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AN EXPERIMENT ON THE WATER BALANCE IN A SALAMANDER[1]

BY

Yoshiaki KUSUKI

Introduction

Most of the important experiments so far reported upon the water metabolism of animals have been carried out with the frog. The vital limit of desiccation of the animal as well as the percentage value of water retained by each organ in a desiccation or thirst experiment was determined by many investigators. However the water balance is rather conspicuous in urodelan Amphibia, since it is sometimes of terrestrial and sometimes of aquatic life and by far most suitable for the study.

Fortunately, a kind of Urodela, *Hynobius retardatus* DUNN, abounds here in the Hokkaido, and serves very advantageously as material for any kind of experimental work in the present project. Accordingly the writer carried out the following experiments with this animal.

Method of Experiments

Hynobius was collected during the season from late March to early April in the vicinity of Sapporo. As the female was small in size and rare in number in this season, only the male was used. Moreover, on account of the sexual act the animal is of complete aquatic life in the early spring so the water content of the body is abnormally high, therefore it was desirable to carry out the experiment in early summer and in autumn. The animals were kept alive in aquaria with running water at a temperature of 10°–11°C.

1) Contribution No. 94 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.

They were fed either on earthworms or on cow liver. But in order to avoid the influence of the food the animal was transferred into the water tank and kept for 5–10 days without food before the experiment. At first the metabolism of water of the animal as a whole was examined by simply drying or sometimes by rehydrating. Secondly, the water retaining capacity of different organs of the desiccated animal was determined.

The difference of weight between the fresh and dehydrated materials is considered mainly to be due to the loss of the water evaporated from the material. Thus the amount of water exchanged is simply measured by weighing the material. The writer applied also the water determining apparatus designed by Dr. KAFUKU and that of Mr. SHINODA for comparison. In this case the weighing method as above was proved to be as exact as the ordinary water determining method.

Water Content of the Total Body

First the water content of the total body was measured by the ordinary weighing method. The animal was dried in a desiccator at a temperature of 95°–100°C. Generally it takes 1 to 2 days in

Table I.

Season	Summer		Autumn	
Number of material	Water content in %	Deviation	Water content in %	Deviation
1	80.9	+0.7	81.0	+1.4
2	80.0	−0.2	79.0	−0.6
3	81.0	+0.8	80.0	+0.4
4	79.7	−0.5	78.4	−1.2
5	78.8	−1.4		
6	81.1	+0.9		
Mean	80.2		79.6	
Standard deviation		±0.8		±1.0

the oven until the weight of the material becomes constant through loss of the constituent water. Table I shows the result.

In the above it is shown that the water makes up from 79.6% to 80.2% of the total weight of the animal. The determination was also carried out by way of either KAFUKU's or SHINODA's apparatus. Table II gives the result.

Table II.

Season	Summer		Apparatus used.
Number of material	Water content in %	Deviation	
1	76.4	−1.4	Kafuku's
2	80.0	+2.2	,,
3	77.3	−0.5	,,
4	79.0	+1.2	Shinoda's
5	77.4	−0.4	,,
6	76.6	−1.2	,,
Mean	77.8		
Standard deviation		±1.3	

By this method nearly the same percentage value of the water is obtained as by the above. A little higher value in Table I is presumably due to the loss of some evaporable component other than water owing to the such high temperature of the oven as 100°C.

Table III.

Number	Water content in %	Deviation
1	84.5	−1.4
2	86.9	+1.0
3	84.	−1.0
4	87.4	+1.5
Mean	85.9	
St. d.		±1.3

For comparison spring animals which are of mostly aquatic life were examined. It is clear from the table (Table III) that relatively much water is contained in the spring animal, as expected.

Vital Limit of Desiccation

Next the animal was kept in a wire cage or in a jar without water in order to discover the vital limit of evaporation. Naturally the rate of evaporation depends upon the weather conditions and it was proved that the rapid loss of water always acts unfavourably for the animal. The weight of the animal was repeatedly taken and the time until the critical moment of life was measured. Some of the animals did not survive even if they were returned to the water after the experiment. The results of the observations have been arranged in Table IV.

Table IV.

