DETERMINATION OF TOTAL BODY WATER OF RABBITS EXPOSED TO HIGH EXTERNAL ENVIRONMENTAL TEMPERATURES USING A TRITIUM LABELING METHOD

L. A. Guseva and M. R. Zel'tser

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Investigation of water metabolism in rabbits unadapted to heat and exposed to a high external temperature, by means of a tritium method, an increase in the water circulation per diem from 13 to 19% (relative to the total body water) or an increase in the intake and excretion of water by 149 ml per diem was discovered. Exposure of adapted animals to heat of the same intensity increased the intake and elimination of water by only 78 ml compared with the control. In both cases the total body water fell slightly.

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The most convenient method of determining the water balance in man is the dilution method using tritiated water as labeled compound. Its concentration is measured by means of a liquid scintillation counter [1, 3-5, 7, 9, etc.].

This paper describes the results of preliminary investigations to determine the total body water of animals by a tritium labeling method during heat stress and adaptation of animals to a high external environmental temperature.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits unadapted and adapted to heat. Control experiments were carried out on the same animals: at room temperature, then after exposure to heat for 2 h (air temperature in the room 42°, relative humidity 25%) and again after training for 2-2.5 months. During the experiment itself the animals received no food or water, but at other times no restrictions were applied.

The water metabolism was determined by tritium labeling. The experimental animals received tritiated water by mouth in a dose of 0.01 μCi (10^{-8} Ci/ml)/g body weight.

After the tritium concentration in the body fluid had reached equilibrium, so that the tritium was uniformly distributed throughout the water compartment of the body, blood samples were taken from the marginal vein of the rabbits' ear. The moment of reaching equilibrium was determined in a special experiment from the curve of increase in activity of the sample. Water was removed from the blood samples by vacuum distillation [5], and 0.5 ml of the water distilled from the blood was dissolved in 10 ml of scintillation fluid of the following composition: dioxan 1 liter, 2,5-diphenyloxazole 6 g, diphenyloxazolylbenzene 275 mg, sublimated naphthalene 112 g.

The sample was poured into a cell with parallel, flat walls made of potassium-free optical glass. The cell was placed in the measuring chamber through a light trap, enabling samples to be replaced without switching off the high voltage, and there it was scanned by two FÉU-53 photomultipliers included in a coincidence circuit to minimize the contribution of noise pulses in the instrumental background. For the same reason, the photomultipliers and measuring chamber were placed in a refrigerator. The total water content in the body was calculated from the results of the measurements by the following formula:

$$V_x = \frac{a_1}{a_2} \cdot V_1,$$

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where V_X represents the total volume of body water (in ml); a_1 the initial specific activity of tritium administered to the animal; a_2 the mean specific activity of the biosubstrate; and V_1 the volume of water administered (in ml).

By measuring the specific activity of the biosubstrate samples taken on days following administration of tritium to the animals (under conditions of comfort and with exposure to heat for 2 h daily) its half-elimination period was determined, characterizing the velocity to water exchange in the body and bearing the following relationship to it:

$$\delta = \frac{0{,}693}{T} \cdot 100,$$

where δ is the percentage of water circulating per diem, a value indicating the fraction of the total body water exchanged in the course of the 24 h; t the half-elimination period (in days). The absolute circulation of water per diem (in ml) is given by:

$$Q = V_x \cdot \delta,$$

where V_X is the total body water (in ml).

EXPERIMENTAL RESULTS

A uniform distribution of tritium in the water compartment of the rabbits was achieved only 90-120 min after administration of the isotope.

The body water content of rabbits kept at a normal room temperature was $70 \pm 1.1\%$ of the body weight, with variations between 64 and 74%. The half-elimination period of tritium oxide was 4.2-6.5 days (mean 5.4 ± 0.26 days). The water circulation, calculated from the rate of its elimination and the toal body water was $13 \pm 0.6\%$, or 258 ± 14.8 ml/day. This volume of water consisted of the water drunk, water entering the body with the food, and that formed in the body during oxidation [6].

After a single exposure to a high room temperature (40-42°), the body temperature of the unadapted rabbits increased by $2.48 \pm 0.11°$ ($P < 0.01$), and the body weight fell by 107 ± 6.2 g ($P < 0.01$), no diuresis occuring. The total body water, expressed as a ratio of the body weight, showed a tendency to decrease compared with the control ($67.6 \pm 1.7\%$). The half-elimination period of tritium oxide fell to 3.8 ± 0.32 days ($P < 0.01$) with variations from 2.5 to 5.8 days.

The decrease in the half-elimination period showed that the circulation of water in the body increased to $19 \pm 1.5\%$, or 406 ± 53.7 ml, i.e., the water intake and elimination increased by 149 g.

It is interesting to note that the body temperature of rabbit no. 4 increased to 41.7°, the half-elimination period of tritium fell to 2.5 days, but the total water content was unchanged; the body temperature of rabbit no. 7, exposed to the same heat stress, increased to 42.1%, the half-elimination period was unchanged, but the total body water decreased. Hence, the increase in circulation of water in rabbit no. 4 to 27% of the total body water, or to 709 ml/diem, decreased the degree of stress on the thermoregulatory reactions, as expressed by maintenance of the total body water level and by a smaller increase in the body temperature compared with that of rabbit no. 7. One of the mechanisms of regulation of the body temperature during heat stress is evidently an increase in the circulation of water in the body.

After adaptation of the rabbits to heat for 60-75 days, the response reaction of the body to the next heat load was less marked than on the first day of exposure to heat. The body temperature rose by $1.2 \pm 0.08°$, the body weight fell only by 40 ± 3.8 g, the circulation of water per diem was $14 \pm 0.45\%$ of the total body water, or 335 ± 14.4 ml, and the half-elimination period of tritium under these conditions was 4.9 ± 0.17 days.

The volume of water consumed and excreted thus increased by only 78 ml over the control level.

Investigations have shown [2, 8] that the body water diminished with age. In the present investigations the increase in body weight during the adaptation period was 695 g, while the total water content fell from 70 to 67% of the body weight. The water content possibly falls because of the accumulation of fat or other solid tissues, resulting from age changes. Further observations are required to solve this problem.

It can be concluded from the results of preliminary investigations that tritium labeling can be used to investigate water metabolism in animals exposed to heat stress. The method is accurate and simple and, in addition, it simplifies determination of the water intake and the taking into consideration of all sources from which water enters the body.

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