THE EFFECTS OF DESICCATION ON THE ROTIFER PHILODINA ROSEOLA

BY

MERKEL HENRY JACOBS

WITH ONE FIGURE

I	Introduction.....	207
II	Historical.....	210
III	Structure and natural history of <i>Philodina</i>	217
IV	Behavior of <i>Philodina</i>	220
	1 Behavior under normal conditions.....	220
	2 Behavior at the onset of desiccation.....	222
V	Visible changes attending a process of desiccation.....	223
VI	Degree of desiccation attained by <i>Philodina</i>	227
	1 The views of Davis and others.....	228
	2 Evidence for the view that a true desiccation occurs.....	231
VII	Effect of desiccation on the life processes of <i>Philodina</i>	236
	1 Injurious effects of desiccation.....	236
	2 Influence of the previous condition of the animal.....	237
	3 Influence of the conditions attending the desiccation.....	238
	a Effect of rapidity of drying.....	238
	b Effect of temperature at which drying occurs.....	242
	c Effect of duration of desiccation.....	244
	d Effect of alternations of moisture and dryness.....	244
	e Effect of intensity of desiccation.....	246
	f Effect of temperature on mortality.....	248
	4 Conditions of the life processes in a dried animal.....	249
	5 Effect of desiccation on egg production.....	252
VIII	Summary.....	260
IX	Bibliography.....	262

I INTRODUCTION

It has long been known that various animals, among them certain of the rotifers, tardigrades, and the smaller nematode worms, can survive conditions fatal to most other organisms. Although normally living in water, or at least under conditions of moisture, these animals may be dried for long periods of time without serious injury. They are found in countless numbers in

the dust from the gutters of roofs and other places where water is accustomed to stand at intervals, and even after prolonged drying the addition of a little water is all that is necessary to start them into activity. While in the dried condition they show no visible signs of life. All movements cease and the body frequently shrinks to a shapeless mass so that it is difficult to distinguish them from the particles of sand among which they are found. When water is again applied the body gradually regains its original form, movements appear, and after a longer or shorter time, depending on the conditions of the desiccation, the animals resume their normal activities apparently none the worse for the experience. Not only may they be dried naturally in the air but they may be subjected artificially to even more extreme conditions. Various observers have kept them in desiccators and vacua for long periods of time and have subjected them to temperatures at which life ordinarily is impossible, without destroying their power of again resuming their normal activities upon the application of water.

The length of time the animals may remain in this state of suspended animation is often considerable; there are well authenticated cases of rotifers, whose usual period of life is probably not more than a few weeks or months at most, which have been revived after a period of desiccation extending over three or four years; one observer even claims to have revived them after fifteen years' desiccation. In the case of the *Anguillulidæ* even longer periods have been recorded; Baker in 1771 succeeded in reviving individuals of *Tylenchus scandens* which had remained in a dried condition in grains of wheat for 27 years.

As to the principal facts just given there seems to be little doubt. They have been confirmed by numerous observers and anyone may repeat for himself with little trouble the experiments on which they are based. In the interpretation of these facts, however, there has been, and still is, much diversity of opinion. What is the actual effect of drying on the rotifer or tardigrade? Is the water contained in its tissues really removed or does the animal have some means of protecting itself against the loss of water, the desiccation being only apparent? If the former be the case, what

is the condition of the dried animal? Are its life processes merely retarded or have they come to a complete standstill? In the revival of a dried animal by the application of water, are we dealing, as many have supposed, with a case of passage from death to life or merely with an acceleration of vital processes which have been continuing all the while but in a greatly reduced state?

These and similar questions have been under discussion for many years and as yet no unanimity of opinion has been reached by zoölogists in regard to them. Every point has been affirmed and denied many times by equally capable men. Much of the discussion on the subject has been pure speculation based on neither observation nor experiment and hence is of little value; however, even the most careful observers have differed radically with each other on many points of importance, the observations of one worker being contradicted by the apparently equally accurate observations of another worker. The result is that the question even at the present day is in a state of the greatest confusion and uncertainty and is still far from being finally settled.

It was with the intention of clearing up some of these points of dispute that the present piece of research was undertaken in the fall of 1906 at the suggestion of Prof. E. G. Conklin. It gives me great pleasure to express at this point my sense of deep indebtedness to him not only for suggesting the subject but for the interest he has taken in the work and for the many helpful suggestions and criticisms he has offered. The experiments were performed in the Zoölogical Laboratory of the University of Pennsylvania during the years 1906-1907 and 1907-1908. During the course of the work a number of points of interest somewhat off of the main line of the investigation came up; some of these points are touched upon in the present paper, others are still under investigation and are reserved for subsequent publication.

The animal worked upon was *Philodina roseola*, one of the Bdelloid rotifers, and in all cases except where otherwise expressly stated it will be understood that the observations apply to this form. *Philodina* was chosen partly on account of the ease with which it could be obtained, making experiments on large numbers of individuals possible, and partly because it shows the phenom-

enon of revival after desiccation in a particularly favorable way. A limited number of observations were made on *Adineta* (*Callidina*) *vaga*, a nearly related form, but these show practically no points of difference from those made on *Philodina*.

II HISTORICAL

In the year 1701, Anton von Leeuwenhoek in searching for new objects to examine under his microscope chanced to take some of the dry dust from the gutter of a roof and on moistening it was greatly astonished to see after a time living animals swimming about actively in the water. Struck with the observation he again allowed the animals to dry and on moistening them the next day with water previously boiled by way of precaution against introducing any life from outside sources, he obtained the same result. Further experiments convinced him that these animals, which were rotifers, probably belonging to the species *Rotifer vulgaris*, might be deprived of water for at least several months without losing the power of recovering their normal activities when water was again supplied. This was the first observation to be made on the phenomenon of desiccation with subsequent revival in animals; strangely enough it attracted little attention at the time. Leeuwenhoek believed that the animals themselves were not truly dried but that they were protected by an impenetrable cuticle from loss of water * * * "*cuticulas ex tam solida conflatas esse materia ut ne minimam quidem permittant exhalationem. Quod si sese aliter haberet, asserere non vereor haec animalcula * * * onni aqua destituta necessario omnia esse emoritura.*"

The next mention of the subject was made in 1743 by an Englishman, Turbervill Needham, who observed minute worms to issue from grains of wheat when water was applied to them. To use his expression they "took life" on the application of moisture. In the same year another Englishman, Henry Baker, again called the attention of naturalists to the rotifers discovered by Leeuwenhoek, but he contented himself with merely repeating that writer's description of them and it was not until ten years later

that he discussed the subject on his own account. Needham's discovery excited considerable attention but was for the most part received with incredulity. That living organisms could be dried and after remaining apparently lifeless for a time be caused to "take life" by the application of water seemed so incredible to the scientists of the day that they preferred either to deny the animal nature of the worms altogether, calling them "filaments animés," "fibres mouvantes" or "etuis pleins de globules mobiles" which were started into movement by the imbibition of water or to assert that they arose by spontaneous generation. It was not until the time of Fontana (1771) and Roffredi (1775) that the animal nature of these anguillulids was established beyond all doubt.

In the meantime other men were making similar observations. Among the earliest of these may be mentioned Trembley (1747), Baker (1753), Schaeffer (1755), Ginnani (1759), Ledermüller (1759), Fontana (1768), Göze (1772), Corti (1774), Müller (1775) and Roffredi (1775). The list of the animals capable of enduring desiccation was also increased during this period. Ledermüller in 1759 had observed the revival of "paste eels," Fontana in 1768 of Gordius, and Spallanzani in 1776 of tardigrades and the anguillulids found in the dust of roofs. Later observers added several new forms and increased the list of rotifers and tardigrades which show this peculiarity.

The history of our exact knowledge of the phenomenon of suspended animation dates from the publication in 1776 of Spallanzani's "Opuscoli di Fisica Animale e Vegetabile." The section of this work relating to the desiccation of animals is a model of scientific research. Spallanzani, unlike most of his contemporaries, tested his theories by actual experiments and these experiments were often carried out with great care and considerable ingenuity. If other workers had used the same amount of care the subject would be in a far less confused state than it is at the present day. Spallanzani first repeated the experiments of Leeuwenhoek and made the observation, which has been confirmed by a number of subsequent workers on the subject, that rotifers can recover their activity after a period of drying only when a certain amount of sand or moss is present; when dried on a clean glass slide they are

invariably killed. He considered this result to be due to the injurious effect of the air on the dried animals, the sand protecting them from its action. He made many other interesting observations, for example, on the effect of high temperatures on animals in different degrees of desiccation, on the number of times they may be dried without being killed and the effect on them of various chemical substances. Unlike Leeuwenhoek he believed that the rotifers could endure the withdrawal of the last traces of water from their bodies. He was able to revive them after subjecting them while in the dried state to the desiccating action of high temperatures and the vacuum. There was no doubt in his mind that the process of desiccation caused an actual stoppage of the life processes and that the animals in the dried condition were to be considered as dead. He entitled his paper, "Observations and experiments on certain marvelous animals which the observer can at his will make pass from death to life."

Spallanzani's work naturally excited great interest and gave rise to much discussion. The statement that animals could be brought back to life after being dead for a time could not be allowed to pass unchallenged by the physiologists of the day and many heated controversies arose between those who supported Spallanzani and those who opposed him. Among those who engaged in the discussion may be mentioned such distinguished naturalists as Haller, Cuvier, Oken, Humboldt, Lamarck, Treviranus, and Johannes Müller. The controversies for the most part, however, were carried on on purely theoretical grounds and had little experimental evidence to back them. From the time of Spallanzani to the time of Doyère, a period of sixty years, although the question continued to be one of the most discussed in the whole realm of physiology, practically no new facts were added to our knowledge of the subject.

On the whole, the opponents of Spallanzani seemed to gain the upper hand. This was largely due to the apparently convincing arguments advanced by two men, Ehrenberg in Germany and Bory St. Vincent in France. Both of these workers not only denied that dead rotifers could be brought back to life but asserted that recovery after true desiccation was impossible. The unde-

niable fact that rotifers, tardigrades, and anguillulids may be obtained from apparently dry sand they explained in different ways. Ehrenberg, on the one hand, contended that actual drying does not occur, the sand protecting them from loss of water "as a woolen mantle protects the Arab from the intense heat of the desert." He believed that in this state of apparent desiccation all of the vital processes continued, even reproduction. Bory St. Vincent, on the other hand, believed that the animals in question could not survive a period of drying even when sand was present and that their apparent revival was due to the hatching of eggs concealed in the sand. "Nous avons quelquefois * * * retrouvé des Rotifères * * * mais ils n'y ressuscitaient pas; ils s'y développaient comme les Daphnies et autres petits entomostracés, dont les ovules sont demurés dans le sol."

