is simply a special case of the starvation principle. What is more interesting is that the process can be indefinitely averted by keeping the creatures well supplied with food: in this case they simply continue to multiply by ordinary binary fission. Prowazek likewise states that he induced conjugation by dividing up the water of the large vessel in which he kept his creatures amongst several shallow saucer-shaped vessels; this is obviously but another method of obtaining a stationary condition of matters where the decrease in food will set in, helped by the evaporation.

Long ago (Balbiani, 1861) it was observed that the stage previous to conjugation was characterised by peculiar behaviour on the part of the infusoria. Some writers refer to a visible state of excitement, a frequent hurrying hither and thither, with intervals of repose. These aspects, which are not constant for all species, have doubtless been exaggerated, and tend to detract attention from the really remarkable features

which are the way in which they play with one another in pairs, stroke one another and feel one another all over with their cilia (*Paramecium*) or frontal cirri (*Stylonichia*) swim about with one another , separate and then return , or go off on the search for another mate. What I would emphasize is the fact that after a pair has thus endeavoured to find out what measure of affinity there is between them , they will break off and start off on the search for mates afresh. This point serves to bring out what is usually little noticed , and in consequence little reckoned with - the individuality of the Protozoa. What constitutes affinity it is impossible to say. There is seldom any equality in size , any identity in shape. The secret of it all lies within.

After a period of such swimming together it will be noticed that an organic connection has been established between the two. In the case of *Paramecium* it commences on the ventral aspect in the region between the mouth and anterior end. In the case of *Stylonichia* there is a fusion on the anterior lateral region. It is always possible during the initial stages of this fusion to shake or pull the creatures apart without serious damage to themselves , in which case however , they very rarely come together again. The damage is repaired in solitude , and then they are ready to search again for mates. But after the fusion has reached a certain degree , e.g. when in *Stylonichia* it is represented by a lateral band whose

breadth is anywhere from an eighth to a tenth of the creature's total length , then the forcible separation is attended with serious damage , and the individual rarely enters into conjugation at all in the sequel.

Bütschli states that the conjugation of the Oxytrichinae is always lateral. The pair lie side by side so that the left lateral surface of the right individual touches the right lateral surface of the left partner. It also frequently happens that at first the right partner lies further forward than the left , so that its frontal area projects beyond that of the left partner. But as conjugation proceeds and the fusion becomes more extended this inequality disappears , and one simply observes that there has been a complete fusion , as far , in a posterior direction , as the mouth.⁺ This fusion involves both creatures in certain cases. Thus the right partner surrenders the left half of its adoral zone , the anterior lateral cirri , its mouth and gullet and one or two of the frontal cirri , while the other foregoes the right half of its peristome , a portion of the related membranelles , the lateral cirri and the greater portion of the frontal ones as well. They have in common one mouth and peristomal area.

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When the fusion proceeds beyond the mouth we are dealing with what is distinguished as Copulation.

with related membranelles which appears to belong to the left partner. The rate of their motion as a pair is considerably slower than that as individuals. Towards the end of the conjugation-period behind the mouth appear the rudiments of a new ciliary and cirral apparatus which gradually spread and press forwards, supplanting the old. This renewal is complete, and later includes the mouth and gullet of both exconjugates. The new peristome is formed somewhat behind the old one: the first hint of it is a slight depression in the ectoplasm. I have noted and drawn one case in which the right partner was applied ventrally to the left partner which adopted the normal position. This abnormality in position was maintained throughout, and had no effect on the ordinary course of the process.

The length of time occupied by the process varies with the species and with the temperature. In the case of *Paramecium caudatum*, Maupas found that at a temperature of 25° - 26° C conjugation lasted 12 hours. At a temperature of 18° - 20° C I found that it usually occupied a good 24 hours. In the case of *Colpidium colpoda* Maupas found that the process lasted 24 hours at a temperature of 20° - 21° C: at a temperature of 15° C I found that the process occupied 47 hours. In the case of *Stylonichia pustulata* Maupas gives 29 hours as the length of the process for a temperature of 15° C: with the thermometer at 20° C it does not take more than the 24 hours.

With all the Infusoria I have had under observation conjugation most generally commences in the small hours of the morning. This also holds true for binary fission: a rough calculation in the case of a culture of *Paramecium caudatum* gave the proportion of examples occurring during the day as about $\frac{1}{2}$ per cent. *Paramecia* in conjugation generally swim about most of the time, but they sometimes remain stationary at the bottom of the vessel and only give evidence of life by a slow ciliary motion. It is generally held that the forms which enter into conjugation are under the normal size, and it is certainly the case that the continued division results in a measurable decrease in size. But in proportion to the time the decrease in size is much greater during conjugation itself since in the majority of cases the mouths are blocked and functionless, or may have in part disappeared, so that fresh food can not be taken in, whilst the contractile vacuoles do not cease to work all the time. On coming out of conjugation the ex-conjugates require to be well fed previous to recommencing division: but here again too rich food-conditions seem to have an inhibitory action upon vegetative^{na} production.

