BODY WATER TURNOVER RATES IN *ANADARA GRANOSA* *

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ABSTRACT

Tritium is used as a tracer to study the body water turnover rates in shell fish *Anadara granota* under laboratory conditions. The body water turnover rates for the animals of different weights were studied. Younger animals seem to have larger turnover rates. The aqueous as well as organic bound tritium were measured and the organic fraction reached to about 5% of the body water tritium concentration at equilibrium in a period of two weeks. [Corresponds to 1·2% of the total body water tritium activity in the animal].

INTRODUCTION

TRITIUM being the only radioisotope of hydrogen, it will be a useful tool to study the physical, chemical and biological processes involving water[1]. Tritiated water and its vapour will be taken into the body water of the organisms by diffusion which might get partially absorbed in the organic parts of body tissue. Preferential retention of tritium in the body tissue depends upon the chemical form when it enters the system (such as through tritiated thymidine) and this could lead to higher doses to the localised organs. Recently two reviews have comprehensively treated tritium and its effects on biological systems and the environment[2,3].

Relatively little information is available on the rate of tritiation of body water in the marine organisms and its exchange to the organic form. Data on tritium are restricted to a few measurements by Koranda[4] and Skauen[5] on the levels of tritium in the loose and bound water of marine animals. Hence, an investigation was initiated in the laboratory to study the rate of incorporation and loss of tritium in the body water and organic portion of shell fish arca (*Anadara granosa*).

EXPERIMENTAL

A glass aquarium of 12″ × 10″ × 10″ dimensions was filled with 10 litres sea water. The pool water was stocked with 15–20 arca of same weight, aerated and temperature recorded daily. The experimental animals were collected from Sewri mud flats, Bombay. The tritiated water was added to the pool water to a concentration of $1 \cdot 5 \times 10^5$ dpm per ml. The animals were given no food during the experimental period.

The organisms and the pool water were sampled at intervals. Each time one animal was dissected, the tissue was dried with filter-paper to remove the adhering water and blood and then vacuum distilled for 1–2 hours at 60–70° C to extract the body water. 0·5 ml of this water was added to 15 ml of dioxan scintillator (6·5 g PPO + 0·13 g POPOP + 100 g naphthalene in 1 litre of dioxan) and counted. 0·5 ml of the pool water was also counted for comparison.

The lyophilised dry tissue was pulverised by shaking with a Teflon ball in a thick-walled glass container and then dried to a constant weight in an oven at 100° C. The dry tissue (100 mg) was then burned in oxygen atmosphere to obtain water of combustion. Known volume of liquid scintillator was added to this water of combustion and counted by liquid scintillation to give organic bound tritium.

Rate of loss of tritium from the body water of the organisms was also studied. The equilibrated animals were transferred to inactive sea water and were sampled periodically for the measurements.

RESULTS AND DISCUSSION

Optimisation of scintillator volume.—Preliminary studies were conducted to study the effect of scintillator volume on counting efficiency. A known volume of scintillator was spiked with 100 λ of tritiated water. The volume of the scintillator was changed gradually and efficiencies calculated (Fig. 1) after the subtraction of background scintillations for the corresponding volumes. The figure reveals a gradual increase in efficiency as the scintillator volumes increase, remains steady and then falls off for higher volumes of the scintillator. The volume corresponding to plateau region (15 ml) was chosen as the optimum volume.

Quenching effects.—0·5 ml of the body water tritium was compared against the same volume of medium sea water. Though the same volume of sample was used in both the cases, the quenching effects due to sea water and body water may vary. Hence, experiments were done with varied volumes to standardise the quenching effects of sea water and body water (Fig. 2). It is found that quenching

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due to sea water is slightly more than that of body water. The quenching constants for sea water and body water are $q = 5{\cdot}71\ \mathrm{ml}^{-1}$ and $q = 4{\cdot}33\ \mathrm{ml}^{-1}$ respectively.

