On the Desiccation Death of the Rotifer, *Philodina roseola*

By

Shun-ichi UMEZAWA

Zoological Laboratory, Faculty of Literature and Science, Kôchi University
(With 11 Text-figures and 1 Plate)

Introduction

It has long been known that the rotifer can survive drying conditions fatal to most other aquatic animals. Although much research has been done on the desiccation resistance of the rotifer, particularly by M. H. Jacobs ('09) and L. M. Hickernell ('17), the capacity of this animal to withstand severe desiccation has not yet been fully ascertained. When the animals are exposed to dry air for a long period of time and then transferred to water they do not suffer fatal injury. Various explanations and hypotheses concerning this are reviewed in Jacobs' monograph. These can be classified into the following three categories: (1) Animals protect themselves against dryness by their own preventive secretion, and this protection is promoted if they are surrounded with sand or other impurities. Moreover, the secretion is affected by the rate of drying, the animals being able to secrete enough under slow drying condition, but not under rapid one. In the former case the animals show a strong resistance to severe desiccation. (2) Even when animals are strongly desiccated, they do not suffer any injurious effects and this state of the animal is called "life-less". (3) Parthenogenetic eggs only may be capable of surviving desiccation, although adult animals can not withstand drying condition.

The present paper deals only with effects of drying on the rotifer, *Philodina roseola.*

Material

Specimens of a common Bdelloid rotifer, *Philodina roseola*, living in Sapporo and in Kochi were used for Exp. 1 and Exps. 2–5 respectively. A comparison was made between the desiccation resistances of *Philodina* in Sapporo and of those in Kochi, under definite drying conditions (temperature 25°–27°C., relative humidity 68–70%). A single rotifer on a slide-glass was subjected to desiccation for various periods of time; its strength of desiccation resistance was indicated by the length of time required to kill the animal. The rotifer in Kochi was more resistant to desiccation, and even after having been kept dry for about 37 hours, several individuals could survive without injury. On the contrary no rotifer in Sapporo was alive after drying for about 90 seconds.

In the first experiment the animals were reared with unicellular algae in a watch glass under laboratory conditions. When the amount of water in the watch glass decreased by evaporation, distilled water was added. In the other experiments the animals, breeding in natural environmental conditions, were used immediately after collection. Before the experiment they were repeatedly washed with distilled water. The animals used were about three or four days old after hatching and almost equal in size.

—— Death-form in *Philodina* ——

When the animals are immersed into water after desiccation, they usually swell out with water imbibed through their cuticle and begin to move. But some of them die during desiccation. The two main death-forms are observed: in one type the cuticle apparently separates from the visceral organs (Fig. 1, a) and in the other it does not(Fig. 1, b and c). The former is designated as "rapid drying death-form", and the latter "slow drying death-form". Photographs of the typical forms are shown in Fig. 1.

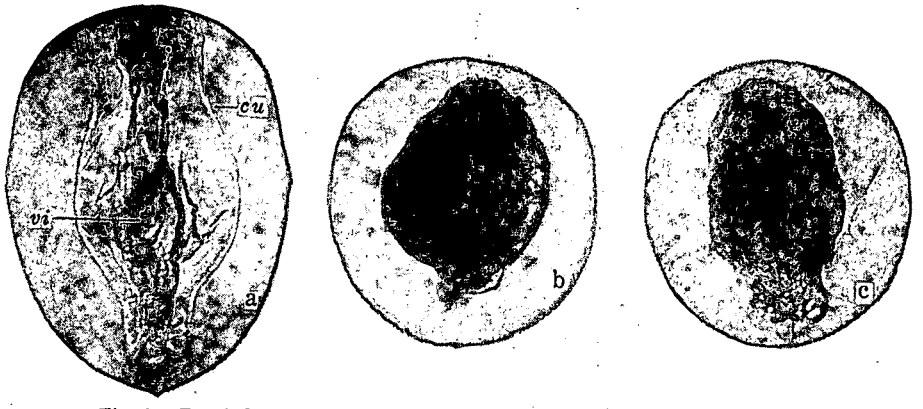

Fig. 1. Death-form in *Philodina*, cu, cuticle; vi, visceral organ. For further explanation, see text.

Exp. 1. Effect of velocity of evaporation on the desiccation death of the rotifer isolated on slide-glass

Apparatus and Method

Desiccation was performed individually on slide-glass under different conditions of temperature, relative humidity and velocity of air flow. The relative humidity was regulated

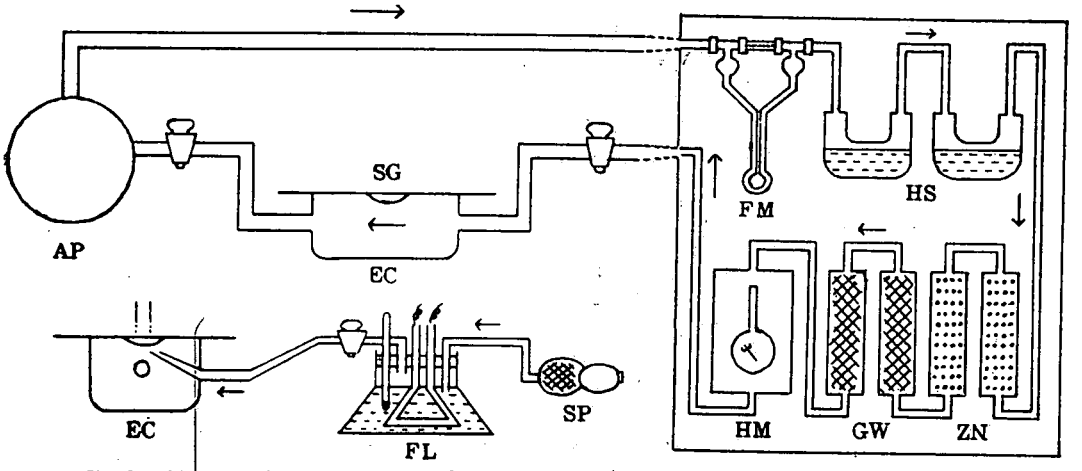

Fig. 2. Diagramatic representation of experimental apparatus. AP, air pump; SG, slide-glass; EC, experimental chamber; FL, flask; SP, spray; FM, flow meter; HS, sulphuric acid; HM, hygrometer; GW, glass wool; ZN, zinc oxide. For further explanation, see text.