We have just stated that the period of conjugation is more or less of the nature of a fast, and that with this there is associated a very marked decrease in size. At the same time there is a marked increase in the size of the excretory globules and in the number of the excretory granules, shewing that the

assimilation processes are not arrested in all their stages. The contractile vacuole functions all along but with a lessened frequency (normal rate for *Stylonichia* with temperature of 20° C , every eight seconds; during conjugation , every twelve to sixteen).

It is a commonplace of Ciliate interpretation that the macronucleus stands in close relation to the vegetative life of the creature. We see in its great size the means whereby the maximum surface is obtained for the exchanges that necessarily take place between it and the cytoplasm. We may also explain in a similar manner those cases where it is long and ribbon-like: (e.g. *Spirostomum ambiguum*) in this form it is most easily brought into relation with the remotest parts of the body. Now it is known that during conjugation there occur lengthenings of the macronucleus or of parts of the macronucleus considerably previous to the later divisions which it is not unreasonable to suppose may admit of a similar explanation. These lengthenings of the macronucleus are associated with the early stages of what is generally regarded as a process of degeneration. But , as Prowazek points out , if this be the case we are dealing with a very intricate process of degeneration , and one that is also remarkable for its constancy. This peculiar behaviour of the macronucleus is no irregular decay or hasty purposeless dissolution. Stein long ago noticed in the case of *Stylonichia* that the two

portions into which the macronucleus divides in the first instance are unlike in size and appearance, the anterior being more extended and the posterior contracted almost into an oval shape. With these initial stages of the macronuclear evolution is also associated an increase of size which leads to further division. The chromatin becomes condensed and fragmentation is still further proceeded with until a number of circular or oval balls alone represent the past macronucleus. That they disappear in this as in most instances has been known for some time, but how, I have been unable to satisfy myself. Bütschli and Prowazek have noticed their expulsion: Maupas believes that they are absorbed.

With the micronuclei on the other hand are associated all the distinctive features of conjugation. Their function is reproductive as contrasted with the vegetative offices of the macronucleus. Their behaviour follows very much the same general plan in all cases in which it has been followed. The micronucleus or micronuclei give rise by division to a definite number of portions, of which all disappear but one which again divides: one of this last pair of micronuclear fragments, crosses over into the partner, and there fuses with the one that remained in it to form a zygote-nucleus, and this by subsequent divisions gives rise to portions that either by fusion or individually develop into new macronucleus and micronuclei. In the case of *Stylonichia pustulata* the

micronuclear evolutions resemble those of *Paramecium aurelia* (as described by Maupas) during the first half , and those of *Onychodromus grandis* during the second half of the process. The micronuclei , probably by imbibition , increase to about double their ordinary size. Then by two indirect divisions they become eight in number: of these seven degenerate and disappear. I have not observed their expulsion and am inclined to believe that they are absorbed. The eighth divides again to form what are generally known as the male and female pronuclei , one of which passes over into the body of the other creature. Union takes place , and then the zygote-nucleus works its way back to about the centre of the creature. From it by means of two indirect divisions , four nuclei are formed of which two become the micronuclei (the posterior ones) while of the other two (anterior) one disappears , and the other develops into the macronucleus. Bütschli , who seems to indulge in an almost Weismannian belief in the immortality of the micronucleus as a whole , is most unwilling (*Protozoa: Vol. I: Pt. III: pp. 1619, 1622*) to believe the evidence that proves the disappearance of seven-eighths of it. When we consider how in the better known cases - *Paramecium* and *Stylonichia* - not only all the macronucleus but the greater proportion of the micronucleus or micronuclei disappear completely during conjugation , and how also the whole cytoplasm is rejuvenated as the result of the process , we see how small a fraction of

the infusorian is immortal in Weismann's sense of the term.

During the last stages of conjugation the pair become much more active in their movements and as a result the line of connection between them gradually shortens. Finally it is reduced to a point: then this vanishes without special effort, and the creatures are free. Not only however, as already remarked, are they now distinctly smaller than when they entered into conjugation, but their cytoplasm is more than ever opaque owing to the continued manufacture of excretory matter. This is gradually worked off, and in forty-eight hours at the most the creatures have regained their normal appearance.

In returning to a short continuation of the consideration of the conditions and cause of fruitful conjugation, we may recall the points already gained. So far as conditions are involved we have seen that the life-history of the infusoria runs in cycles, beginning and ending with acts of conjugation, and that it is necessary that each individual go through a definite number of divisions till it reaches the state of puberty. Thereafter it must be brought into relationship with the descendants of some exconjugate other than that from which it has sprung. We also learned that the immediate cause is inward, and that infusorians that have arrived at the period of puberty may be induced to conjugate by starvation. Now in this complicated process of conjugation which has hitherto been regarded as the primary feature of Ciliate life, the micronucle-

ar evolutions which have been outlined above are fairly constant for most species. The difficult point to believe is that of the eight, for example, portions into which the micronucleus (or micronuclei) divides, the sole survivor should be decided by its position alone, viz. that one which happens to be nearest to the mouth of the creature.⁺ Still the observations which establish the quantitative equivalent of these eight corpuscles make it as difficult to accept any other explanation of the matter. These observations have not yet reached the accuracy of those upon Metazoan forms in relation to the reduction of the chromosomes, but it is very evident that in the disappearance of these seven micronuclear corpuscles we have something analogous to the extrusion of polar bodies previous to the fertilisation of the metazoan egg. In attempting to find an answer to the question what significance is to be attached to this reduction in volume? Maupas could only reply that it was really impossible to say. Molecular forces, he believed, played a certain role in these phenomena: perhaps this reduction in volume modified these forces and gave more delicacy to their actions and effects. If, however,

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It is even still harder to believe that of the eight apparently equal corpuscles into which the zygote-nucleus divides (*P. caudatum*), position alone determines which shall become macronuclear and which one micronuclear: and yet we seem shut up to this.