The next worker to take up the subject in a scientific manner was the French naturalist Doyère. So great was the authority of Ehrenberg and Bory St. Vincent and so plausible their reasoning that he had been led to doubt the accuracy of Spallanzani's observations or at least to regard the matter as worthy of further investigation. He accordingly undertook a series of experiments, published in 1842, with the result that Spallanzani's conclusions were in the main confirmed. He found, however, that the animals are not always killed in the absence of sand, a certain proportion of those dried on a clean slide recovering, although requiring a much longer time. Furthermore, he showed that it is not the exposure to the air that injures the rotifers and tardigrades in this case as Spallanzani had supposed, since animals dried in the air and then placed in a vacuum showed a lower mortality than those dried directly in the vacuum. He concluded that the rapidity of drying is an important factor in the effect of the desiccation. He furthermore showed that rotifers may be revived after an apparently almost perfect desiccation. He found that they could endure a sojourn of 17 days in a desiccator followed by 28 days in an air pump with a pressure of 5-6 centimeters of mercury and that after thorough drying in the sunlight a temperature of 140° C. or more could be resisted for a brief period. He concluded, therefore, that the last traces of water might be extracted without destroying the power

of revival, and that, since life processes are impossible in the absence of water, in the dried animal we are dealing with a case of life *in potentia* as opposed to life *in actu*. These contentions were supported by observations made several years later by the physicist Gavarret, who subjected rotifers to the action of a vacuum of only 4 mm. pressure for 51 days, sulphuric acid being present to absorb all traces of free moisture, and yet was able to revive them by the application of water.

About the same time Pouchet, followed by Pennetier and Tinel, obtained results which were diametrically opposed to those of Doyère. Numerous carefully conducted experiments led these naturalists to believe that a true desiccation is invariably fatal in the case of rotifers, tardigrades, and anguillulids just as in other animals. They found in all of their experiments that these animals when dried on a glass slide either with or without a small quantity of sand are killed in the course of a few days even at ordinary temperatures and that individuals dried under more natural conditions cannot resist an hour's exposure to a temperature of 100° C. Pouchet even found that rotifers and tardigrades obtained in a dried condition from natural sources were all killed in three months when exposed to the air in a sunny place. All of these results were presumably due to the loss of water by the animals when exposed to unfavorable conditions.

Since the views of Pouchet, Pennetier, and Tinel on the one hand and Doyère and Gavarret on the other, differed so widely and since both seemed to be based on equally careful experiments, it was decided, instead of wasting time in fruitless discussion, to submit the matter to arbitration. Accordingly a commission was appointed in 1859 by the Société de Biologie consisting of MM. Balbiani, Berthelot, Brown-Séquard, Dareste, Guillemin, Ch. Robin, and Broca, chairman, to hear the evidence presented by both sides, to perform any experiments of their own they might consider necessary, and to present the result of their deliberations in the form of a report to the society. This commission discharged its duty with the greatest thoroughness. It held forty-two regular sessions without counting the times when a few of its members met for discussion. The examination of the evidence

presented by both sides in the form of experiments occupied several months and the final report of the commission presented in March, 1860, covers a hundred and forty printed pages. This report on the whole sustains the contentions of Doyère, not because of any inaccuracies in Pouchet's results, but because they are largely negative in nature while Doyère's are positive. It concludes with the words, "des animaux * * * aménés au degré de dessiccation le plus complet qu'on puisse réaliser dans l'état actuel de la science, peuvent conserver encore la propriété de se ranimer au contact de l'eau." Although the language employed in the report is rather guarded, it evidently was the opinion of the commission that life under these conditions could exist only *in potentia*.

The results obtained by this commission as well as those of Doyère and Spallanzani are contradicted by the observations of some of the more recent workers on the subject. In 1873 H. Davis, a member of the Royal Microscopical Society and a student of rotifers published a paper which has since been largely quoted and in which he stated his conclusions that in *Philodina*, at least, the desiccation when followed by revival is only an apparent one, the animal being able to protect itself by means of a gelatinous secretion against the loss of its body fluids. He demonstrated how such a secretion could be effective by showing that grapes covered with a thin coating of gelatine remained in a juicy condition for a long time even in the vacuum of an air pump while grapes not thus treated soon lost their water and assumed a shriveled appearance. The fact that rotifers dried with a small quantity of sand are capable of recovery even after a prolonged exposure to extreme conditions while those dried on a clean slide for a much shorter time are not, had always been more or less puzzling to naturalists since it was first observed by Spallanzani. It received a ready explanation on Davis' theory, it being assumed that in the former case where drying was slow the animal had time to protect itself by pouring out a secretion, while in the latter case drying was so rapid that the animal, not being able to form its usual protection, was killed. This explanation was so simple and so in accord with the facts known for other animals that it was immediately accepted by many and at the present day is still frequently quoted.

Certain of the most recent investigators have gone even farther than Davis. O. Zacharias ('86), for example, on the basis of several observations made on a species of *Philodina* has tried to discredit the whole subject of the revivification of desiccated animals. According to him, rotifers can resist drying no better than other aquatic animals; when withdrawn from water they are invariably killed. Like Bory St. Vincent he considers the many cases of supposed revival to be due to the hatching of eggs which by virtue of their thick shells are able to resist drying the same as the eggs of insects or other animals. The fact that revival is commonly supposed to occur in the presence of sand he regards as strong confirmatory evidence since it is to be supposed that concealed in the sand there might easily be many eggs which would escape the notice of the observer. He concludes his paper with the words * * * "es einzig und allein die *Eier* sind durch welche die continuierliche Generation ensfolge aufrecht erhalten wird."

F. Faggioli ('91) as the result of a number of observations and experiments on several species of rotifers comes to the same conclusion. Like Zacharias he regards the stories of the revival of animals submitted to conditions of desiccation as myths based on imperfect observations. Perhaps his position may best be summed up in the following quotation from Fredericq ('89) which he cites in his paper. "Les Rotifères et les Tardigrades adultes meurent sans retour quand on les desseche. Mais les oeufs qu'ils ont generalement dans le corps ne sont pas dans le même cas. Ces oeufs conservent leur vitalité malgré l'absence d'eau. Places ensuite dans un milieu convenable et humide ils se developpent avec rapidité et donnent naissance à une nouvelle génération de jeunes animaux que l'on avait à tort considérés comme résultant de la reviviscence du corps de leurs parents."

It is seen from this short historical review that the entire subject is in a very unsatisfactory state. On scarcely any point is there any general agreement. All observers, perhaps, will admit that it is possible to obtain living animals from sand that is apparently dry but further than this there is no consensus of opinion. One body of observers represented by Bory St. Vincent, Zacharias and

Faggioli claim that the animals come from eggs concealed in the sand in the dead bodies of the parents, the latter having of necessity been killed by the exposure to the conditions of dryness. Another body, represented by Leeuwenhoek, Ehrenberg, Pouchet, Davis, and Hudson, although admitting that adult rotifers may survive even prolonged conditions of desiccation, consider that they are able to do so only in virtue of some effective means of preventing the escape of water from their tissues, the condition of the animal in the dried state therefore not differing in any essential respect from its normal state. Still others, among them Spallanzani, Schultze, Doyere, Gavarret and Preyer consider that the desiccation is a true one and may continue theoretically up to a point of absolute dryness without injury to the animal. They either state specifically that the animal in this condition is lifeless or tacitly assume such to be the case. Other observers, while admitting that desiccation may proceed very far, consider that the life processes never come to a complete standstill although they may be very greatly retarded.

It is the purpose of the present paper to consider the evidence for these various views and to attempt to reduce the subject to a state of greater certainty than that in which it now rests. The two points which will chiefly be considered are first, whether or not the animal suffers a true desiccation and, second, what is the state of the vital processes of the animal in the absence of water. Before proceeding to the observations and experiments on which the conclusions of the paper are based, a brief account will be given of the structure, natural history, and behavior of *Philodina* to render more intelligible that which is to follow.

III STRUCTURE AND NATURAL HISTORY OF PHILODINA

Philodina roseola is one of the commonest of the rotifers, belonging to the order *Bdelloida* of which the common rotifer, *Rotifer vulgaris*, is also a member. It is widely distributed throughout the world, being frequently found in small basins in the rocks in which water periodically collects; a favorite habitat is in the stone urns in cemeteries. It is usually associated with the uni-

cellular alga, *Spæhrella lacustris* (*Hæmatococcus pluvialis*), on which it feeds and from which it appears to derive the red color which gives it its specific name. In size, it is microscopic, being barely visible as a minute speck to the naked eye. Adult individuals when fully extended measure from 0.35 to 0.5 mm. in length and when contracted from 0.1 to 0.15 mm. The newly hatched young are about five-eighths as long as the adults which they resemble closely except in being colorless and in having a more transparent alimentary tract.

The members of the group Bdelloida are peculiar among the rotifers in their method of locomotion. In addition to their swimming movements which resemble those of other rotifers, they are able to creep over the surface of solid objects like a leech, hence the name applied to them. These creeping movements are rendered possible by the telescopic structure of the body, which may readily be extended or contracted, and by the presence at its anterior end of a so-called proboscis by means of which adhesion to solid objects is possible. The proboscis of *Philodina* is fairly stout and when the trochal disc has been withdrawn within the body, as it always is when the animal is creeping, it appears to form the anterior end of the body. When the trochal disc, which consists of two ciliated lobes which the earlier observers mistook for wheels, is extended, the proboscis is drawn back so as to lie behind and dorsal to it. In creeping, the animal first fastens its foot by means of a sticky substance secreted by the cement glands contained in it; the proboscis is then attached and the foot drawn forward, the body shortening and arching itself, like that of a leech or a "measuring worm." The foot is then again attached and the body extended. These movements may occur with considerable rapidity although the progress of the animal is not so fast as when swimming. As a rule a high temperature and fresh water favor swimming movements and low temperatures and foul water creeping movements.

Externally *Philodina* is covered with a fairly thick cuticle which in the regions of the head and foot is divided into a number of segments by alternate stiff and flexible portions. By an inrolling of the flexible regions the stiffer parts are allowed to fit within one

another and a sort of telescoping of the body is thus permitted. When fully contracted the head and tail are drawn entirely within the large segment which covers the middle of the body and both ends of the latter are puckered together as if drawn in with a string. The animal in this condition is shaped like a lemon and is well fitted to resist the injurious effects of desiccation. The part of the cuticle that is outermost, that is, that which surrounds the middle of the body, is thicker than the parts that cover the head and tail. It is also somewhat different in chemical nature as is shown by the fact that when living animals are placed in a weak solution of methylene blue it is only part of the body that takes the stain. This is true either of contracted animals or those that are creeping or swimming. In the latter case a blue band appears about the middle of the body, the head and foot remaining colorless. The fact that that part of the cuticle which alone is exposed at the time of drying should be of a different nature from the remainder is probably significant. Perhaps it may be more impermeable to water and thus check the rapidity of evaporation. Whether or not it is a complete protection will be considered in another place. Within the cuticle and the hypodermis which secretes it are found the muscles; these are especially well developed in *Philodina* on account of its habit of contracting and extending the body. They are both longitudinal and circular, the former serving to draw the head and foot together at the time of contraction and the latter by exerting a pressure on the fluids of the body to cause their extension. In a rotifer dried slowly enough to retain its natural form, the position of these muscles may be observed in the form of slight thickenings beneath the cuticle.