Number of material	Loss of weight (%)	Time used (hours)	Days survived after return to water.	Remarks
1	36.6	7	1/2	desiccated in wire cage
2	38.9	24	died	in jar
3	36.2	24	7	,,
4	35.9	7	3	in wire cage
5	35.9	6	5	,,
6	37.5	24	7	in jar
7	34.0	7	7	in wire cage
8	38.6	17	3	in jar
9	28.1	6	7	in wire cage
10	40.2	7	7	,,
11	23.4	1	died	,,

7 days show that these animals survived longer than a week.

The effect of desiccation varies to some extent with different individuals. However it is seen that under a favorable condition the animal can lose nearly 40% of its body weight in this experiment; sometimes it can endure almost 50% loss of body water. On the contrary, the rapid loss, for example, in Nos. 1, 4, 5, and 11, generally exerted a harmful effect even in the low percent loss of water which ordinarily causes no fatal effect in slow evaporation.

Water Content of the Body at the Vital Limit of Desiccation

With a view to ascertain whether the loss of body weight during the above desiccation is simply due to the evaporation of water, the water content of desiccated animal was measured as shown in the following table (Table V).

Table V.

Season	Summer			Autumn		
No.	Loss of weight (%)	Water content (%)	Condition after desiccation	Loss of weight (%)	Water content (%)	Condition after desiccation
1	37.7	66.0	alive	35.1	68.5	alive
2	36.9	67.7	,,	35.9	69.7	,,
3	41.8	64.9	dead	35.3	72.2	,,
4	38.2	67.1	,,	33.4	68.7	,,
5	39.7	64.0	,,			
Mean	38.9	65.9		34.9	69.8	
Theoretical values[1]		67.3			69.1	

Supposing that the water content of the normal animal is 80% and that weight decrease depends solely upon the water evaporated, then the water remaining after desiccation is easily calculated. This gives the theoretical values in the table. The empirical data agree well with the theoretical, indicating that there is no occurrence of water composition at the cost of reserved organic materials.

1) The calculation is as follows.

W .... initial body weight.
A .... the percent of water contained in normal group.
B .... loss of weight in %.
X .... the percent of remaining water.

If we assume that B is equal to loss of water, so X must be as follows.

$$X = \frac{W.A - W.B}{W.100 - W.B} \times 100 = \frac{A-B}{100-B} \times 100$$

Result of Rehydration after Desiccation

After the animal had suffured desiccation, losing body weight to about 30–35%, it was again transferred into the water where it remained 5–6 days until the body weight became stationary, recovering about 2/3 of the original weight. To obtain the theoretical value[1] the weight of solids which were lost during the rehydration by way of shedding of the skin and excretion of feces was measured. The next table gives the result.

Table VI.

Loss of weight (%)	Recovery of weight (%)	Water content (%)	
		Empirical	Theoretical
33.6	21.0	78.7	78.0
33.4	20.0	79.7	77.9
32.0	23.6	80.0	79.1
31.5	23.7	78.0	78.8
29.5	14.3	80.0	78.9
32.0%	20.5%	79.3%	78.5%

The fact that the empirical value 79.3% is higher than the theoretical value 78.5% indicates that there occurs some loss of weight by abnormally high metabolism during the rehydration experiment. The value is, however, by no means very significant.

Water Content of Organs

1. *Water content of organs in the fresh animal.* The water content of the following organs was determined. It is worth while to

1) The method of calculation is as follows.
W .... initial body weight.
L .... loss of body weight not recovered by rehydration.
Ls .... weight of solid lost during rehydration.
X .... theoretical value of water content after rehydration.

$$X = \left(\frac{W \times \frac{80}{100} - (L - Ls)}{W - L} \right) \times 100$$

note first the technique of preparation of the material in order to gain the exact conception of the organs used.

Blood—Pericardial lymph was absorbed with filter paper and then ventricle was cut to allow the blood to flow.

Tail—Tail behind the cloaca was used.

Skin—Body skin except the tail part.

Muscle—Only a part of dorsal muscles (epiaxonic muscle) and that of hind limbs (M. caudali-pubo-ischiadico-tibialis) were used.

Liver—The mesentery and the gall-bladder were removed.

Stomach and intestine—The lower part of the oesophagus and a part of the anocloacal connection were included.