Richard Hertwig's (on his own confession) halting results (Ueber die Konjugation der Infusorien: Abh. der Bayr. Akad. d. Wiss: II: Cl. XVII:) about a reduction in the number of spindle-fibres and corresponding chromatic elements during the final division in the case of *Paramecium caudatum* be confirmed , then the explanation of the disappearance of these micronuclear corpuscles will fall into line with that of " reduction" as it is understood in connection with the maturation and fertilisation of the metazoan egg.

We see the true inwardness and object of conjugation when we recognise that the nuclear apparatus that each exconjugate gets as the result of the process acts on all the organism , as Maupas says , like a regenerating ferment , absolutely restoring it to the previous normal condition. In the composition of the zygote-nucleus out of two equivalent micronuclei of different origin we have exemplification of the fundamental principle of fertilisation , but the important point in connection with the ciliata is the resulting renewal - " Verjüngung " - as Bütschli has it , of the conjugating pair. Now this is obviously , as we have shewn , in direct auxiliary relation to the previous or vegetative life of the infusorian, which is therefore the primary and more important phase of its existence: conjugation is but secondary and subservient to the phase of binary fission.

Engelmann^r, then, Maupas and Bütschli who see in conjugation a process of "reorganisation" or "rejuvenation" lay correct emphasis on the vegetative phase of ciliate life as primary. During the main portion of the creature's life there is constant gradual waste and degradation. We have seen that in^{In} the case of the products of a single division there is often a definite inequality in size and often change in the position of the macronucleus. It would appear difficult accordingly to avoid the conclusion that there is a corresponding inequality in the macronuclei themselves, and even if this were minimal in the first instance, its tendency would be to increase. Hence we may suppose that as the result of continued division disproportion will arise not only in the case of the quantities of the two constituents of the nucleus, chromatin and achromatin, but in the relations of the macronuclear and cytoplasmic masses. Conjugation is then, finally, induced, as much in the interest of the individual as of the species; the disproportions are removed, the waste is repaired. This conjugation effects by the substitution of an entirely new macronucleus and of a micronucleus new only in part but thoroughly remade, in place of the old. The infusorian is now enabled to exercise the functions of assimilation, growth and division with all the energy of a newmade creature.

Ueber Entwicklung u. Fortpflanzung der Infusorien. Morphol: Jahrb. I: 1876: pp. 573-635.

My purpose is not, ^{however,} accordingly, to minimise the importance of conjugation but to keep it ⁱⁿ its proper secondary place. For in opposition to the view of the workers referred to above are set forth those of men like Gruber who lay great insistence upon conjugation and elevate it to the place of most importance in the life-history of the Infusorian, because they see in it the origin of variation, and so the ultimate source of new species. Now the point that I wish to make is that Bütschli's reply to this position is, in part at least, weak and unsatisfactory, but, on the other hand the whole force of Gruber's contention is removed by the demonstration that there is variation in binary fission itself.

Bütschli's reply is rather to Weismann's similar view that as the result of the admixture of the characteristic qualities of two individuals such as takes place in conjugation, new variations arise in the descendants. He says that his experience tends to show that this union of qualities of two individuals in conjugation so far from being a source of increased variability rather tends to the neutralisation of characteristic qualities, and even to their enfeeblement. But this is hardly the experience of ordinary breeders, and much evidence of a contrary nature could easily be gathered. His illustration (Protozoa Vol. I:Part III:p.1639) moreover, is far from forceful, because in considering the conjugation of the descendants of one distinct union, which may be supposed to have identical "Keimplasma", he is illustrating by the conjugation of near relatives which ^{there is} ~~we saw~~ ^{is} reason to believe was

ultimately unfruitful , and therefore useless for purposes of discussion. Accordingly I believe that the refutation of Gruber's position is to be found in the fact that variation is involved in binary fission itself. This then leaves the vegetative phase of Ciliate life its ^{primary} ~~ordinary~~ position , and enables us to see in conjugation simply a device whereby the waste involved in that process can be refunded.

As yet I have not made any extended examination of the amount of variation through successive generations of binary fission , although the figures 210 , 60 , 100: 215 , 65 , 110 which represent the measurements of the second generation for the member , 220 , 50 , 105 , of the 6th pair (*Paramecium caudatum*) shew that the tendency remains.

PART IV

ENCYSTMENT.

