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# On the Constitution and Mode of Formation of "Food Vacuoles" in Infusoria, as Illustrated by the History of the Processes of Digestion in Carchesium polypinum

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# IX. *On the Constitution and Mode of Formation of "Food Vacuoles" in Infusoria, as illustrated by the History of the Processes of Digestion in* Carchesium polypinum.

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[Plate 34.]

In the course of some unpublished experiments on the rôle of acid in protozoan digestion, I had occasion to use coagulated white of egg as a food for certain colonial Vorticellidæ. This substance, diluted with water before coagulation, and therefore given to the animals as a finely granular precipitate, is usually found after enclosure in the form of smooth oval or spherical masses (Plate 34, fig. 2, *C.s.*), which are widely different both in refractive characters and in size, from the minute irregular particles offered for ingestion. So striking is the contrast, and so constant is its occurrence with this form of food, that I attempted to watch the process by which the spherical ingesta are shaped: in this attempt I was struck by the clearness with which some characteristic features of the process are demonstrable in *Carchesium*, and realized that the very act, with the performance of which I was especially concerned, was apparently bound up with the operation of a mechanism undescribed in existing descriptions of digestion in the Protozoa. This led me to record, in the paper which follows, the result of sequent observation of the phenomena of ingestion, digestion, and ejection in *Carchesium;* for, while the intracellular solution of food is as truly *without* the cell substance as in the case of *Amœba;* while, indeed, there is no fundamental fact established for the digestion of that animal which does not find a parallel here,—the digestive cycle is shortened, unfamiliar details of the process are striking, and even the more familiar events occur with a dramatic vividness which makes them almost strange.

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The animals I watched (*Carchesium* and *Epistylis*) were kept in hanging drops of water, and were thus under no unusual pressure. Various solutions and suspended particles were added at different times to the water of these hanging drops, and, in order to examine the results of the addition of each substance, I used, as a rule, Oc. 3, Obj. F. (ZEISS) or Oc. 3, Obj. $\frac{1}{12}$ oil immersion (ZEISS or LEITZ), working sometimes by daylight, sometimes by artificial light. The focal length of these objectives does not allow the examination of animals in any great depth of encircling fluid; but such signs of lesion as extreme convexity of the peristome, the development of an enormous contractile vacuole, definition of the outline of the nucleus, are so prompt in their appearance in these Vorticellidæ, and so unmistakable, that it is possible, by the use of recurrent immersions in abundant water, to watch the same colony for many successive days.

*Carchesium polypinum* grows in pedicellate clusters of varying size; the disadvantage which (from an observer's point of view) attends the marked contractility of the stalks of a healthy colony is compensated by the comparatively large size of the polypes, and the transparency of their cell substance. Various species of *Epistylis*, more manageable because they are mounted upon relatively rigid pedicels, are often too opaque for accurate study.

Any interest which the food of *Carchesium* may have, centres almost exclusively round the events which mark its actual progress through the cell substance of the animal; it is hardly to the point, then, to discuss here the varying and somewhat fanciful terminology which former writers have applied to the funnel-shaped oral tube which collects and guides matter before its ingestion. It seems needful to say, however, that two points have formed the subject of much discussion; the first is the position of the mouth, the second is the internal continuation of the oral tube. In fig. 9 I have duplicated the diagrammatic representation of the isolated "digestive canal" in *Epistylis flavicans* given by GREEF,* in order to bring out, by corresponding letters, the differences in nomenclature which part him from some preceding writers. According to GREEF, the external opening of this canal may be termed the *mouth*; a slightly sinuous *pharynx* follows, spacious at first but narrowing considerably in its course, and a small dilated sac, the *œsophagus*, ends this internally. Since waste matters are ejected constantly from a ridge which lies between the middle and outer thirds of this pharyngeal tube, it is clear that the entrance of food and the exit of débris take place for a certain distance through a common passage. It may be on this ground that some writers, notably CLAPARÈDE and LACHMANN,† place the mouth internally of the anal ridge (*cf.* fig. 9), and give the name of *vestibule* to the outer one-third of the *pharynx* of GREEF. The tube as it narrows internally is, according to them, the *œsophagus*, and the terminal dilated sac the pharynx. In this paper I adopt the terminology of GREEF, which at least follows the sequence of regions in a true alimentary canal; his theory that a collapsible tube curves for some distance from the œsophagus into the substance of the animal, I cannot support,

* R. GREEF, Archiv f. Naturgesch. (WIEGMANN), vol. 37, 1871.

† CLAPARÈDE ET LACHMANN. Études sur les Infusoires.

from observations made on *Carchesium*. It may be possible that there is structural difference between the genera (I have said that the statements of GREEF refer to *Epistylis flavicans*), for different specimens of *Carchesium* do seem to show slight variations in the extent of the pharynx; but the existence of anything like a permanent, far-reaching tube is disproved, I think, by evidence which can be brought forward more suitably later. As a rule, the pharyngeal tube (fig. 5) placed somewhat obliquely, ends about midway to the basal attachment of the polype; it is ciliated throughout and separated from the oval œsophagus by a slight annular constriction. Into the œsophageal sac, all ingesta are whipped by ciliary action, and they start from its most internal point on their intracellular career as constituents of the vacuole of ingestion. It is this career which I propose to trace, grouping its minor details in sequence about such as are more important, and postponing any discussion of their significance until the tale is told. And it may be well to preface the whole description with a brief account of those events in the gullet which are immediately antecedent to ingestion.

## EXTRACELLULAR PHENOMENA.

The ingestion of particles by *Carchesium* is in a certain sense selective, but the selection depends in no way on the nature of the particles, but only on their size. According to GREEF,* the stream of solid granules which may enter the mouth of *Epistylis flavicans* is so directed by two delicate valvular membranes that some of its constituents gain the internal parts of the pharyngeal tube, while some are discharged at once to the exterior. These membranes are figured as occurring at that knee-like bend which characterizes the pharynx in *Epistylis*, and it is quite possible that in *Carchesium*, where the immediate rejection of most particles primarily swept into the mouth is clear enough sometimes, the directing action becomes almost that of a strainer. I have followed the ingestion of a compact solid, measuring $6\mu$ by $3\mu$, but any fragment of enclosed matter which is larger than this, exceeds it in one diameter only, or is one of such loose flocculi as make up a freshly fallen precipitate of alizarin sulphate. Among ingesta which are linear and relatively long, we must count some bacterial filaments and the acicular crystals which alizarin sulphate forms with lime in water. In the great majority of cases, when the polype deals with these ingesta, short rods gather in the gullet and help to form the vacuole of ingestion; I think it reasonable to suppose, therefore, that the oral and pharyngeal cilia have some power of breaking up these slender threads, in addition to the selective action by virtue of which they hinder the entrance of particles which exceed a certain size. In some rare cases one can watch the enclosure of a bacterial filament (equal in length, perhaps, to the polype which encloses it) without any preliminary fragmentation. The act is carried out by the protoplasm of the animal, independently, it may be, of

* R. GREEF, *loc. cit.*

any immediate secretion of fluid; and the end of the bacterium first enclosed is coiled upon itself, sometimes even while the free posterior end, which helps eventually to thicken the coil, lies freely in the pharynx. The process is relatively slow, and occurs so rarely that there is some temptation to regard it as a distortion of normal ingestion; there seems little room for doubt, at least, that the most acceptable particles are very minute,—Indian ink suspended in water, the smaller fatty globules of milk, carmine, also finely divided, and the coagulation precipitate from diluted white of egg to which I have referred above.

While, however, it is clear, from these facts, that the ingesta of *Carchesium* are made up of particles which vary considerably in size, the connection between this variation and the fashion of their preliminary accumulation in the gullet is less evident. The lowest pharyngeal cilia, acting downwards from the annulus which I have described, beat the solid matter within their reach into the most internal extremity and then into the body of the œsophageal sac (which may dilate considerably, fig. 5, *ing.*). But the admixture of water, or rather the medium in which the animal is living, is the feature which varies in prominence with some apparent eccentricity. On the whole, irregular particles, and especially nutritious particles, lie in a very fluid vacuole of ingestion. Exceptions to both these statements occur, however; thus, minute bacteria have sometimes but scanty fluid surroundings, and the grains of Indian ink may be gathered so closely that the accompanying fluid is hardly appreciable, or may, on the contrary, lie in a vacuole so well marked that Brownian movement is obvious.

## Intracellular Phenomena.

### The Successive Phases of an Act of Digestion.

The preliminary accumulation of particles or building up of the vacuole of ingestion just detailed is a process which lasts through 25 to 40 seconds in an active animal, and may be drawn out to minutes in lethargic forms; but it is by a relatively sudden discharge from the extreme end of the œsophagus that the vacuole or its anterior half* is intruded into the neighbouring protoplasm. The vacuole is sometimes spindle-shaped, sometimes almost spherical, or it may have any form intermediate between these two extremes (fig. 5K and fig. 7); the spindle-shape is probably to be associated with general functional depression; other modifications in form appear to depend on the size of the particles enclosed. The most common shape (fig. 2B) (broadly elliptical, with acutely drawn anterior and posterior ends) is associated with the most minute ingesta, while larger ingesta (fig. 7, *b*) seem to force a wider temporary rupture in the protoplasm into which they enter. When ingestion is complete, the vacuole does not pause, but

* In speaking of vacuoles which have been watched from the moment of enclosure, I use the terms *anterior* and *posterior* to indicate respectively that end of the vacuole which was first in progression, and that end which was enclosed last.

performs what I may, perhaps, call a movement of *progression;* that is to say, it passes with a steady, gliding motion towards the basal attachment of the polype. This progression never carries the vacuole beyond the concavity of the band-like nucleus (which body, indeed, seems to define the path of ingesta in the basal region of *Carchesium*), and, dying away, it gives place to a phase of *quiescence* which is generally well marked. The vacuole pauses after turning through one or even through two right angles; it pauses, that is to say, with its long axis at right angles to, or parallel with, the long axis of the polype (figs. 2 and 5).