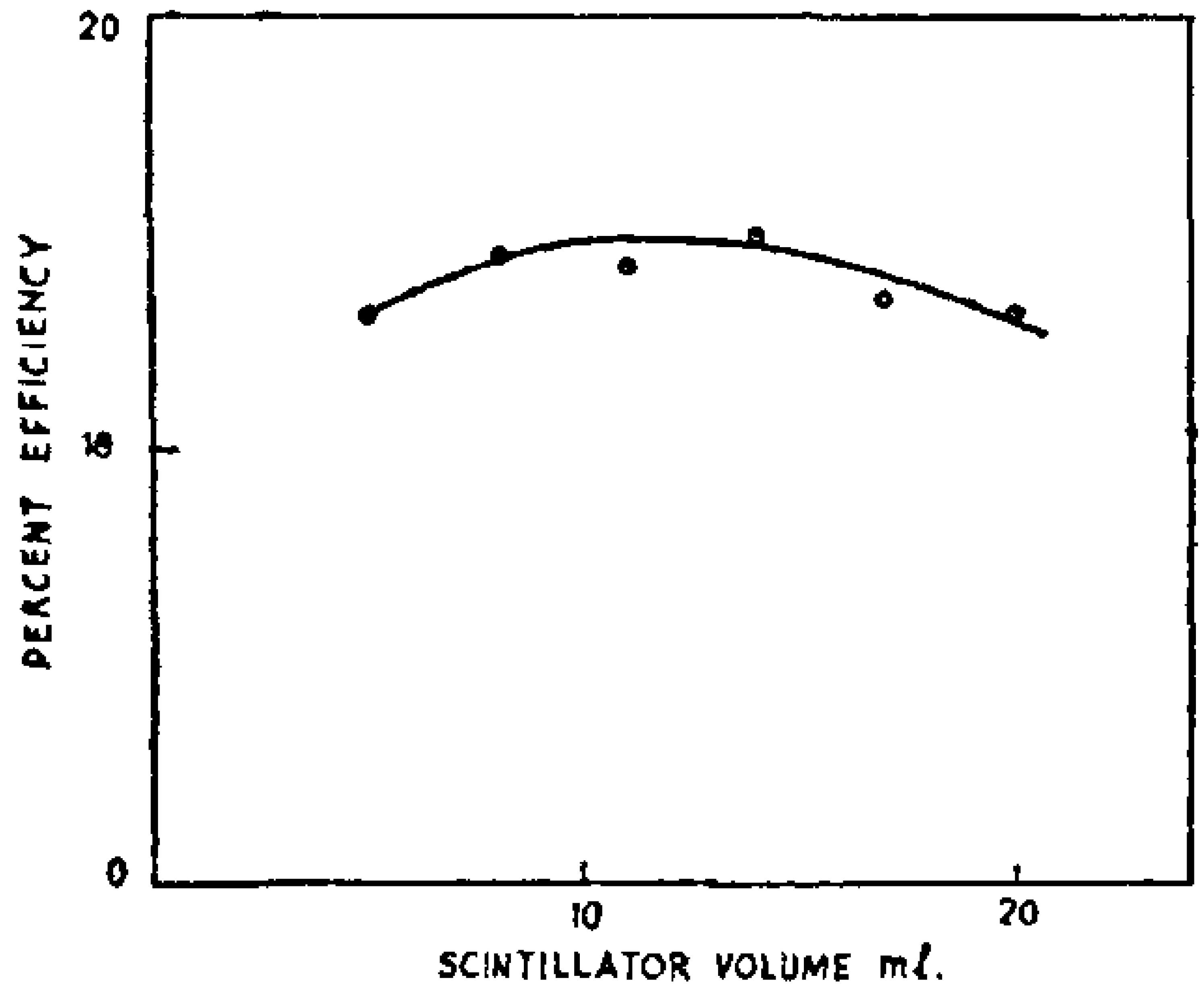

FIG. 1. Optimisation of scintillator volume.

