PHYSIOLOGY OF THE CYSTMENT EXAMINED ON THE COLPODA FASTIGATA

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The cystment is a well-known incident in the life of the unicellular animals. Its attraction, its cytological and physiological significance roused the interest of a great number of investigators. Investigations first aimed at the conditions of the en- and excystments. Such factors were looked for that induce the unicellular animals to form thicker, for the most part stratified ecto- and thinner endocysts and to leave it under proper circumstances.

The opinions regarding the nature of the factors producing the formation of cystment are divergent, sometimes contradictory. Its reason is to be attributed to the various experimental conditions and circumstances of the observations. Consequently at present it is untimely to draw conclusions relating to factors known as controlling the en- and excystmen such as quantity of food (3, 8, 15, 16, 19, 25), the presence of excretion products of the microorgans (3, 13, 14, 17, 18, 22) and of decomposition products of protein (14, 21), changes in the quantity of oxygen (8, 13, 18), in temperature (13, 16, 21, 18) and in pH (13), osmotic phenomena (3, 13) etc and be considered as a union. Still more complex becomes the problem by the suggestion wherely the cystment of the protozoa is considered to be a resting cycle which occurs even if the environmental conditions are favourable to the active life. On the basis of the examination of soil protozoa VARGA (26) of the Hungarian investigators, and myself too (4, 5), advocate this suggestion. I supposed that the frequent drying of the soil could bring about such properties of the protozoa after a long period. This hypothesis roused partly my interest and prompted me to deal with the physiology of the cystment.

The phenomenon of the cystment is followed by the radical change in the structure and vital processes of the protoplasma. The phenomena of life are partly and some are entirely inactivated. The protoplasma loses water (10, 11, 21) and sometimes shrinks to its 1/8 (10). The enzymatic activity of the produced plasmagel, the respiration (20) and the metabolism are considerably lessened. The process in some of the protozoa can experimentally quite easily be produced, but can be also easily reversed. To study the succession of the

changes is very important from the view point of the life and structure of the protoplasma. This is an other reason calling my attention to the process of the cystment.

The aim of this paper is to give an account of the connection between the cystment of the *Colpoda fastigata* Kahl and the culture medium substances of the changes and modification of the *viability* of the encysted animal, of the factors of the cystment, of its cyclic or continuous character.

Material and methods

Problems referring to the cystment have been studied on *Colpoda fastigata* Kahl taken from the soil, cultured from one cyst. It is easy to cultivate, well cysted, thus very suitable for examinations. The experimental animals were bred in aqueous extracts of different roots, as root-extracts proved to be significantly more efficacious for cultivation than any other part of the plant (4). Moreover they considerably stimulate the excystment in strong dilution.

Various cultures were used. The single series of these cultures are denoted with capital letters for the sake of perspicuity (»*A*« consisted of culture 4—6 and »*C*« of culture 2—2).

»*A*«-series: root-extract cultures of *Colpoda fastigata* taken from the root of single plant-species. These cultures had been in my earlier investigations examined to see what effect have the aqueous extracts of the root on the *Colpoda fastigata* and bacteria and on their numerical formation and on the encystment (4). Shortly after the encystment the single cultures kept in Petri-dishes were wellnigh simultaneously dried. After a year and a half all of them were poured with the decoction of the mixture obtained from the root or bulb resp. of the *Pulmonaria officinalis, Salvia nemorosa,* and *Colchicum autumnale* each of them well stimulating the excystment. Thus in the different root-extract cultures such as — *Achillea millefolium, Allium angulosum, Aristolochia clematitis, Datura stramonium, Cichorium intybus, Daucus carota, Mentha longifolia, Ononis spinosa, Nonea pulla, Reseda lutea, Salvia nemorosa, Solanum dulcamara,* and *Verbena officinalis* — produced cysts could excyst due to the common effect of the root-substances. From the rate of the excystment the common effect of the bacteria present in the earlier cultures, the metabolic products of *Colpoda fastigata* and of the root-extracts on the cysts and on their viability could be concluded. Data obtained by earlier method (4) are referred to 0,5 ml of the culture fluid on the basis of the quotient of the size of the cover-glass and of the field of vision.