Up to this point it cannot be said that simple inspection reveals any constant change in the vacuolar contents. Solid particles, when they are minute enough to be present in numbers, are distributed uniformly in the medium which holds them; small Infusoria, if by chance they are enclosed, are active. And although, in some cases, movement at and near the centre of the vacuole ceases, or becomes, at least, less readily appreciable, yet more frequently Brownian movements, or the wider excursions of motile bacteria are obvious still. When, however, the phase of quiescence has persisted for some seconds (in healthy specimens), there is a striking rearrangement of the contents of the vacuole; the change is of such a nature that the solid particles lying scattered until this moment are gathered centripetally, and clear fluid passing centrifugally from among them surrounds the central composite mass. To this phenomenon I propose to apply the term *aggregation*,* since its salient feature is the approximation of particles which were separate initially; it is so striking upon occasion, and at the same time so far modified by the nature and disposition of the ingesta, that the following somewhat detailed description seems justified.

Case 1.—*The aggregation is single; one central mass represents the scattered granules of the preceding stages.*

The large majority of the ingesta of *Carchesium* illustrate this form of *aggregation;* at the same time they vary so much among themselves in size, shape, and density, that unity of type in the action is sometimes obscured by secondary modifications. I will choose certain ingesta which seem especially fitted to illustrate this association of fundamental likeness with superficial difference, and will linger a little time to consider the fate of each.

**Finely divided Coagulated Proteid.**

*Carchesium*, fed with the coagulation precipitate separated from the diluted white

* It is with some reluctance that, in applying the term *aggregation* to this phenomenon, I use a word to which DARWIN has given a different and definite technical meaning in his work on *Insectivorous Plants.* No other word, however, describes with equal accuracy the striking rearrangement of solid particles which takes place so suddenly in a vacuole of ingestion, and, by the light of later work on the secretory activity of plant cells, the description of change in the cells of the tentacles of *Drosera*, given with such faithfulness by DARWIN, must be considered, on the whole, as of classical rather than immediate interest. Partly on these grounds, and partly in deference to clearer judgment than my own, I have spoken of the centripetal clustering of ingested particles as "aggregation" throughout the pages which follow.

of fresh eggs, may be regarded as ingesting minute, irregular particles of nutritious matter suspended in water, or rather, in a very dilute solution of salts; some soluble organic matter is probably present too, when the colony is living in an impure medium. In this case the fluidity of the vacuole of ingestion is generally well marked, the faint pink tint, familiar to histologists as belonging to thin protoplasmic films which are separated by fluid, is distinct during the phases of progression and quiescence, and the dancing particles of proteid are distributed uniformly. But when the phase of quiescence is over, their movements cease; those which lie peripherally leave the boundaries of the vacuole, and, with a general and relatively rapid centripetal motion, the central coherent mass is formed. Nor is this union temporary; the individuality of the granules is gone for ever, and time and further change only tend to perfect the apparent homogeneity of the composite solid.

This process is seen most satisfactorily with high powers of the microscope,* and the absolute displacement of each solid particle is of course exceedingly small. I find that in no recorded case have I estimated the distance traversed in *aggregation* as greater than 6·2 $\mu$, and so marked an excursion as this is rare. The phenomenon is very striking, however; and, I may add, the difficulty of offering a really satisfactory explanation is great; close observation, if it does not surmount this difficulty, allows one to add the following details to a general preliminary statement.

1. The *aggregation* is almost invariably excentric; I have mentioned that the vacuole of ingestion may turn through one, or even through two right angles before the movement of progression dies away; clearly then, its most anterior point may pause at any spot along the circumference of a semicircle arching upwards from the concavity of the nucleus (fig. 1, 2$a$). Yet, however marked the preliminary shifting, it is from the anterior end that the greatest centripetal movement takes place; the mass of gathered granules settles towards that region of the vacuole which entered last from the œsophagus, though even here there is fluid separating it from the surrounding protoplasm.

2. The *aggregation* often begins at one point—not necessarily the most anterior,—but runs round the vacuole so rapidly, that it is only just possible to pronounce the displacement of particles lying along different radii of the vacuole not simultaneous, but successive. And in certain cases the synchronism is perfect, and gives the impression that a force is at work which gathers up all outlying particles, fusing them or establishing a substantial link between them in such fashion that the retreating border of solid matter is not shadowy, but highly refractive and sharp, showing well against the encircling rim of clear fluid (fig. 3K).

3. The first and most striking *aggregation* cannot be regarded as ending for ever all movement of the solid matter involved in it. In the first place, the freshly-formed composite proteid mass is not always homogeneous. It is, indeed, a mass and

* ZEISS, Oc. 3, Obj. F. LEITZ, Obj. $\frac{1}{12}$ im.

not a shell of gathered particles, still, there is sometimes localized admixture of fluid; little rifts, pinkish in tint, hint that tiny drops of fluid have been imprisoned by the first coming together of the irregular particles of proteid. In the second place, actual measurement shows that for some seconds after the first spasmodic clustering the ingested matter shrinks; this shrinkage is gradual, it persists through the next phase, which may be distinguished in the digestive process, and points (as does the gradual disappearance of the drops of imprisoned fluid which have just been mentioned) to a slow but closer gathering of the particles which were moved at first with relative swiftness.

**Indian ink suspended in water.**

The fate of this form of matter within the substance of *Carchesium* is suggestive, for the particles of Indian ink are extremely minute and practically insoluble in water and saline solutions, and in these points they resemble the proteid precipitate just described. At the same time the nutritious character of boiled white of egg establishes a sharp distinction between the two substances, the clearness of contrast being marked in proportion as each body is unmixed with other matter. The Indian ink may be gathered into the vacuole of ingestion with such energy that the admixture of fluid is hardly noticeable, or it may (and this is more commonly the case) pass through the phases of progression and quiescence of the vacuole, in active Brownian movement. When it is a question of the latter alternative, the Brownian movement is stopped by a centripetal gathering of particles as unmistakable (in vigorous polypes) as the primary *aggregation* of finely divided proteid. Only this difference reveals itself on observation, that some scattered grains of Indian ink are at times left outside in the general clustering and lie peripherally in the expressed fluid, whereas I can quote no instance in which all the solid particles of proteid present in a vacuole of ingestion are not gathered in during *aggregation* to the composite mass which is formed.

When, on the other hand, Indian ink particles fill the vacuole densely, even from the moment of its formation, then the displacement in *aggregation* is not well-marked; but the edge of the enclosed mass becomes sharply defined against a narrow rim of clear fluid which encircles it like an aureole. In no case is there immediate separation of the particles which have come together, despite the absence of cohesiveness shown by Indian ink grains suspended in water; a well-marked composite mass is formed, a mass interesting, as well on account of the fashion of its formation, as on account of its further history within *Carchesium*. The details of this history, however, can be dealt with more suitably later; so, with a simple notice of the facts that the secondary shrinking, so noticeable during the ingestion of proteid, is hardly appreciable when pure Indian ink is enclosed, while the excentric character of the aggregation may be marked, I turn to describe forms of ingested matter which have characters not touched on before.

**Particles, nutritious and innutritious, which exceed in size the constituents of the above-mentioned ingesta. Carmine grains, pigment grains (ultramarine), the smaller fatty globules of milk, some bacteria, and small monads.**

In Plate 34, fig. 17, $b$, $b_1$, I have sketched complex ingesta of carmine and bacteria, and the two diagrams illustrate many of the points which characterize the forms of matter—in some ways so diverse—which I have grouped together here. In the first stage represented ($b$), *aggregation* has taken place recently, and it is clear that the actual displacement of matter has been slight. In the stage drawn two minutes later ($b_1$), the contraction, or shrinking, is accentuated, the enclosing fluid is clearer, and the outline of the ingested mass well-defined. These statements hold for most cases in which the vacuole, entering from the œsophagus, is well filled with fairly large particles; the primary rearrangement tends to be unimpressive; it merges into what I have called secondary shrinking, and the whole process is deliberate. When, however, such particles are present scantily—when a few micrococci, or scattered individuals of bacterium termo, are enclosed, then the *aggregation* is striking; and a single spirillum, a single monad, or a single globule of fat, when each is ingested with minute particles, sparsely distributed, may move inwards, quite markedly, from the external limit of the vacuole (fig. 7, $a$, $a_1$). The fate of milk globules deserves special notice; I find that the fat of milk is no more available for the nutrition of *Carchesium* than for that of *Amœba* or *Actinosphærium*,* yet the ingestion is eager and persistent. The tiny globules can never, of course, fuse to a homogeneous mass, and (being, indeed, generally densely packed from the œsophagus inwards) they do not, as a rule, illustrate at all vividly the typical spasm of primary aggregation, or gradual following shrinkage. But their undoubted coherence into mulberry-like masses is striking (fig. 7, $c$), and if it be urged that the proteids of milk in solution (fresh), or precipitated (albumen in boiled milk), may have their share in effecting this union, still the facts seem to demand the existence of some homogeneous unifying substance, its outline joining the peripheral fatty globules, while those that are more internal lie embedded in it. It is natural to try to test the validity of such an inference by reference to the fate of ingested proteid and Indian ink. I am anxious, however, before entering upon any discussion of the meaning of *aggregation*, to increase the list of facts upon which such discussion may be based, and I turn to record the results of some observations of a second main modification of the phenomenon.