Brain—The brain was taken out of the skull and cleaned with filter paper.

Table VII.

Water content of organs in fresh condition

Organ	Summer animal		Autumn animal	
	Water content %	St. d.	Water content %	St. d.
1 Blood	84.1	±2.3	90.4	±2.0
2 Gall-bladder	—	—	86.2	±1.9
3 Brain	84.8	±0.8	85.0	±0.8
4 Eye	82.0	±1.1	82.4	±0.6
5 Heart	81.9	±1.3	82.0	±0.9
6 Muscle	80.1	±0.9	82.0	±0.7
7 Stomach-intestine	81.4	±1.1	80.8	±0.7
8 Lung	80.9	±1.9	80.5	±0.9
9 Skin	77.8	±1.2	80.2	±0.9
10 Kidney	80.0	±1.1	80.0	±1.1
11 Tail	75.7	±1.7	79.6	±0.8
12 Spleen	78.3	±1.3	78.8	±1.7
13 Wolffian-duct	77.8	±2.4	77.1	±1.1
14 Remainder	73.5	±0.4	74.6	±1.4
15 Testis	75.4	±3.2	74.4	±7.4
16 Liver	73.4	±2.0	71.5	±2.0

Heart, lung, testis, Wolffian duct and kidney—They were removed easily without special care.

The remainder part—The parts including the skeleton, connective tissue and a large part of the muscle.

The table (Table VII) shows the result of measurement using the same method as before for the whole body. The figures given in the column of summer animal of the table are the averages of 11 different individuals and in the autumn animal are those of 7 respectively.

There is not much difference between the animals in different seasons. The blood, brain, eye, heart and muscle show higher percentage value of water content than the whole body while the tail, spleen, testis and liver have less water than the body as a whole.

Table VIII.

Water content of organs in desiccated condition

Organ	Summer animal		Autumn animal	
	Water content %	St. d.	Water content %	St. d.
1 Brain	83.3	±1.7	81.6	±0.8
2 Blood	70.6	±1.8	77.1	±2.0
3 Heart	76.8	±1.4	76.9	±1.5
4 Eye	73.2	±1.5	75.5	±0.5
5 Gall-bladder	—	—	75.0	±1.9
6 Lung	73.9	±1.9	73.2	±2.2
7 Kidney	73.7	±1.5	72.9	±0.4
8 Stomach-intestine	72.3	±1.4	72.9	±0.8
9 Muscle	69.1	±1.9	71.5	±1.2
10 Spleen	70.5	±2.9	71.2	±1.0
11 Testis	67.8	±6.3	68.7	±6.8
12 Tail	61.6	±2.2	67.3	±0.8
13 Wolffian-duct	67.1	±2.2	66.9	±2.5
14 Liver	67.9	±1.5	64.2	±3.0
15 Skin	60.5	±4.1	63.9	±1.1
16 Remainder	59.8	±0.2	63.1	±1.1

2. *Water content of organs in desiccated animal.* It is interesting to see the distribution of water in a desiccated animal. For this purpose the animal was desiccated gradually until the loss of body weight reached 36.2% in the summer animal and 34.3% in the autumn animal. The table (Table VIII) was obtained from the average of 10 individuals in the summer animals and 7 in the autumn ones.

A glance at the table shows that the different organs do not lose their constituent water equally. Only the brain retains an amount above the average of body water while all others are under the average in water content. The skin is most remarkable, as it ranges from the 9th in order in a fresh condition down to 15th in order after the desiccation. For the sake of convenience of comparison, the difference of percentage value of water between the fresh material and the desiccated condition was calculated. The table (Table IX) shows the result.

Table IX.

	Organ	Summer animal	Autumn animal
1	Skin	−17.3	−16.3
2	Blood	−13.5	−13.3
3	Tail	−14.1	−12.3
4	Remainder	−13.7	−11.5
5	Gall-bladder	—	−11.2
6	Muscle	−11.0	−10.5
7	Wolffian-duct	−10.7	−10.2
8	Stomach-intestine	− 9.1	− 7.9
9	Testis	− 7.6	− 7.9
10	Spleen	− 7.8	− 7.6
11	Lung	− 7.0	− 7.3
12	Liver	− 5.5	− 7.3
13	Kidney	− 6.3	− 7.1
14	Eye	− 8.8	− 6.9
15	Heart	− 5.1	− 5.1
16	Brain	− 1.5	− 3.4

Again it is quite clear that the brain and the heart lose the least amount of water as compared with the other organs. The skin and the blood are particularly affected by dehydration. The comparison of different organs shows another fact; namely more water is contained in the organs which lose water more easily by desiccation than in the others.