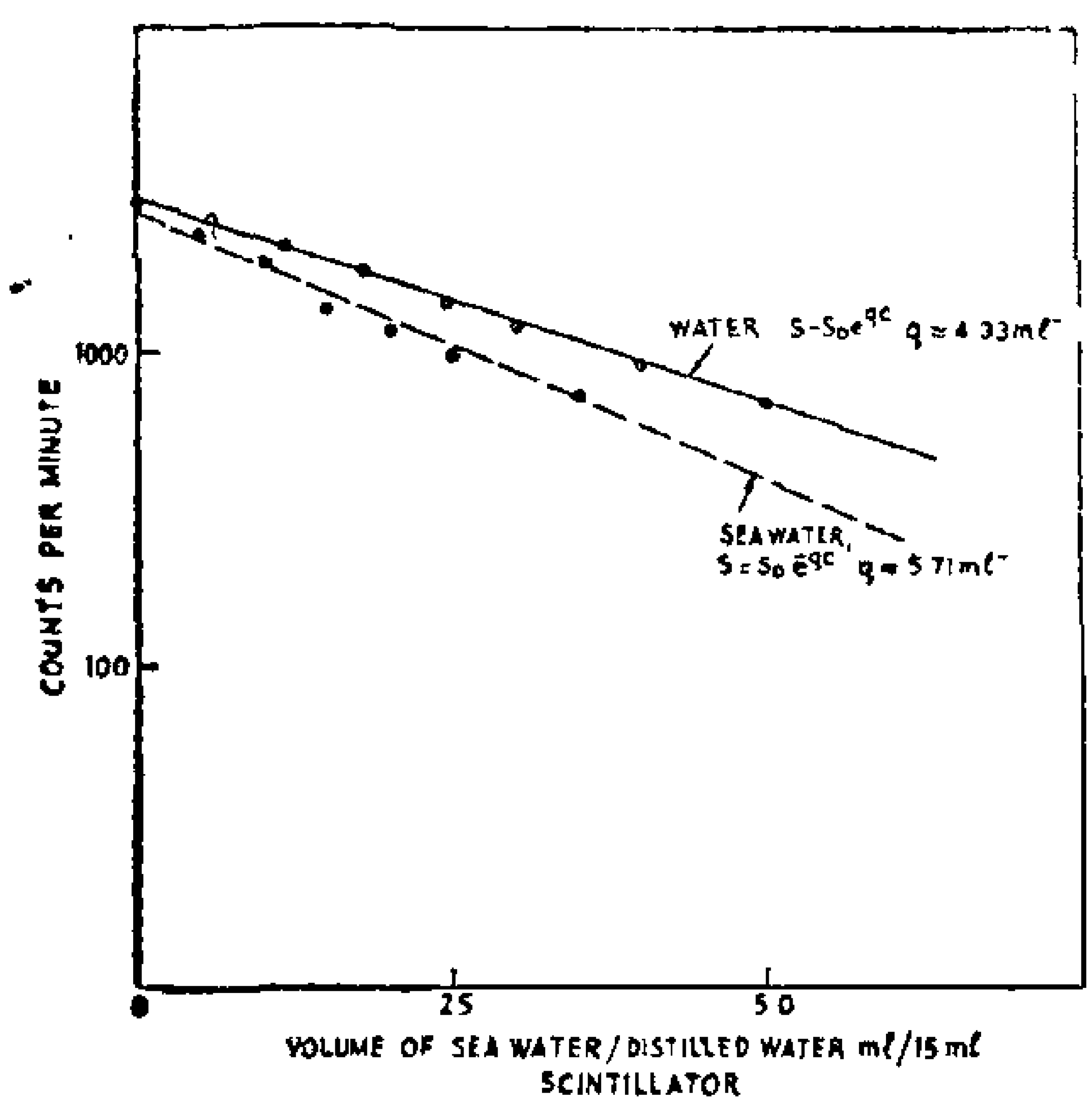

FIG. 2. Quenching effect with sea water and distilled water.

Tritiation of organisms.—In order to assess the efficiency of vacuum distillation technique of extracting body water, the tissue after distillation was kept in an oven at 100° C for 24 hours. The weight loss after keeping in oven was determined and compared with the efficiency of vacuum distillation technique (Table I). Samples weighing upto 5 gm tissue weight need only 1–2 hours whereas higher weights need more time.