»*B*«-series: Root-extract cultures of *C. fastigata* made of root mixtures of various plant species. Plants for examinations: *Aristolochia clematitis, Daucus carota, Oenothera biennis* and a little *Colchicum autumnale.* Into one part of the cultures, the fluid in the ratio of 2 : 1 of the root extract culture was filtered where previously a great number of the *C. fastigata* had been encysted. In such cultures the common effect on the cystment of the compounds of the active bacteria, as well as of the *C. fastigata* produced during its life and cystment, could be measured. The pure root-extract cultures were used as control. In the single cultures fresh cysts were inocculated (6 in a field of vision of 0,7 mm diameter) at the beginning of the experiments. They rapidly excysted. Their encystment, quantitative formation could be well observed.

»*C*«-series: Hay-decoction cultures. In these cultures the encystment of the *C. fastigata* was examined in the presence of other kind of ciliata. In the fresh cultures the combination and number of the protozoa at the time of the inocculation was as follows:

a) *Paramecium caudatum* (7 pieces) and *Pyxidium asymmetricum* no. sp. free-swimming *Peritricha,* becoming permanent (6 pieces).

b) Free swimming *P. asymmetricum* (10 pieces) and *C. fastigata* (10 pieces).

c) Paramecium caudatum (12 pieces) and *C. fastigata* (15 pieces).

»*D*«-series: I have examined the cystment of the *C. fastigata* in tap-water and in distilled water. The animals in tap-water were four times centrifuged, for observations in distilled water three times in tap-water, in distilled water twice. Following centrifugation the tap-water cultures contained 300 and 600, while those in distilled water 900, 1200 respectively per 0,5 ml.

Result of the examination

1. Strikingly few data are available as to the viability of single protozoa in cyst condition. As the problem is in close connection with my investigations, I made some informatory examinations also in this respect.

It is commonly known that protozoa live in great number in and under the mosses, so do the *Colpoda*. When the moss is dried, they get encysted, consequently active protozoa may be recovered from such material after a shorter period if flooded with water.

To my examination the moss, brought partly from the *Mátra* by *Ábrahám* and partly obtained from the *Bükk*, was used. The moss from the *Bükk* was flooded two years following drying, while that from the *Mátra* three years. The former material was densely populated first by *C. fastigata* after 48 hours. From the *Mátra* moss beside a few *Testceae (Euglypha alveolata, Corythion dubium, Trinema lineare, Difflugia globulus)* the *Colpoda flavicans* appeared in moderate number.

An enlightening result was shown by the root-culture of diverse plant species from *Pápakovácsi* in 1952 wherein the *Colpoda cucullus, fastigata, flavicans, inflata, maupasi,* were represented in a fairly considerable number. At the end of 1953 beside the *Blepharisma elongata ciliata* only a few amoeba, flagellata and testacea were present. This picture hardly changed later. At the beginning of this year the culture has been poured over with root decoction whereupon several ciliata excysted. *(Tetrahymena pyriformis, Drepanomonas revoluta* and one amoeba, the *Vahlkampfia limax).* No active *Colpoda* could be detected in the culture.