Of cysts in Ciliate life it is usual to recognise two kinds - Reproductive and Protective. The former is associated with multiplication by division; the latter is for protection during unfavourable external conditions such as drought. It may indeed be questioned whether the distinction is far-reaching. Both cysts are essentially protective, and though it is commonly stated that protective cysts are more durable and for the most part are possessed of thicker sheathing, yet in those forms where one has had the two classes under observation, this difference has not always been so marked as to attract special notice. In the face of Rhumbler's time-honoured paper on *Die verschiedenen Cystenbildungen und die Entwicklungsgeschichte der holotrichen Infusoriengattung Colpoda*, it may appear somewhat rash to commit oneself to such

+ It is hardly necessary to remark that some observers (Balbiani, Fabre-Domergue) would distinguish a third class of cyst - Digestive - grounding their action on certain peculiarities of *Amphileptus Claparedii* St. Butschli shews that the cyst so formed differs in no particular form from the ordinary reproductive one.

a statement; but I have on more than one occasion noted that his well-marked "dauer-cyst" ⁺ also functioned as a reproductive cyst, and I am confirmed in my views on this point by a private communication from S. Prowazek, who states that he has re-conducted Rhumbler's experiments and is unable to agree with many of his conclusions. The paper in which Prowazek attacks Rhumbler's positions is not yet (Nov. 1900) published.

The following observations relate only to the Protective cysts proper; those connected with Reproduction did not come especially under one's notice. The conditions under which protective cysts are formed are very various, and as yet but imperfectly understood. Of prime importance amongst them is, however, probably, drought. In an analogous manner encystment has often been induced by gradual evaporation off slides (Cien^kowski, Fabre-Domergue). Some forms (Oxytrichae, etc.) encyst on the slightest provocation, and one can easily keep them alternating between the ordinary free-swimming

⁺ Rhumbler distinguishes between the Theilungscyst and the Dauer-cyst by saying that the latter has not the characteristic opening of the former. This may or may not be; but I have observed division set in immediately after the soaking of the hay which shews that what had functioned as a Dauercyst was also a Theilungscyst or vice versa.

condition and the initial stages of encystment by exposing them on a slide to the free air , then adding fresh water to make up for that lost by evaporation , re-exposing and so on , though not indefinitely. Matters are however carried too far when this slide-evaporation-process , conducted as carefully and tediously as you like , is made a test of a creature's ability to encyst. This would , nevertheless , appear to be Fabre-Domergue's position on the question of the prevalency of the faculty of encystment amongst the Ciliata. It is probably a fair statement to say that as the result of his experiments on encystment conducted with slides exposed to the open air , he concludes that this process is restricted to comparatively few of the Ciliata.⁺ Bütschli , on the other hand , after reviewing all the evidence , maintains that the lack of ability to form cysts is the exception amongst the group in question. My own opinion is that all the Ciliata would be found to possess the faculty if only the suitable conditions could be discovered. This view I hold strongly for two reasons: (1) there is no a priori objection to it of which I am aware; cysts are known of members of the different orders and sub-orders of the Ciliata: (2) in the course

⁺ Fabre-Domergue. Recherches anatomiques et physiologiques sur les Infusoires ciliés: Ann. des Sci. nat.: (s.) Zoologie T.V:1888.

of Bütschli's paragraphs on this subject , reference is made to various forms that were not known to encyst: of two of these - Paramecium and Spirostomum - I have succeeded in obtaining the cysts.

But to return: another well-known cause of encystment is lack of food , - exhaustion of the food-supply , e.g. Schizomycetes. Conversely , a superabundance of putrefaction with its resulting bacteria may cause Ciliates to encyst in self-defence. Thus one has often noticed encystment supervening during what proved to be the last stages for the time being of a culture , when one can only suppose that the excessive quantities of bacteria caused through their metabolism noxious changes in the gas relations that were necessary to the Ciliate's free existence. In short , any fouling , or more intimate change of the medium such as freezing , if not brought about too quickly, may lead to encystment. So far , however , as I have observed , the season of the year has in itself no effect upon encystment.

It would appear however that too much stress is laid on these merely external causes of encystment. All the stimuli towards encystment do not necessarily come from without. In this connection Bütschli is unconsciously almost teleological when he suggests that amongst certain parasitic Ciliata , encystment is their method of reaching another host.⁺ I have

⁺ We may note at this point that Bütschli emphasises the fact

more than once remarked that the conditions of encystment lie as much with the individual as with its environment. Subjected to like conditions, all the forms did not respond by encystment: sometimes only a small percentage. Thus during some experiments in vital staining, several Oxytrichae were put with *Paramecium caudatum* in a watchglass filled with a nutritive ~~medic~~^{um}ine to which, in the first instance, one drop of picrocarmine was added. At a certain stage in this gradual process, some of the Oxytrichae encysted. No more picrocarmine was added, and it was found after a day or so that only some 25 per cent in all had encysted. They were all subjected to the same external conditions, but there were other internal conditions not common to them all which produced the result stated. I am at a loss to explain otherwise cases of encystment on a small scale amongst strong cultures of *Stylonichia*. The view that binary fission is mere duplication tends to obscure the fact of the individuality (although this is too grand a word) of the Protozoa. Encystment, unlike Conjugation, is rarely epidemic.