3. *Water content of organs in the rehydrated animal.* Eight autumn animals were desiccated until they lost 34.5% of their initial body weight and then they were brought back into the water. By this rehydration experiment they recovered 23.3% of body weight on the average. Each organ was examined as in the other cases. The table (Table X) gives the figures of average of 8 different individuals.

Table X.

Water content of organs of rehydrated group

Organs	Water content (%)	St. d.
1 Blood	87.5	±1.4
2 Gall-bladder	86.1	±2.7
3 Brain	85.3	±0.6
4 Eye	82.2	±0.5
5 Muscle	81.6	±1.4
6 Heart	80.8	±1.0
7 Kidney	80.8	±0.6
8 Stomach-intestine	80.2	±0.8
9 Lung	79.9	±0.9
10 Skin	79.6	±1.5
11 Tail	78.0	±1.4
12 Spleen	77.7	±1.2
13 Wolffian-duct	76.9	±2 3
14 Testis	76.0	±7.3
15 Remainder	73.7	±0.8
16 Liver	71.7	±1.9

By comparison of the above table with Table VII, it may be seen that almost all organs have recovered water content to the initial condition. This means that the skin and the blood serve most actively in the water regulation of the animal.

Body Fluid in Desiccation

We have left unexamined the body fluid which might be strongly affected by desiccation. It was measured in the autumn animals as the following table shows. Desiccation was continued until the animal lost about 35% of its body weight.

Table XI.

Weight of body fluid in grams.

Normal group	Desiccated group	Rehydrated group
1.084	0.366	—
0.896	0.492	0.764
0.912	0.381	0.960
0.950	0.452	0.953
1.046	0.567	1.000
0.928	0.354	0.806
1.309	0.361	1.000
Mean 1.018	0.425	0.914
Average body weight in grams 7.117	7.202	7.842
Percent of body fluid 14.3%	5.9%	11.7%

As is seen from the table more than 50% of body fluid is lost during the desiccation process. It is remarkable that the normal amount of body fluid can not be recovered in the rehydration experiment.

Conclusion and discussion

The observation shows that the water content of the entire body in *Hynobius* is about 80%. Nearly the same value was found in frogs by other authors namely; 77.3% to 80.5% by BEZOLD ('57), ca. 80% by DONALDSON ('10), 80.8% by HALL ('22), 79.3% by UEKI ('24) and 82.14% by SMITH and JACKSON ('31). Also in salamanders BEZOLD ('57) showed nearly the same value, 79.6% and 80.2%. Naturally there must be some difference of water content according

to the species of animals, and to some extent to the season. DONALDSON ('10) observed the changes of water content in the frog from April to January of the next year. According to him the water content decreases to a minimum from August to September and it reaches to a maximum in January. The same tendency was shown in the present experiment with spring, summer and autumn animals. Even in the same season the water content slightly differs according to the individual.

Hourly weight fluctuation of an individual frog in the course of a day was studied by GUTHRIE ('14). He reported that maximum hourly variation was as much as 10% of the body weight. Obviously this depends upon the rate of water intake through the skin and the output of water as urine, and upon the temperature and humidity of the time as well.

As to the vital limit of desiccation there have been many studies on various kinds of animals. The vital limit of desiccation varies according to the different species of Amphibia and even in one and the same species in accordance with the individual. In general Amphibia lose a considerable amount of water (30% to 50% of initial body weight) before death. Under the most favorable condition, it can reach even to 59% (STEINBACH '27). The value 30% to 50% measured by other investigators coincides with 35% to 40% which is obtained in the present experiment. As in the case of the frog which was studied by KUNDE ('57) and DURIG ('01), rapid desiccation is fatal for *Hynobius* in which only 20% loss causes death. In the present experiments the optimum duration of desiccation was 7 to 24 hours. DURIG ('01) reported that the desiccated frog sometimes attained to a more than normal weight when returned to water again, but in the present writer's observation, rehydrated salamanders did not recover the original weight, regaining about 2/3 of weight lost.