with sulphuric acid solution of different concentrations. The main parts of the apparatus are shown in Fig. 2, where air is caused to flow in the direction of the arrow (→) by air-pump (AP). Water vapour in the air flowing in the apparatus was removed by sulphuric acid solution (HS). The sulphuric acid vapour involved in the air during passage over the sulphuric acid solutions, was removed by filtering through zinc oxide (ZN) and glass wool (GW). Hygrometer (HM) is placed just before the experimental chamber (EC). The velocity of the air flow can be controlled by the cock with aid of the flow meter (FM). The experimental chamber consists of a dish with three sidearms; and its upper side is closed by a slide-glass (SG) with stop cock grease. Two of sidearms are passages for the air and the other is connected to the flask (FL) which contains hot water, and the slender tip of the latter arm is bent upward so as to be situated just beneath the center of the slide-glass. When spray (SP) is pressed, the moisture in air pressed out from the tip is condensed onto the center of the slide-glass.

An animal with a bit of distilled water was placed on slide-glass. After water surrounding the animal was completely evaporated, a definite amount (0.00006 cc.) of water was added again,* which spread around the animal as far as slide-glass was kept throughly clean. In the drying experiment an animal was placed in experimental chamber (EC), adhering on the bottom surface of the slide-glass, and then the dry air was permitted to circulate through (EC). After a while the water surrounding the animal was completely evaporated. The time required for evaporation of the surrounding water was taken as a relative desiccation velocity. After a definite time of drying, a drop of cold water was placed on the upper outer surface of the slide-glass of (EC), and then moist air in (FL) was permitted to flow into (EC). By this procedure a drop of water was condensed; it covered the whole surface of the animal. After this the animal was transferred into water and an individual capable of moving within about twenty-four hours was reckoned as being alive.

Results

The desiccation was made under various conditions in temperature and relative humidity; temperatures employed are 13°, 23° and 33°C., relative humidities 32%, 54% and 80%. The velocity of air flow is constant. The percentage of recovery in each

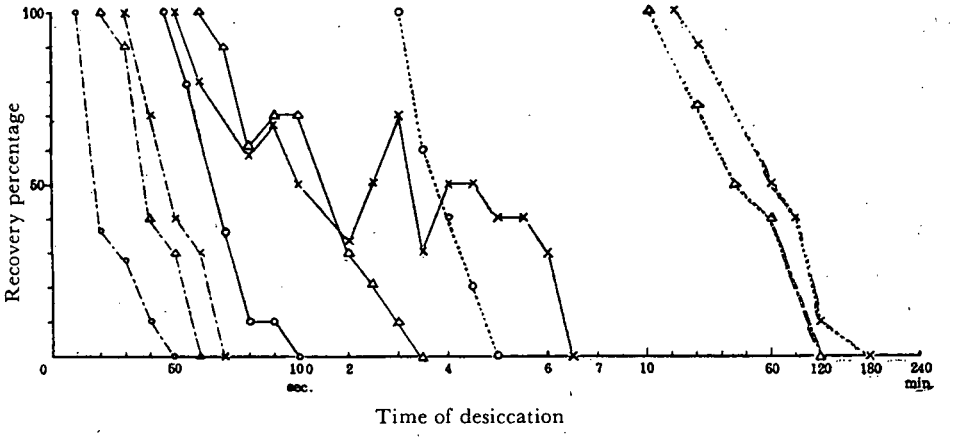

Fig. 3. Relation between the percentage of recovery and time of desiccation under various drying conditions. Temperature; ···13°C., ----23°C., -·-33°C.; relative humidity; ○32%, △54%, ×80%.

* The animal is not injured when no loss of water in the body occurs.

is shown in Fig. 3. The time required for killing the animal under relative humidities 32%, 54% and 80% at 13°C. was 5, 120 and 180 minutes respectively; at 23°C., 100, 200 and 390 seconds respectively; at 33°C., 50, 60 and 70 seconds respectively. The lower the relative humidity is at a fixed temperature, the sooner the desiccation death occurs, and the higher the temperature is at a fixed relative humidity, the sooner death comes. From these results it seems probable that the injurious effect of desiccation increases with the grade of drying.

Almost all of the individuals killed by desiccation showed the typical "rapid drying death-form".

—— The rate of evaporation of water from a droplet on the slide-glass under a definite drying condition ——

When a droplet of water hanging from the bottom surface of a slide-glass is exposed to an atmosphere of relative humidity R less than 100%, the droplet diminishes in size by evaporation, keeping semi-spherical form. If the radius of the droplet, the constant of evaporation and the density of water are denoted by r, k and ρ respectively, r decreases at the rate

$$-dr/dt=k(100-R)/\rho,$$

because water is lost by evaporation from a unit area of the droplet surface per unit time by an amount equal to $k(100-R)$. Then a droplet of which the radius is r_0 initially will disappear in the time

$$t_0=\rho r_0/k(100-R).$$

The author observed experimentally t_0 on a number of droplets having the same initial radius $r_0=0.0306$ cm and determined by means of the above formula the constant of evaporation k as shown in Table 1. The values of k were found as expected to be nearly constant for the same temperature but to decrease with the decreasing temperature.

If it is assumed, therefore, that the amount of water lost from the animals under a definite drying condition is equal to that from the free surface under the same condition, the amount of evaporated water from an animal at the critical point of the drying process can be calculated by means of k. The calculations under the various drying conditions are shown in Table 1.

Table 1. The relative amount of water lost from the animals at the critical point of the drying process

Temp. (°C)	R. H. (%)	t_0 (sec.)	k	Fatal limiting of the drying process on the animal, t_r (sec.)	The relative amount of water lost from the animals, $k \times t_r$ (gr/cm²/sec¹)
33	32	60	0.0000075	50	0.00038
	54	84	0.0000078	60	0.00047
	80	195	0.0000080	70	0.00056
23	32	115	0.0000040	100	0.00040
	54	205	0.0000033	200	0.00066
	80	550	0.0000030	390	0.00117
13	32	240	0.0000019	300	0.00057
	54	330	0.0000019	7,200	0.01368
	80	490	0.0000030	10,800	0.03240

As indicated in Table 1, the amount of water lost from the animal at the critical point

of drying is almost equal in values in each case, except the cases of temperature 13°C. and relative humidities 54% and 80%, in which this amount is exceedingly great. Consequently, it is considered that the amount of water which may be required to maintain the minimum vital activity may be approximately constant independently of the evaporation velocity under a rapid drying condition, but increases under a slow desiccation.