Case 2.—*The movement is double; two processes, or, more probably, two steps in one process, may be distinguished, unlike in duration, and, to a certain extent, in result.*

The composite mass is made up of a central portion, shaped by the first rapid gathering, and a cap-like or spherical border of solid substance deposited later (fig. 8, 1$d$, 2$c$). In one series of experiments this double movement constantly followed the ingestion of the white of stale eggs coagulated after dilution. The

* M. Meissner, 'Zeitsch. f. Wiss. Zool.,' vol. 46. 1888 M. Greenwood, 'Journ. of Physiol.,' vol. 7.

feeble coagulation, which I obtained by boiling, in this case, contrasts strongly with the dense precipitate thrown down by heat from a solution of fresh white of egg. And since it is known that when eggs are kept, albumoses and peptones make their appearance and increase, and that albumen coagulates feebly in alkaline solutions, it seems probable that the opalescent fluid, obtained by boiling the stale "white," offers for the attack of *Carchesium* a constituent which has hardly been considered as yet—nutritive matter in solution. Associated with this soluble food, we have, in the case under consideration, a scarcity of suspended particles, and the relative importance of each factor in producing the phenomenon I am about to describe is not easy to estimate.

The first centripetal movement is a vigorous reproduction of the process as it has been described above. The cluster of proteid particles is excentric in position, its constituent granules, probably because they are scattered initially, come together with much energy, and at the same time may leave among themselves the little fluid-filled rifts described before. This admixture of fluid is so striking sometimes that the ingested body may be termed vacuolate; its border is always sharp and unbroken; its size (and *à fortiori* its mass) is less than the ordinary size of a food ball, shaped by recent *aggregation*. This difference is of course a difference of degree, but a qualitative peculiarity becomes obvious almost at once in the fluid of the vacuole of digestion. I have tried to represent this in fig. 8, and to show that after the first gathering of granules slight opacity in the outlying fluid hints at the appearance of fresh solid matter. There is, in fact, a second movement shortly; little bullæ of clear fluid appear round the edge of the vacuole (fig. 8, 1*c*), and the opacity of its contents becomes slightly accentuated; later, a gelatinous mass retracts, with slow centripetal motion, and settles round the "nucleus" formed by the first quick movement (1, *d*). The substance separated thus by the second action is generally homogeneous, and often difficult to see; in rare cases, the preparatory turbidity of the vacuole, of which I have spoken, is exaggerated to granularity, and when this is so, the shrinking solid is granular too (fig. 8, 2*c*). I have said that this second retraction is slow, indeed it may be many minutes after ingestion before the double sphere lies fully in clear fluid; rarely, however, there is an approach to the rapidity of primary *aggregation*. I think that the rate of movement, the extent of retraction, and the consistence of the second shrunken mass depend upon the existence of a certain balance, in the proportion of soluble and suspended matter first ingested, and upon the amount or energy of the substance causing retraction, which is passed from the surrounding protoplasm into the vacuole. Thus, double *aggregation* can be made single by the addition of an overwhelming quantity of finely divided substance to the fluid, of which soluble matter was an important constituent before; on the other hand, a few inert particles, such as those of carmine or Indian ink, do not hinder the moulding of the double mass, but are recognizable, for the most part, near its centre—they are involved, that is to say, in the first sudden movement.

Again, the degree of the second retraction varies; as a rule the shrinking is so marked that in the stage of *storage** the double character of the ingesta is hardly perceptible; more rarely there is a loss of vacuolar fluid, while yet a granular "nucleus" lies clearly within its gelatinous investment—the mechanism of retraction has failed partially, either because of unusual lack of vigour, or because of excessive resistance in the substance which is to be moved.

Any specimen of *Carchesium*, in which this double clustering of ingested matter is demonstrable, can, as I have said, be induced to perform the single movement by suitable change of food; it would seem, then, that we are dealing rather with inconstant stimuli than with inconstant response of cell substance. And the whole variation is valuable, mainly, I think, because the slower changes which characterize it are of help in the attempt to analyse the quicker, usual movement.

The somewhat detailed description which I have given of this phenomenon of *aggregation* may tend to obscure the fact that it is, in *Carchesium*, but one phase in the sequence of digestive change; that all the important transformations immediately bound up with solution of food are yet to follow. It ends the period of quiescence, during which most ingesta tarry within the basal curve of the nucleus; and it precedes a second mimic peristaltic movement, which, since the ingesta pass once more towards the peristome (though on the side of the polype opposite to that on which they entered), may perhaps be distinguished as the movement of *retrogression* (fig. 2, B, *a*, *b*). This is slower than the initial *progression* from the œsophagus, and is usually characterized by the slight continued shrinking of the ingesta on the one hand, and, on the other, by the enlargement of the vacuoles which surround them. These changes are indicated in fig. 2, A, B, fig. 7, *b*, *b*, and, though they vary under changed conditions, may be said to accompany the transit of all particles, whether they are relatively large or minute, insoluble or nutritious. But when *retrogression* has carried the vacuolate ingesta almost to the level of the peristome, there are indications of varying fate; and the cell-substance of *Carchesium* shows that discriminating reaction to the nutritious or innutritious character of enclosed matter for the reality of which I have pleaded before in the case of *Amœba*.

**Nutritive ingesta,** following the course of the red line in fig. 1, pass towards the more deeply-seated substance of the animal (I speak now of typical acts of digestion), and may pause at any point in the area shaded with transverse lines, or may move through it slowly. In either case they lose the fluid which surrounds them, and with its disappearance there is a total intermission of digestive activity; the ingesta pass into what I would call the stage of *storage;* this may persist even for hours. Throughout this phase the enclosed masses, which have origin in coagulated proteid, are smooth, and oval or spherical; many ingesta, not of distinct experimental origin, and therefore doubtful in nature, form rather dense wrinkled masses, and in clusters

* *Vide infra.*

of ingested bacteria the individuals can still be distinguished as bright points, or (after proper treatment) as deeply-stained dots. Ingesta, whatever their nature, have reached the extreme point of solidity, of shrinkage; and a polype, after vigorous feeding, is chalk-white by reflected light, and, by transmitted light, studded with relatively opaque patches, however transparent may be its own cell-substance.

Eventually, however, the storage stage is ended;* there is no constant or noticeable locomotion of the ingesta, but vacuoles re-form around them at any point over a large area. And this freshly-secreted fluid has a powerful solvent action on proteids; even coagulated proteid succumbs, and the semi-mucilaginous investment of bacteria does not guard them from speedy loss of discreteness. In figs. 3 and 4 diagrammatic representations of this part of the digestive process are given, the ingested matter being coagulated proteid in fig. 3; in fig. 4, bacteria.

In fig. 3 one food-mass out of many is chosen for reproduction, and the first sketch is made immediately after *aggregation* is accomplished. The movement has been inconsiderable, and only a small amount of fluid surrounds the composite mass in whose substance there is just the indication of tiny drops of imprisoned fluid. At *a*, the stage of storage is reached; the fluid of the vacuole, separated out by *aggregation* and developed more clearly during the movement of retrogression (of which there is no picture here), has disappeared, and the proteid sphere is denser and more clearly defined than at any other moment during its intra-cellular career. *b*, *c*, and *d* represent stages in development of the digestive vacuole, and solution of its contents. At *e*, the proteid matter is obviously reduced in amount; the vacuole holding it is still very large, and has approached the area from which discharge takes place.

Fig. 4 differs in two points only. In the first place, the actual enclosure of the ingesta was not seen; it is not certainly there, but only (from comparison with known cases) probable in a high degree that they are clustered masses of bacteria; in the second place, the time data given here help one to realize the vigour of digestive action, even on enclosed matter which does not promise ready solution. It will be seen that in less than an hour after the first formation of a digestive vacuole (*a*) there is marked reduction of the food-mass attacked, while at the same time no scattering of its substance can be made out. These two features characterize every energetic digestive act, but they vary in prominence. Thus, if many insoluble particles are bound together by but a slight admixture of nutritious matter, then in the later stages of digestion such particles may be set free; this is often the case when Indian ink is ingested with proteid (fig. 5, *a*), and may be seen rarely when *Carchesium* is fed with milk. Or, again, the reduction in size may be inconspicuous when the insoluble matter mixed with any food-stuff is bulky, or is so constituted that no close packing of its particles is possible (fig. 7, $b$, $b_1$).

I should like to emphasize another change which is at least as constant an accompaniment of digestion as continued cohesion of food particles or reduction in size of

* Some actual time details are given later.

the mass which they compose—I mean increase of transparency, loss of solidity and of firm outline. I have tried to represent this in the figures of *Carchesium*, but with slight success, for in reality it is so striking that there is but little tendency to confuse ingesta before and after the stage of storage, though in both cases (typically) they are vacuolate. Indeed, as it has been pointed out that after the first loss of vacuole the extreme point of concentration is reached, so I may add that never is there such shadowy outline, never such sharp contrast in refraction between accidental insoluble particles and the apparently mucilaginous basis by virtue of which they cohere, as in advanced digestion.