The rate of uptake and release of tritium in the body water of arca (animal weight 36 g, wet weight 6·9 g) is represented in Fig. 3. The organisms

TABLE I

Efficiency of water removal by vacuum distillation

Waight of animal g	Tissue weight g	moisture content vac. distill. % (2 hrs)	moisture content by oven at 100° C% (24 hrs)
9–12	1·4–2·3	82·1 ± 3·9	85·0 ± 1·9
23–26	3·5–4·3	78·4 ± 0·1	80·5 ± 1·4
26–29	4·9–5·3	75·3 ± 4·2	77·6 ± 1·4
29–32	5·0–6·4	77·6 ± 1·9	78·4 ± 1·7
32–35	5·2	77·1	77·3
38–	6·9	72·7	80·0

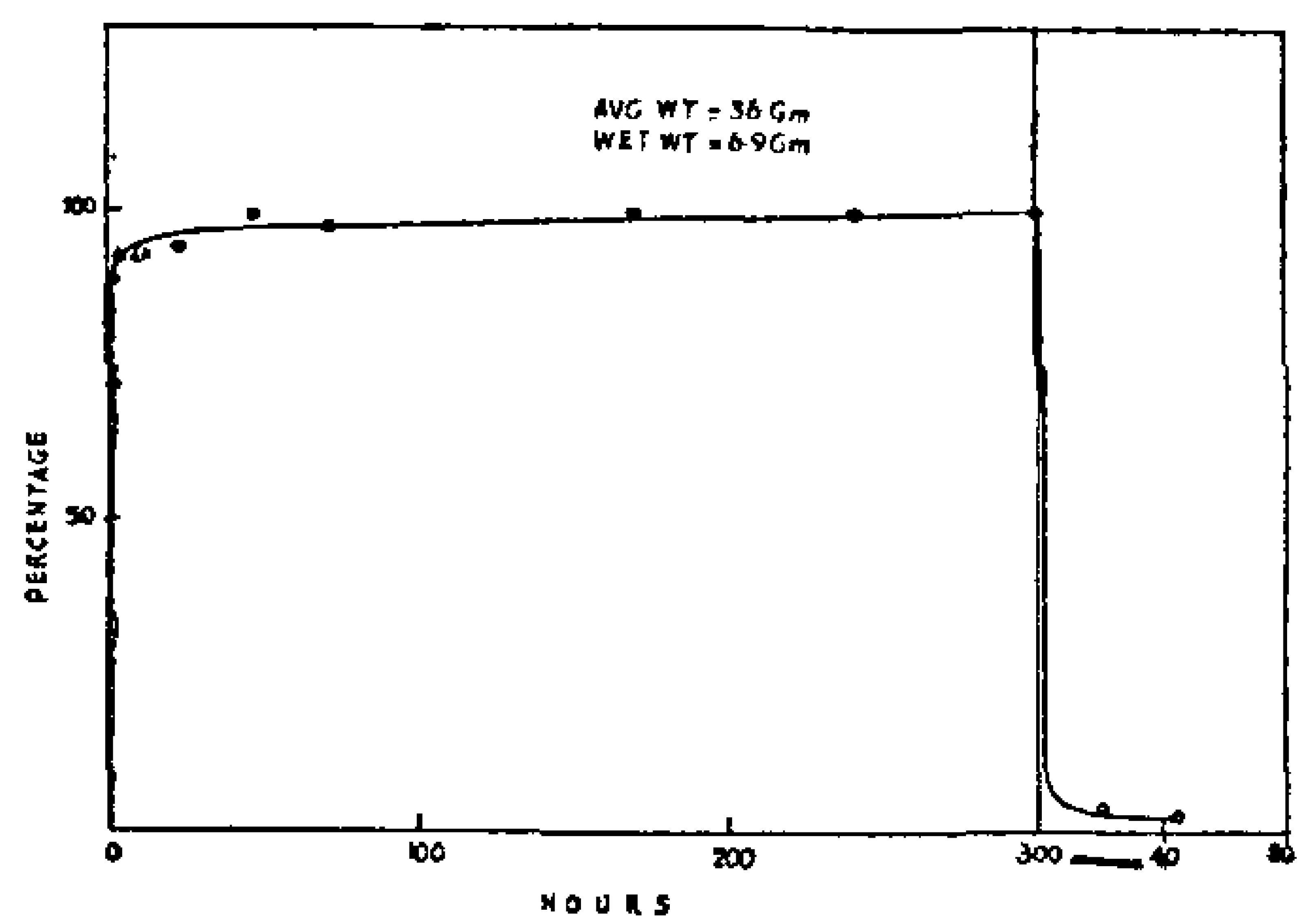

FIG. 3. Uptake and release of HTO by *Anadara granosa.*

reached ~92% of the medium activity within 3–4 hours. The body water tritium gradually increased further in 48 hours and then followed the decrease in medium water during the remainder of the experiment. The equilibrated animals when transferred to the inactive medium released its tritium quickly within few hrs, the release rate was the same as that of uptake.

Younger animals (animal weight 20 g, tissue weight 3 g) reached 92% of the medium activity within half an hour of exposure. The release experiments indicated that the animals released their body water activity to the inactive medium at the same rate as that of uptake (Fig. 4).

Rate of uptake.—The rate of uptake of tritium can be computed from the following equation,