On the other hand, the rate of the evaporation of water from the free surface is in proportion to the saturation deficiency of air. The time required for drying a definite amount of water (0.00006 cc) from the free surface under each given drying condition was calculated (Table 2).

Table 2. The time of vaporization of 0.00006 cc of water

Temperature (°C)	Relative humidity (%)	Saturation deficiency (mm.)	Experimental (sec.)	Theoretical (sec.)
33	32	25.66	60	60
	54	17.36	84	88
	80	7.55	195	204
23	32	14.33	115	108
	54	9.73	205	158
	80	4.22	550	364
13	32	7.64	240	220
	54	5.39	330	286
	80	2.25	490	684

The disagreement of the measurement values with the calculated ones seems to be probably due to error caused by uncertainty in measurement of mass of a water droplet.

Exp. 2. Effect of velocity of evaporation on the desiccation death of the rotifer on filter paper

In order to ascertain more precisely the effect of evaporation velocity on the rotifer the experiments were performed again by three method. (1) Animals were desiccated on slide-glass under the condition of temperature 24°C. and relative humidity 88%. (2) Animals were placed on a piece of filter paper with a bit of distilled water and then desiccated under the same drying condition. (3) Animals placed on filter paper in the same manner were rapidly desiccated in P_2O_5–desiccator at 24°C. After a definite period of time they were transferred into water and individuals which had been capable of moving within about twenty-four hours were counted.

Results

The manner of the evaporation of water from the filter paper must somewhat differ from

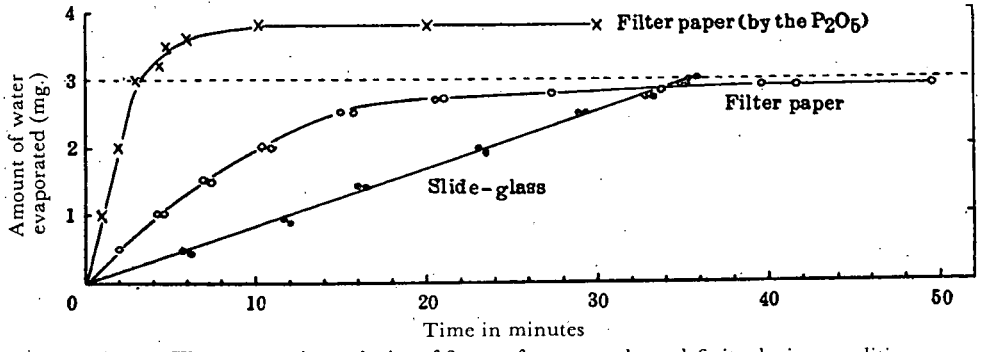

Fig. 4. The evaporation velocity of 3 mg. of water under a definite drying condition (temperature 24°C., relative humidity 88%).

the glass surface. Some experiments undertaken to demonstrate this difference, which results are summarized in Fig. 4, prove apparently this expectation to be true. At the beginning of the desiccation the rate of evaporation of water from the filter paper is more rapid than that the glass surface. But it decreases exceedingly with increase of the desiccation time and finally the evaporation stops at a higher level than that from the glass surface. It is certain that even after stoppage of evaporation under ordinary drying condition a small quantity of water still remains in the filter paper. Contrary to the case of the filter paper the evaporation velocity from a water droplet placed on glass is uniform throughout the desiccation period and water completely evaporates. The desiccation in P_2O_5-desiccator (Fig. 5) is very vigorous and even moisture retained in the filter paper which has been equilibrated under the conditions of temperature 24°C. and relative humidity 88%, quite disappears.

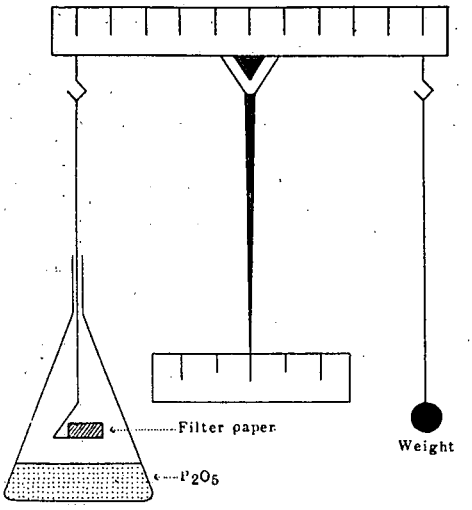

Fig. 5. Method of determination of the amount of water evaporated from filter paper in the P_2O_5-desiccator.

This difference in evaporation of water from glass surface with that from filter paper exerts different effects on the survival of the rotifer.

Table 3. The effect of desiccation on the rotifer, being isolated on slide-glass or on filter paper

	Drying condition	Time of desiccation (hr.)	No. of individuals	No. of individuals died from desiccation
Slideglass	24°C., 88%	0.5	19	1
		1	10	1
		2	15	5
		15	15	5
		20	20	15
		24	20	19
Filter paper	24°C., 88%	1	17	0
		2	10	0
		15	19	0
		24	20	0
		120*	10	0
	P_2O_5-desiccator at 24°C.	1	10	0
		2	13	0
		12	10	2
		24	16	1
		35	10	5
		48	10	7

* The drying conditions in this case are as follows; temperature 23°–25°C., relative humidity ranging from 88% to 75%.

As showen in Table 3, when desiccated on the slide-glass under the condition mentioned in (1) or on the filter paper in P_2O_5-desiccator the number of survival decreases in proportion to the length of the period of drying time. On the contrary, the animals placed

on the filter paper can survive without injury for a long period of time. As to the cause of this, perhaps some protective action of a small quantity of moisture retained in the filter paper may play an important role.

In this experiment most of the animals showed "slow drying death-form", except in the case of slide-glass in which a few individuals presented "rapid drying death-form".