Complete solution of ingested matter is extremely rare in *Carchesium*, especially when it is a question not of the relatively pure nourishment provided by experiment, but of the mixed diet of a struggling life; after a varying time,* then, insoluble remains of all kinds pass from the more central position where the digestive ferment has attacked them (fig. 1., solution) and come to lie in what I have called the area of *discharge* (fig. 1, discharge). According to GREEF,† all fæcal matter is expelled into the pharynx (at about the junction of its external and middle thirds) from a ridge, running transversely to the long axis of the polype in this region, and round about this narrow area the vacuolate food masses are grouped when digestion is at an end. And here there is occasional fusion of vacuoles, so that two or more ingesta are placed in common fluid surroundings (fig. 6, *d*). This phenomenon may, indeed, occur at any period in the digestive cycle; thus, in fig. 6, *c*, I have represented a fusion of four ingesta effected during *retrogression*, and the fusion of vacuoles of *ingestion* is not infrequent. But the coalescence is rather to be associated typically with advanced digestion than with these early stages, and recalls the fashion in which, in *Actinosphærium*, many ingesta may be passed to the exterior from a common excretory vacuole.‡ In *Carchesium* the occurrence is rarer, and it may, moreover, be obscured by a characteristic want of synchronism in the ejection of fluid and solid matter; as a rule, nutritive ingesta reach the area of discharge in well-marked vacuoles, but these are almost invariably reduced in size and may disappear before extrusion of the solid matter (fig. 3, *e*.). The assumption that the fluid lost thus as a discrete vacuole does, indeed, pass from the animal, and not into the protoplasm which secreted it originally, may be regarded as hasty, and so bewildering are ciliary movements and the constant shifting of the whole polype that the fate of anything as inconspicuous as is fluid tends to be obscure. But apart from *à priori* considerations, which lead one to assume the existence of waste matter here, the somewhat sudden character of the disappearance and an occasional preliminary ejection of free granules or viscid flocculi serve as some direct evidence of outward movement. After the diminution or disappearance of its fluid surroundings the solid residue is discharged, passing as if slipped into the

* *Cf.* below, p. 372.

† R. GREEF, *loc. cit.*

‡ M. GREENWOOD, 'Journ. of Physiol.,' vol. 8, p. 283.

pharynx. Here it pauses until such time as some of the currents called into being by the vigorous action of surrounding cilia sweep it to the exterior.

I have said in an earlier part of this paper that **innutritious matter** is swallowed by *Carchesium* with undiscriminating eagerness, if its constituent particles are small. Indian ink grains and coagulated proteid mixed equally in the surrounding water give rise to ingesta, in which both substances are mingled impartially. Further, it is quite usual in vigorous polypes to get a replacement of one set of ingesta by others of unlike nature, when the suspended particles proper to each group are made to preponderate successively outside the animal. And in experimenting thus, I have never been able to note any clearly selective ingestion; coagulated proteid may replace carmine or bacteria, and may be ousted in turn by Indian ink or milk; it is the quantity or distribution of matter that determines its entrance rather than its availability for subsequent digestion. Even after enclosure, the onward movement of progression is characteristic, and, the phase of quiescence being ended (usually) by a typical movement of aggregation, retrogression follows. Finally, after a pause in the "area of *solution*," discharge takes place from the anal ridge. Only in the duration of these several events, and in the really important details, the wrappings to this bare thread of movement, can we trace the fact that innutritious matter does not stimulate the cell substance of *Carchesium* so effectually as does digestible food.

In the first place, the time of enclosure is short. I will not anticipate the more detailed statement on this point which is to follow, but will only say that this diminution does not affect the first periods I have distinguished. The time taken up by progression and quiescence is, on the whole, very slightly longer than when true food stuffs form the ingesta. But sojourn in the area of solution and the area of discharge is shortened, and 40 to 60 minutes after the first enclosure, ejection may have been even considerable.

In the second place, it is noticeable that very many non-nutrient ingesta are discharged without that re-formation of vacuole which marks the actual solution of digestible matter. Deprived of fluid after the movement of retrogression is over, they acquire no fresh fluid from the surrounding protoplasm, and they are set free finally as spherical or more commonly elliptical masses (fig. 2, A, fig. 16, *d*). But I cannot leave this statement, contrasting as it does with the history of true digestion, without a modification which at first sight is damaging to its effect. It happens sometimes that ingesta, moulded from insoluble pigments, never show complete loss of the vacuoles which have been brought out by *aggregation*. Not only does the fluid persist, it increases in amount, and in such case the peripheral grains of pigment (I speak now of finely particulate matter) are freed into the fluid of the vacuole and show typical Brownian movement (fig. 5, *a*). There is, however, in no case thorough disintegration of the ingested mass; whatever change sets free the granules is circumferential only, for a central solid remains and shows on ejection no lessened cohesiveness. This phenomenon is not extremely rare, but may be termed exceptional. I hope to discuss later, its relation to the view formulated above, that the secretion of the true digestive fluid is selective, and to show that the two are apparently rather than really discordant.

**The length of time during which Carchesium retains ingesta, and its distribution among successive phases of digestion.**

When a group of polypes is left overnight in water holding coagulated proteid in suspension, it is usual in the morning to find each polype crowded with ingesta in the stage of storage (Plate 34, fig. 2, *C.s.*). And this state of things may persist yet longer if the animals and their surroundings are left undisturbed, whereas, mounted and watched by transmitted light, they soon show signs of digestive activity; vacuoles are formed round some of the ingesta at least, and the changes which I have described above as characterizing solution, follow. On the other hand, this long enclosure is by no means inevitable, and digestion may follow ingestion very rapidly. Thus in fig. 3, in which actual taking in of boiled white of egg went on under the microscope, the stage of storage (*a*) was but of short duration, for all the digestive changes which succeeded it (figured at *b*, *c*, *d*, *e*) were sketched in little more than one hour from the time when ingestion began. It may even be that there is no suspension of digestive activity at all; the fluid which the movement of aggregation defines is never lost in this case; on the contrary, it increases in amount as digestion proceeds, and the assumption that its constituents change seems justified. The promptitude with which secretory activity may manifest itself under changed conditions is represented in fig. 4, and this figure and fig. 2 *c.* further serve as illustrations of that temporary unlikeness in the fate of like ingesta to which reference has been made above. Digestion may be localized at first with apparent caprice; some food masses are taken and others left, though there is no final failure of activity but only delay.

It will be gathered from these statements that there is some difficulty in fixing within narrow limits the time during which *Carchesium* retains its nutritive ingesta; it would seem indeed that many factors cooperate to produce variations in the onset and duration of true digestive activity, and that the characters of the ingesta and the varying condition of the animal are equally potent with changing external conditions.

Among external conditions, however, there can be little doubt, I think, that **light** has a stimulating action. It is true that the necessary limitations of a "hanging drop" suggest that other influences, less readily appreciable, act upon specimens that are being watched under the microscope; still when due care is paid to irrigation and aëration, one is impressed by the constancy of a certain sequence of events. If the ingesta are in an advanced stage when observation begins (this happens with recently caught *Carchesium* which often holds greatly changed bacterial food) then there is speedy *discharge;* food is digested, or it may be, hurried to premature ejection when the animals are mounted during the *storage* intermission of secretory activity. Light does not produce appreciable lesion in the substance of *Carchesium;* the nuclei are unaffected, and even under artificial light* there may be vigorous ingestion: rather, it stimulates to advance in the digestive cycle,—to secretion, or it may be to ejection.

Apart from the influence of light, the presence of **fresh food material** has some effect on the attitude assumed by *Carchesium* to foreign matter already within its substance. It is too much to say that ingesta of one kind will replace those of any other kind, but ejection always appears to be promoted by fresh ingestion, especially when the preexisting ingesta have been enclosed for some time. In the case of *Amœba* I noticed the tendency to reject carmine or grains of starch when such highly nutritive ingesta as monads became available, and a slightly different adjustment of the same complementary acts, shows itself in *Carchesium* in an increased tendency to eject débris when many fresh ingesta are being formed. The difference consists in this, that nutritive matter is not clearly prepotent, at least in vigorous animals; the expediting action of renewed ingestion on (*ex. gr.*) the discharge of bacterial remains is as obvious when the newly enclosed matter is Indian ink, as when it is finely divided proteid.

Last among the external influences which affect the duration of enclosure I am inclined to place **mechanical stimulation**, such disturbance, that is to say, as is bound up with sudden change of environment and with manipulative interference. This is not often dissociated from change in illumination, and for this and other reasons I am ignorant of its true importance. But ejection sometimes begins synchronously with remounting, manifesting itself with a vigour which lessens presently, and I often had occasion to associate the discharge of effete matter in *Amœba* with mounting or transference of the animal; it seems difficult, then, not to regard some obscure contact stimulus as one more complication tending to hasten discharge.

It remains to consider the relation of ingested matter not to variation in external conditions but to the state of *Carchesium* at any moment. It is readily conceivable that, changing in condition, the animal would react changingly to constant stimuli, and that in this fashion variations in the manifestations of digestive activity would occur. Any attempt to estimate the value of this factor, however, must follow an examination of the way in which variation characterizes not only the whole time of enclosure but each succeeding phase.

1. *Extra-cellular phenomena.*—In an earlier part of this paper I described the way in which particles are gathered into the pharynx and gullet of *Carchesium* to aid in forming the vacuole of ingestion. If we disregard the wide intervals which may separate successive periods of feeding, and take no account for the moment of partial and ineffective accumulation of solid matter in the œsophagus of a lethargic form, it

* Any specimens examined by artificial transmitted light are exposed to rise of temperature in the medium in which they lie, as well as to intense illumination. I have found that signs of lesion appear very constantly with any considerable rise of temperature, but below this point I can draw no clear distinction between the effect of light and that of heat.





