THE RATE OF URINE PRODUCTION OF *ANODONTA CYGNEA*

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INTRODUCTION

It is difficult to measure directly the rate of urine production in the fresh-water lamellibranch *Anodonta cygnea* because the excretory pore is fragile and in an inaccessible position. Picken (1937) measured the rate of filtration from the heart into the pericardium by opening the pericardium from the dorsal side. Under these conditions he found a mean rate of filtration, in ten animals, of 237 ml./day, at 17° C. The average weight of the animals, without the shell, was 50 g.

This paper reports the results of the indirect measurements of the filtration rate and the rate of urine production in intact animals. The rate of filtration was measured by the inulin clearance. The rate of urine production was inferred from the initial rate of loss of weight of animals placed in diluted sea water isotonic with the blood.

MEASUREMENT OF FILTRATION RATE

All the animals used in the experiments weighed about 100 g., with the shell. Inulin concentrations were measured by the colorimetric method of Roe, Epstein & Goldstein (1949).

Preliminary determinations were made of the inulin space of *Anodonta*. One ml. of distilled water containing 30 mg. of inulin was injected into the foot. After 4 hr. the animal was drained for 5 min., dried and weighed. The animal was then opened in a clean glass dish and two blood samples were taken from the heart. The total inulin content of the animal and the inulin concentration in the blood were then measured. The shell was dried and weighed so that the inulin space could be expressed in terms of the wet weight of the animal without the shell. The mean inulin space of four animals was 55% of the wet weight without the shell. This value will include the contents of the pericardium and the excretory organ so that the blood content will be slightly less.

The inulin clearance rate was measured as follows. One ml. of distilled water, containing 30 mg. of inulin, was injected into the foot. The animal was then left for at least 6 hr. in clean water to allow the inulin to become uniformly distributed about the haemocoele and to allow the pericardium and excretory organs to be flushed out with the inulin filtrate. The animal was then washed and transferred to 500 ml. or 1 l. of clean water. After 4–12 hr., when the concentration of inulin in the water had risen to a convenient level, the animal was removed, dried and

weighed, and the concentrations of inulin in the blood and in the water were measured.

The following conventions will be used:

b_0 = initial concentration of inulin in the blood in mg./ml.

b = concentration of inulin in the blood after t hours, in mg./ml.

c = concentration of inulin in the water after t hours, in mg./ml.

C = volume of water in ml.

B = volume of blood, as measured by the inulin space, in ml.

v = volume of filtrate produced per hour, in ml.

If it is assumed that b does not fall during the experiment then

$$cC = bvt. \tag{1}$$

A more accurate value of v can be obtained if allowance is made for the fall in b during the experiment.

After the first hour $$b_1 = b_0\left(1 - \frac{v}{B}\right).$$

After the second hour $$b_2 = b_1\left(1 - \frac{v}{B}\right)$$

$$= b_0\left(1 - \frac{v}{B}\right)^2.$$

After t hours $$b = b_0\left(1 - \frac{v}{B}\right)^t. \tag{2}$$

As the amount of inulin in the system is constant

$$cC + bB = b_0B. \tag{3}$$

As c, B, b and B are known, b_0 and v can be calculated from (2) and (3). As long as vt is small compared with B a large error in B has only a small effect on v, and the value of v calculated is similar to that obtained from (1).

The results of seven experiments, at four different temperatures, are given in Table 1. The inulin clearance rate at 18° C. for a 100 g. animal is about 1 ml./hr., and from the difference in rate between 0 and 18° C. the Q_{10} value appears to lie between 2·0 and 2·5.

MEASUREMENT OF URINE PRODUCTION

If an *Anodonta* is placed in a solution isotonic with the blood the osmotic inflow of water through the surface should be temporarily eliminated but the production of urine may be expected to continue for a short time.

The freezing-point depressions of the blood of twenty *Anodonta* were measured by Ramsay's method (Ramsay, 1949). The mean freezing-point depression was 0·0785° C. 4% sea water, freezing-point depression 0·080° C., was used as the isotonic solution.

It is possible to obtain fairly consistent weighings of *Anodonta* when the conditions of weighing are standardized. The shells of the animals were wedged open

by short lengths of glass tubing. Before weighing the animals were drained for exactly 1 min. in an upright position, and the shells were wiped dry. Weighings were made to the nearest 0·01 g. Under these conditions the standard deviation of nine successive weighings of an 80 g. animal, in tap water, was 0·15 g. The experiment was performed as follows. Animals which had previously been kept in tap

Table 1. *Inulin clearance of* Anodonta cygnea

Temperature (° C.)	Total weight of animal (g.)	Inulin clearance (ml./hr.)
0	94	0·26
0	101	0·23
10	109	0·30
16	124	0·44
18	134	1·05
18	124	1·20
18	113	1·30

Table 2. *Change of weight of* Anodonta *in an isotonic solution*

Time in min. ...	0	15	30	45	60	75	90
Weight in g., animal I	95·64	95·44	95·38	94·71	94·96	94·35	93·73
Weight in g., animal II	66·57	65·99	65·24	64·57	64·38	64·15	63·70

Table 3. Anodonta cygnea. *Rate of loss of weight in isotonic sea water*, 15° C.

Body weight (g.)	Loss in first hour (g.)
96	0·95
66	2·19
65	0·72
59	0·16
53	0·88
97	1·71
90	1·99
67	2·43
Mean 74 g.	Mean 1·38 g./hr. S.D. ± 0·8

water were placed in the isotonic solution and weighed every 15 min. for 90 min. The results were plotted graphically, and the rate of loss of weight obtained from a straight line drawn through the seven points. Extended experiments showed that the rate of loss of weight started to fall after about 90 min.

RESULTS

The details of the first two experiments are given in Table 2, and the final results of eight experiments are summarized in Table 3. The consistency of the results is not good; nevertheless, they give a figure of 1·9 ± 1·1 ml./hr. for the urine production of a 100 g. animal, which is of the same order as the figure for inulin clearance.

DISCUSSION

The results of the inulin clearance experiments show that the rate of filtration in the intact animal is about 1 ml./100 g. total weight/hr., at 18° C. This is much smaller than in animals in which the pericardium has been opened (Picken, 1937). The difference may be due to a slight pressure inside the intact pericardium. The rate of filtration varies with temperature with a Q_{10} of about 2. The mean rate of urine production inferred from the loss of weight experiments at 15° C. is 1·9 g./100 g. total weight/hr., but the spread of the results is rather wide. Florkin (1938, Table 3, Exp. 6) found that at 17° C. a 120 g. *Anodonta* lost 0·8 g. in the first hour, in an isotonic solution. The experimental errors in the loss of weight experiments are greater than in the inulin clearance experiments, but the results show that the rate of urine production is of the same order as the filtration rate.

One of the objects of this investigation was to obtain some estimate of the rate of urine production of *Anodonta* for purposes of certain theoretical investigations which are described elsewhere (Potts, 1955).

SUMMARY

1. The inulin clearance rate of a 100 g. *Anodonta* varies from about 0·2 ml./hr. at 0° C. to 1·0 ml./hr. at 18° C.

2. The rate of urine production, inferred from the initial rate of loss of weight in an isotonic solution, is $1{\cdot}9 \pm 1{\cdot}1$ ml./hr. at 15° C., and is thus of the same order as the inulin clearance rate.

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