Exp. 3. Effect of duration of desiccation on the rotifer

As shown in the preceding paragraphs, the number of dead animals increases with the time of the drying, and the mechanical injury which may be caused by rapid drying is not so seriously fatal, because of the fact that no "rapid drying death-forms" are observed even in the animals desiccated in the P_2O_5–desiccator. It is assumed, therefore, that some changes resulting from dehydration may take place within the body during the desiccation. In this experiment the effects of drying-duration on the desiccation death of the rotifer were dealt with.

Method

At the beginning of the experiment the animals were repeatedly washed with distilled water and then transferred onto pieces of filter paper with a small amount of distilled water. Some pieces of filter paper with the animals were dried until the water contained in them almost disappeared under the conditions of temperature 29°C. and relative humidity 75%*, and then transferred into desiccators which contained P_2O_5, $CaCl_2$ and anhydrous $CuSO_4$, respectively. The other pieces of filter paper were dried under the laboratory conditions. Then, all the pieces of filter paper were transferred into water at various intervals. The individuals which began to move within twenty-four hours were counted as surviving. About twenty individuals were used in each case.

Results

The laboratory conditions during the experiments were as follows: temperature 29°–34°C., relative humidity 75–95%, and saturation deficiency 1.80–9.44 mm. Results obtained are illustrated in Fig. 6.

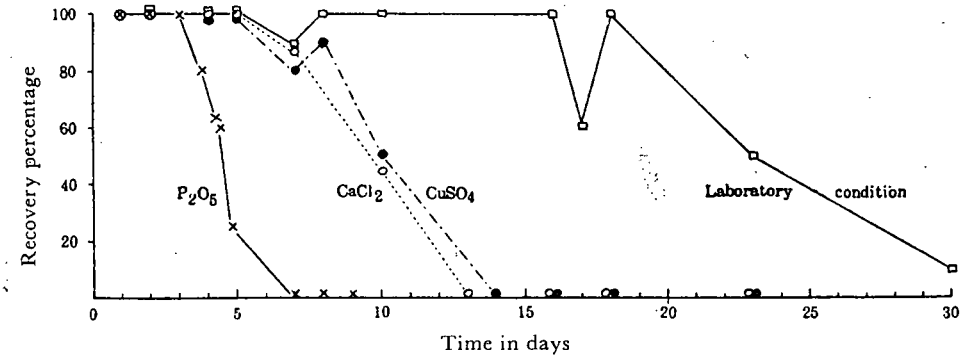

Fig. 6. Relation between duration of desiccation and recovery under each drying condition. About twenty individuals are used at each point.

* The water content of the filter paper in this case was equilibrated for about ten minutes.

As shown in Fig. 6, the fatal limit of time, when dried in the P_2O_5-, $CaCl_2$- and $CuSO_4$-desiccators, is 7, 13 and 14 days respectively. On the other hand, when the animals are dried under the laboratory conditions, a few can live after thirty days. Thus, the desiccation death occurs in order of P_2O_5, $CaCl_2$, $CuSO_4$ and laboratory condition. In all cases, especially in P_2O_5, recovery rate decreases with increase of duration of desiccation.

The body tint of the animals which had been placed on the filter paper was usually brick red, and sometimes gradually faded away during desiccation under the laboratory condition.

—— Water content of filter paper ——

The rate of evaporation of water from free surface is in proportion to the saturation deficiency of air. Water of filter paper may evaporate according to this law. But the results of the previous experiment show that in ordinary drying condition a small quantity of water remains in the filter paper. In this experiment one series of pieces of filter paper was kept under laboratory conditions with various saturation deficiencies and the other series was put into the desiccator of P_2O_5, $CaCl_2$ and $CuSO_4$. After about twelve hours changes in their weights were measured (Fig. 7).

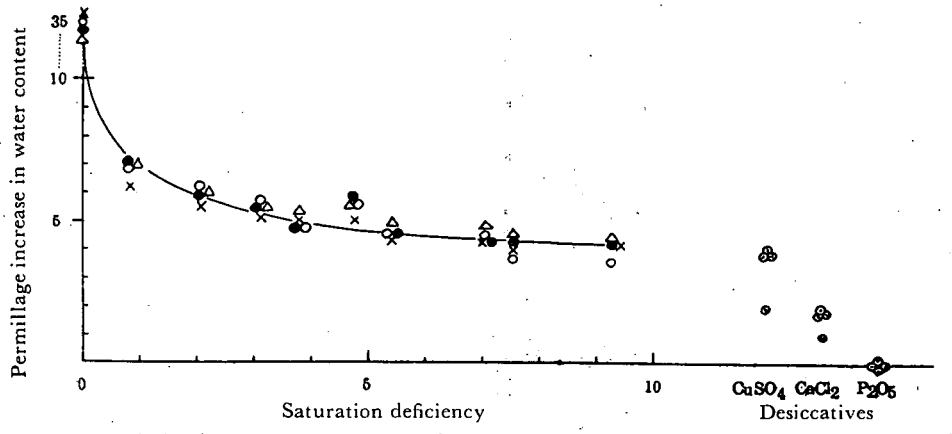

Fig. 7. Relation between water content of filter paper and saturation deficiency and desiccatives. The water content in the P_2O_5-desiccator is considered as zero.

The results illustrated in Fig. 7 indicate that the water content of the filter paper decreases with the increase of saturation deficiency. The water content of the pieces of filter paper in the desiccators decreases in the following order: $P_2O_5 < CaCl_2 < CuSO_4$.

Exp. 4. CO_2-output during desiccation

In the preceding it was suggested that in the rotifer the loss of a certain amount of water from the animal body was a main cause of death from desiccation. This amount of water, in rapid drying, seems to be nearly constant independently of drying rate, but it seems to increase in slow drying in virtue of physiological adaptation against dehydration. It was also found that the body tint gradually faded away in the course of desiccation. Consequently it becomes very important to make clear whether there is any change of the metabolism in the animals being under desiccation. In this experiment carbon dioxide output during desiccation, which may be considered as an indication of metabolism, was

measured. In order to ascertain, further, the relation between the recovery and the drying-time under this experimental condition, another comparative experiment was performed.

Apparatus and Method

Carbon dioxide in the air flow is caught by a droplet of barium hydroxide solution (0.1 M) in which carbon dioxide changes into barium carbonate, precipitating as minute crystals owing to its low solubility. Consequently rough comparison can be made of the amount of carbon dioxide in the air flow by measuring the amount of the minute barium carbonate crystals.