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becomes clear that there is considerable regularity in this preliminary act. Such variations as occur belong rather to different polypes at different times than to any one animal during one period of feeding. In vigorous ingestion the time which passes between the internal discharge of two succeeding vacuoles is usually 40 seconds; greater energy shortens the interval to 30 seconds, and in some cases, which are yet quite healthy, it is drawn out to 1 minute or rather more.* And since there is practically no intermission of ciliary activity, the time which separates the acts of ingestion may be regarded as measuring, too, the creation of the vacuole which is to move. Whilst watching any one polype I have never seen a sudden confusion or exchange of these time limits, such, for example, as an adoption of the 30 seconds interval for one of 1 minute; the rhythm is approximately regular, but in different animals its rapidity varies.

2. *Progression.*—It will be remembered that the position taken up by the vacuole of ingestion during *quiescence* is not quite constant, in other words, there is a journey of variable length through the substance of *Carchesium* before progression is at an end. And the time spent in this journey varies, but not, I think, directly with the extent of locomotion; it may be that a vigorous impulse effecting ingestion lasts long, carries the food mass far and moves it rapidly, and that a faint impulse acts sluggishly and fails soon. I give 10 seconds as the characteristic duration of normal *progression*, but the time may be lengthened to 14 seconds, or may be shortened to 5 or 6 seconds.

3. *Quiescence.*—The phase which follows progression is, to a certain extent, its complement; thus, it lasts typically for 9 seconds, may be shortened to 5 seconds or lengthened to 25, the variations tending to be inversely as those of the movement of progression. The correlation is incomplete however, so that the total time elapsing between *ingestion* and *aggregation* is somewhat inconstant; it may vary from 11 seconds to 37 seconds, but is commonly 20 to 24 seconds.

4. *Aggregation.*—Primary *aggregation*, when it is vigorous, is practically instantaneous. It is one of the most constant periods in this drama of digestive change, and among some hundreds of observations I have records of hardly more than a dozen which show any marked divergence on this point; $\frac{2}{5}$ second, $\frac{4}{5}$ second, or even 1 second represent the most frequent degrees of extension. I have one case in which $1\frac{4}{5}$ second passed before *aggregation* was achieved, and yet another case is recorded in which the lethargic character of the movement was so pronounced that I leave it for special consideration later.

5. *Retrogression.*—When primary *aggregation* is over, the digestive vacuole pauses for a variable time before the development of the slow retrogressive movement, and it is not easy to see how the impulse to retrogression arises. A certain displacement would, of course, result from the recurring arrival of fresh vacuoles of ingestion, but this movement is determinate in direction, and its onset does not necessarily coincide

* R. GREEF describes successive acts of ingestion as occurring after an interval of a quarter of an hour.

with the advance of new food material. In periods of vigorous feeding ingesta are passed back and reach the area of solution in 1 minute; this time may be doubled in animals which are apparently quite healthy, and, as I have tried to make clear in another place, the path is sometimes shortened, and sometimes shows irregular extensions of which no account is given in the purely diagrammatic scheme of fig. 1.

6. *Storage.*—Passing into the large central area where food is stored as well as digested, the ingesta of *Carchesium* enter on the most variable period of their sojourn within its substance. After gradual loss of the fluid which first became obvious during *aggregation*, they may lie for 12, 18, or 20 hours showing no sign of change; on the other hand, I have seen vigorous re-formation of a vacuole, and the onset of solution only 30–60 minutes after the first loss of fluid. This shortening of the storage stage is associated with digestion under continuous observation, and finds its extreme expression in the entire omission of any pause and loss of vacuole; *solution*, in this case, follows *retrogression*.

7. *Solution.*—This phase, which may be regarded as giving point to all the preceding ordered marshalling of ingesta, is much more constant in length than is the stage of antecedent storage. It is obvious that the matter upon which the fluid of each vacuole has to make its attack varies in intrinsic digestibility, in admixture with innutritious foreign particles, and in amount and density. Thus, there is undoubted qualitative difference between the substance of bacteria and coagulated white of egg. Without attempting any rigid definition of the time limits of the solvent process, then, I will only say that characteristic transparency of the ingesta is unmistakable 15 or 20 minutes after the vacuole has re-formed, and that within an hour the change in aspect—often with great reduction in size—is very marked. In like manner, when the storage stage is omitted and we deal not with a freshly-formed vacuole, but with enlargement of one pre-existent, and (presumably) with variation in its constituents, signs of solution are striking within an hour.

8. *Ejection.*—As forming the last period in this history of intracellular phenomena, I group together the actual rejection of matter and its antecedent stay in what I have called the area of discharge. The preliminary pause is sometimes long and the transference of the débris of ingesta from the area of solution may be slow and irregular, even when all appreciable solution is at an end. Hours may, indeed, intervene here, while, on the other hand, I have seen the much changed remains of nutritive ingesta discharged within 1½ hour after enclosure. This is the stage which is shortened most readily by mechanical, and possibly by chemical stimuli; it is indeed not uncommon when disturbances of such a nature are set up, to see mucilaginous débris, apparently the accumulation of hours, discharged in irregular succession. The act of ejection is rapid, but each mass of waste matter is passed out into the pharynx without any constant accompaniment of fluid, and may linger there for some seconds, even for minutes, before it is finally set free.

Up to this point I have been speaking of the fate of nutritious ingesta with or without admixture of innutritious substance; the case in which the nutritious element is minimized,—it may be, abolished, needs one word of description. When carmine grains, particles of Indian ink, or of ultramarine blue are taken in by *Carchesium*, there is no appreciable change in the first responsive events, and to the end of the movement of retrogression the time relations of the different periods are practically unaltered. Later, however, two divergences from the programme I have just sketched are striking. In the first place, the whole time of enclosure is shortened. This has been stated in the general description of the fate of innutritious matter given on p. 367, and I will merely add, in the first place, that constant observation, mechanical disturbance, and all those conditions which tend typically to abbreviate the digestive cycle are especially potent here and often promote vigorous discharge. Ejection may indeed be considerable, 50, 40, or even 35 minutes after the beginning of ingestion. In the second place, it is mainly the stages which correspond to *storage* and *solution*, which are curtailed or obscured. I have before alluded to the fact that there is unusual persistence of fluid in rare instances, but these can hardly be regarded as exceptions to the statement that loss is not followed by renewed secretion; that there is no active re-formation of vacuoles if the direct descendants of the vacuoles of ingestion disappear. In the great majority of cases the pigment masses lie as if stored until they pass to the area of discharge before ejection. And here the time relations of succeeding acts approximate again to those which characterize the end of true food stuffs. There is nothing distinctive in their journey to the area of discharge nor in the act by which they are ejected.

In the following Table I have gathered together most of the numerical results which have just been discussed; it will be seen that rather pronounced variability interferes with definite statement in some cases; I have therefore given, where it is possible, the time which I regard as belonging typically to each event, and the extreme variations from this, which I have recorded.

| | Total time of stay in substance of *Carchesium*. | Extra-cellular gathering of ingesta. | Progression. | Quiescence. | Aggregation. | Retrogression. | Storage. | Solution. | Ejection and preliminary stay in area of discharge. |
|---|---|---|---|---|---|---|---|---|---|
| Shortest time recorded | 30 mins. (innutritious matter) 1 hr. or $1\frac{1}{2}$ hr. (nutritious matter) | 30 secs. | $5\frac{1}{5}$ secs. | 5 secs. | Instantaneous | 50–60 secs. | Omission or 30 mins. | 50 mins. | 10–20 mins. |
| Longest time recorded | 30 hrs. | 65 secs. | $14\frac{2}{5}$ secs. | $25\frac{2}{5}$ secs. | $1\frac{4}{5}$ sec. | ? 5 mins. | ? 22 hrs. | ... | Some hours |
| Typical time recorded | ... | 40 secs. | $10\frac{2}{5}$ secs. | 9·04 secs. | Instantaneous to $\frac{2}{5}$ sec. | 1 to $1\frac{1}{2}$ min. | Some hours | | |

**The significance of some of the phases through which ingesta pass in the substance of Carchesium.**

*The meaning of aggregation.*—The striking feature of this phenomenon is undoubtedly the movement of suspended particles, the fundamental feature is the existence of a force which moves them—a force constantly centripetal, though not always perfectly symmetrical in action, and operating instantaneously in times of vigour.

The constantly sharp outline of the mass of moving particles, whatever the actual displacement of its constituents, the persistent central cohesion, not only of possibly glutinous matter, but of oil drops or grains of Indian ink, and an occasional lack of synchronism* in action, forbid the hypotheses that aggregation is due solely to the centripetal discharge of fluid into the vacuole from the surrounding protoplasm, or to the separation and contraction of a highly elastic lining film. On the other hand, all these facts are in harmony with the view that the phenomenon is essentially one of shrinking—that there is rapid retraction of some viscous matter which entangles in its substance, and thus aggregates any solid particles present in the vacuole of ingestion. This hypothesis finds further support in the considerable staining power of food masses formed from coagulated proteid, as contrasted with the reaction of the proteid particles before ingestion, in the persistent shrinking exhibited by ingesta until they are stored, destitute of vacuoles, and in the phenomenon of double *aggregation* (*v. sup.*). And certain secondary modifications of the movement, which are observable at times, offer no hindrance to its adoption. Thus, in some specimens of *Carchesium* which are apparently lacking in vigour, a food mass formed by *aggregation* may cling for a time to the walls of the vacuole by delicate viscous threads springing from, and finally retracted to, its poles (fig. 6, *b.*); at other times so much fluid is entangled in a food mass as it forms that it is clearly, if temporarily, vacuolate, and in yet other cases the central particles contained in a vacuole of ingestion may show lingering movement, while retracting hyaline substance separates them from the periphery of the vacuole. These modifications occur rarely, and I regard each as an expression of partial failure in the action of the mechanism which underlies *aggregation*; there is, as the case may be, localized delay in the retraction of the viscous matter, or localized lethargy in its maturation.