We may therefore conclude that the causes of encystment

that in this group (Ciliata) encystment is never the result of copulation or conjugation as is frequently the case at any rate amongst the Mastigophora.

are more complex than would appear at first sight , and that in any specific instance we are unable to indicate them except along the very broadest lines. Very definite changes will occur in the medium - differences in concentration - as the water slowly evaporates; this will entail some change in the nature of the food-supply , although not necessarily in its amount) , and in response the protozoan encysts. Accordingly it seems probable that the proximate causes of encystment will be traceable to the food conditions. If this be so , it may be possible to over-emphasize the importance of mere evaporation (drought) as an immediate cause of encystment. The protozoan often encysts long before there is any hint of complete evaporation in response to some difference in its food supply or in the concentration of the medium.

There are not many Ciliata that will stand Fabre-Domergue's test of letting them dry on a slide in the open air in order to produce encystment; it is a much more gradual process than that. Even when one employed watch-glasses under cover there seemed to be a certain unnatural tension on the body wall of the protozoan due to the manner in which it was , as it were , held to the glass by the evaporating water. Consequently I found it helpful to cover the glass surface with a very thin layer of pond mud or even of cotton wool - something which presented an uneven ground surface - so that the protozoan was relieved from what appeared to be the attraction of

a smooth surface and left free to respond to its own strains and stresses consequent on the last stages of evaporation. At any rate by this device I was enabled to obtain dried Paramecia - quite apart from any question of encystment - such as I was unable to obtain by evaporation however gradual from a plain slide or bare watch-glass. Some of these dried Paramecia are figured on Plate VI.

With regard to the encystment of Paramecium, Bütschli in his Protozoa simply states what was true at the time, viz. that no cyst of that particular form had been described. In the ~~Traité~~ ^{descriptive} de Zoologie published in 1899 Fabre-Domergue, who is entrusted with the Ciliate type, is dogmatic as ever - "Paramecium n'enkyste pas." In a popular text-book which has deservedly reached a third edition (Outlines of Zoology by Professor J. Arthur Thomson) this statement may be found: "specimens of Paramecium may be readily and abundantly obtained by leaving fragments of hay to soak for a few days in a glass of water. A few individuals have been lying dormant about the plant; they revive and multiply with extraordinary rapidity." (3rd Edition, p.90). I am further informed by the author that he has himself picked the forms off the hay, but he states nothing as to their condition. By "lying dormant" one might understand some such dried up Paramecium as is figured on Plate VI; if so I can only state that in no single case did any of those so obtained revive after a

later reapplication of moisture. If he referred to encystment, it is strange that he did not describe the cysts that Bütschli maintains were unknown.

Fabre-Domergue's dogmatism can be easily disposed of on a priori grounds alone. If Paramecium does not encyst how does he explain the fact that it is so frequently met with in hay and other infusions? This would appear to be a well-authenticated fact, although I must state that as the result of many trials I only succeeded once in obtaining Paramecium in this way, which indeed was the cause which set one to work from the other end. It would seem then that to account for the presence of Paramecium in the hay infusion we must suppose it to be on the hay, windborne ⁺ thither in an encysted condition, which it leaves under the influence of the water and thereafter proceeds to multiply very rapidly.

In an article entitled "Die Protozoenkeime im Regenwasser" which appeared in the Biolog. Centralblatt, Dr. G. Lindner relates his success in obtaining various germs and cysts from rain-water which he collected in Cassel. Perhaps the "unstalked backward-swimming" Vorticellidae that he was able

⁺ It is also possible to suppose that Paramecium cysts formed at one locality are borne upwards by the wind and are brought down again by the rain elsewhere.

to rear from this medium have the greatest systematic interest. But he goes on to say that on several occasions he raised a species of *Paramecium* which he identifies with that commonly known as *P. putrinum*. Of these germs or cysts he gives no description. I have attempted to reproduce his figures on Plate VI, where the *Paramecium* indeed looks as if it were "lying dormant" and dried up, but is described as "encysted" in the original figure letter press. Lindner also succeeded in raising *Stylonichia mytilus* from a cyst, and I can agree with him in saying that it is a comparatively short-lived creature.

On one occasion I obtained cysts of *P. caudatum* after establishing the method already described with watch-glasses. The preliminary steps were much the same as were observed in the case of encysting *Stylonichia*, although the change in form in the case of *Paramecium* was not so marked as in the latter. On Plate VIII I have figured the various stages in the encystment of *Stylonichia mytilus*. Previous to encystment both forms become more and more sluggish in their movements. The vital energy seems either in process of storage or of dissipation. The cytoplasm loses its clear transparent character - this is especially noticeable in the case of *Stylonichia* - and becomes concentrated, opaque and yellowish. With this must be associated an increased passage of fluid through the contractile vacuoles with no corresponding intake.

Gradually all movement ceases. In the *Stylonichia* series the motion associated with stage b was still in direction and manner much like the ordinary, although considerably slower; often however the creature revolved slowly round its long axis for spells at a time, a motion probably due to its peculiar shape at this time. Stage c saw further slowing and a vibratory or quivering motion added; stage d was practically motionless.

The paramecium secretes a delicate gelatinous wall. Its shape varies from approximately oval to approximately circular. As in this instance I had but two or three cysts whose whole history I wanted to investigate, none were preserved by the ordinary means. I am therefore unable to state decidedly whether the wall is single or double: I am inclined to believe however that there is but the one. Prowazek, in an article to which reference will be made later, says that the cysts of *P. bursaria* which he found had a distinctly double membrane, but he figures the membrane as single. Within this cyst the Paramecium, unlike *Stylonichia*, still continues to revolve, but with ever decreasing energy, which expresses itself likewise in the slackening of the action of the contractile vacuole to once in 42 seconds.