$$\frac{dI_{rb}}{dt} = GR - (K + B)\, I_{rb}$$

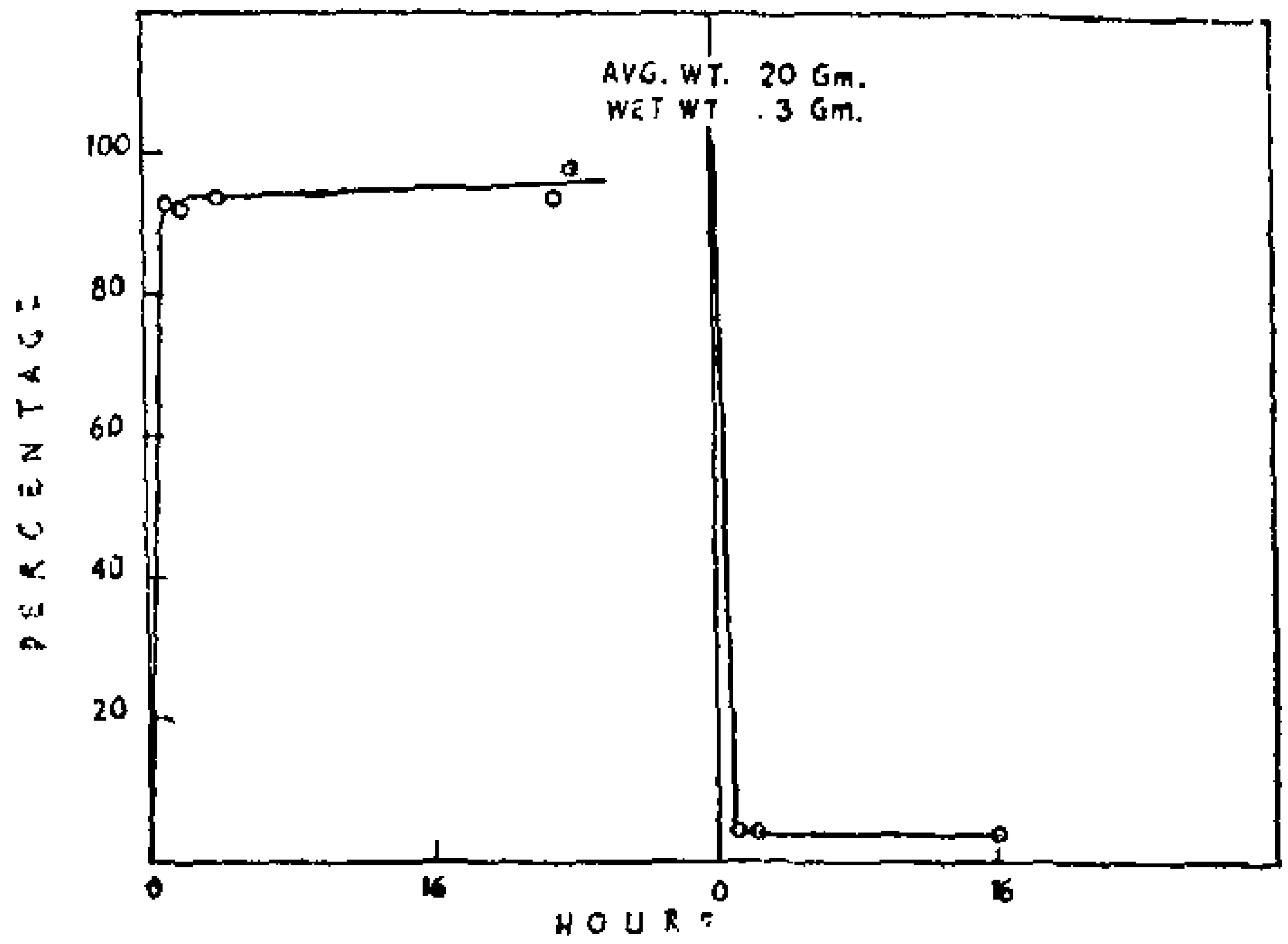

FIG. 4. Uptake and release of HTO by *Anadara granosa*.

where I_{rb}, G, R, K and B are radioactive atoms in the whole animal, specific activity of the medium, rate of uptake, radiological and biological decay constants respectively. Since K is very small for tritium compared to B, the equation can be rewritten as

$$\frac{dI_{rb}}{dt} = GR - BI_{rb}.$$

At equilibrium, $GR = BI_{rb}$. From the release experiments for younger animals B is calculated to be $3 \cdot 06\ hr^{-1}$. Incorporating this value in the equation at equilibrium condition, R is calculated to be 2·3 ml/g tissue wt/hr. Experiments were also carried out to study the relation of rate of uptake of the animal to its weight. Results are given in Table II. It is observed that the percentage uptake of tritium is more or less independent of the weight up to about 25 g. However for larger sized animals the per cent uptake falls down. The rate of uptake is seen to be a function of time or in other words to the probability of opening up of the animal. The animals when exposed to half an hour or one