The various internal organs are much the same as those of other rotifers. The mastax is well developed and the walls of the stomach are thick and glandular. They usually appear more or less yellowish, greenish, or sometimes a deep brick red, depending on the amount of pigment present in the cells of *Sphærella* on which the animals are feeding. The nephridia are fairly conspicuous and under the higher powers of the microscope it is easy to observe the peculiar flickering movement of the flame cells. A contractile vesicle is present into which the nephridia discharge their products.

The method of reproduction of *Philodina* is of interest. There are two ovaries, one on each side of the alimentary canal, and each is combined with a large vitellarium. The eggs undergo a partial development within the body of the mother and at the time of laying are relatively large and are protected by fairly thick shells. The point of special interest in connection with the reproduction of *Philodina* is that there are no male individuals; this is also true of the other members of the group *Bdelloida*. Although these animals are among the commonest of our rotifers and have been under observation for over two hundred years, males have never been observed, and it seems reasonably certain that they do not exist. Parthenogenesis appears to be the only method of reproduction.

The eggs hatch two or three days after being laid and the young from the first behave exactly like the adults which they closely resemble in most respects. They seem to be somewhat less resistant to desiccation, but there is much individual variation and the difference is not very great in any event. They feed freely and grow rapidly; egg laying may begin seven or eight days after hatching at which time they have not yet reached their full size.

As to the length of life of *Philodina* under favorable conditions, unfortunately nothing definite can be said except that it is considerably longer than has been supposed in the case of other rotifers. *Hydatina* is said to live about two weeks; in the course of these experiments rotifers isolated at the time of hatching have been kept alive for more than six weeks, and under more favorable conditions they would probably have lived still longer.

IV THE BEHAVIOR OF PHILODINA

I Behavior under Ordinary Conditions

The behavior of *Philodina* is more complicated than that of most rotifers owing to the two different modes of locomotion. Ordinarily the animals are found in one of the following four states, (1) swimming, (2) creeping, (3) attached by the foot and feeding or (4) contracted. The causes which determine the succession

of these states in a given individual have not yet been worked out in detail although the problem presents a number of points of interest. A swimming individual behaves in much the same manner as the forms described by Jennings ('04); orientation in response to any stimulus takes place by random movements resembling those of an infusorian. In a creeping individual, on the other hand, orientation is much more direct; the process resembling that which occurs in a planarian. Any strong stimulus causes the animal to stop and make a few testing movements with its proboscis; orientation either towards or away from the stimulus then occurs directly without the intervention of random movements. If the stimulus be very strong, the animal contracts at once, this reaction being caused by a variety of stimuli such as heat, injurious chemicals, hypertonic solutions, mechanical shocks and the onset of desiccation. It is interesting to note that in this one animal we have at one time the method of reaction of an infusorian and at another that of a typical metazoan. As has already been mentioned, the temperature and the purity of the water seem to be two of the factors which determine whether creeping or swimming shall prevail, although probably they are not the only ones concerned. Feeding movements may occur under almost any conditions; they are especially marked after a period of desiccation.

The reaction of *Philodina* to light is of some interest. Normally it is almost indifferent. When a number of individuals are present in a dish they tend to become scattered to all parts of it regardless of the direction of the source of light. If now they be disturbed, either by jarring the dish, drawing the water through a pipette a few times, or adding a few drops of fresh water, they immediately move towards the side of the dish away from the light, either creeping or swimming according to circumstances. This reaction is very striking and never fails to occur. In the course of these experiments it was put to a practical use whenever it was desired to obtain rotifers free from sand. A small amount of the sand from the large culture was placed in a small dish and the water agitated with a pipette, being given a spiral motion so as to carry the sand to the center of the dish. In a few moments most of the rotifers had invariably collected in the clean water on the

side of the dish away from the light where they could be removed by means of a fine pointed pipette. If left undisturbed for thirty or forty minutes they again spread out into all parts of the dish.

2 Behavior at the Onset of Desiccation

At the onset of desiccation, no matter under what circumstances it occurs, the animals all show a general restlessness. If they have been quietly feeding, for example, they withdraw the corona and begin to make active creeping movements. These movements are more or less at random and thus differ from the responses made when creeping, to more definitely localized stimuli. The negative phototaxis, which is shown so markedly under certain conditions, is always abandoned when the water begins to disappear. A group of rotifers which has collected on the side of the dish away from the light is quickly broken up, each individual starting to creep in the direction in which it happens to be turned at the time and continuing in this direction until it comes either to the edge of the drop or to a place where the surface film lies so close to the slide that it cannot creep under it. It then stops, contracts, makes one or two testing movements with the proboscis, and starts off again in a new direction until brought to a stop in a similar way. Contact with solid objects such as grains of sand does not cause the creeping movements to cease as Davis and others have assumed. So far as can be observed the behavior of rotifers dried on a clean slide and those dried with a quantity of sand is essentially the same. In both cases they continue to creep as long as it is possible to do so and when such movements become impossible they contract into a more or less spherical mass and dry wherever they happen to be. As has been mentioned the movements are purely at random. Frequently a number of rotifers may be seen in the field of the microscope at once, all creeping in different directions. When two rotifers meet they pay no attention to each other but continue on their way just as before. There is no instinct that leads them to gather together in groups for mutual protection as Hudson and others have asserted. Where such groups are found the cause is merely that they have all re-

mained in the evaporating drop as long as possible thus being brought together as the latter decreased in size. When drying occurs under a cover glass or with sand, the contracting surface film of the drop may frequently be seen to sweep a number of rotifers together and by further contraction press them into a compact mass; the part the rotifers themselves play in the process, however, is purely a passive one.

On the whole, the method of response to the first stages of desiccation shown by *Philodina* is an advantageous one. It is true that the random movements sometimes lead single individuals into places where they are caught by sudden evaporation of the water and dried without any protection at all. On the other hand, the majority of the rotifers, by continuing to move as long as possible, tend to find their way to the places where the water lingers longest and where drying, when it does occur, is most gradual. If they stopped at the first grain of sand they encountered they would frequently be dried under far less advantageous circumstances.

V VISIBLE CHANGES ATTENDING THE PROCESS OF DESICCATION

When the water has so far evaporated that creeping is no longer possible, the rotifer contracts in the manner already described, drawing in both the head and the foot and then puckering the two ends of the body as though they were drawn together by a purse string. This part of the contraction is accomplished by muscular action and occurs before the water surrounding the animal has evaporated. Before the final contraction occurs the head may be rapidly extended two or three times as if to test the external conditions. When the process of drying is very slow contraction may continue still further, the animal becoming noticeably smaller even before all of the water has disappeared. Under these conditions irregular wrinkles do not appear although the puckering at the two ends is discernible. When dried more rapidly, even when in contact with a large quantity of sand the animal assumes a more or less irregular form, both longitudinal and transverse wrinkles appearing in the cuticle and the internal organs shrinking away

from the latter. Although the animal is not necessarily injured by this irregular shrinking it is more likely to be than in the case where it contracts regularly.

Loss of water from the body is very rapid after the last trace of the surrounding film has disappeared. Wrinkles appear almost immediately in the case of individuals dried on a clean slide. In one minute the body may have become fairly irregular in outline and in two minutes assumed the characteristic appearance of desiccation. Although further loss of water and shrinkage occur the change in size and appearance after two or three minutes is not very noticeable since the cuticle has assumed its final wrinkled form and further contraction is confined to the internal organs. In rotifers dried more slowly the contraction is more evident since the cuticle and hypodermis are pressed together against the internal organs and follow them in their shrinkage. That muscular action is responsible for at least a part of the contraction is shown by the fact that dead rotifers when allowed to dry assume very irregular forms, the closing of the ends of the body above described not occurring.

The amount of shrinkage that occurs under favorable conditions is very considerable. It may easily be followed by keeping single individuals under the microscope during the drying process and making camera drawings of them from time to time. In those that are dried slowly and thus contract regularly it is easy to compare approximately the volumes at the different times. Such an examination shows that in the case of slow desiccation with sand, which is one of the most favorable methods, the animal may shrink to one-third or one-fourth of its original volume.

When water is added to rotifers that have been dried for a time a very rapid swelling occurs. In one minute the animal has doubled in volume and in five or often less has reached its normal size. From this time until movements appear the visible changes are slight and consist in changes of form apparently due to the slow relaxation of the muscles that have drawn in the ends of the body. When those at the anterior end relax the animal becomes pear shaped; the foot may be extended either before or after this occurs. In rotifers which have been dried rapidly and which are therefore

much wrinkled, the first change that occurs is a filling out of the wrinkles, the amount of swelling apparently being slight since the greater part of the shrinkage has occurred in the internal organs, the cuticle having stuck fast to the slide or to a grain of sand. That a considerable swelling occurs, however, is easily observed when a group of rotifers has dried together, the individuals being pushed apart as the water is absorbed. The usual time required for all of the wrinkles to disappear is about five minutes, although it may frequently be considerably less.

The point at which the water enters may be determined by staining rotifers *intra vitam* with neutral red before drying them and then adding water which has been made weakly alkaline by the addition of a small amount of sodium bicarbonate. As the latter touches the organs which have been stained red it changes them to a bright yellow color and thus furnishes a very exact means of observing the method of penetration. Such an experiment shows that the water enters largely at the two ends of the animal and to a much less extent through the cuticle surrounding the body. Its entrance is practically instantaneous.

The time required for movements to appear varies greatly with the circumstances attending the process of desiccation. The times required under a number of different conditions are given in another section of this paper. Under the most favorable conditions they may appear in five minutes, as was the case with a number of individuals which had been dried slowly on filter paper; usually seven to ten minutes are required and sometimes an hour or more. The first movements to appear are muscular contractions within the body, although sometimes the flame cells of the nephridia begin to beat before muscular movements can be observed. In any case the beginning of the activity of the nephridia is one of the first signs of the return of the normal life processes. Care must be taken not to confuse with true muscular movements the jerky movements exhibited immediately after water has been applied which are due only to changes in tension set up by the imbibition of the water and are not indicative of life. In all cases where the time required for movements to appear is mentioned it is understood that it is the distinctively vital movements that are meant. Soon after the first movements have appeared the foot

is extended, often with great suddenness. This is apparently accomplished by the contraction of the circular muscles of the body which thus set up a pressure which causes it to be protruded. Even in individuals which have been killed by drying the foot becomes more or less extended but this is probably due to the absorption of water by osmosis.

After the first extension of the foot, movements separated by periods of rest may recur at intervals for some time before the animal fully recovers and creeps away. Complete recovery sometimes occurs in as short a time as ten minutes, but under certain conditions it may require several hours or even a day. Most workers have introduced a source of error into their observations by failing to keep their rotifers for a long enough time. In the present series of experiments, the animals were always kept at least twenty-four hours before being pronounced dead.