The cytoplasm seems very condensed and yellowish, food particles are expelled and lie around free in the cyst. The macronucleus is distinctly visible, and oval in form. In

this condition the cysts remained for some time. The ultimate cessation of motion has some corresponding changes in the ciliation. In connection with this point Bütschli seems to favour the view that in such cases the cilia drop off; but in no instance of encystment have I observed anything to confirm this view. All that I have noticed seems to suggest absorption. During encystment there seems to be a more or less thorough reorganisation of the cytoplasm, so that the creature that emerges from the cyst when the latter again finds itself in a favourable environment is very different from that which entered it. Unfortunately I missed the actual process of escape which took place at the end of four or five weeks, and will now refer to a communication of S. Prowazek's which appeared in the Zool. Anzeiger, Bd. XXII, p. 339. The paper is dated July, 1899, exactly one month after the encystment process that I got with *P. caudatum*. He relates that he found certain round, clear, practically refractive cysts, surrounded by a yellow-green distinctly double membrane, which enclosed several excretory granules. He found the cysts in moss in exposed places in certain woods. In several cases he was fortunate enough to see the opening of the cysts which he thus describes, together with one or two other details. "The oval macronucleus seems at the commencement to be fairly distinctly marked off from the plasma and is granular: countless small vacuoles arise in the

entoplasm; cyclosis begins but at first in a retrograde manner and with irregularity. The cyst-wall now shews more distinctly a sort of thinning at one point, - the plan^{ce} of the old cyst opening or pore connected with the vacuole. Later the creature takes in water and the nucleus does not appear to be so functionless; the wall is stretched and raises itself. The vacuole empties slowly: its wall is indented, and the indentations correspond to the later star-figure. The rate of evacuation for a temp. of 19° C is every 2.40 minutes. The presence of cilia can only be determined by a wave-like activity after evacuation.....

The creature now endeavours to reach the thin portion of the cyst-wall by backward rotations. Before it crept out the vacuole was very widely expanded and did not contract: then a very violent activity set up in every part of the creature, it pressed itself against the cyst-wall, and suddenly slipped out, whereupon the vacuole emptied itself completely. The plasma later seemed more opaque and yellower. The cilia still beat somewhat irregularly and individuals amongst them were quite stiff. The whole process lasted about half an hour."

It is however in the case of the larger form *Stylonichia* that one gathers the impression of intense concentration as the main feature of encystment. Here one observed how all the excretory matter and the coarser remains of food matter

were gradually extruded , and in every way the creature seemed to draw itself together. This caused a certain amount of expansion in the middle , and at the same time a certain limitation in the ciliate and cirrhal areas especially towards the extremities. One could even observe a splitting tendency in some of the cirri. In his Viennese Protozoan Studies Prowazek emphasises the distinction we have already hinted at in the encystment of holotrichous Ciliata as compared with that of oxytrichous forms like *Stylonichia* , viz. that while the former , as we saw in the case of *Paramecium* , secrete a cuticle within which the creature rotates , (and as the cilia beat for the most part one after the other from before backwards the appearance presented within the cyst is that of a circling wave) , in the case of *Stylonichia* there is no definite rotation , but cilia and cirri alike beat slowly and irregularly , and finally the whole outer cuticle of the creature forms the cyst-wall. The actual formation of the cyst-wall occurs as a gradual rising or swelling-up of the cuticle , which commonly commences on the dorsal aspect and thence spreads all over the creature which is now approximately circular in form. The cirri still retain at the commencement of this process a certain amount of activity , but they become transformed into the larger spines that are in evidence in stage d of Plate VIII. In this way the entire cuticle becomes changed into an irregularly prickled protective

wall. I may add that during the initial stages of encystment the contractile vacuole emptied itself about every 20 seconds. A stained specimen shewed a macronucleus unlike that of the normal creature in appearance, although I am unable to interpret the difference. Up till practically the final stages it was always possible to prevent encystment by putting the creature into another culture or by changing the concentration of the medium. After it has been encysted some little time this is likewise the method by which it can be prevailed upon to decyst. Previous to this a new contractile vacuole is formed whose earliest contractions are not more frequent than one in two and a half to three minutes. It seemed to arise at a point where a superfluity of fluid had collected. Gradually the contractions increased in rate till just before escape they were within 5-8 seconds of the normal rate. The creature now shews considerable activity within its prison and in the course of its revolutions which are not all the time in the same direction it appears to strike the walls of the cyst with considerable violence which probably has the effect of weakening its texture. Eventually a narrow slit appears, possibly along a line corresponding to some old opening such as that of the mouth. Through this slit the creature works itself although the process may drag out over a couple of hours.