*The Origin of the Retractile Substance.*—The formulation of this hypothesis as an interpretation of the phenomenon of aggregation leads naturally to the darker problem; how and where is the retractile substance formed? The continued ingestion of rich food by *Carchesium* does clearly increase the granularity of its cell substance, but the converse activity of secretion brings about as little undoubted

* In fig. 6, *a*. $a_1$, a marked instance of this intermittent action is illustrated; after most of the particles were stilled, Brownian movement persisted at the poles of the vacuole; finally, the last vagrant granules were gathered in, forming cap-like additions to the main mass.

structural change here as in the case of *Amœba* or *Actinosphærium*.* A polype, in which fifty successive acts of *aggregation* have been observed, does not, save for its abundant ingesta, differ from one that is fasting. It is rather on *à priori* grounds, then, that I am inclined to localize the formation of viscid matter in the period or periods which precede *aggregation*. The period of *quiescence*, which has so dramatic an ending, is in itself devoid of obvious event; there is then too great readiness, perhaps, to associate with it, in thought, some phasic heightening of obscure molecular activity. As a matter of fact, *quiescence* tends to vary inversely as *progression*, in other words, a fairly constant interval elapses between the moment of ingestion and the aggregation of ingested particles. And, throughout this interval, there is typically continuation of " proper " movement or of Brownian movement with undiminished energy, though rarely I have seen considerable excursions of the peripheral particles in a vacuole round an apparently viscid central mass. I conceive that the retractile substance does not, when first formed (possibly immature), offer an effective hindrance to the oscillation of fine particles or the movement of small organisms, that it may vary in amount and condition, thus offering a varying resistance to movement, and that it is probably accumulated in the vacuole of ingestion during the period of quiescence, although the possibility of earlier formation cannot be excluded.

Lastly, it may be asked what initiates the act of shrinking? Why does the viscid substance in its first retraction move with such vigour? I would suggest that the phenomenon is a modified clotting action. In all perfected clots there is extra-cellular interaction of two bodies, or it may be, reconstitution of one body, and with the chemical change physical change is associated—a separation of solid matter, varying somewhat in character, a subsequent shrinking, more or less pronounced.

* I may add that here (once more, as in the case of Rhizopods) the data are lacking yet, which would enable me to correlate any change in the rhythm of the contractile vacuole with the waxing or waning of digestive activity. All the observations which I have made lead me to associate incompleteness or paralysis of the characteristic pulsations with such lesions of nutrition as occur when aëration is deficient, or when *Carchesium* is poisoned with carbonic acid, that is to say, with phases in the metabolic cycle more remote than those concerned with the solvent processes of digestion. I hope at some time, and after more experiments, to discuss this point.

To any one familiar with the structural characteristics of the *Protozoa*, it will seem gratuitous to insist on the sharp contrast which the phenomenon of *aggregation* offers to the rhythmic movement of a *contractile vacuole*, so little have the two processes in common. I have pointed out in a former paper (' Journal of Physiology,' vol. 8, p. 264,) that in *Amœba* "the true digestive vacuole shows no sign of contractility," and the statement holds in the case of *Carchesium* without qualification. The gathering together of ingested particles, which is so striking and so sudden in *aggregation*, only throws into clearer relief the unbroken line of cell-substance bounding the vacuole; on the other hand, the typical rhythm of the *contractile vacuole* owes its very existence to the spasmodic approximation of the vacuolar walls, which advance, obliterating for the time the appreciable fluid-filled cavity. A *contractile vacuole* is, without exception, destitute of foreign enclosures; the process of *aggregation* is bound up inseparably with the movement of recently ingested solid particles.

Some evidence has been brought forward for the belief that such a separation of solid matter takes place in the digestive vacuole of *Carchesium*, and it may be conceived that there is an extra-cellular (but intra-vacuolar) discharge of matter which clots and which is predestined, by virtue of its proper constitution, not to gradual increase of viscidity, but to a quick shrinking, almost synchronous with its first deposition, and as obvious as the other contents of the vacuole will allow. It may be said that this conception is too hypothetical to be of value, but I would urge that the contrast between the fluidity which precedes *aggregation* (in the vacuole of ingestion) and the viscidity which it initiates is very sharp, and that the quickness of retraction—no necessary part of typical clotting—may be determined to some extent by a new factor in the vacuolar fluid. When delicate indicators of the presence of acid* are given to *Carchesium* the maximum change of tint which they show is associated with the stage of storage; the onset of change dates from the phase of quiescence and becomes more marked during the movement of retrogression. In other words, the acid reaction of the digestive vacuole is perceptible about the time that *aggregation* is accomplished, and though I have failed to detect an increase of size in the vacuole at the instant when movement is striking, it is clear that a secretion of fluid, even important in character, might, if diffused over the surface of the vacuole, affect any one diameter but slightly, or that localized secretion coexisting with localized absorption might leave the total bulk of accumulated fluid unchanged. Bearing in mind the points which have been mentioned more than once, the sharp demarcation of aggregated matter from the fluid which surrounds it, the onset and occasional accentuation of change at the periphery of the vacuole of ingestion, I venture to put forward the view that the acid fluid does not act by sharing directly in the stilling of motile ingesta, which is so characteristic here, it rather heightens the instability of previously secreted matter, so that clotting is induced.

The whole mechanism of retraction, then, may be thus conceived. At the moment of aggregation, substance which has been secreted during the phase of quiescence, probably in an immature condition (*i.e.*, as immediate antecedents of the final body), undergoes a change which may be regarded as a specialized form of clotting. Shrinking as it clots, it entangles solid particles which lie near. At the same time there is an access of acid fluid to the vacuole of ingestion, and it seems probable that this change of medium is advantageous to the effective retraction of the clot or even helpful to its first formation.

### The Biological Value of Aggregation.

It remains to consider the relation borne by the phenomenon which I have described

* The presence of acid in the digestive vacuoles of *Protozoa* has been proved by METCHNIKOFF ('Ann. de l'Inst. Past.,' 1889) and LE DANTEC (*Ibid.*, 1890). I have discussed its relation to the process of digestion in *Carchesium* in a paper which is yet unpublished.



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to general protozoan digestion. Is it, we may ask, peculiar to such a highly specialized infusorian form as is *Carchesium*, or is it raised from the level of the merely interesting, to be dignified as a forcible illustration of some fundamental and often hidden process? It is not easy to offer direct experimental evidence in favour of the latter view, but the body of indirect evidence which tells in its favour is not inconsiderable.

1. In records which describe the digestive process in the *Vorticellidæ* there is unanimous reference to the spherical masses of food which, circulating in the endoplasm, are so striking optically; and GREEF* in his "Investigations into the Natural History of the *Vorticellidæ*," gives the following brief account of their formation in *Epistylis flavicans*. The freshly ingested food mass is spindle-shaped, and passes towards the base (attached extremity) of the body, arching round again to pass anteriorly (orally) to the level of the point of ingestion. Here a little knob arises at the pointed end of the mass, and immediately the whole is gathered together to a spherical lump. Other observers are even briefer in description; the variations in size of ingesta, the presence or absence of encircling fluid, the elliptical shape of the vacuole of ingestion, all these are noted or form the subject of discussion, without any minute account of the details of successive phases. It is clear, I think, that the ingesta have been observed, as a rule, after *aggregation* is over, but that the statement given by GREEF is really an abbreviated account of the actual process in *Epistylis flavicans*.

2. In Infusoria, other than the *Vorticellidæ*, the references and descriptions also deal with ingesta, in which the welding process is complete. Thus EHRENBERG† based his celebrated "polygastric theory" on the very general occurrence of "Magenzellen," describing them in forms as unlike as *Monas* and *Stentor*. It has long ago been shown that the "Magenzellen" are spherical food masses; that the method of their formation has been left unrecorded is hardly surprising when we remember that these infusoria are often vigorously motile and sometimes opaque, and that details of the process of aggregation have been lacking hitherto, in a transparent, stationary form like *Carchesium*.

3. In discussing the formation and history of digestive vacuoles in ciliate infusoria, BÜTSCHLI‡ draws a distinction between those animals which ingest relatively large food masses and those in which particles, generally minute, are swept into the pharyngeal tube by ciliary action. He points out that the concomitant ingestion of water is obvious in the latter case, and the vacuoles, in which ingesta lie, are distinct; when the food masses are large, fluid surrounds them clearly only some time after the act of ingestion. I refer to the distinction thus drawn, because it throws some light on the early history of ingesta in *Rhizopods*. In these animals there is no disturbing

* R. GREEF, *loc. cit.*

† C. G. EHRENBERG, 'Die Infusionsthierchen,' Leipzig, 1838.