As to the water content of different organs, the data given by the other authors differ more or less from each other. On the whole, the kidney, lung, eye, and testis show relatively higher per-

cent of water in the cases of others than in those of the writer. So far as the testis is concerned the chief source of discrepancy comes probably from degeneration after the breeding season. In general there is agreement in the following points, that is, the blood, heart, brain, eyes, muscle and kidney contain relatively more water than the other organs, while the skeleton and liver contain relatively less. This fact leads one to think that excepting those organs which contain an aqueous secretion, for example, the gall-bladder etc., the animal organs of higher metabolism are provided with more water than are those of lower metabolism. There are some other facts which support this consideration; embryo or young animals which are very active, contain more water than old, less active animals. In the breeding season, in other words, in the most active season, the water content of the whole body rises markedly.

(Desiccated experiment) DURIG ('01) who studied only on the heart, liver, kidney, muscle and brain of the frog, stated that the muscle loses water most strikingly, while the brain and heart do so relatively less than others. UEKI ('24) investigated the frog which was subjected to various degrees of desiccation and reported that the organs did not lose water at the same rate as the whole body. According to him the blood (—19.4%) and skin (—11.8%) lost water markedly, and the spleen (—1.8%) and brain (—3.1%) less than other organs. A similar tendency was found by SMITH and JACKSON ('31). In their study the blood (—11.6%), skin (—10.2%) and stomach-intestine (—9.8%) decreased in water content strongly and the muscle (—7.6%), and testis (—7.5%) lost moderately, while other organs lost less than 5%; above all the bone, heart, brain and eye were nearly unchanged.

These findings almost agree with those reported in this paper. The histological study reported by STEINBACH ('27), shows a structural peculiarity of skin of *Salamandra muculosa*, i.e., the skin of this animal is rather thick and when desiccated, it loses its thickness

more markedly than the skin of the frog. This is also the case in *Hynobius*.

In all the tables given above only percentage value of water content was shown. However the actual amount of water lost during the desiccation is easily seen by calculation as shown in the footnote.[1)]

Table XII.

Organ	Loss of Water in %	Organs	Loss of Water in %
Blood	58.1	Eye	28.2
Skin	45.2	Lung	27.2
Gall-bladder	44.8	Spleen	26.4
Tail	37.6	Kidney	26.2
Muscle	36.8	Testis	25.2
(Entire body)	(34.3)	Heart	22.1
Remainder	31.2	Liver	20.4
Wolffian-duct	30.8	Brain	18.5
Stomach-intestine	29.2		

It has become still clearer that the blood, skin, tail and muscle lose water to a larger degree than other organs and this in turn shows that by these organs the main water regulation is undertaken.[2)] Besides these organs, the body fluid (lymph) plays a great role in the storage of water, as in *Hynobius*, the body fluid accounting for 24.5% of the loss of water of the total body. According to SMITH and JACKSON ('31), in *Rana pipiens*, even 45.8% of weight loss comes from the loss of lymph. It was shown that the liver, which acts as a food reservoir, does not serve for water storage, that the brain and heart are the most important organs to maintain

1) The method of calculation is as follows.

A .... Water percent of organs of normal group.
C .... Water percent of organs of desiccated group.
X .... Water loss in percent of initial organ weight.

As shown before,

$$\frac{A-X}{100-X} = \frac{C}{100} \text{ so } X = \frac{A-C}{100-C} \times 100$$

2) This conclusion shows a rough agreement with a finding of ENGELS ('04), who studied on the dog and stated that the muscle is the most important organ of water storage.

the animal life and therefore lose less water in desiccation. Contrary to the case of *Hynobius* no detectable change of water of the blood takes place in Mammalia by desiccation as is shown by COHNHEIM ('09).

There is some interest in the fact that the organs which show marked water loss during desiccation, suffer also a relatively greater loss in weight during inanition.

As to the rehydrated group the results of other authors, as well as the herein reported ones, show that all organs return to normal amount of water content except for a slight loss of weight by decomposition of body material on account of the higher metabolism.

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