The apparatus used is shown in Fig. 8, where air flows in the direction of the arrow (→). Both carbon dioxide and water vapour in the air flowing in the apparatus were previously removed by soda lime (SL), natron asbestos (NA) and sodium hydroxide (NH) in the glass tubes and flasks. Glass wool (GW) is added to the circulatory system to remove dust in the air. Respiratory chamber (RC) and experimental chamber (EC) are connected between the air cleaner (SL–GW) and vacuum-pump(VP). Reservoir (RE) and manometer (MM) are connected just before the vacuum-pump. The velocity of the air flow can be controlled by a capillary (CA) inserted between (MM) and (EC). The respiratory chamber is a deep dish with two sidearms and its upper side is closed by a cover glass (CG) with stopcock grease. A desiccative, P_2O_5 or $CaCl_2$, is placed at the bottom of (RC) to dry the animals. A piece of filter paper to which some rotifers have adhered is put on the bottom surface of (CG). When the desiccative is placed in the chamber, the air, from which moisture has been previously taken away more or less by natron asbestos and sodium hydroxide, is desiccated further in the chamber. The air dried only by natron asbestos and sodium hydroxide is denoted simply as dry air in the following. The experimental chamber is set on the table of a microscope. The chamber is a dish with three sidearms, and a cover glass is tightly sealed with vaseline on the upper side. Two of the sidearms (a) and (b) are air inlet and outlet respectively, the other (c) is connected to the reservoir of 0.1 M of barium hydroxide solution, and the slender tips of (a) and (c) are upward bent to the center of the cover glass. When the gum-cap (G) is pressed, a droplet of barium hydroxide solution pressed out from the tip of (c) is attached onto the center of the cover glass. CO_2-free air flowing in through sidearm (a) runs against the hanging droplet of barium hydroxide solution (HD) and then passes on through sidearm (b) to the vacuum pump. The air in (RC), when both stop-cocks are turned on, is permitted to enter into (EC).

About twenty rotifers were repeatedly washed with distilled water. After that they were placed on a piece of filter paper with a bit of distilled water and kept at room temperature until the filter paper dried up; then the filter paper was placed in (RC) in the way described above. After these procedures both stop-cocks of (RC) were turned on, permitting the CO_2-free air to pass through it; after a while they were turned off. Thus the animals having been in this manner placed in the respiratory chambers, two of which contained P_2O_5 and $CaCl_2$ respectively and the other no desiccant, were allowed to respire for twenty-four hours. After twenty-four hours the air in (RC) was permitted to enter into (EC). Then, a droplet of the barium hydroxide solution was placed on the bottom surface of the cover glass of (EC). A part of carbon dioxide given off by the dried animals in (RC) was caught by the droplet, and the minute crystals of barium carbonate appeared in the

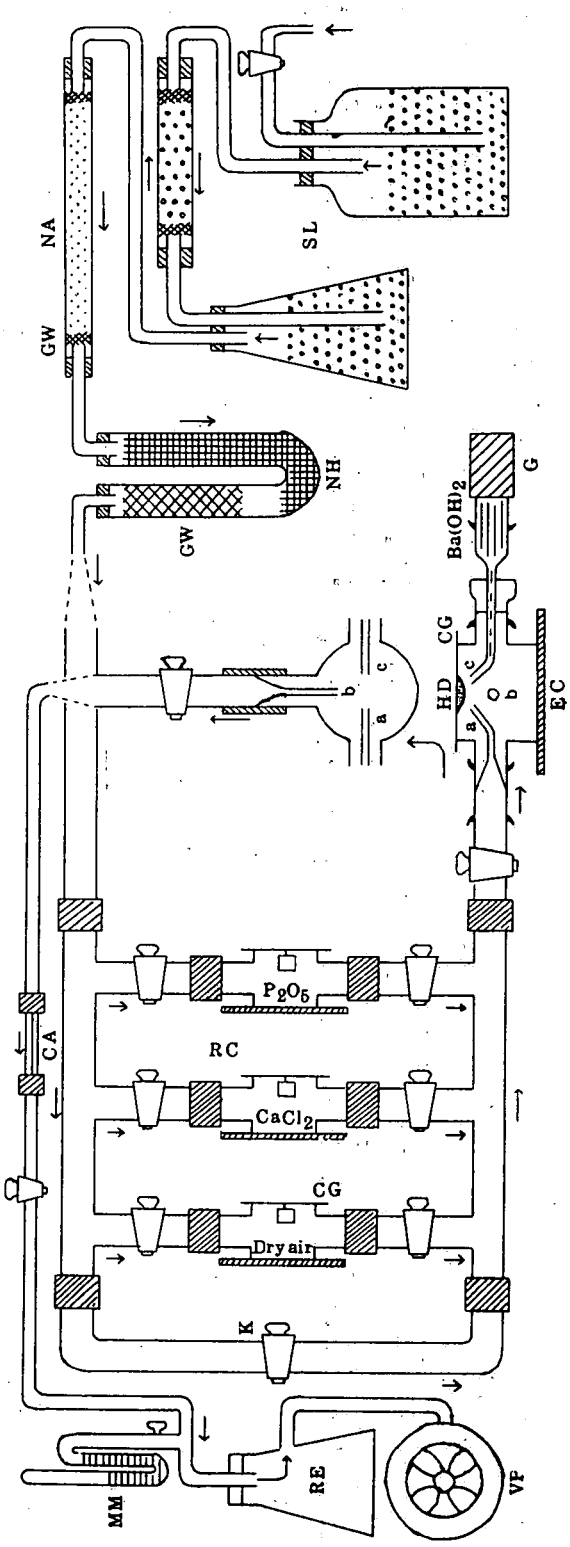

Fig. 8. Diagramatic representation of experimental apparatus. MM, manometer; RE, reservoir; VP, vacuum pump; K, stop cock; CA, capillary; CG, cover glass; RC, respiratory chamber; HD, hunging droplet of barium hydroxide solution; EC, experimental chamber; G, gum-cap; GW, glass wool; NH, sodium hydroxide; NA, natron asbestos; SL, soda lime; a, b and c, tips of sidearm. For further explanation, see text.

droplet were examined under the microscope. After examination of crystals both stop-cocks of (RC) were turned on to permit CO_2-free air to enter into (RC) and then were turned off. In this way the test was made repeatedly for six to twenty-eight days. At the end of the experiments the dried animals were transferred into water again and individuals which were capable of moving within about twenty-four hours were counted.