‡ O. BÜTSCHLI, "Protozoa" (BRONN'S 'Klass. u. Ord. d. Thierr.').

excess of motility, indeed, the act of ingestion has been described in some detail; but there is, I think, no published account of the process of aggregation. I would urge, however, that there is formation of a viscous substance, which unites scattered ingesta during their stay within *Amœba,* and that its presence, hidden by the fact that the food here is often massive, is indicated by the following experimental results: (*a*) In watching the process of digestion in *Amœba*, I have often seen the enclosure of such active infusoria as monads. And in the notes on these observations (made some years ago*) I find frequent mention of the fact that a monad continued to move in the vacuole of ingestion for 5, 7, or 10 minutes, and thus was suddenly and finally reduced to quiescence; the statement seems to afford a parallel to the sequence of *progression*, *quiescence*, and *aggregation* in *Carchesium* (2). (*b*) Again I was struck, even at that time, by the cohesion of ingesta during solution. I may perhaps illustrate this by reference to an experiment in which a monad and a green protococcus were received into one vacuole of ingestion. The complex mass was watched for six hours, and, after the first onset of change, there was no break in the union of the two bodies; *e.g.*, at the end of the third hour the monad "found a small semicircular mass, fitted as by some force over one part of the circumference of the protococcus;" at the end of the sixth hour the relative position of the two bodies was unchanged. (*c*) Lastly, I may say that this cohesion was not confined to true food stuffs, but probably characterized such insoluble particles as grains of carmine and Indian ink. Thus I described the finer granules of those bodies as moving after ingestion "not freely, but gathered into groups by a basis of hyaline substance which is generally spherical and often difficult to observe." It seems not improbable that it was a secretion and subsequent retraction of viscous matter quite comparable to that formed by the protoplasm of *Carchesium*, which gave the "basis" its spherical form and united the scattered grains of pigment.

If this indirect evidence be allowed to have weight, it supports the hypothesis that the secretion of a viscid retractile substance round ingesta is a widely spread process in *Protozoa*. The *Coelenterata*, where the digestion is localized for the most part in the body cavity, show, as is well known, marked gland cells in their endoderm, and the digestive fluid apparently takes origin in the intra-cellular solution of secretory granules; in *Protozoa* no such formed secretory products can be detected in the substance of a resting cell; the outpouring of the body, which is destined to clot and shrink, is coextensive with their absence.

It is possible, I think, to examine still further, the relation of aggregation to the whole process of digestion. In an earlier paper I have advocated the view that, while the ingestion of solid matter is promiscuous in *Amœba*, the later history of ingesta varies according as they are nutritious or insoluble. In the latter case, they lie in the

* 'Journn. of Physiol.,' vol. 7, p. 269.

endosarc, not surrounded by fluid ; nutritious bodies, on the other hand, are digested by the fluid poured around them. In *Carchesium*, nutritious matter is aggregated, bathed in acid fluid, and then, by gradual loss of surrounding vacuole and continued shrinking, reaches its maximum density in the storage stage. Later, it swells under the action of freshly secreted fluid until it is difficult to draw the line between swelling and solution, and possible to pass from the fluid periphery of a digestive vacuole, through increasing viscidity, to a fairly solid central nucleus. It is after the secretion of this second fluid that food stuffs are dissolved. Innutritious matter shows quite comparable accumulation of fluid and subsequent loss with maximum approximation of its particles, especially if ingestion be carried on in the dark. Mounting at this stage provokes ejection, but not, as a rule, fresh secretion of fluid. In fact, the great majority of experiments support the view that the cell substance is, on the whole, discriminating in its secretion of fluid with which the solution of food is immediately associated, but that the formation and clotting of viscous matter in the vacuole of ingestion is called forth by ingesta of all kinds.

Since the food ingested naturally by *Carchesium* is very largely made up of bacteria, and may therefore be actively motile, it is conceivable that there is need of some speedy and effective action on the prey, and that this necessity has concentrated the secretion of the potent retractile substance and generated the habit of uniform response to all ingested matter. On the other hand it may be that indiscriminate aggregation is arrived at in quite another fashion; the viscid matter is so intimately related to the actual process of digestion, that no entering vacuole, whatever its contents, is sent on unprovided with its share of so important a factor.

Either of these suppositions is in harmony with the last point I wish to mention as suggestive of the far-reaching significance of aggregation, I mean its time relations to the other events in the digestive cycle. Once aggregated, the most active bacteria move no more; when ingesta have attained to the stage of storage they are at the point of maximum shrinkage, and are at the same time ready for subsequent solution. We find, in accordance with these facts, that the time elapsing between *ingestion* and *aggregation* is more nearly constant than any other period in the process of digestion, and that, on the other hand, the storing of ingesta may be as vigorous in character as it is varying in duration. I have counted one hundred stored clusters of bacteria in one polype of *Carchesium*, and it has been mentioned above that no interval or an interval of many hours may be introduced between the end of the movement of retrogression and the onset of solution. The possibility of such excessive accumulation is to be associated, probably, with the wide intervals which may elapse in the free state between successive periods of feeding; and there is obvious fitness in the introduction of variation here, for the ingesta are reduced to inertness, and to their minimal size, and therefore make less demand than at any other moment on the functional activity of the animal in which they lie.

*Note.*—It remains for me to mention a modification of one phase in the digestive process to which reference has been made above. This modification is an extreme case of lethargy in the aggregation of particles. A specimen of *Carchesium* was mounted for observation at 12 noon, and for fifteen minutes showed vigorous ingestion of Indian ink; at the end of this time, coagulated proteid (probably mixed with some bacteria) was substituted for the pigment, and some further ingestion followed. Observation was then suspended for four hours, and at the end of that time ingesta were being formed by persistent or renewed activity; the particles which helped to make up the vacuoles of ingestion moved so vigorously, that I think they must have been, in the main, motile bacteria, and, watching for the act of aggregation, I saw that five, six, seven, even nine seconds elapsed after the onset of centripetal movement before there was complete quiescence. A homogeneous rim or shell of colourless substance seemed to encircle the particles which moved freely within it until such time as the stilling force was felt even to the centre of the mass.

This variation illustrates an extreme case of initial localization of the retractile substance; the exceptional factors which are obvious here and may help to give the phenomenon its exceptional characters are, preceding secretion of some magnitude on the one hand—on the other, vigorous movements of ingesta; it may be then that the formation or outpouring of the forerunners of the viscid matter was languid, or that, this being so, the actively motile particles determined, in some fashion, its localized formation; on the next day the same polypes showed instantaneous *aggregation*, so that the whole condition was only temporary.

## Summary.

1. *Carchesium polypinum* offers in many ways a particularly good field for the study of some of the processes involved in protozoan digestion; ingestion is often eager, digestion may be rapid, and the especially transparent cell substance which characterizes this animal allows the observation of both.

2. One striking feature of any specimen examined after the administration of abundant nutriment is the presence of numerous spherical masses of food; these may number one hundred in one polype of *Carchesium*, they show remarkable solidarity, and, on the whole, uniform size, and as the solid particles ingested are invariably minute, the formation of the relatively large succeeding ingesta is a matter of some interest.

3. Continued watching shows that each spherical food mass springs from one vacuole of ingestion, and in the following fashion: the vacuole discharged internally from the œsophagus by some obscure impulse, and made up of water, and (as the case may be) amorphous matter, motile particles or inert particles, passes towards the base of the animal. It pauses internally at the curve made by the nucleus in this region, and without further locomotion, or after slight rotatory movement the finely divided solids which it contains undergo a sudden and striking rearrangement. All the granules present are shifted centripetally, all individual movement is stilled. A composite solid, lying in clear fluid surroundings, represents the scattered particles of a moment ago.

3a. To this centripetal shifting the term *aggregation* may be applied conveniently. It is demonstrable most effectively in vacuoles which are made up of much fluid, and but little finely divided solid

matter; it is masked sometimes by the number or size of the particles involved. Further, when the ingested matter has a certain definitely complex composition (and there is some evidence for the view that a soluble food stuff must be present), there is a more pronounced modification of action; two centripetal movements of matter may be distinguished—the first, a quick rearrangement by which most of the discrete particles present are gathered to form a central "nucleus"—the second, a prolonged retraction of matter separated out symmetrically or asymmetrically from the clear vacuolar fluid, and fitting finally like a shell or cap over the mass first laid down.

3B. From the point of time at which the centripetal gathering of substance takes place the fluid of the digestive vacuole begins to show an acid reaction.

4. The spherical food masses thus welded from scattered fragments then journey through the substance of *Carchesium* in a fairly constant fashion, but for a variable time; they are stored occasionally for some hours, while at times the beginning of digestion follows at once on the act of aggregation. All the ingesta present are not, of necessity, digested synchronously, but this may be regarded as invariable—that the storage of matter for any length of time before digestion is accompanied by loss of fluid surroundings, and that solution is effected in a fluid medium. Stored ingesta are constantly dense and shrunken; solution implies swelling, transparency, the persistence or re-formation of a well-marked vacuole.

5. Digestion may take place at any point throughout a relatively large part of the central substance of *Carchesium*, but the region from which insoluble matter is rejected, is, like the place of ingestion, definite. A vacuole of ingestion passes into the protoplasm from the extreme internal point of the œsophagus; effete matter is passed into the pharynx at the junction of its external and middle thirds from some spot in a ridge running transversely to the long axis of the polype in that region.

6. The ingestion of matter by *Carchesium* is indiscriminate when the particles concerned are sufficiently small, and nutritious and innutritious substances exhibit alike the striking centripetal clustering which has been described; the intra-cellular sojourn of innutritious bodies is curtailed however, the vacuoles in which they lie at first tend to disappear quickly, and there is but rarely that re-formation of fluid which is so nearly concerned in the solution of true food-stuffs.

## Explanation of Plate.

Fig. 1. Plan of *Carchesium*, to show the typical path of nutritive ingesta; purely diagrammatic. The red line marks the course from which (for the sake of clearness) some possible complications are omitted. Arrows indicate the direction of movement; the points at which striking actions occur are numbered in order.