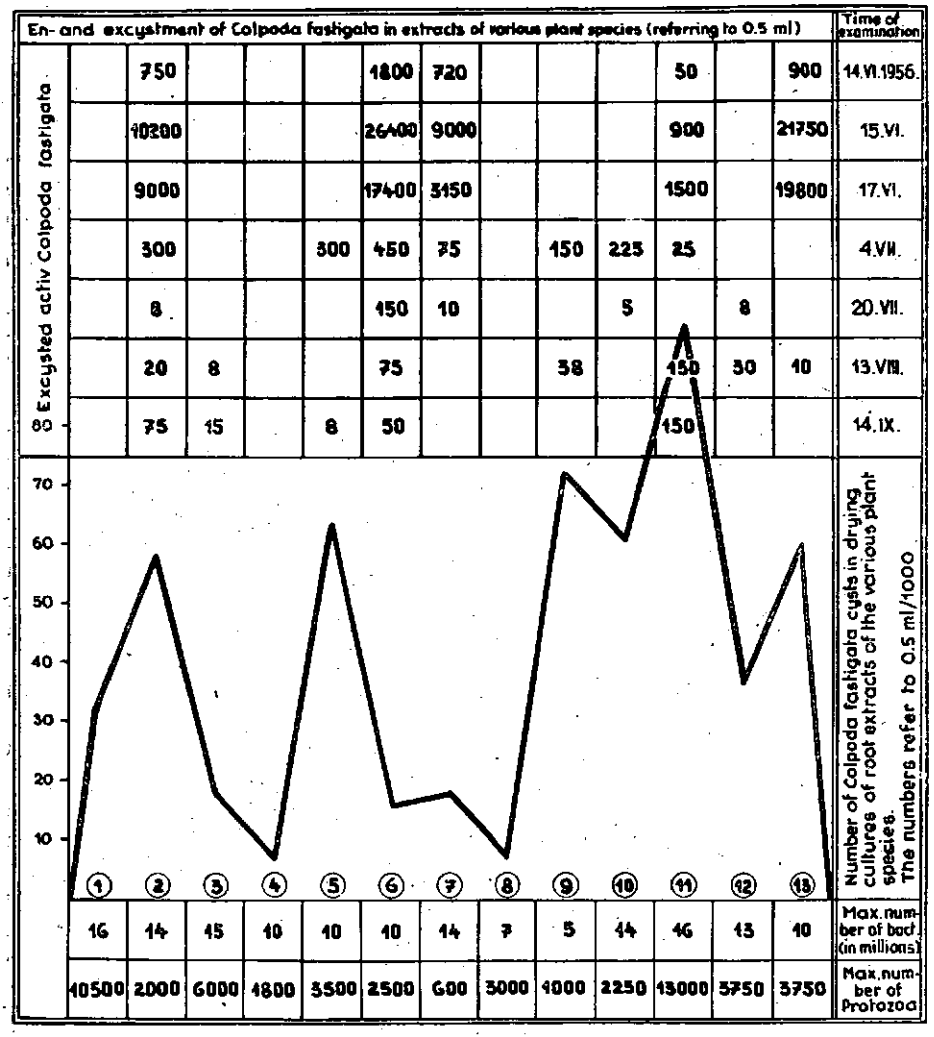
2. Examining the »*A*«-series i. e. the dried cystic cultures diluted with the root-extracts I started with the quantitative data obtained before drying the microorganisms *(Table I.).* The data of the table and the graphs therein show as follows:

a) In the single cultures a considerable difference is to be noted between the highest number of protozoa experienced prior to the drying period and the actual number of cysts found at the drying. The number of the cysts is several times that of the maximal active protozoa. It is natural as beside the protective cysts an enormous big number of reproductive cysts appeared.

The correlation of the formation of these cysts is changed with the age of the culture. The number of the reproductive cysts decreases with the aging of the cultures whereas that of the protective cysts increases. The reproductive processes in the cysts are mainly characteristic of the well fed, large sized animals. While the smaller (26—30 μ) forms are divided into two or possibly four, the larger ones into 8, may be also into 16. The largest forms (76—82 μ), anyhow quite exceptionally, were divided into 32 parts. Fairly frequent phenomenon that the single organisms produced by division do not excyst, but encyst again within the cyst.

b) In some of the cultures the number of the active protozoa is relatively low as compared to that of the cysts (e. g. *Allium angulosum, Nonea pulla, Reseda lutea).* In these cultures the root-extracts, bacteria and their products favourably influenced the reproductive processes within the cyst.

c) In other cultures *(Achillea millefolium, Aristolochia clematitis, Ononis spinosa)* the number of cysts, relatively with a high number of active protozoa



Explanation: O-Root-extracts of *1-Achillea millefolium, 2-Allium angulosum, 3-Aristolochia clematitis, 4-Datura strammonium,* 5-*Cichorium intybus,* 6-*Daucus carota,* 7-*Mentha longifolia,* 8-*Ononis spinosa,* 9-*Nonea pulla,* 10-*Reseda lutea,* 11-*Salvia nemorosa,* 12-*Solanum dulcamara,* 13-*Verbena officinalis.*

Numbers above the graph: Excystment due to the same root-extracts *(Pulmonaria officinalis, Colchicum autumnale, Salvia nemorosa).*

was low, which is suggestive of unfavourable effects of the extracts, may be of the bacteria and their decomposition products on the reproductive processes.

d) Cysts in dry conditions over a year and a half behaved very differently as to the effect of wet root-extracts. Animals encysted in the root-extracts e. g. *Achillea millefolium, Datura stramonium* and *Ononis spinosa* did not encyst at all in the new root-exracts. It follows that in the cultures of *Datura* and *Ononis* the root-extract as compared to the bacteria exerts its effect primarily and without doubt unfavourably on the viability of the animals (as it was unfavourable to the increase of the bacteria and *Colpoda). (Table I.).* Moreover the gelification of the protoplasma, its contraction following water loss is much more pronounced than in the other cultures. In the dried cultures, when flooded, a considerable number of decomposed cyst-remains, recognizable only by their outlines, was found.