The species of *Spirostomum* whose cyst was investigated in isolation was ambiguous. I have not yet succeeded in

getting out the cyst of *S. teres*. Previous to encystment this form likewise contracts but slowly, and a rudely pear-shaped form is acquired, suggesting that there is greater freedom to contract at the anterior extremity of the creature, while owing possibly to the size and firmness of the walls of the contractile vacuole, the latter cannot at once adapt itself to a globular body. This however is gradually done and at the same time there is a quieting of ciliary motion. With regard to the ultimate fate of the cilia, the only indications were those of absorption. The outermost of the two distinct coats (ectocyst) is now laid down; later, in one specimen, the outer rim was differentiated clearly into what then appeared to be a third zone. Of the two principal zones, the inner is darker than the outer. The creature lies quite freely within the cyst which has an average diameter of 180μ . The colour of the cyst is yellowish-green. Latterly excreta and other detritus began to adhere to the ectocyst, due either to its gluey character or to the glutinous nature of the fluid that is expelled by the contractile vacuole and escapes to the exterior by oozing through the cyst-wall. The nucleus was observable as a long ribbon-like body lying partially coiled upon itself. It was distinctly noticeable in the living cyst, and when later it was stained after complete failure to bring the creatures out, it responded characteristically to borax-carmin.

Amongst the numerous Ciliata , the encystment of the oxytrichous forms has been studied with most care , but even here much still remains to be done. The result of gradually adding borax-carminc in small amount to the medium in which I was keeping several *Oxytricha pelionella* was that on the 19th day of the month , or five days after the commencement of the process of colouring the medium , some of them encysted. On the 22nd the ectocyst was well-marked and stained: the contractile vacuole emptied itself every 25 seconds. On the 23rd the pink ectocyst had shrunk in breadth at its narrowest point by about 2μ , and there was the same amount in breadth of ectocyst. The contractile vacuole evacuated every 35-45 seconds , and during the systole one could easily trace the star-shaped arrangement of feeding canals so well known in the excretory system of *Paramecium*. On the 24th the ectocyst had attained a thickness of $2-3\mu$: it was somewhat clearer naturally than the ectocyst , which maintained very much the same appearance. The creature still continued to circulate actively within the cyst , and the contractile vacuole maintained its evacuation rate of from 35-45 seconds. There is no doubt that certain waste products were still expelled to the exterior: the fluid had the same pinky tinge as the ectocyst. During the next few days the circulation of the creature still continued , mainly from left to right , but the contractile vacuole only operated every 85 seconds.

Ultimately all movement , whether of the whole or of a part , ceased , and the creature appeared to occupy all the space in the interior of the cyst. I do not consider that even now all the waste products had been ejected , as numerous fine green granules apparently of this character were still in evidence. In this condition of rest , when the whole interior cavity was filled by the creature , the cysts were kept for several days. They were then changed into a more favourable environment , and gradually a reorganisation of the body began to take place - a process which I am not prepared to state had not already commenced during the preceding period of apparent rest. A gradual revivification took place; the contractile vacuole set to work again , firstly at great intervals; excretory granules and other waste products - solid matter - were expelled and lay in the fluid within the cyst , for the tight fit that characterised the resting stage was no longer in evidence , and as the creature became more and more re-formed it appeared to take up less and less room. Its rotations which soon recommenced were again mainly from left to right , with occasional reversion from right to left. At times the movements became excessively violent , and the creature no longer took up any constant position in the cell. In the earlier stages of revivification it was quite impossible to orientate it: only later - about the third day of the four or five that this new process occupied - did its outlines

really become clear. As in the case of *Stylonichia* it is probable that the violence with which it circulated and beat against the cyst-wall loosened the texture of the latter, and a slit appeared through which the creature worked its way in a process that sometimes occupied a full half hour. The stained cyst-wall with its two zones remained. Potash did not dissolve it. Iodine darkened the contents of living cysts and tinged the walls with blue.

One had also occasion to follow this process of revivification in the case of cysts of *Urostyla grandis*. These had an average diameter of 50-60 μ . The cyst-wall seemed thinner in comparison and more transparent than that of any of the other forms already mentioned. It had a slightly bluish tinge in reflected light, the *Urostyla* was itself of an intensely bright green, while great brown blotches of excretory matter commonly floated around in the enclosed fluid. Previous to escape the contractile vacuole emptied itself every 25 seconds, and the nucleus was clearly visible. The ciliary action was likewise very intense; every portion of the body seemed to be alive. In addition to rotation of the whole, wave-like motions of the outer surface passed right along and round the creature. Part of the cyst-wall gave way completely, and the *Urostyla* was free.

The only observation that I wish to make at this time about *Colpoda cucullus* is that of conjugation within 48 hours

of decystment. On the received theory of conjugation, this would seem to show that after all, encystment, though primarily protective, may be - probably is all the time - deleterious to the vital energy of the creature.

(b) Fragmentation of Amoeba.