1. Ingestion.
2. Quiescence.

2*a*. Aggregation.
3. Solution.
4. Ejection.

These points must be regarded only as the centres of areas which bear respectively the same relation to the process of digestion. I have tried to denote this by variations in shading, which, however, must be regarded as indicating the position of the areas, rather than as defining their extent.

Area of progressive movement—shaded in rings.

Area of quiescence—shaded in crosses.

Area of retrogressive movement—shaded in dots.

Area of solution (ingesta linger here during digestion)—shaded in transverse lines.

Area of discharge (ingesta gather here before ejection)—shaded in transverse and longitudinal lines.

In all the figures, *nuc.* = nucleus.
*ph.* = pharynx.
*œs.* = gullet.

Arrows indicate the movement of vacuoles, or of substances in a vacuole.

The ingesta are often arranged diagrammatically to avoid overlapping, and, in figures 3, 4, 5, and 7, are drawn with a camera lucida under Oc. 3, Obj. F. Zeiss, and afterwards reduced. The figures are all taken from *Carchesium polypinum*, but I have attempted no reproduction of the structural details of the animal, nor of such specialized portions of its substance as the peristome and the ciliary wreath. The outline of the polypes is drawn to scale in order that the localization of the ingesta and their size may be realized.

Fig. 2. A.—Ingestion of Indian ink suspended in water.

To show (1) usual shape of vacuole of ingestion (*ing.*).
(2) change produced by primary aggregation (*K*).
(3) ejection of a mass of Indian ink, from which outside grains have been freed (1).
(4) ejection of a mass entirely coherent, as after *aggregation* (1*a*).

B.—Concomitant ingestion of Indian ink and precipitated white of egg

*ing.*, vacuole of ingestion.

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*a*, *b*, *c*, *d*, secretion of fluid by means of which the coagulated proteid is digested. The undigested grains of India ink remain, and in (*d*) approach the area of discharge.



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C.—Ingestion of coagulated proteid.

*ing.*, vacuole of ingestion; proteid particles lie scattered throughout the vacuole.

*s*, stage of storage.

*d*, one proteid mass reduced in size by subsequent digestion.

Fig. 3. To show digestion of coagulated proteid in *Carchesium polypinum*.

*k*, aggregation has just taken place.

*a*, stage of storage.

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*c*, *d*, reduction under solvent action of fluid.

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Fig. 4. To show digestion of a solid mass, probably of bacteria, which had been for some hours in the stage of storage.

*s*, stage of storage; observation began at 11.45 A.M.

*a*, *a′*, two ingesta sketched at 12 noon.

*b*, one of these ingesta. Sketched at 12.10 P.M.; shows slight increase in transparency, and swelling.

*c*, 12.18 P.M. Reduction in size begins to be marked.

*d*, 12.45 P.M. Digestion has produced far-reaching change; the undigested residue still forms a coherent mass, now with shadowy ill-defined edges.

Fig. 5. To show modifications of the typical digestive act.

*ing*, vacuole of ingestion, containing stale white of egg and Indian ink.

*K*, primary aggregation; nearly all the grains of pigment have shared in the centripetal movement, but the vacuole left outside is not clearly fluid.

$k_1$, second phase of double aggregation; a cap-like mass of substance fits over the first pigmented accumulation; clear fluid lies outside.

*s*, the same mass after secondary shrinking; storage stage.

*a*, later; digestion has begun, and some of the grains of pigment are set free into the fluid vacuole.

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*l*, *mu*, *ir*, from other experiments.

*ir*, to show distorted aggregation, produced sometimes by the ingestion of solid particles with a viscid nutritive solution.

*mu*, ingestion of Indian ink with some mucilaginous matter which was visible obscurely from the moment of enclosure.

Fig. 6. Ingesta of *Carchesium polypinum*.

*a*, $a_1$, to illustrate interrupted aggregation; in *a* the terminal grains of Indian ink are still in movement; in $a_1$ the aggregation is complete, but the grains gathered in last are not yet distinguishable from the rest.

*b*, vacuole of ingestion. *Aggregation* is just complete; slender viscous threads join the central mass of proteid to the polar boundaries of the vacuole.

*c*, ingesta lying in a common vacuole; the result of fusion of vacuoles during *retrogression*.

*d*, similar fusion of vacuoles towards the end of the stage of solution.

*c*, ejected mass of Indian ink; there is a central, solid nucleus and some (?) mucilaginous basis unites the outlying granules.

Fig. 7. Ingesta of *Carchesium polypinum*.

$a$, $a_1$, to show extensive centripetal movement of two fat globules enclosed in a very fluid vacuole of ingestion. Arrows mark the direction of movement.

*b*, carmine and bacteria drawn after *aggregation;* movement has been slight, and the composite mass is bulky still.

$b_1$ (the same mass drawn after two minutes); shrinking has gone further, the fluid of the vacuole is separated more clearly.

*c*, ingestion of milk; the fat globules are held together by some almost invisible basis.

Fig. 8. Ingesta of *Carchesium polypinum*, illustrating the phenomenon of double aggregation.

*a*, vacuole of ingestion.

*b*, after primary *aggregation*; the first solid matter separated out leaves the vacuolar contents slightly opaque.

*c*, the second slow movement begins.

*d*, the second movement is over, and a double mass lies in clear fluid.

*a*. In this sequence the second *aggregation* involves a movement of *granular* matter.

*b*, after primary *aggregation*.

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Fig. 9. Adapted from R. GREEF.

Diagrammatic representation of the alimentary canal in one of the *Vorticellidæ;* to contrast the nomenclature of GREEF with that of LACHMANN.



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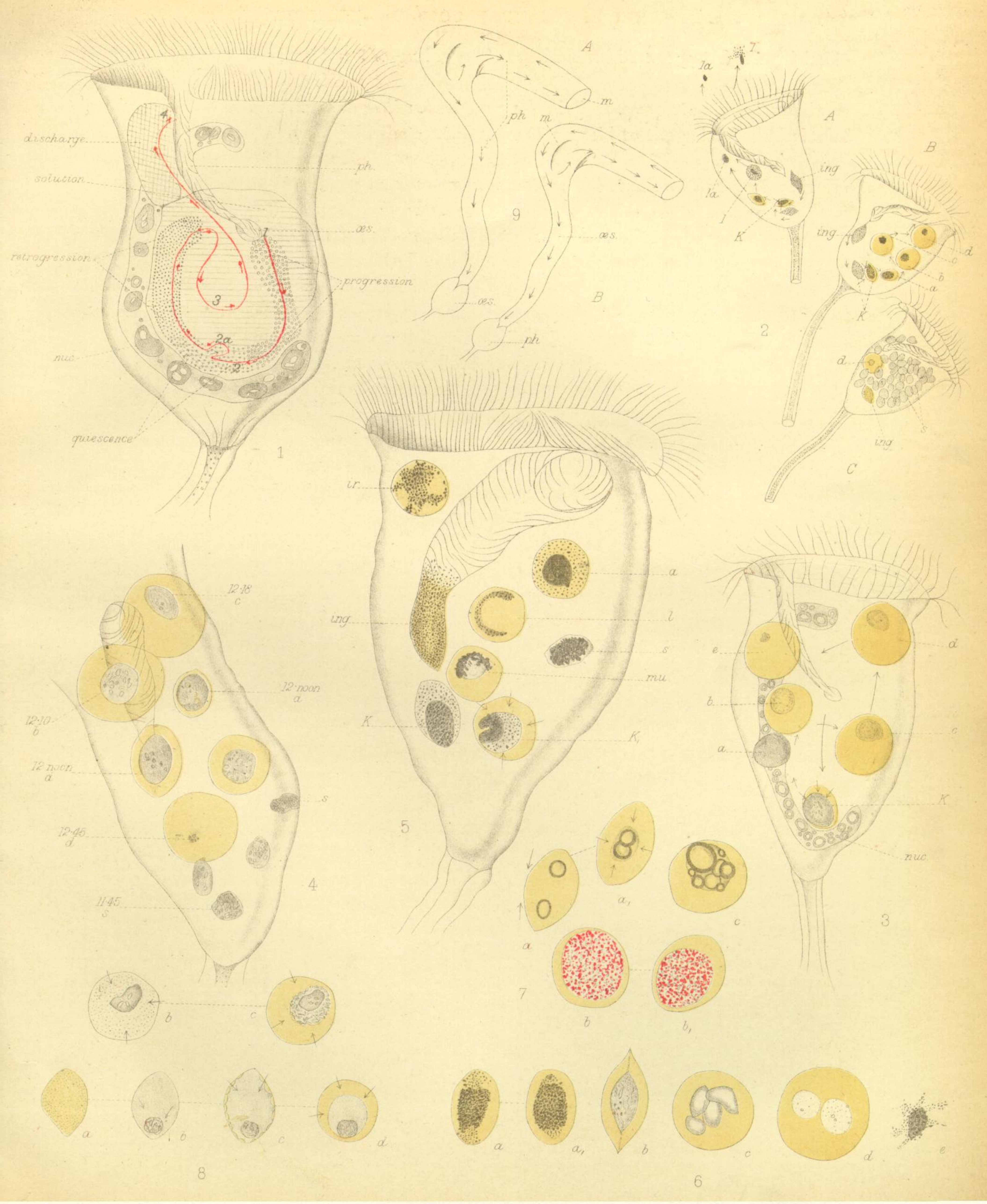
W.G. del. Edwin Wilson, Cambridge.



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