In the culture of the *Achillea millefolium* the number of the bacteria and active *Colpoda* was high, that is the original culture was favourable for the increase of the microorganisms. The cysts of the *Colpoda* produced despite the stimulatory effect of the new root-extract did not excyst. Presumably such bacteria products are accumulated in the aged culture that injuriously affect the protoplasma of the *Colpoda.*

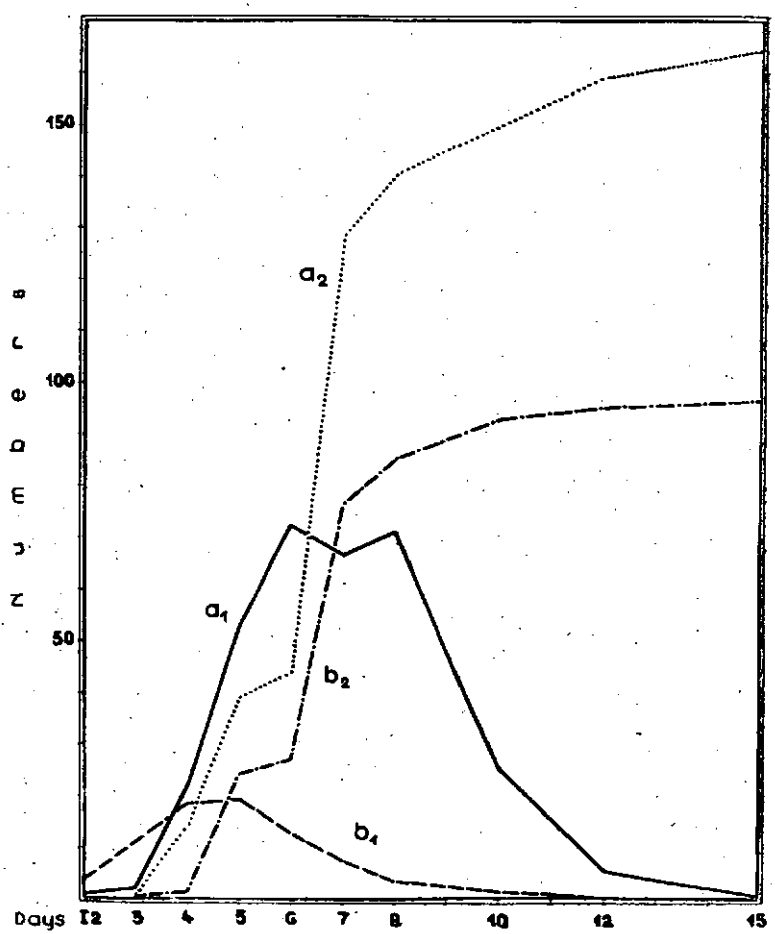
A marked encystment could be noted in almost half of the cultures after 24 hours flooding. Other cultures required several days *(Allium angulosum, Solanum dulcamara).* It was striking and gave an impulse to examine the time of the different types of cysts of the *Colpoda.* Thus the fluid of a month and a half old culture was pipetted and the cysts remained dry, were flooded with mixed root-extract at 16 o'clock. The result of the excystment is shown in the following table:

Type of cyst	Total	Time needed for excystment (hour, minute)												Not excysted
		16^{51}	17^{05}	17^{15}	17^{30}	17^{45}	17^{55}	18	18^{5}	18^{15}	18^{30}	18^{45}	19	
Thin shelled	45	1	5	8	12	18								1
Medium thick shelled	12						1	1	3	2	2		1	2
Thick shelled	22											1		21
Very thick shelled	2													2

It can be seen that the thin shelled cysts excysted almost without any exception, the majority of the medium thick shelled ones, those of the thick shelled hardly and the thickest shelled ones (the largest) not at all. They remained unchanged for the following hours, even for days. In the cultures of the Allium and *Solanum* cysts of the *Colpoda* of such thickness survived 1,5 year following drying while other types did not; their excystment, however, required several days.

The culture was diluted with further fresh root-extract on the day following examination. The new reproductive cysts produced since the previous day and a part of the productive cysts excysted with remarkable rapidity. In some of the encysting forms, rotating on the same spot, the movement slowed down, then stopped, sometimes divided into two, sometimes not, essentially, however, activated without encystment. Several examinations proved that a single drop of fresh root-extract sufficed for certain encysting forms to stop or to reverse the process of the encystment.

In the »*B*«-series i. e. in the cultures of pure root extract *(a)* and in that containing the decomposition products *(b)* the encystment of the *Colpoda* significantly differed (Graph.).