After the rotifers have recovered they may show the effects of the desiccation in various ways. Sometimes the body is more or less crooked and distorted, at other times the cilia appear injured and do not beat normally. In other cases the animals remain contracted and make no movements although they are not dead as is shown by the fact that they undergo no disintegration. That some of the life processes such as oxidations still continue may be shown by placing upon them a drop of a solution of neutral red which has been rendered weakly alkaline by a very small quantity of sodium bicarbonate. Living rotifers which are excreting carbon dioxide change the color of the solution and are rapidly stained pink or red; dead ones do not show this reaction. A complete transition is therefore shown from individuals which are killed through those in which movements never appear but some of the life processes continue for a short time and those which have been more or less injured to those which completely recover and show no visible signs of injury.

The facts just mentioned may be considered as an answer to the views of those who doubt the whole phenomenon of recovery after desiccation, among whom Zacharias ('86), Fredericq ('89) and Faggioli ('91) may be named as some of the more recent workers. The evidence advanced by these men is purely negative in char-

acter. In certain cases they failed to observe the recovery of dried rotifers and they, therefore, conclude that such recovery is impossible. On the other hand, many observers have contributed an abundance of positive evidence, of which that just given is typical. It is possible for anyone, by using the proper precautions, to dry rotifers and see for himself the gradual return of movements up to the time of complete recovery. It is certain, therefore, that the return of all of the normal vital activities in animals which by drying have been rendered motionless and apparently lifeless is not one of the myths of zoölogy as many have supposed. This does not imply that the rotifers which have been subjected to the conditions of dryness have actually undergone a true desiccation. From the evidences so far presented it is possible that they have not. This point will be discussed more in detail in the next section.

It is necessary to observe that all rotifers are not equally resistant to a process of drying. The only well authenticated cases of recovery after desiccation are found in the family Philodinidæ (Philodina, Rotifer and Adineta) in species which normally live in places where they are exposed to drying at frequent intervals. Perhaps other rotifers possess this power, but that such is the case has not been proven. A number of experiments made to determine this point with *Megalotrocha* and several of the Loricata gave purely negative results, no cases of recovery being observed. The observations of Lance ('94) are of interest in this connection. He found in the tardigrades that only those species could resist desiccation which live in places where they are exposed to it under natural conditions.

VI DEGREE OF DESICCATION ATTAINED BY PHILODINA

One of the most disputed points in connection with the entire subject of suspended animation in rotifers and other aquatic animals is the degree of desiccation they may attain without injury. Some workers hold that there is no limit to the amount of drying that may occur, while others just as strongly contend anything resembling a true desiccation is necessarily fatal. They believe that in

all cases in which recovery occurs the loss of the body fluids has in some way been prevented. Among those who have held this latter view, perhaps the most important name is that of H. Davis ('73) who was the first to give a plausible explanation of the means by which desiccation could be prevented. Since his views have found a wide acceptance and are frequently quoted at the present day, they will be considered at some length and an attempt made to determine their truth or falsity.

1 The Views of Davis and Others

Davis as well as many others before him, had noticed that rotifers dried on a clean glass slide are killed by the process while if a little sand or moss is present recovery almost always occurs. He supposed that in the latter case the rotifers had time to protect themselves by secreting a gelatinous waterproof cyst which effectually prevented evaporation while in the former case, having no protection, they were dried and consequently killed. According to his view a true desiccation of the animal is always followed by death and in all cases of recovery desiccation has been prevented. He showed that grapes may be effectually protected against drying by means of a thin coating of gelatine and assumed that in the case of rotifers exposed to desiccation we are dealing with a phenomenon somewhat similar.

Others had claimed that the sand has a direct protective effect. Ehrenberg, for example, had compared it to the woolen mantle of an inhabitant of the desert and others had considered that it always holds in its interstices sufficient water to prevent complete desiccation. But Davis and Hudson rightly objected that sand at 100° C., at which temperature dried rotifers may survive for a short time, would be but poor protection against loss of water in the case of such a soft bodied animal, and Davis' explanation that the rotifers are covered by an actual waterproof capsule has the merit of being the first one to take this fact into consideration.

Nevertheless, there are many reasons why this view cannot be accepted. In the first place the evidence for the existence of such a gelatinous secretion is extremely slight. Davis did not see it

clearly himself and merely states in support of this view that several of his correspondents after reviving dried rotifers observed a quantity of sticky material adhering to them. This is not surprising since the cement glands at the tip of the foot are continually secreting a sticky substance by means of which the animal fastens itself to solid objects and which under certain conditions may be poured out in considerable quantities. The mere presence of a small amount of this substance seems like rather slender evidence on which to base such far-reaching conclusions.

Hudson ('89) is more definite in his statements. In describing the appearance of a group of rotifers dried on a piece of paper he says, "Each *Philodine* is the center of a patch of glutinous secretion which meets the similar patches surrounding its neighbors in a succession of straight lines; so that the whole group has quite a tessellated appearance. Here and there where fibers pass over or through a group long tongues of the secretion stretch from the animals to the fibers." The appearance observed by Hudson is a very common one in groups of dried rotifers; the interpretation he makes of it, however, is entirely wrong. What he thought to be the "glutinous secretion" is nothing but the cuticle of the animal from which the internal organs have shrunk more or less in the process of drying and which so closely resembles a secretion of some sort as to be easily mistaken for it on superficial observation. In the present experiments many careful observations were made on rotifers during the processes of drying and subsequent revival with the result that absolutely no evidence could be obtained that any such substance is secreted at the time of drying or dissolved when water is added. By adding a little methylene blue to the water before the rotifers dry, it is easy to follow the process in detail. This stain colors no part of the living animal but that portion of the cuticle which is outermost when it is contracted. If the animals are not allowed to remain in it too long there are no injurious effects and normal recovery occurs. Rotifers thus stained and then placed in clean water before being dried show very clearly all of the changes that occur during the process. If any secretion were poured out in the manner described by Davis and Hudson it should be visible outside of the stained cuticle.

Such, however, is not the case. The cuticle itself at the time of drying becomes the "glutinous patch" and on the addition of water resumes its original appearance.

If we assume that the peculiar staining reaction of this portion of the cuticle is itself due to a protective secretion of some sort we still see that the explanation of Davis cannot hold. The staining reaction is the same in all animals, creeping, swimming, and contracted, and appears very quickly. Even in rotifers killed instantly by a drop of boiling water the blue color appears just the same as in living ones. The secretion then, if such it be, is present in all individuals at all times and is not produced only on special occasions as Davis asserts and as it should be if his views were correct.

Another objection to Davis' theory lies in the behavior of the rotifers at the time of drying. Davis held that the failure of rotifers dried on a clean slide to protect themselves was because of their behavior under such unusual conditions. To quote from his paper, "The rotifers in crawling excitedly over the slide as they generally do trying to find more water or protection in their usual refuge—sand and dirt—part with much of their adhesive covering and the evaporation of the small quantity of water is so rapid that they have no time to settle down quietly as usual while more covering is secreted; they roam about almost to the last minute when they are overtaken by drought and shrink hastily into a ball to dry and perish." Davis evidently had observed the behavior of rotifers dried on a slide without sand; he just as evidently had not observed their behavior when dried with sand. If he had, he would have observed no differences of any importance. Whether a rotifer is dried on a plain slide, under a cover glass, or with sand its behavior is essentially the same. It keeps on creeping until the water has so far evaporated that it can creep no farther and then it contracts and dries more or less rapidly according to circumstances. When the water begins to disappear it does not stop when it comes to a quantity of sand and quietly encyst itself as he supposed. In almost every case it keeps on creeping the same as before and often dries up at some distance from the sand. That most of the rotifers are eventually found in

contact with the sand is due entirely to the fact that as the water disappears their movements become more and more restricted until they finally come to an end in its vicinity where the water lingers longest. It is true that the presence of sand is beneficial in the drying process but, as experiments to be mentioned later show, this is due entirely to the greater slowness of the drying under these circumstances and not to any preparations the animal makes to resist desiccation. That the presence of sand is responsible for no essential difference in behavior is shown by the fact that rotifers dried with a small quantity of sand show practically as great a mortality as those dried with none at all. If the mere presence of the sand enabled the rotifers to encyst themselves normally, we should not expect this to be so. If, on the other hand, the effect is merely one of the rapidity of evaporation, the fact finds a ready explanation.

It is seen, therefore, that the two points of originality in Davis' view, namely, the presence of protective secretion and a difference in the behavior of the rotifers when dried under different conditions are both based upon too slight evidence and are not confirmed by more careful observations. So far as his arguments go, there is no reason to believe that rotifers exposed to conditions of dryness can escape a true desiccation. On the other hand, in addition to this negative evidence there is much positive evidence in favor of the view that an actual drying does occur.

2 Evidence for the View that True Desiccation Occurs

In the first place, it must be noted that during the process of desiccation there are very marked changes in the size and form of the rotifers. Shrinkage always occurs and there may be considerable distortion of the body if the drying is rapid enough. According to Davis' view we should expect such shrinkage only in rotifers dried on a clean slide which are killed by the process. The fact is, however, that it occurs in all cases. When rotifers are very slowly dried, with or without sand, they retain their original form very perfectly and fairly accurate estimates may be made of their loss in volume during the drying process. From careful

measurements made on several different individuals before and after the addition of water it was determined that rotifers which had contracted to one-third or one-fourth of their original volume and remained in this condition for several weeks or a month were perfectly capable of being revived. A number of careful measurements showed that under all conditions of drying—on a clean slide, with sand, on filter paper, etc.—a very considerable shrinkage occurs, the exact amount being dependent on the conditions of the desiccation. How can such results, which may be obtained by anyone with very little trouble, be reconciled with Davis' theory that loss of water is prevented by an impermeable secretion? If the water is retained within the body how can such loss of volume occur?

It might be suggested, although such a supposition sounds rather improbable, that loss of water occurs up to a certain point but that by the time this point is reached formation of some sort of a protective covering has gone far enough to prevent its further escape. That a protective covering impermeable to water is not present is shown by the following experiment. Several rotifers were dried for a month under the most favorable conditions possible; at the end of that time they were examined and seen to be considerably shrunken. A small piece of wet filter paper was then placed in the dish in which they were contained in such a way as not to touch them, and the dish was covered with a piece of glass. Camera drawings were made of the rotifers at intervals of one minute to determine the effect of the moist atmosphere. A distinct swelling was observed in one minute and in five minutes the volume had increased perhaps 50 per cent. The dish was then uncovered and shrinkage immediately occurred. When water was added the rotifers recovered normally. This experiment, therefore, shows conclusively that in rotifers dried in such a way that recovery is possible after a desiccation lasting for a month the covering of the body is freely permeable to moisture. It is certain, therefore, that no such protection as that demanded by Davis' view is present.