hour to the tritiated water, reached 92% of the medium activity if they have opened up during that time interval. Thus the rate of water intake for arca can be as high as 2·3 ml/g tissue/hr and this indicates that the animal renews its body water in single opening up of the shell when the body water volume is of the same order of uptake. This is true for the animals of 20 g weight and less. Under the same conditions, the rate of intake of water for 30 g and 40 g animals are 1·99 and 1·73 ml/g tissue/hr.

Organic bound tritium.—The build-up of organic bound tritium is represented in Fig. 5. The organic tissue had accumulated to a level of 6×10^3 dpm per g dry weight which is about 5% of the body

TABLE II

Per cent uptake of tritium in varied sizes of arca

Weight of the animal g	Tissue weight g	Activity medium cpm/0·5 ml	Body water cpm/0·5 ml.	Time of exposure mts	Uptake %
7·81	1·40	12620	11681	30	91·9
11·54	2·62	12620	10773	30	85·3
13·54	2·46	12620	11145	60	88·3
17·50	2·73	29946	27918	60	93·2
19·82	3·39	9720	7362	60	75·8
21·10	4·22	12620	11628	60	92·1
26·48	3·76	12620	11740	60	93·0
30·46	4·26	9720	7900	60	81·3
39·10	7·22	26042	18404	60	70·6