This enquiry was first prompted by a practical detail. It had been noticed on more than one occasion that when tubes of Amoebae which had been ordered from a contractor for purposes of class demonstration were opened, no Amoebae were forthcoming. I had occasion to isolate *Amoeba proteus* on slides in a damp chamber for the purpose of studying their life-history more minutely, keeping them in pairs, and subjecting them to various changes of environment. During a course of gradual starvation, it happened that on one morning there was no evidence of the Amoebae on the slides. Careful examination disclosed no evidence of death by accident - no remains were visible. On two distinct occasions such disappearances took place in the course of the third night after the Amoebae had been isolated in four or five drops of water with a minimum of food, to which medium a drop or so of distilled water alone was added in the course of the succeeding days. Further examination disclosed the presence of minute Amoebae in great numbers at a distance of from .04 to .15 mm. from the

surface of the water. A magnification of 105 revealed them as tiny translucent specks: under a magnification of 625 they were found to vary in diameter approximately from 2μ to 5μ (i.e. within 12 hours of formation). Three days afterwards they averaged from 6 to 12μ . In the youngest forms it was well-nigh impossible to detect the presence of any protoplasmic contents beyond the nucleus and contractile vacuole. The most marked feature was the very distinct differentiation of ectoplasm and entoplasm. The former was pale blue and absolutely hyaline: the tinge of the latter was slightly darker. Very often the ectoplasm formed a sort of halo round the entoplasm when the forms were seemingly motionless, but the commonest shape was that of a fan as depicted on Plate. III. At this early stage marked pseudopodia were rarely formed: more or less blunt protuberances or swellings of the ectoplasm were often in evidence, but its movements were generally total. I am not aware what are the proportions of ectoplasm and entoplasm in the full-grown Amoeba, but in these young forms the quantity of ectoplasm from a surface view was largely in excess of the entoplasm, the proportion often being more than 2 to 1: in the adult, at any rate, the proportions are entirely reversed. I have never observed any case of conjugation amongst these young Amoebae. They often touched and moved round one another, but no fusion of ectoplasms was ever detected. Their two chief characteristics seem^ed to be,

a) the extreme differentiation between ectoplasm and entoplasm, and b) the fact that all their movements were conducted at or near the surface of the water over which they seemed to creep; never, so far as one could see, did they sink to the ground surface of the slide. Near the surface of the water they fed latterly on bacteria, and only after three or four weeks did they take to the normal ground life of the Amoeba, being carried down possibly under the influence of gravity. From these facts one is inclined to imagine that originally the Amoeba was a surface form.

We now come to the method of production of these minimal Amoebae. On two distinct occasions they were obtained in the case of *Amoeba proteus*. On the second of these I was prevented from making the necessary observations during the night. Owing to the complete absence of any hint of the process in the case of this species I had supposed that there was some fragmentation comparable to Gruber's idea of "Zersplitterung" in the case of *Opalina*, (Vorläuf. Mittheil. s. *Biolog. Centralb.*: IV: 1885: p. 715:). In this particular case Nussbaum has shewn that what Gruber attributed to "Zersplitterung" was but the result of a rapid succession of ordinary transverse fissions (*Arch. f. mikrosk. Anat.*: Bd. 26: 1886:); but such an explanation is out of the question in the present instance owing to the number and minute size of the daughter forms. As after these two or three experiments I was unable to obtain

further specimens of that particular species I am unable to make any definite statement.

Further experiments were conducted with *Amoeba princeps*. The same procedure was followed and on several occasions I have obtained a like result. The staining of forms that one considered to be about to fragment shewed no fragmentation of the nucleus such as one had expected ^{previous} to the formation of the daughter *Amoeba*. Further, in this case it was often possible ^(on the slide) ^{had taken} to observe where the process ~~took~~ place, for the species is larger than *proteus*, and extruded diatoms and other waste matter, being accumulated in greater quantity, were left behind. I had occasion, however, to remark the presence of the "Glanzkörper" described by Greef in the case of *Pelomyxa palustris* (Archiv f.m.Anat:X:), whose chief peculiarity is their shining appearance. Their shape and size vary within certain limits, but for the most part they are roughly circular. The matter of which they are composed is homogeneous and yet shews a certain differentiation into two zones. Greef figures one of these "Glanzkörper" with what would appear to be two protuberances, but he describes them as depressions. I am very doubtful of his interpretation, for the object more naturally resembles one of the bodies in fission. During the later stages of the creature's existence when being slowly starved, these "Glanzkörper" seek the surface all round. I have observed them work their way out of

the entoplasm into the ectoplasm. Here they remained for a while: then the ectoplasm in which one lay formed itself into a blunt pseudopodium at that point into which the body moved: the ectoplasm then suddenly ruptured and the "Glanzkörper" was free. I never saw them discharged through the contractile vacuole. They then float and exactly in the same horizons as the young amoebae that have already been described. The fact that their disappearance synchronised with the appearance of these small amibes suggests that they are a species of internal bud. But the circumstance that I have not yet been able to follow the transformation of the one form into the other makes the explanation of their origin no more than possible. None the less the fact is not altered that these tiny Amibes are formed, and that in their formation we have some method of reproduction of the group that is not yet generally recognised, far less understood.

I have figured some of these Amoebae as they appeared at different stages after their origin. (Plate III).

* Figures of Lacrymarian cysts are also given for purposes of comparison. Previous to encystment this form settles down, the intense activity of the extensible neck decreases and the latter is contracted till the appearance of the creature is similar to that given on Plate IX, 4.5. Gradually a perfectly spherical outline is assumed and the first membrane secreted. The cysts which have a diameter of 30μ possess

* This paragraph should have been included at p.113.

a distinctly double wall of which the outer on the third day was three to four times as broad as the inner, and had a slightly laminated appearance. Their combined breadth varied from 5-7 μ . The cyst contents were condensed and granular in appearance.