It might perhaps be objected that in the above experiment the swelling observed took place mainly in the superficial part of the

body—perhaps even in a special secretion surrounding it. The following experiment shows clearly that this was not the case. A number of rotifers were stained *intra vitam* with neutral red and then carefully dried. Several were tested to be sure that revival occurred normally on the addition of water. Others were then subjected in the dish in which they were dried, to *moist* ammonia fumes, being kept under observation all the while. Almost immediately the internal organs, which had stained deeply in the neutral red, began to change color and in less than a minute had become yellow, showing thereby the penetration of the ammonia. This experiment, like the preceding, shows that the cuticle of normally dried rotifers is not impermeable to gases and to water vapor and that it therefore cannot protect the animals from desiccation. It is quite possible and even probable that the cuticle, especially the thicker part about the middle of the animal which covers it when it is contracted, is useful in retarding evaporation. Experiments to be mentioned below show that too rapid evaporation is injurious. But although it may retard evaporation it cannot prevent it and it seems certain, therefore, that so far as any external covering is concerned there is no bar to complete desiccation of the animal.

That rotifers which have been exposed to conditions favoring desiccation contain very little water is shown by simple physical methods. Spallanzani and Doyère both noticed that such rotifers are so brittle that they break into pieces when pressed with the point of a needle. The same result was obtained in the course of these experiments and it was also observed that no water could be obtained by exerting pressure on the cover glass covering the rotifers, provided that the latter were examined immediately after removal from the desiccator. When kept in a damp atmosphere for a short time they absorbed sufficient water to be detected by this method of treatment, and this probably accounts for the results obtained by Davis, who claims to have been able to squeeze water from rotifers which had been dried for three weeks in a vacuum, since he admits that the water was obtained only after *repeated* pressure had been applied. The fact that repeated pressure was necessary at all shows that the amount of water present could have been but slight, and it leaves room for the objection that there was

time for moisture to be absorbed from the atmosphere. That only a small amount of water is sufficient to give the appearance he describes may be proved by pressing under a cover glass small shreds of filter paper or pieces of apparently dry plant tissues. There is also a possibility that what Davis imagined to be water was only a thin film of air which under certain conditions arising in microscopic work closely resembles water.

The fact that desiccation is often very complete may be shown by chemical means as in the following experiment. A number of rotifers, stained *intra vitam* with neutral red, were allowed to dry under the most favorable conditions possible and were then placed in a desiccator over night. One lot was moistened and found to be normal in every respect. A second lot was exposed to ammonia fumes rendered as dry as possible by means of calcium chloride and a third lot exposed to the same fumes after having been allowed to remain in a moist atmosphere for five minutes. The third lot changed color from red to yellow almost instantly; the second lot retained their red color. That the effect in the latter case was not due to the inability of the ammonia to penetrate the cuticle was shown by the fact that a number of the rotifers which had been purposely crushed also retained their color. The failure of the characteristic reaction between ammonia and neutral red in the case of rotifers dried in a desiccator must be considered to indicate that they retain very little water.

There is also much indirect evidence that the desiccation is a real one. Some of this evidence will be mentioned later but it may not be out of place to refer at this point to the resistance shown by dried rotifers to high temperatures. Gavarret and others have found that rotifers in water are killed by a temperature of 51°C ., giving all the evidences of heat coagulation. When partially dried and in a moist atmosphere they are killed at 81°C . and after thorough drying they may resist temperatures of 100° – 110°C . Doyère even found a rotifer which after being dried in the sun for several weeks was not killed by a very short exposure to 153°C . How can such results be explained on Davis' theory that the body fluids are retained within a watertight cyst? Does the protoplasm at the time of the formation of this cyst undergo some mysterious

change which causes it to resist the coagulating effect of high temperatures while still retaining its water? Such a supposition is on its very face highly improbable. On the other hand if we believe that there is an actual loss of water these facts fall in line with the observations of Lewith and others that in egg albumin with loss of water the coagulation temperature rises from 56° to 145° C. There is every reason to believe, therefore, that in *Philodina* we have to do with a true desiccation of the body. This is shown by the chemical and physical tests mentioned above, by the great amount of shrinkage that occurs at the time of drying, by the fact that the cuticle is freely permeable to gases and to water vapor, and by the additional fact that coagulation is not caused by relatively high temperatures. Furthermore, it has been shown that the only plausible explanation that has ever been given of a method by which loss of water could be prevented is based on insufficient observation and is not borne out by the facts. We must not assume, however, as many have done, that the desiccation is ever an absolute one. Even with the most perfect desiccating devices known it is impossible to remove the last traces of water at ordinary temperatures. The chemist recognizes the fact that in organic analysis it is necessary to heat the substance which is being analyzed almost to the charring point to remove all of the water it contains. With rotifers this is impossible since long before this point is reached certain irreversible changes have occurred which cause the death of the animal. It is certain that no rotifer has ever lived after an *absolute* desiccation. It is useless, therefore, to speculate whether or not life would be possible after complete removal of water, since such a condition cannot be attained by the means in our possession without destroying the very structures on which life depends. But the fact that an animal with no means of protecting itself against loss of water save the hygroscopicity of its tissues may remain capable of resuming its normal vital activities after a sojourn of weeks or even months in a vacuum or a desiccator is in itself a striking one and one which must necessarily enlarge our conceptions of the properties of living matter.

VII EFFECT OF DESICCATION ON THE LIFE PROCESSES OF
PHILODINA

It has been seen that recovery is possible in the case of rotifers which have attained a considerable degree of desiccation. It remains to consider in more detail the effect of this desiccation on the life processes of the animal. Are the latter brought to a complete standstill or are they merely retarded? Is the dried rotifer dead or alive—or neither? Does the process of desiccation have any injurious effects and if so what is the nature of these effects? Questions such as these have occupied the attention of physiologists for many years and in the following section an attempt will be made to at least partially answer them. The last one—having to do with the injurious effects of desiccation—will be considered first since it appears to throw some light on the others.

I Injurious Effects of Desiccation

It has been the testimony of practically all observers that desiccation is always more or less injurious. This is shown by the fact that when large numbers of individuals are used some deaths always occur, even under the most favorable conditions of drying, and by the further fact that desiccation may not be repeated indefinitely. Spallanzani was the first to observe the latter point. He found that in certain rotifers experimented on by him all were killed after the sixteenth drying. In the course of this work Spallanzani's experiment was repeated with a small number of rotifers, the latter being dried once a day with a small quantity of sand. The following table shows the number of dead and alive on each successive day:

TABLE I

<i>Date</i>	<i>Alive</i>	<i>Dead</i>
December 31.....	11	0
January 1.....	8	3
January 2.....	5	6
January 3.....	2	9
January 4.....	2	9
January 5.....	2	9

<i>Date</i>	<i>Alive</i>	<i>Dead</i>
January 6.....	1	10
January 8.....	1	10
January 8.....	1	10
January 9.....	1	10
January 10.....	1	10
January 11.....	0	11

2 Influence of the Previous Condition of the Animals

The preceding experiment is typical of a considerable number performed and shows unmistakably that desiccation is a more or less harmful condition which some rotifers are better able to resist than others. That the resisting power of different individuals may be influenced by the conditions to which they were exposed previous to desiccation is shown by the following experiment. A number of rotifers were placed in a glass dish with a little clean water and were starved for three days. A number of others were taken from the original culture and both lots dried under exactly the same conditions and kept for the same length of time (four days). Water was then applied and the percentage which recovered noted. The following table gives the result of the experiment:

TABLE II
Effect of Starving

	Number of individuals	First movements (average for three rotifers)	Percentage of recoveries
Well fed individuals.....	15	19 minutes	80
Starved individuals.....	20	no movements	0

This experiment shows the great caution that must be used in comparing results obtained with rotifers taken from different cultures or the same culture at different times. In all of the following experiments the rotifers compared were as similar as it was possible to obtain them and furthermore fairly large numbers were dealt with so as to eliminate as far as possible this source of error.

*3 Influence of the Conditions Attending Desiccation**a Effect of Rapidity of Drying*

Since the time of Spallanzani it has been the almost unanimous testimony of all observers that rotifers dried on a clean glass slide, even for short periods of time, are killed, while when a little sand or moss is present this is not the case. These factors have been variously explained. Spallanzani thought that the sand was necessary to protect the animals from the injurious action of the air. Ehrenberg and others that it held in its interstices sufficient water to prevent actual desiccation and Davis that in its presence the rotifers were able to protect themselves by means of a waterproof cyst. All of these views have been shown to be untenable. That it is not contact with the air that kills the animals is shown by Doyère's experiment in which rotifers and tardigrades dried in the air showed a lower mortality than those dried in a vacuum. That the sand in itself cannot serve as a protection against evaporation is shown by the fact that it may be heated to a temperature of 100° C. or more without killing the rotifers contained in it. That Davis' explanation is not the correct one is shown by the fact, discussed at some length above, that even in rotifers dried with sand a true desiccation occurs. We are, therefore, forced to the conclusion that the favorable effect of the sand is due rather to a retardation of desiccation than to its entire prevention.

To test this view three lots of rotifers were taken from the same culture and the first dried without any sand, the second with a small quantity, and the third with a large quantity. All were then kept at room temperature under exactly the same conditions for four days, at the end of which time water was added and the rotifers kept under observation. In the following table the first column gives the number of individuals experimented upon, the second the average time required for movements to appear in a given number and the last column the percentage that eventually recovered. The number chosen for the second column was usually 10 per cent of the entire number of individuals and except where otherwise mentioned the determinations were made on this

basis. The choice of 10 per cent, of course, was entirely arbitrary but this number was found to be convenient and to give fairly reliable results. In making the last determination it was necessary to keep the animals for at least a day to be sure that no cases of revival should be missed. For this reason, in this and all similar experiments, the rotifers were dried in shallow glass dishes which could be covered and thus the loss of the water added at the end of the experiment prevented. A great fault to be found with much of the previous work on the subject is that ordinary glass slides were used with which on account of evaporation the rotifers could not be kept under observation for more than a few hours. Doubtless this is the reason why so many observers have been unable to find evidences of revival after drying without sand.

The results of the experiments, made with these precautions, are given in the following table. It will be seen that the time required for the first movements to appear is inversely proportional to the percentage of recoveries; in other words, the slower the movements are in appearing the greater is the mortality. This relation was shown in a large number of experiments to be quite general and knowing one quantity it is possible to predict in a rough and approximate way the other.

TABLE III
Effect of the presence of sand

	Number of individuals	First movements	Percentage of recoveries
Dried with large quantity of sand	49	12 minutes	82
Dried with small quantity of sand	65	45 minutes	17
Dried without sand.....	58.	No movements in 40 minutes	5

These experiments are in full accord with the view that the protective effect of the sand is due to the greater slowness of the drying process when it is present. If the mere presence of sand in itself were sufficient to cause the rotifers to settle down and secrete a capsule about themselves we should expect that a small quantity would be almost as effective as a large quantity. This, however,

is not the case as the above figures show. The difference between the effects of drying with a small quantity of sand and with none at all is usually not so great as would appear from the table, since in the first experiment the mortality is unusually high. As a rule, almost the same result is obtained in the two cases.