Explanation: Formation of active forms of a_1 *Colpoda fastigata* and that of a_2 cysted in fresh root-extracts.
Formation of active forms of b_1 *Colpoda fastigata* and that of b_2 cysted in mixture of the fresh root-extracts and filter of aged culture-fluid. (2 : 1).

A part of the active forms was equally overfed, non-viable in the cultures *a* and *b*. The other animals formed the reproductive cysts in large number. The difference of the two cultures was expressed by the larger number of the active forms in the *a*-cultures than in *b*. (Examination of numerous cultures proved the insignificance of the concentration of the root decoction.) The difference was shown also by the quantity of the cysts produced. In the cultures *a* and *b* there is a marked difference in the proportion of the active forms and resting cysts. Namely in the *b*-culture the quantity of the resting cysts, related to the active forms, is significantly larger than in culture *a* for in the culture *b* the ratio of the reproductive processes occured in the cysts was more pronounced.

4. To examine the cultures of the »C«-series the earlier experiences gave rise whereby in the cultures of mixed protozoa the single species relatively change rapidly each other and the average number of the cysts is low while in cultures of one species the individuals are present in larger number for 1—2 months and the amount of the cysts is remarkably high (4). Thus we can assume that the common presence of the different species may influence the vital processes, so it may the encystment as well.

The study of the cultures to approach the question resulted as follows:

a) The *Paramecium caudatum* living in culture for a longer time and well-nigh sterile by repeated centrifuging meant a peril to the permanent free-swimmers *Pyxidium asymmetricum* if cultured together. The swimmers perished without exception after 48 hours.

b) Swimmers of *P. asymmetricum* and the *C. fastigata* are well cultured together. The cystment of the *Colpoda* essentially occured as in the culture without swimmers.

c) The cultured *P. caudatum* had a marked effect on the cystment of the *Colpoda* in common culture, inasmuch as the animals were encysted in 24 hours (Fresh *Paramecium caudatum* from sewage-water under similar circumstances did not considerably affect the encystment of the *Colpoda).*

5. In the »*D*«-series i. e. in the tap-water and in the distilled water the encystment of the *Colpoda fastigata* showed a peculiar feature. In tap-water they were encysted after 3—6 days. In distilled water more than half of the animals perished due to cytolisis. Those survived have been taxed to the utmost by the hypotonic medium that is by the concomitant increased osmoregulation. Despite, active forms could be stated in considerable number even with tap-water culture. In fact the concentration of the distilled water, has after two weeks in the culture. This seems to be paradoxical when compared been somewhat changed by the plasma and the plasma-substance of the cytolised animals and so enabled the animals alive to get some food. Though the water remained still hypertonic, the cystment occured which is to be attributed to this favourable condition.

Beside the unusual environment the following were observed:

a) Cysts were formed in about 40% of the survived species after cytolisis. The cyst-shell is mostly smooth as contrasted with those produced in root-extract cultures. It is not infrequent to see rugouse shell of a new cyst within a smooth shell.

b) The function of the contractile vacuola is pronounced for days even in the animals still in the shell. The cavity of the vacuola in some of the animals is agape for a while and slowly a moderate contraction is shown.

c) The plasma of the cysts quickly shrank; in some to half of the original size within 3 days even in one or two to a $^1/_4$.

d) From a few cysts long, thin forms were encysted (sometimes divided) bearing fairly well the hypertonic environment, even there were such that could encyst after some days, then excysted again.

Discussion of the results

1. Viability and lifetime of the cysted Colpoda fastigata

To study the physiology of the cystment numerous investigators had used plant-extracts. BARKER and TAYLOR (2) stated that the protozoa specifically excysted owing to the effect of both the animal and plant extracts as well. JOHNSON and EVANS (16) noted that the extract of *Elodea* considerably lengthened the time required to the encystment of the *Woodruffia metabolica*. HAAGEN, SMIT and THIMANN (14) found the fractions of hay-decoction, while PRATER, HAAGEN and SMIT (14) the fractions isolated from corn-leaves and combined with Co-factors to be very effective for the excystment of cysts. As to the development, survival of the protozoa, the root decoction was found to be the most favourable of all the different ones such as straw, hay, fresh leaves, rice and wheat grains in my examinations (4). For the time being my aim was not to deal with the isolation of the substances in the root-extracts. Following my earlier investigations seemed logical and timely to raise the question: whether the root-extracts qualitatively differing had any effect and influence on the active protozoa living therein, on their cysts and on the viability, lifetime of these protozoa?