—— Relation between the concentration of carbon dioxide and the crystal formation of barium carbonate ——

The relation between the concentration of carbon dioxide in the flowing air and the amount of barium carbonate crystals formed in the droplet of barium hydroxide solution was preliminarily examined under definite physical conditions by means of the experimental apparatus described above. Air containing carbon dioxide in a certain concentration was filled in the respiratory chamber (RC), and then permitted to enter into the experimental chamber (EC) in which a droplet of the barium hydroxide solution had been suspended on the bottom surface of the cover glass. A part of the carbon dioxide in the air being caught by the droplet, the minute crystals of barium carbonate precipitate in it. Photomicrographs of the crystals of barium carbonate formed under the various conditions are shown in Pl. I, Fig. 12, a–f. These figures show that the amount of the barium carbonate crystal is in proportion to the concentration of cardon dioxide in the air flow. Therefore, it is possible to estimate roughly the concentration of carbon dioxide in the air in the respiratory chamber by comparison with the quantity of the crystal of barium carbonate under any given definite conditions.

Results

The daily amount of carbon dioxide given off by the animals during desiccation under the various conditions was estimated in succession, and the results are summarized in Fig. 9, where the recovery percentage in the other series of experiment is also illustrated. Pl. I, Fig. 12, g–i shows photomicrographs of the crystals of barium carbonate. During the experiments the temperature ranged from 23° to 30°C. The velocity of desiccation is in the following order, $P_2O_5 > CaCl_2 >$ dry air.

In the early period of the desiccation a considerable number of crystals of barium carbonate was formed in every twenty-four hours. After that period the curve show the tendency for the crystal formation to decrease with lengthening of the duration of desiccation. Finally crystals have been hardly formed for a few days, which means the stoppage of respiration, even though some of the animals can still survive without injury for some days, except in the case of P_2O_5. On the other hand, the recovery percentage diminishes progressively with the length of drying, but the decreasing of the recovery percentage always lags behind that of the crystal formation. That is to say, the animals can withstand the desiccation, even when the crystal formation is exceedingly small or nil.

Now, the tendency of decrease in the crystal formation is in proportion to the rate of drying as in the case of desiccation death. The higher the drying velocity is, the faster the crystal formation decreases, but there exist irregular changes in CO_2-output per day, especially in the case of dry air.

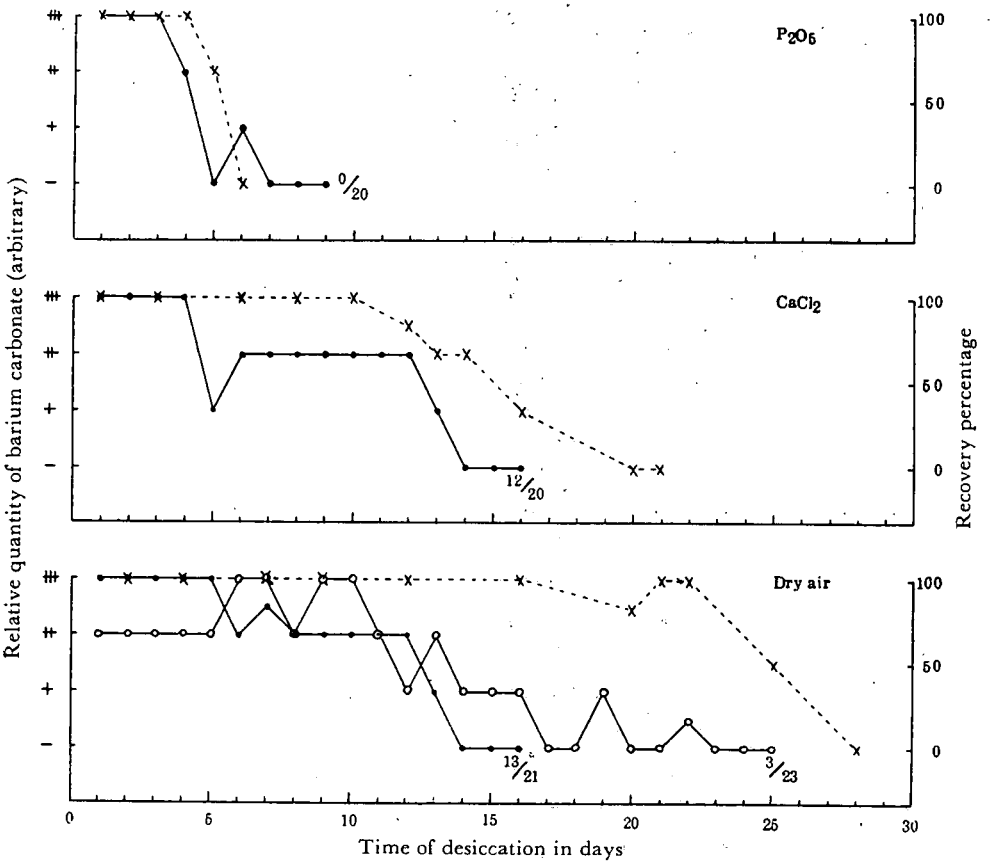

Fig. 9. Relation between the quantity of crystals of barium carbonate and the duration of desiccation in P_2O_5, $CaCl_2$ and dry air (•—• or ○—○), and relation between the recovery and the duration of drying (×---×). The crystals were examined at interval of twenty-four hours. The recovery rate is shown by the ratio of the number of individuals surviving desiccation to the total number of individuals used, as 3/23, etc. In the recovery curves about twenty individuals are used at each point.

Exp. 5. Colour change of the body tint during desiccation

As mentioned previously the body tint of the rotifer gradually fades away during desiccation. In connection with the change of the metabolic activity during desiccation, to clarify this phenomenon seems to be important. For this reason the following several experiments were performed.