The effect of the rapidity of drying is brought out in a striking manner in the following experiment. Two lots of rotifers from the same culture were dried at the same time at room temperature, the one in a calcium chloride desiccator and the other in a partly covered dish containing a piece of moist filter paper. In the first dish the drying required twenty-five minutes; in the second about twelve hours. The drops of water in the two cases were as nearly the same size as possible and neither contained any sand. After the drying had taken place both were kept for three days in the desiccator, attaining presumably the same degree of dryness.

TABLE IV
Effect of rapidity of drying

	Number of individuals	First movements minutes	Percentage of recoveries
Dried slowly under cover.	60	7	75
Dried uncovered in desiccator. . .	51	39	12

It is seen from these figures that by allowing the drying to proceed slowly enough a large percentage of the rotifers may recover even when dried for several days in a perfectly clean glass dish. This result is opposed to most of the observations that have been made and is not in accord with Davis' theory. It must also be noticed that even where the drying was extremely rapid a certain number recovered when by using a covered dish rather than an ordinary slide they could be kept a sufficiently long time after the application of water.

Any method of retarding evaporation seems to have the same effect. Rotifers dried on small pieces of filter paper in which evaporation is necessarily slow may be preserved in good condition for very long periods. Even after a month movements appear

on the addition of water in five to seven minutes and creeping individuals are sometimes observed in ten or fifteen.

These experiments, therefore, seem to show that the rapidity with which the first part of the drying occurs is of great importance in determining the ultimate effect of the desiccation on the animal. They also serve to explain the discrepancies that appear in the accounts of various observers. Spallanzani, Pouchet, Davis, Hudson, Leidy, Zacharias, Faggioli and others state very positively that a few hours drying on a clean slide is invariably fatal. Doyère, on the other hand, is just as positive that such treatment is not necessarily harmful. When we examine the accounts given by these experimenters of their method of procedure we find that Doyère dried his animals very slowly while the others did not take this precaution, hence the disagreement in their results.

When we compare rotifers dried slowly with those dried rapidly we can see why the latter method of procedure should be injurious. A rotifer dried slowly, although shrunken to a fraction of its former size, preserves perfectly its original form and shows no irregular wrinkles on the surface of the body. The body wall fits tightly about the internal organs and the muscles adhere to it closely. One dried rapidly, on the other hand, is very irregular in appearance. The internal organs are more or less shrunken away from the cuticle which itself is often very much distorted, and sometimes has the appearance of an irregular gelatinous secretion. Frequently when water is added it may be seen that some of the muscles have been torn by the stresses set up by the rapidity of the drying and it is to be supposed that under these conditions the delicate internal organs would also suffer greatly. The injurious effect of the rapid drying therefore seems to be a mechanical one. Even those individuals which are not permanently injured by the first drying, since they are very susceptible to changes in moisture, if kept exposed to ordinary atmospheric conditions would be far more likely to be harmed than those which at the start contracted into a compact and symmetrical mass. This may explain the fact that rotifers dried without sand cannot be kept for such long periods of time as those dried under more favorable conditions.

b Effect of Temperature at which Drying Occurs

From the results obtained in the preceding experiment it might be thought that rotifers dried at a high temperature would show a greater mortality than those dried at a low one on account of the more rapid evaporation of the water. Strangely enough, this is not the case. In two parallel experiments in which all of the conditions are the same except the temperature at which the drying occurs, the rotifers dried at the higher temperatures always show the higher percentage as well as the greater rapidity of recovery. This relation was found many times. The following are two typical experiments. In the first one a moderate amount of sand was present; in the second the rotifers were dried in clean dishes, without any foreign matter. In both cases after the drying was complete, the dishes were allowed to remain covered at room temperature for four days before the application of water.

TABLE V

Effect of temperature at which drying occurs. Sand present

	Number of individuals	First movements minutes	Percentage of recoveries
Dried at 20°	49	12	82
Dried at 40°	55	4	94

TABLE VI

Effect of temperature at which drying occurs. No sand

	Number of individuals	First movements	Percentage of recoveries
Dried at room temperature fairly rapidly	58	no movements in 40 minutes	5
Dried at 40° in desiccator	52	20 minutes	65
Dried at 40° in covered dish	57	7 minutes	93

These experiments do not form an exception to the rule stated above that rapid drying is more injurious than slow drying. *Other things being equal* rapid drying is the more injurious. This is seen in Table V. The rotifers dried in the desiccator at 40° showed a

greater mortality than those dried slowly in a partially covered dish at the same temperature although in spite of the rapidity with which they were dried they were much less injured than those dried at 20°. We may conclude, therefore, that while rapidity of desiccation is a very important factor in determining the mortality, it is not the only one and it may even be obscured by certain others such as the one mentioned.

Attention must be called to the extremely high percentage of recoveries and the rapid appearance of movements in the case of the rotifers dried slowly at the high temperature. Although no sand or other solid matter was present and although the drying lasted for four days, fifty-four out of the fifty-seven rotifers recovered normally. This is as high a percentage as is usually obtained under natural conditions. When we recollect that almost all workers on the subject have failed to get any cases of recovery after even a few hours' drying in the absence of sand and that Doyère who is almost alone in having observed such recovery admits that in his experiments the mortality was always great and recovery very slow, we see the great importance of the external conditions under which desiccation occurs.

It is difficult to say just why the high temperature should have a beneficial effect. It cannot be that by promoting chemical changes it causes the formation of a protective secretion since rotifers dried at the high temperature appear even more shrunk than those dried at a low one and furthermore because they swell with equal readiness in a moist atmosphere showing the perfect permeability of their cuticle to water vapor. Perhaps the high temperature by favoring greater muscular activity may cause a greater contraction at the time of drying and thus prevent the injurious stresses set up in the tissues of a partially extended animal when the water leaves it rapidly. However this may be, it is interesting to note that under natural conditions the water in the urns in which *Philodina* lives must frequently reach a fairly high temperature at the time of its evaporation from exposure to the sun's rays and perhaps the greater resistance shown by this rotifer when dried at a high temperature is due to an adaptation to the conditions under which it lives in nature.

c Effect of Duration of Desiccation

The effects so far considered have been those due to the conditions under which drying occurred. It has been seen that the rapidity of the drying has a very important relation to the ultimate effect of the process and that the temperature at which it occurs is also of importance. There are other effects, however, which do not depend on the conditions under which the desiccation occurs, for example, it has long been known that the duration of the desiccation must be taken into account. Other things being equal, the longer the rotifers are kept the smaller are their chances of recovery. This fact has been noticed by numerous observers; Davis was unable to revive rotifers that had been kept dry under natural conditions for a year. The following table, based on experiments made on rotifers dried fairly rapidly in the absence of sand illustrates the relation that exists between the duration of the desiccation and the mortality. The rotifers all came from the same culture and were dried under exactly the same conditions at the same time.

TABLE VII
Effect of duration of Desiccation

Time of drying	Number of individuals	First movements minutes	Percentage of recoveries
5 minutes.....	40	11	98
1 hour.....	27	14	89
24 hours.....	32	22	84
2 days.....	25	35	60
4 days.....	33	68	54

d Effect of Alternations of Moisture and Dryness

Since the conditions of drying in the preceding experiments were the same in each case the difference in mortality observed must be due to some progressive change or changes that continue throughout the entire period of desiccation, the only variable factor being that of time. The question arises as to the cause of the

larger number of deaths in the longer period of drying. Is there a gradual loss of water which eventually injures the animals? Does slow metabolism gradually destroy the organs of the body in the absence of food? Do injurious waste products accumulate which cannot be eliminated? Or do changes of temperature and moisture result in mechanical injury to the tissues?

That changes in moisture and changes in temperature insofar as they cause changes in the relative humidity of the atmosphere have an injurious effect may easily be shown. In the following experiments two dishes containing rotifers from the same culture were dried under exactly the same conditions, no sand being present in either. Both were placed in an oven at a temperature of 40°. The first one was kept covered, its condition, therefore, remaining uniform, and the second one alternately turned upside down over a small dish of water and right side up over the same dish, being thus exposed to alternating moist and dry conditions. This process was repeated five times during a period of an hour and a half. In no case were the rotifers touched by the water. As in the other experiments the time required for the appearance of movements and the percentage that recovered were noted.

TABLE VIII
Effect of alternations of moisture and dryness

	Number of individuals	First movements	Percentage of recoveries
Dry 1½ hours.....	44	9 minutes	48
Alternately moist and dry 5 times.....	55	no movements	0

This experiment is interesting in several ways. In the first place, if death depended on loss of water as assumed by Davis and others we should expect the mortality to be less in the second case in which the rotifers had scarcely an opportunity to become thoroughly dried than in the first in which evaporation was unchecked. Instead, we find the reverse to be the case. In the second place the experiment shows in a physical way watsiologh

certain experiments mentioned above show in a physical way, namely, that the cuticle of dried rotifers is freely permeable to water vapor, their death being caused by changes in the amount of moisture.

Since rotifers are so easily killed by rapid changes in moisture content it must be supposed that in nature this is at least one of the factors responsible for the higher mortality during long periods of desiccation. The causes of the injury as in the case of rapid drying is probably mechanical, resulting in destruction of the delicate internal organs and the cells composing them. Rotifers living under natural conditions where much sand and organic matter is present would not be subjected to such rapid changes as those kept in small dishes and hence the mortality would naturally be expected to be lower. The protective effect of sand, therefore, is due not only to prevention of too rapid drying at the start but to prevention of too rapid changes in the moisture content of the animals after desiccation has occurred.

e Effect of the Intensity of the Desiccation

The mechanical injuries to the tissues caused by the swelling and shrinking under different atmospheric conditions are not the only causes of death during a prolonged desiccation. Under perfectly uniform conditions either in the dry air of a desiccator or in air thoroughly saturated with water vapor the same relation exists between mortality and duration of the desiccation. Other things being equal, a long desiccation is more injurious than a short one. Since the external conditions remain the same the injury must be due to some progressive change occurring within the body of the animal. Conceivably this change might be (1) a gradual loss of water (2) an accumulation of injurious waste products or (3) metabolic processes by which either the reserve supplies of food or the body tissues themselves are slowly used up.