The result of the examinations on the cultures of the »*A*« series gives a clear-cut answer to the question *(Table I.)*, as in some of the dried cultures being diluted with the same root-extracts after a year and a half no excystment occured, while in other media a considerable number of *C. fastigata* was activated after one day. Anyhow it is true that the metabolic product itself of the microorganismus of the media is a significant factor regarding the cystment. This, however, is no account for the failure of the excystment in the root-extracts of the *Datura stramonium* and *Ononis spinosa* in the presence of suitable stimulatory substances after one and a half year, when in the root-extract of the *Daucus carota* or of the *Verbena officinalis*, under the same circumstances numerous excystments occurred already on the first days. There is an other question here, that is, whether such a remarkable change of the viability of the animals due to the effect of some substances is being developed during the active life or is the result of an injurious effect bearing on the cyst condition? Both possibilitis are to be taken into consideration.

In the cysts — especially with the young, thin shelled animals — there appears the slowly dilating contractile vacuola due to the effect of the fresh root-extracts within a few minutes which means beyond doubt, that the substances stimulating the excystment penetrate the ecto- and endocysts within a

very short time. The penetration of the injurious substances present in the culture solution may be also asumed. The statment of BODENHEIMER (6) et al, that the cysts in wet soils are less resistant than those in dry soils, can be related to such process. The activity of the plasma reduced to minimum in consequence of the gelification, may be unfavourably affected by the permanent or long-lasting presence of certain deleterious substances solved in the wet soils. This assumption is supported also by my own observation. The different species of *Colpoda* — both *fastigata* and *cucullus* — could not be activated after four years flooding the culture containing them all the time, with root-extract. Whereas DAWSON and HEVITT (9) induced the cysts of the dried *Colpoda cucullus* to excyst even after 5 years. I could activate the *Colpoda flavicans* obtained from dry moss after 3 years.

The conclusion may be drawn that the viability, the rate of the excystment of the cysts are essentially determined by the circumstances of life. Thorough examination is needed to elucidate the length of the time by which gel plasma, shrunken in its shell, remains viable. In the case of the *Colpoda*, it is on no account 14—16 months as it is stated in one of the papers available (10).

2. Cystment in the presence of substances injurious to the life of the protozoa

In relation to the cystment of the protozoa it is wellnigh every time possible to demonstrate such substances which, if do not involve any immediate damage to the animal, may deleteriously affect the active life. They are e. g. the decomposing organic matters, the metabolic products ot the living organisms or the so-called »killed« substances (13) so far qualitatively little known etc.

a) The role played by the decomposing organic matters, and metabolic products as well is indicated by the examination of the cultures in the »*B*«-series *(Graph.)*.

In the *b* individuals of these cultures it is quite striking the effect of the metabolic and decomposition products filtered from the aged cultures: the active protozoa and cysts are fewer than in the pure root-extract cultures. This could be expected taking into consideration the examinations of other investigators (17, 24). Besides is still problematic the fact that in cultures rich in decomposition products there is relatively a high number of cysts as contrasted with the low number of the active protozoa. As the phenomenon is closely connected with the division the question arises: what is the cause of the increased reproductive processes in the cysts in the presence of the decomposition products? To all appearance the presence of these products as well as that of the toxic substances stimulates the *Colpodae* to a prompter increase for the sake of their existence and only afterwards produces more gradually resting cysts.

b) On the basis of the results of the examinations made on the »*C*«-series cultures, may be rightly assumed that in the cystment a role of interaction is played by the protozoa living near each other. It was noted that in the presence of the *Paramecium caudatum* cultured in the laboratory the permanent swimmers of the *Pyxidium asymmetricum* rapidly perished while the *Colpoda*

fastigata cultured well together, quickly encysted. Such a prompt effect can not be ascribed to common metabolic product: rather to some »paramecin«-like substance that is known in certain strains of the *Paramecium aurelia* (13) and is lethal to other protozoa (»killed«). It is to remark that *P. caudatum* brought from natural sweet-water, freshly centrifugated had no such effect. Such substance could be likely produced during the culture. The presence of such and similar factors are to be accounted for both in the cultures of protozoa and under natural conditions. The short span of life of certain species, the interchange with other species may be thus explained.

c) From the above statements follows that the shells mean no full defence for the encysted animal. Injurious substances solved in fluid may penetrate them to a certain extent. This is likely the reason why in cultures rich in metabolic products or in wet soils are the cysts less resistant. The penetration of these substances, however, seems to decrease with the extent of the plasma gelification i. e. is in inverse ratio to it. This is shown as follows:

The contractile vacuola, the functional intensity of which can be considered to be the rate of the osmoregulation, works, anyhow, slower and slower after the encystment of the *Colpoda fastigata*. Thus the shells of the cysts do not prevent the water from penetrating the protoplasma. Still more remarkable is the phenomenon in the hypotonic environment where the vacuolar function can be noted considerably longer following encystment, naturally in a strongly reduced form. As the gelification of the plasma progresses, the penetration of water is decreasing and finally no osmoregulatory activity is shown by the contractile vacuola. So the penetration of the environmental solutions can be considered almost ceased as such. The pronounced shrinkage of the protoplasma which can be considered as a biosineresis, becomes now still more pronounced. I would emphasize this because some workers (11, 21), are inclined to attribute the plasma shrinkage to an increased pulsation activity. In my opinion the osmoregulation and biosineresis are radically different processes, two different phenomena of the water metabolism of the unicellular animals. In the case of biosineresis if water is released, the contractile vacuolae have no role whatever in its transportation from the plasma. An other question is how do some substances stimulating excystment such as root-extracts or injurious, toxic substances, get into the gelified protoplasma after the discontinuance of the osmoregulation? To this question no answer can be so far given. The fact is that the gelifaction of the protoplasma is a very significant factor from the defence-point of view as against the chemical substances. The presumed »killed« substance did kill every member of the *Pyxidium asymmetricum*'s swimmers within 48 hours which encysted very difficultly and slowly, at the same time prompted a rapid gelification of the *Colpoda fastigata* specimens which encyst very well and quickly and would have perished without this process. Thus the protoplasma is able to avoid the injurious effect of the »killed« substance.

Hence it is suggested: the more pronounced are the biosineresis and gelification, i. e. the more ineffective the polar roots become due to the bonds formed, the less viable is the protoplasma. Such shrunken animals, — observed in many a case — could be prompted to excystment only after days even with strongly active substances. Contemporaneously its defence is growing stronger

as the inactivation of the polar roots markedly slows down the penetration of the substances injurious to the plasma. Therein, among others, I suppose, is the great biological significance of the gelification concomitant to the cystment, i. e. of the formation of the plasma-gel.

3. Cystment in absence of demonstrable injurious substances

The cystment of the C. fastigata in tap- and distilled water is noteworthy because it can not be directly connected with injurious factors. This supports the opinion of investigators attributing the encystment to the lack of some indispensable substance.

Reference has already been made that symptom of encystment, formation of protective cysts may start in the presence of demonstrable food. In fresh root-extracts 3—4 days following inoculation, large number of the active Colpodae, their reproductive, then protective cysts appear. In the opinion of BARKER and TAYLOR (1) such a crowding is a considerable factor of the encystment with the *Colpoda cucullus*. This motivation, however, does not turn the attention to the reason. namely, giving 1—2 drops of the root-extract to the crowded, overpopulated culture was sufficient to stop both forms of the encystment, to reverse them and to reactivate the animal. It follows that the substance or substances important to life of the cultures are consumed by the great number of the active forms. The lack of these substances or their reduced quantity may prompt the Colpoda to encystment. GARNYOBST (12) supposes vitamins of vital importance, but may be attributed to such trace elements that are required to the function of the enzyme. Anyhow so far no investigations have been made.

4. Contrary processes of cystment in the same culture

The en- and excystment frequently occurred simultaneously and parallelly in the cultures of the *Colpoda fastigata*. It may be assumed to all appearance that in some of the animals the resting period begins, induced either by the environment or some inner stimulus. On the other hand it is possible, a process marking the end of the resting period which naturally results in the excystment. To approach this phenomenon has so far failed. It can be explained as follows:

The enviroment for the ex- or encysting animals is only apparently identical. Namely, the encysting animal is in contact with the culturemedium only by the thin pellicula. The cysted animal is separated from the environment by the cystshell consequently it is better delimited. A peculiar inner environment is being formed during the shrinkage of the protoplasma, during its biosineresis. Namely, the plasma may give up not only dehydrated water but also substances absorbed earlier. Hence within the cyst-shell such substances may be produced which stimulating the encysted animal may act as an excysting factor. This may be concluded from the phenomenon experienced in the hypotonic medium, where in general the more rapid sineresis, the releasing originally bound substances respectively, induce the excystment within a short time (in spite of the unfavourable environment to the active life). This phenomenon was twice, thrice repeated.