The rotifer used were reddish in most cases. The animals were repeatedly washed with distilled water and then placed on a piece of filter paper with a bit of distilled water. After drying at room temperature, the body tint was examined under a definite artificial illumination. The gradation of tint was judged by the comparison with the standard colour chart. Then one half of the pieces of the filter paper the animals were exposed to desiccation under a light condition and the other half under a dark condition. At various intervals the gradation of the tint was estimated in the same way as mentioned above. Finally the number of the survivals was counted in the usual way.

The body tint faded away during desiccation (Fig. 10). Fig. 11 indicates that the sur-

vival percentage and the grade of fading of the body tint decrease in parallel with the advance of drying. This fading of the body tint occurs more quickly under light condition than under dark condition. When the body tint remarkably faded away, the animals

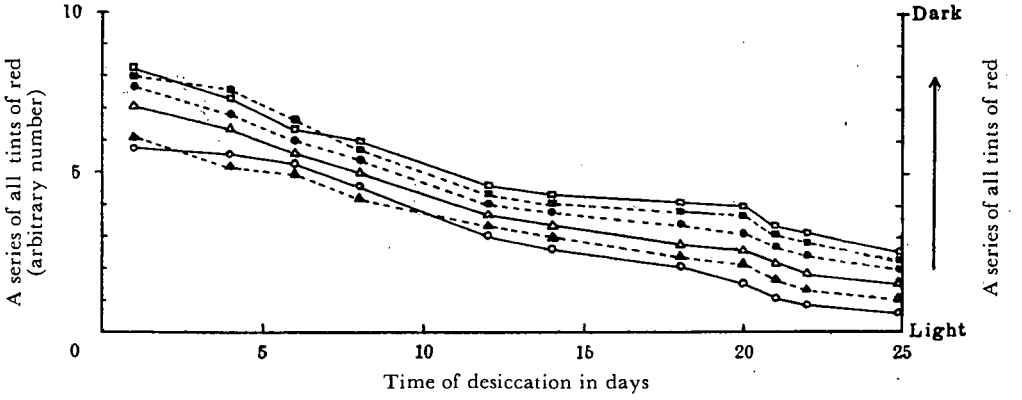

Fig. 10. Colour change of body tint during desiccation.

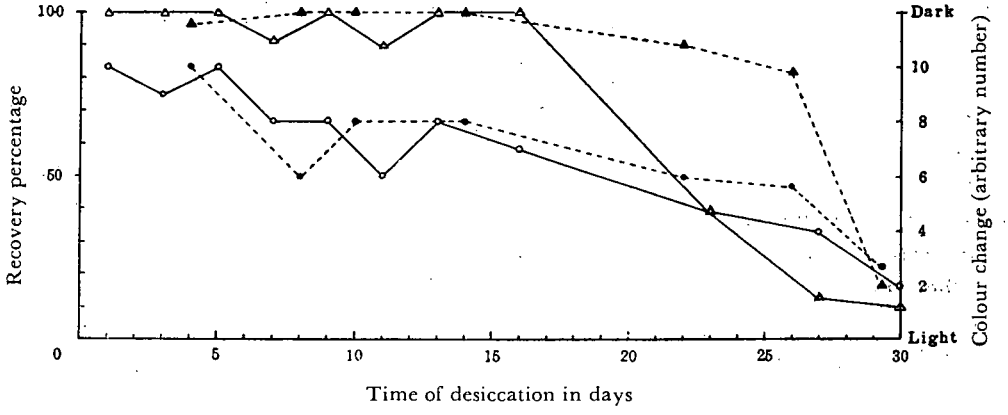

Fig. 11. Relation between the recovery (△ and ▲) and the colour change of body tint (○ and ●) in the animals being desiccated under a light (△ and ○) and a dark (▲ and ●) condition at temperature of 25°-30°C.

were no more alive.

And further some simple chemical tests were made as to the coloured matter in the animals. The animals were individually treated in small dishes with the three following reagents. (1) Concentrated solution of sulphuric acid; the external portion of the animal body became hyaline, then took reddish hue, changing immediately into a deep blue, and finally the deep blue hue gradually faded away. (2) Iodine-potassium iodide solution;* the animal body changed to dark purple after treatment. (3) Diluted solution of potassium bichromate; the body tint of the animal was gradually decolourized. These results indicate that the coloured substance may be carotinoide in nature.

Conclusion

Concerning the desiccation of the rotifer, Jacobs thought that water in the animals was

* The solution consists of iodine 1, potassium iodide 7 and water 100.

removed through their permeable cuticle, and the rapid drying exerted an injurious mechanical effect on the animals. According to Hickernell, the membrane protective against desiccation is formed neither at the beginning nor in the course of desiccasion. He surmised that desiccation death may be due to one or a combination of the following causes: mechanical injury due to too rapid drying, starvation resulting from a lack of reserve food material, the poisonous effect of metabolic products and insufficient time to adjust the nuclear-cytoplasmic reorganization before drying. According to the present author's unpublished data, the eggs could not survive such degree of dryness which is not lethal to the adults.

The experiments in which the effect of evaporation velocity on the rotifer was examined show that the drying velocity has a very important effect on the survival, rapid drying being more injurious than the slow one. That is to say, the desiccation death of the animal occurs quickly in rapid drying and later in slow one.

The calculation of the relative amount of water lost from the animals just at the moment when the death occurs in drying process shows that this value seems to be approximately constant independently of the velocity of the evaporation under the rapid drying condition. Besides this, in the individuals killed by rapid drying, the type of desiccation death-form was occasionally found to be "rapid drying death-form". Accordingly, it may be safely said that the desiccation death in the rapid drying occurs owing to some mechanical injury and to the dehydration of an amount of water which may be necessary to maintain the minimum living activity. When the animal was slowly desiccated this fatal critical value of the amount of water is much higher than in the rapid desiccation. This seems to indicate that the amount of water lost from the animals which may determine their mortality increases in virtue of increasing of physiological regulation against dehydration.

When the rotifer, being usually brick red or pink, is dried, its body tint gradually fades away during the drying and when it fades away remakably, that is an indication of approaching death in all cases. With respect to the colouring substances present in the animal, some chemical tests suggest that these may be carotinoides. In general, carotinoides combine with fat to form vital pigment in the animal body and is caused to fade away by oxidation.