There are many reasons to believe that the first of these possibilities is of little importance. The loss of water after the first part of the drying has occurred would be extremely slight and under ordinary conditions would come to an end as soon as an

equilibrium had been reached with more or less moist surrounding atmosphere. In a desiccator, to be sure, evaporation could continue almost indefinitely, but it has been seen from the experiments of Doyère, Gavarret, etc., that even after the most intense desiccation possible by physical means at ordinary temperatures many rotifers may recover, while they would surely have been killed if kept long enough under conditions in which the same degree of desiccation could never have been attained. Furthermore, in an atmosphere saturated with water vapor the drying is very slight and yet the same relation between mortality and duration of desiccation is noticed as in a case where thorough drying may occur. All attempts made in the course of this work to show by experiment that the intensity of desiccation in itself, and apart from other factors, has an effect on the mortality gave negative results. It could not be proved that an intense degree of desiccation is more injurious than a moderate one except insofar as a greater mechanical injury results when the water is extracted rapidly from the tissues. Presumably, the harmful effect of very perfect desiccation would be but slight if the water could be extracted slowly enough to avoid mechanical injury to the vital organs. Indeed, it seems certain that under some conditions a complete or nearly complete desiccation is far less harmful than an imperfect one. Rotifers kept in a moist atmosphere actually show a greater mortality than those kept in a dry one. The following experiment makes this point clear. Four dishes containing rotifers from the same culture were dried under exactly the same conditions (40° C. and no sand). All were kept for four days, one covered at room temperature, one covered at 40° , one in an atmosphere saturated with vapor at room temperature (average 20°) and one similarly saturated at 40° .

TABLE IX
Effect of moisture. Temperature constant (20°)

	Number of individuals	First movements minutes	Percentage of recoveries.
Moist atmosphere.....	48	9	38
Dry atmosphere.....	54	8	85

TABLE X
Effect of moisture. Temperature constant (40°)

	Number of individuals	First movements	Percentage of recoveries
Moist atmosphere.....	47	no movements	0
Dry atmosphere	45	14 minutes	71

It is seen from the tables that those kept in the moist atmosphere showed a far higher mortality than the others. The percentage that recovered was only 38 per cent as against 85 per cent at room temperature and 0 per cent against 71 per cent at 40°. We are forced to conclude therefore that death is not due to loss of water but to some process that is promoted by the presence of water. This being the case, what becomes of the view of Davis and others that rotifers protected from desiccation live while those exposed to it die? If a rotifer be protected by a waterproof and airtight cyst and be moist inside anyway, why should the presence of a little moisture in the air surrounding it be so injurious? This and other similar experiments show how untenable such a theory is

f Effect of Temperature on Mortality

It has been seen that rotifers dried at a high temperature show a lower mortality than those dried at a low one. If, however, the high temperature be allowed to continue for any length of time it has a harmful effect. This relation suggests that certain metabolic changes may be a source of injury to the animals since chemical reactions in general, including those which go on in the bodies of living organisms, take place far more rapidly at high than at low temperatures. The fact that the moisture causes an increase in the mortality, the greatest number of deaths occurring at the high temperature in the moist atmosphere, confirms this view. For convenience in comparison the two preceding tables are rearranged so as to make the amount of moisture the constant factor, the temperature being the variable one.

TABLE XI

Effect of temperature. Moisture constant
Moist

	Number of individuals	First movements	Percentage of recoveries
Kept at 20°	48	9 minutes	38
Kept at 40°	47	no movements	0

TABLE XII

Effect of temperature. Moisture constant
Dry

	Number of individuals	First movements minutes	Percentage of recoveries
Kept at 20°	54	8	85
Kept at 40°	45	14	71

4 *Condition of the Life Processes in a Dried Animal*

It has been seen that the causes of injury to *Philodina* during a process of drying are various in nature. We can distinguish between the mechanical injuries resulting from too rapid desiccation or from sudden changes in the moisture content of the tissues and the injury which is due to slow changes within the body of the animal which continue under the most uniform external conditions. The fact that these changes are greater at high temperatures and under conditions of moisture than at low temperatures and in the absence of water makes it probable that they are chemical in nature and represent a certain amount of metabolism in the body of the animal. The question naturally arises whether death in the cases cited was due to an actual destruction of the tissues or to some more indirect cause. The former alternative is that adopted by Ritzema Bos in the case of *Tylenchus*. Many considerations make this view improbable in the case of *Philodina*. In the first place, both old and young individuals may be kept in an apparently healthy condition for at least a week in water free from any organic matter that might serve as food. In this case, where active movements continue all the while and where

the body is thoroughly saturated with water, destructive metabolism would necessarily be far more rapid than in the cases mentioned. If death does not occur in the former case it would be far less likely in the latter. The death of rotifers kept for four days in a moist atmosphere, therefore, cannot be explained as being due to destructive metabolism alone. It might be objected that the process of drying in some way lowers the vitality of the animals so that they are more readily injured by changes of this sort. That this is not the case is shown by the following experiment. Thirty-nine rotifers were dried in the same way as those used in the last mentioned experiments. The dish containing them was then inverted over another dish of water in such a way that the moisture could condense on it in small droplets. In this way each rotifer became surrounded by a minute drop of water which, however, in most cases was too small to permit creeping, although allowing a certain amount of movement. They were allowed to remain in this condition for four days, during which time, of course, no food could have been obtained even though feeding movements had been possible. When water was added, all began to creep at once and seemed perfectly normal in every respect. The experiment was repeated with twenty-eight rotifers and again 100 per cent of the animals recovered, creeping movements beginning almost immediately. It will be observed that in these two cases the animals had been dried and by the addition of a very small amount of water placed in conditions favorable to metabolism. Although no food was present all of the animals recovered. Of those dried in the same way and kept for the same time at the same temperature with only so much water as they could absorb from the air, only 38 per cent recovered—and this was a somewhat higher percentage than was obtained in other similar experiments.

We have to deal, therefore, with an interesting condition. Rotifers dried and kept dry recover normally; those dried and placed in a small quantity of free water do likewise; those dried and kept in an intermediate state are mostly killed. What is the cause of this condition? If we examine a rotifer that has been placed in a moist atmosphere after being dried we find that although in appear-

ance it is plump and moist there are absolutely no traces of movement either in the muscles or the contractile vesicle. The animal is to all appearances dead, although the experiments mentioned show that certain metabolic changes in all probability are occurring. In the case of animals surrounded by even a very small quantity of free water movements may frequently be observed and the activity of the nephridia may often be clearly seen. In the dried animal, while there are no movements of any sort, metabolic changes must necessarily be very slow. It is possible, therefore, that in the case of the rotifers surrounded by the moist air that death may be caused by the fact that only a part of the functions of the body continue while the others are suspended, metabolism, for example, going on but the excretory organs being unable to remove the waste substances set free. Perhaps, under these conditions, owing to the impossibility of free interchange of liquids between the different parts of the body, some of the organs are especially affected by the metabolic changes that occur. Whatever the nature of the processes with which we are dealing may be, it seems certain that a state in which rapid chemical changes may continue while some at least of the other vital processes have been suspended is one of great danger to the animal. This danger may be overcome either by reducing the chemical changes that occur by further drying or by causing the resumption of the other vital processes by the addition of a little free water.

It is interesting to note that the suspension of many of the normal activities of the animal occurs very suddenly. If a rotifer drying on a clean slide be watched under the microscope and a drop of water added at the instant when the last film of water surrounding it is about to disappear, movements are seen to be resumed within a few seconds; if the water be added a second after the film has disappeared no movements occur for several minutes. The actual amount of desiccation in the two cases is almost the same; the effect produced is very different. As the last traces of the surrounding water leave the animal there is a sudden suspension of certain of the life processes. The suddenness of this suspension as well as the fact that it occurs before actual drying of the tissues of the animal has begun makes it probable that we are dealing

with a phenomenon under the control of the nervous system. We have to distinguish, therefore, between metabolic processes, which continue as long as any water is present, and other life processes which come to a sudden stop at the very onset of desiccation.

This brings up the question, so often discussed in past years, as to whether a rotifer which has been dried as thoroughly as possible may be said to be living or what Preyer terms anabiotic, that is, lifeless but capable of life as opposed to lifeless and incapable of life. The truth probably lies between the two extremes. We have no reason to believe that there is a complete cessation of all metabolic changes, as Preyer and others have asserted, since so long as any traces of water are present such changes are possible even though they may be greatly reduced in extent, and it is well known that it is impossible to remove all of the free water from organic substances at ordinary temperatures. On the other hand, there appears to be a complete suspension of many of the normal vital processes. Those which depend on movements, either muscular or ciliary, are clearly impossible, and in all probability many others whose nature is more obscure cannot continue in the almost complete absence of water. In a dried rotifer, therefore, we have a cessation of the functions of many of the organs as such, while a certain amount of metabolic change in the tissues of the body as a whole still continues. Whether an animal in such a state is to be called living or lifeless depends entirely on our definition of "life."

5 Effect of Desiccation on Egg Production

During the course of the experiments carried on on *Philodina* it became apparent that a definite relation exists between the periods of drying and those of egg production. It was noticed that at certain times eggs and egg containing individuals were very numerous and that at other times they were almost lacking. Instead of a continual production of eggs as might be expected in a culture containing individuals of all ages and all degrees of maturity it was found that the eggs were produced at almost the same time by all the members of the community regardless of their size and age. On examining the records kept of the condition of the

various cultures, it was found that in every case the period of maximum egg production had been preceded by a period of desiccation and furthermore, that each desiccation of any length of time had been followed by an increase in the reproductive activity. This relation seemed to present so many points of interest that more exact observations were made extending over a period of several months. In addition, certain experiments were arranged in which other factors which might have an influence on the result were kept under control. The following is an account of the observations made on culture II during January, February and March, 1908.

The culture was started in December, 1907. After being dried several weeks, water was applied on January 2. Beginning with January 8 differential counts were made every few days to determine the percentages respectively of eggs, newly hatched young, and adults showing developing eggs. These counts were made from random samples of fifty or more individuals taken from different parts of the dish. Although the results thus obtained could from their nature be only approximate, the fact that several counts made on the same day usually agreed very closely and that smaller cultures in which the entire number of individuals could be observed showed exactly the same changes makes it certain that the figures given represent fairly accurately the condition of the culture as a whole. In Fig. I the percentages of the different classes of individuals have been plotted as ordinates over the dates of observation and curves drawn to represent the changes that occurred in the condition of the culture from day to day. The breaks in the curves represent the periods of desiccation.

Reference to the table will show that aside from a few irregularities due to the entrance of the element of chance the curves for the three periods show a general similarity. The curve representing the number of egg containing individuals shows a maximum about four days after the application of water, that representing the number of eggs shows a maximum in each case after an interval of six or eight days and that representing the number of young after ten or twelve days. These figures agree closely with those obtained where a small number of rotifers were kept under

FIG. 1

observation. The three maxima follow each other in regular order, as should be expected, the maximum for the development of the eggs within the body coming first, then that for the deposited eggs, and finally that for the newly hatched young. The two most noticeable irregularities in the curves occur in the January series. The first is in curve 1 where the maximum comes after the maximum of curve 2 instead of before it. This is probably due to the fact that no records were kept between January 2 and January 8. If they had been, a maximum in all probability would have been found during this period. The second irregularity, that in the curve representing the number of eggs, on January 25, was due to finding a cluster of five eggs, the eggs usually being laid in groups. A careful search failed to disclose any more eggs and hence this apparently high percentage did not represent a general condition in the culture, being merely one of the errors of chance that cannot be eliminated from a method of this sort. However, taken as a whole, the curves show in a striking manner the relation between desiccation and egg laying.