5. The problem of periodicity of the cystment

BEERS, C. DALE (3) fed the *Didinium nasutum* with *Paramecium caudatum* poorly in one culture and well in an other one. In the latter the *Didiniums* did not encyst, while those fed poorly (112 generations) encysted within 64 days. This experimental result gives rise to doubt whether it is worth speaking about periodicity of the encystment. My investigations led me to this conclusion too.

During culturing the *C. fastigata* the animals have been often inoculated. Occasionally the same culture has been diluted with root-extracts, without changing the basic material. The excystment in these cultures was sporadic. The more frequent the dilution was, the more rare was the formation of the resting cyst. On the other hand — as mentioned above — a drop of fresh root decoction sufficed to stop the encystment in process and to reactivate the animals.

Accordingly can not be stated either in the case of the freshwater *Didinium* or in that of the *Colpoda* in the soil that the commencement of the encystment means a necessary resting condition which occurs independently of the environment. Above experiments prove that the encystment means the natural defence of the protozoa, it is a defence against the lack of substances indispensable to life as well as against the presence of substances injurious to life.

Summary

The physiological symptoms regarding the ex- and encystment have been examined on *Colpoda fastigata* taken from the soil and cultured from one cyst. The animals lived well on root-extract and cysted. The extracts, at the same time, considerably stimulated the excystment even in high dilution, consequently proved to be very suitable media to the examinations. Observations were made partly in tap- and partly in distilled water. Result were:

1. Firstly informative examinations were carried out regarding the viability of *Colpoda* cysts living in different environment.

a) Colpoda fastigata excysted in great number after 2 years from dried mountain-moss due to wetness, *C. flavicans* from other moss excysted in small number after 3 years.

b) The various Colpoda cucullus, fastigata, flavicans, inflata, maupasi, from mixed roots en- and excysted due to water, did not activate, however, even with root-extracts after 4 years either (Cysts permanently in fluid).

c) Colpoda fastigata cultured in qualitatively different root-extracts have not encysted alike and the excystment of the cysts showed significant difference *(Table I)* in the same root-extracts a year and a half after drying the cultures. In some of the cultures (e. g. *Ononis spinosa)* the cysts did not excyst at all after a year and a half, they perished. According to the quantitative data of the cystment the single root-extracts affect not only the protoplasma of the active *Colpodae,* but also that of the cysted ones, sometimes they injure it.

2. Substances injurious to life-functions of the *C. fastigata* significantly affect cystment.

a) In fresh root-extract the ratio of the numerous active and cysted forms is lower than in the root-extracts containing toxic products, as well as inju-

rious metabolic products, wherein the number of the active and cysted forms is considerably lower. It seems that in the latter case the *C. fastigata* respond to the injurious substances first with the formation of increased reproductive cysts and produce a greater number of protective cysts only later *(Graph).*

b) Cultured *Paramecium caudatum* that killed the permanent swimmers of the hardly cysting *Pyxidium asymmetricum* with their chemical substances, prompted the *C. fastigata* to encyst within 48 hours in other cultures. It is very likely a »paramecin«-like, »killed« substance. At the biosineresis-like change of the rapid encystment, of the gelification of the protoplasma the inactivation of the polar-roots of the plasma produces such a defense for the animal that assures its subsistance for a longer time. The defence may be enhanced by the shrinking of the plasma, the viability, however, is reduced.

3. The encystment occurs even in the absence of the injurious substances. In distilled water the *Colpoda,* surviving the cytolysis, produced both protective and reproductive cysts. This phenomenon may be ascribed to the lack of vitally important vitamins or to that of trace elements. The vast encystment in the highly populated root-extract cultures may be explained in this way, i. e. by the rapid use of such substances.

4. In the cultures of the *C. fastigata* the ex- and encystment frequently occurred parallelly. It can be assumed that during the biosineresis of the plasma the substances formerly absorbed are released and thereby is formed a peculiar inner environment stimulating the excystment within the cysts which in fluid medium may result in full excystment (In destilled water where the shrinking of the plasma was more rapid, the excystment was also more rapid, even repeated two-, three times).

5. The cystment can not be considered a periodical process, independent of the environment. The phenomenon can be made reversible in any phase and the inactive state may be ceased any time. Having all the conditions needed to the life of the protozoa or injurious substance is absent, no encystment occurs.

The natural defence of the protozoa is expressed by the cystment; it means defence against the lack of substances of vital importance as well as against the presence of substances perilous to life.

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