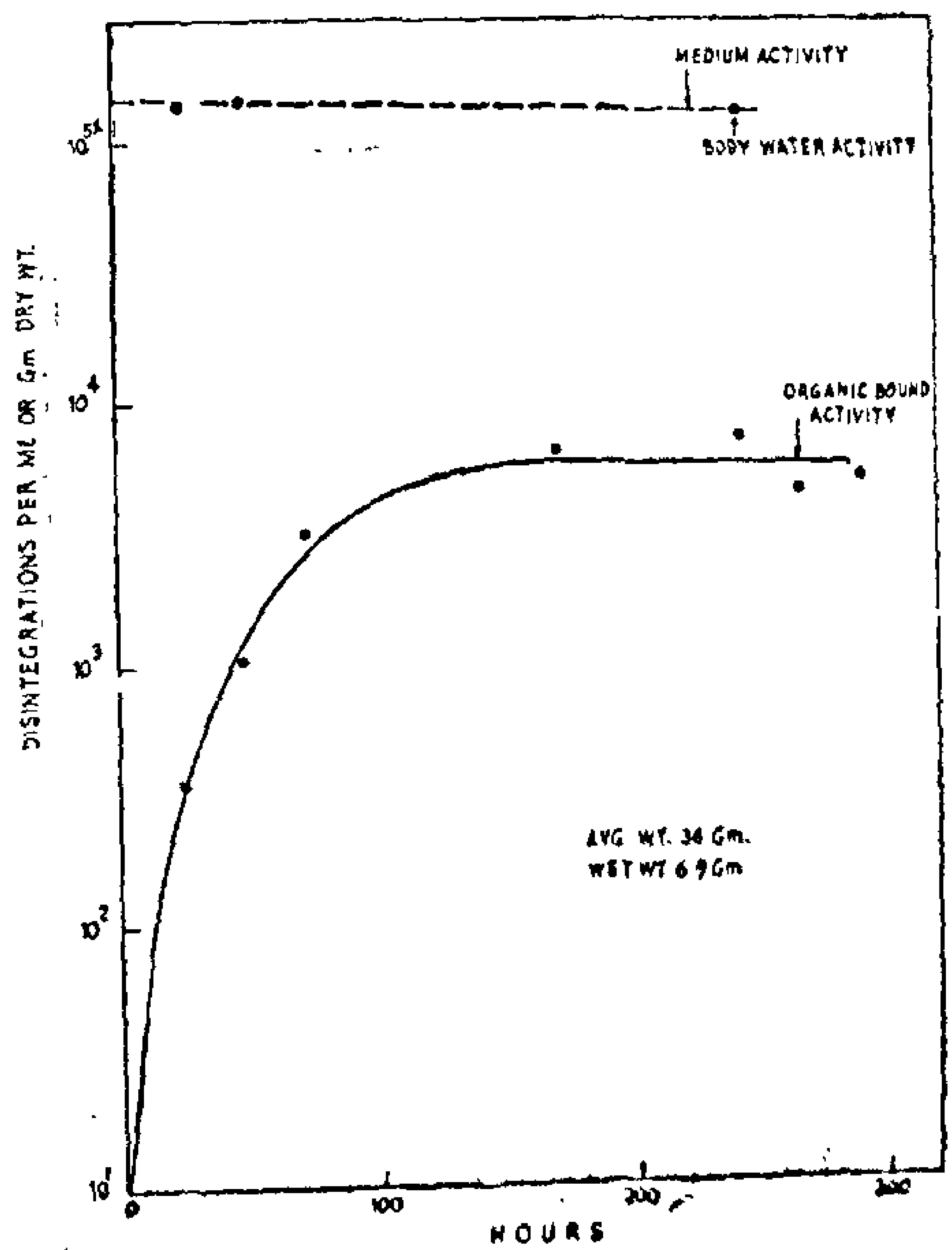

FIG. 5. Organic bound tritium in the uptake of HTO by *Anadara granosa*.

water tritium concentration. This corresponds to 1·2% of the total body water tritium activity in the animal. Skauen[5] found only 10% incorporated in the body tissue of fish, compared to body water tritium concentration. Thompson and Ballou[6] and Patzer[7] reported values of 30% and 20% tissue bound tritium in rat liver and intestine respectively. The retention of tritium in the body tissue depends upon the chemical form and in the present case the animals are fed with tritiated water, hence its retention depended upon the turnover rate of water in the system. Since a high turnover rate is found for arca ($B = 3\cdot06\ hr^{-1}$), major amount of tritium will not be retained in the system.

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