It might perhaps be objected that inasmuch as the periods of desiccation came at approximately equal intervals, their apparent influence on egg production might be due merely to a chance coincidence with a regular periodicity in the production of eggs which is independent of external conditions. That this objection has no force is shown by the fact that in other cultures dried at very irregular intervals exactly the same results were obtained and furthermore that in cultures allowed to continue for a long time without drying no such periodicity was observed. In one such case the culture had not been permitted to dry for several months. During the latter part of this period the percentage of eggs had fallen almost to zero, it being a rare occurrence to find even a single egg. The culture was then allowed to dry for two weeks. Ten days afterward the percentage of eggs had increased to over fifty where it remained for a few days and then again diminished until a week or two later it was at zero.

A number of experiments were made on small numbers of individuals. By keeping them in a fairly small drop of water in a covered dish an accurate count could be made of the number of

eggs produced. In one such case thirty rotifers which had laid no eggs for some time previously in less than a week produced fifty-five eggs. Similar results were obtained in other experiments. The length of time necessary for the appearance of eggs was about the same as that required in the large cultures.

A factor which must be considered as a possible cause of the phenomenon just described is the food supply. Associated with *Philodina* there are always found large numbers of the unicellular plant, *Sphærella lacustris*. The rotifers feed to some extent on the partly grown cells but much more largely on the small microzooids which are produced in large numbers after each period of desiccation. It might be thought, therefore, that the effect observed was due not to the direct influence of the desiccation but to its indirect influence in causing an increased food supply. To decide this point, experiments were tried in which the rotifers after being dried were placed in water free from microzooids. In all such cases, although there was no increase in the food supply, egg production was brought about just as before. The following experiment is a typical one selected from a number showing similar results.

Six rotifers were taken from a small culture in which no eggs had been laid for several weeks, and dried on a clean piece of filter paper for twelve days. On March 7 they were placed in a dish with water and as soon as they had revived the filter paper was removed. No food of any sort was present. In one of the rotifers the ovary was slightly enlarged, in the others it showed no signs of developing eggs. On March 8 the alimentary tract of all had acquired a glandular appearance but contained no food. Traces of egg formation were observed. On March 9 three contained fairly well developed eggs. On March 10 all six contained eggs and two individuals contained two eggs—a rather unusual occurrence. On the 11th one of the rotifers had died but the other five all contained eggs. On the 12th seven eggs had been laid, on the 14th eleven and on the 16th twenty-one, of which four had hatched. By the 17th ten of the twenty-one eggs laid had hatched and by the 21st all had hatched, no new ones being laid after March 16. On the 21st only one of the five rotifers showed any signs of

an egg in the ovary and this one did not develop. No further egg laying occurred.

This and similar experiments show that the production of eggs is not due to the better food supply after a period of desiccation; since egg production takes place in the absence of food. Furthermore, in the cases where a few individuals were kept under direct observation the formation of eggs was sometimes observed to begin before the microzooids had been produced. That the food supply is not without effect, however, is shown by observations such as the following. Seven rather small rotifers showing no trace of eggs were dried on filter paper for three weeks and then placed in a dish of clean water without any food whatever. The development of eggs started just as before and in three days all except one contained partially grown eggs. At this point, however, development ceased, the eggs becoming no larger. Six days later microzooids were added from another culture. The rotifers fed rather freely on them and in three days all contained large eggs. An egg laying period then ensued which did not differ from those already described. This experiment shows that although an abundant food supply is not the factor that starts egg production, a sufficient supply of food must be present in order that the latter shall continue. This is especially the case in smaller rotifers in which the reserve supply stored in the tissues of the body is not great.

Another factor that must be considered is the amount of oxygen present in the water. It is conceivable that the failure of old cultures to produce eggs is due to an exhaustion of the oxygen supply of the water and that the greater activity after a period of drying is due to a renewal of this supply. To this objection it may be answered that cultures in which *Sphærella* is present in abundance and in which, therefore, considerable oxygen must be set free in the presence of the light, behave exactly in the same way as cultures in which *Sphærella* is altogether absent. Furthermore, the addition of fresh water when the culture is almost dry or even after a very short period of desiccation produces no noticeable effect. This last mentioned fact speaks against the view that a diminished production of eggs in old cultures occurs as the result of the accumulation in too large quantities of injurious waste

products. No matter how large the volume of water added no increase in egg production occurs without a fairly long desiccation. In cases where rotifers were transferred to other dishes and placed in fresh water there seemed to be no noticeable increase in reproductive activity.

Although desiccation is able to cause the formation of eggs at times when they would not otherwise appear it is not always necessary for their production. In an experiment made to determine the length of life of *Philodina*, several individuals, raised from the egg without any desiccation, when about a week old began to lay eggs and continued to do so for several weeks. Furthermore, it must be noted that under more natural conditions than can be obtained in the laboratory, eggs seem to be produced more freely than in the cases mentioned. Their production does not seem to be so clearly related to a period of desiccation. This may be partly due to the greater variety in the conditions met with out of doors in a large stone urn and partly to the more abundant food supply which would permit the effects of a single drying to last for a longer time. However this may be, and whether or not desiccation is the only factor concerned in the production of eggs the experiments described leave little room for doubt that it is a factor and under certain conditions a most important one. Under these conditions the process of desiccation serves as a stimulus to cause the production of eggs at times when otherwise they would not be produced. The drying in some way starts into activity the previously resting germ cells and inaugurates a process of growth and division which may continue for a considerable time after the initial stimulus has ceased to act. This fact is of some interest when considered in connection with certain other well known phenomena relating to the development of both plants and animals.

It is well known that most cells cannot continue to grow and divide indefinitely, without receiving certain stimuli from without. The behavior of some of the Protozoa furnishes a striking example of this point. Several workers have shown that in *Paramœcium* after a number of generations division becomes slower and finally ceases unless an appropriate stimulus be supplied. In

nature, this takes the form of a conjugation with another individual, but as Maupas, Calkins, and others have shown, other stimuli may serve the same purpose. After division has ceased it may again be started by a change in food or by various chemicals. Perhaps the list of stimuli that produce this effect is larger than we now suspect. Many examples might be given of the effect of external stimuli in causing growth to proceed beyond its usual limits. The production of plant galls by the stings of insects is one of the best known cases. Possibly the formation of certain pathological growths in animals is due to similar causes. Another example is furnished by the regenerative processes that occur in both plants and animals after injuries, in which resting cells are stimulated into activity.

In the case of the germ cells, which are potentially immortal the effect of outside stimuli is also clearly seen. In the life of nearly every egg cell, after a period of active growth and division comes a stage in which further development is impossible without some outside stimulus. Usually this stimulus is supplied by the entrance of the sperm. The process of fertilization has for its purpose two objects, the one being the introduction of new hereditary qualities and the other the stimulation of the resting protoplasm of the egg to develop. That these two processes are distinct is shown by the fact that under certain conditions development may be made to occur in the absence of the sperm. Cases of artificial parthenogenesis have been reported in many groups of animals in which fertilization is the rule in nature. The echinoderms, molluscs, annelids, and insects all furnish examples, and even in some of the vertebrates the early segmentation stages have been obtained by means of appropriate stimuli. Artificial parthenogenesis may be brought about in a variety of ways. Heat, chemicals, and hypertonic salt solutions have all been used with success. Furthermore, as Loeb has shown, a combination of two of these methods may be more effective than one alone. Experiment will doubtless show new methods and new combinations of old methods to secure the same result.

Is it not possible that in the case of *Philodina* the process of drying may furnish a stimulus comparable to those just mentioned,

the desiccation serving to start the development of the resting germ cells? Analogous cases are known in plants. Many seeds are known to be incapable of germination until they have been dried for a certain length of time. Drying is said to start into activity the resting buds of trees. In *Sphærella lacustris*, which is always found associated with *Philodina*, microzooids are always produced in large numbers after a period of desiccation. Furthermore, in Loeb's experiments on artificial parthenogenesis, the extraction of water from the eggs by hypertonic solutions furnished a very effectual means of starting developmental processes. Loeb has shown that the important factor in this case is the actual loss of water rather than its reabsorption when the eggs are again placed in normal sea-water. There seems to be no doubt, therefore, that under certain conditions loss of water may be an active agent in starting processes of growth and development in resting cells.

It is interesting to note, although the fact may have no significance, that the group of rotifers to which *Philodina* belongs, which are the only ones of the rotifers normally subjected to frequent periods of desiccation, at the same time are the only ones in which sexual reproduction has been entirely lost. The thought suggests itself that in the presence of such great changes in the external conditions the stimulus furnished by the union of the sex cells of different individuals is not so necessary as in most other animals. On the other hand, it must be noted that in the aphids parthenogenesis is said to continue as long as the external conditions remain uniform, males being produced only when there is a change in these conditions. Be this as it may, the fact remains that desiccation in certain rotifers is able to bring on a period of reproduction. Whether or not this is the only factor involved it undoubtedly is an important one and one which may be found to have a wider significance than we now suspect.

VIII SUMMARY

1 Rotifers of all ages may recover all of their normal activities after an extended period of desiccation.

2 The tissues of the body become truly desiccated, as is shown by the amount of shrinkage that occurs, by physical and chemical tests for water, and by other more indirect methods. The desiccation, however, must not be considered an absolute one, it being impossible to remove the last traces of water at ordinary temperatures.

3 The cuticle of *Philodina* under all conditions of desiccation is freely permeable to water vapor and gases. No evidence of a waterproof cyst can be found.

4 The presence of sand, etc., is not necessary that recovery shall occur as many have claimed. By proper conditions of drying almost all of the individuals recover after a desiccation of at least four days in the absence of all solid matter whatever.

5 The process of drying is always more or less injurious under any conditions, as is shown by the fact that some deaths almost always occur and that it may not be repeated indefinitely.

6 The chances of recovery of a given individual depend on its previous condition as well as on the circumstances under which drying occurs.

7 The rapidity of the drying process has a very important effect on the mortality, rapid drying being more injurious than slow drying. It is apparently immaterial by what means the process of drying is slowed.

8 The injurious effect of rapid drying seems to be due to mechanical injuries to the vital organs and the cells composing them caused by the rapid extraction of water.

9 The final intensity of the desiccation makes little difference in the mortality provided it be attained gradually enough.

10 Other things being equal, a fairly high temperature at the time of drying is favorable, a low one unfavorable.

11 Alternations of moisture and dryness are very injurious.

12 Rotifers kept at a high temperature show a greater mortality than those kept at a low one.

13 Rotifers kept in a moist atmosphere show a greater mortality than those kept in a dry one.

14 Metabolic changes in the tissues probably continue in dried rotifers though very much retarded. The functions of the various organs as such are suspended.

15 The suspension of these functions occurs very suddenly, possibly owing to nervous control.

16 Desiccation usually brings on a period of reproductive activity.

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