Jacobs showed that the integument of the dried rotifer was at all times freely permeable to gases. Hickernell stated, from the fact that even when the rotifers were kept in an evacuated desiccator for varying periods of time they could survive, that metabolism might proceed in the absence of air and further suggested that many animals, when deprived of their normal supply of moisture, could still keep alive for a while by means of the oxidation of the substances of the tissues.

In order to ascertain whether metabolism still takes place in the dried condition, the carbon dioxide output during desiccation was examined. According to the results, even when any carbon dioxide output is hardly observed for a few days, some of the animals can survive without injury for some days. From this fact and further from the fact that the body tint attributable to carotinoides gradually fades away during desiccation, it is clear that metabolism still proceeds, though in much retarded rate, in the animal being subjected to desiccation.

When the rotifer is dried up under slow drying condition, the anaerobic or aerobic respiration might still continue. The fadeaway of the body tint implies that fat metabo-

lism takes place in this case. In this way the dried rotifer seems to be able to replenish a part of the water lost from the body with metabolic water.

From the facts mentioned above the following conclusion may be drawn. The main cause of the desiccation death of the rotifer is the loss of the amount of water which may be necessary to maintain the minimum living activity. Mechanical injury brought about by rapid drying also becomes important as a cause of death when the desiccation velocity is high.

Summary

1. The desiccation death of the rotifer occurs quickly in rapid drying and later in slow one.

2. In rapid drying the amount of water lost from the rotifer seems to be approximately constant independently of the evaporation velocity. In slow drying, however, this value becomes much greater than in the rapid. It is implied that this increase may be resulted from metabolic water formed in the animal during desiccation.

3. Carbon dioxide output still takes place even in the dried state, but it decrease with increase of the time of drying. Even when any carbon dioxide output is scarcely observable for a few days, some of the animals can survive without injury for some days.

4. The body tint, being usually brick red or pink, fades gradually away during desiccation, this change in colour occurred more quickly under light condition than under dark condition. When it remarkably fades away, no animal can survive desiccation. Colouring substance in the rotifer may be carotinoides.

5. The desiccation death of the rotifer occurs owing to the loss of an amount of water which may be necessary to maintain the minimum living activity. Mechanical injury due to rapid drying is also important as a cause of death when the desiccation velocity is high.

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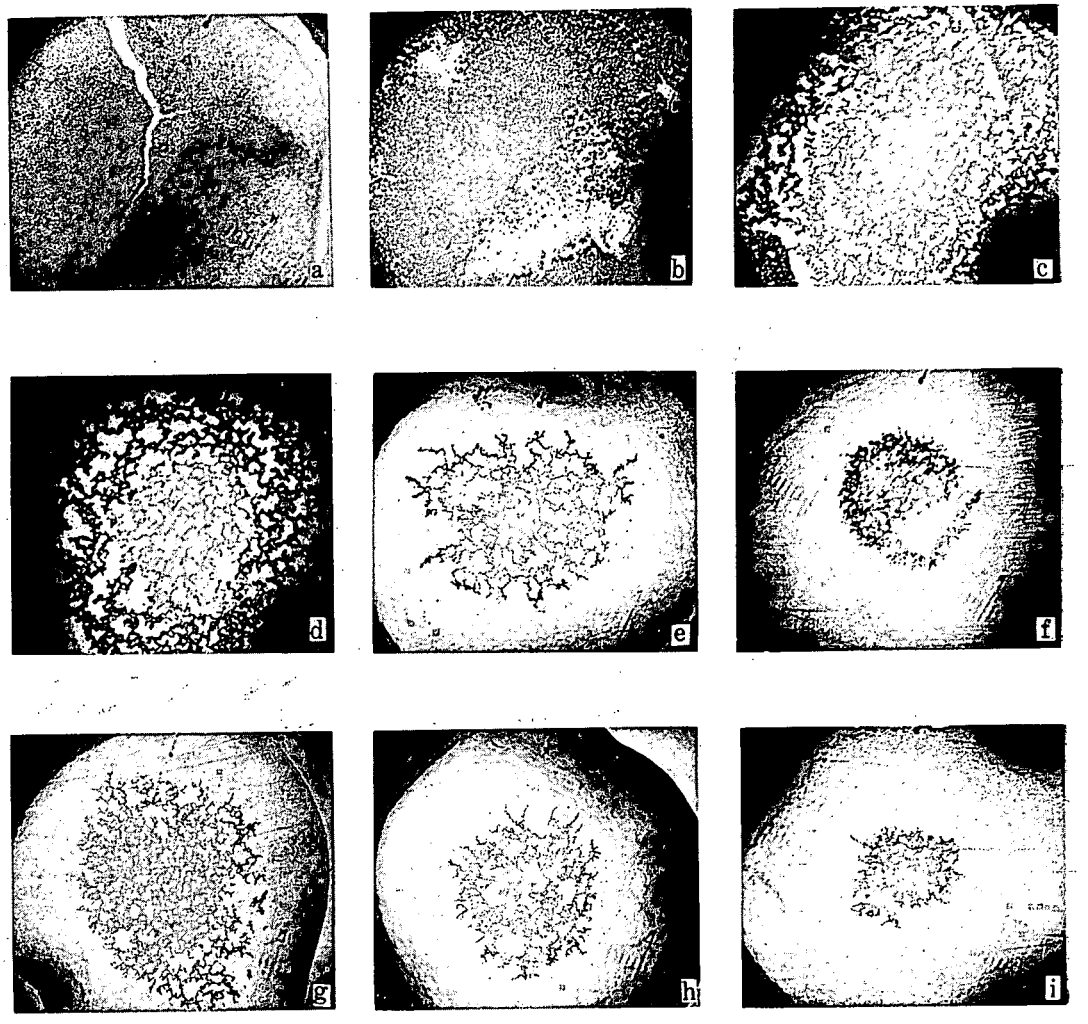
Fig. 12
× 100



Explanation of Plate I

Fig. 12. Photomicrographs of crystals of barium carbonate. Crystals formed by the contact of carbon dioxide in concentration of 0.0002%, 0.00004%, 0.00001%, 0.000006%, 0.000004% and 0.000002% in the air are shown in a, b, c, d, e and f respectively. In the examination of carbon dioxide given off by the animals in a day the quantities of crystals being denoted as ⫲, ++, + and − according to the degrees, g, h and i show ⫲, ++ and + respectively.