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ESTIMATION OF TOTAL BODY WATER (VIRTUAL TRITIUM SPACE) IN THE RAT, CAT, RABBIT, GUINEA-PIG AND MAN, AND OF THE BIOLOGICAL HALF-LIFE OF TRITIUM IN MAN

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Isotopes of water (deuterium oxide and tritium oxide) have been used for the determination of total body water in certain mammalian species (Hevesy & Hofer, 1934; Pace, Kline, Schackman & Harfenist, 1947; Soberman, Brodie, Levy, Axelrod, Hollander & Steele, 1949; Haigh & Schnieden, 1956; Langham, Eversole, Hayes & Trujillo, 1956; Fallot, Aeberhardt & Masson, 1957). However, the techniques involved in these determinations have limited use, being either difficult, expensive or time-consuming. Recent advances in scintillation counting techniques (Haigh, 1957) have made it much easier to estimate one of these isotopes of hydrogen, namely tritium. The purpose of the present paper is to describe how far such scintillation counter techniques can be applied to the determination of tritium in urine and plasma and the estimation of degree of error involved. The use of such techniques for a comparative study of total body water in a number of mammalian species and of water turnover under tropical conditions in one species, namely man, is also presented.

METHODS

0·5 ml. of tritiated water or processed tritiated plasma or urine (see below) was added to 8 ml. of absolute ethanol and 10 ml. of scintillation solution (3 g 2:5-diphenyloxazole/l. sulphur-free toluene). The mixture was shaken and then centrifuged at approximately 1500 rev/min for 2–3 min. 5 ml. of the supernatant fluid was placed in the special counting container supplied with the N612 Ekco Scintillation Counter. This scintillation unit was kept at −20° C in a deep-freeze cabinet and its amplifier set at maximum gain. It was connected to a N530E Ekco Automatic Scaler unit which was set at H.V.1200 and Discrimator bias of −5 V. The scaler received a stabilized current from a constant-voltage stabilizer (Servomex AC 2 Mark IIB). Unless otherwise stated, all specimens were counted for 1000 sec and were allowed to cool in their counting containers for 1000 sec at −20° C before counting.

Preparation of urine. 3·5 ml. of urine was mixed thoroughly in a test-tube with 0·5 g of activated charcoal. The resultant sludge was filtered through a Whatman No. 2 filter paper and 0·5 ml. of the filtrate was then added to 18 ml. of the alcohol-scintillator mixture, shaken and centrifuged, and a 5 ml. portion taken for counting.

Preparation of rat, cat, guinea-pig or rabbit plasma. 0·5 ml. of plasma was added to 18 ml. of the alcohol-scintillator solution mixture. After thorough shaking the mixture was centrifuged. 5 ml. of the clear supernatant fluid was then withdrawn and placed in the counting container before counting.

Preparation of human plasma. Since it was found that the above method of preparation for rat plasma gave a coloured (orange-yellow) supernatant liquid with human and baboon plasma, another method was also investigated. The plasma was precipitated with 10% trichloroacetic acid (Langham *et al.* 1956) and 0·5 ml. of the clear colourless filtrate was mixed with 18 ml. of alcohol-scintillation fluid mixture. 5 ml. of this final mixture was used for counting.

Recovery experiments. In order to determine the degree of quenching resulting from these procedures for plasma and urine, known amounts of tritiated water were added to urine, plasma or distilled water and the activities of these solutions (after treatment, if necessary) compared. The final tritium concentration ranged from 50 to 150 μc/ml. In no instance was the urine or plasma diluted by the tritiated water by more than 10%.

Determination of virtual tritium space

Rat. Male or female albino rats weighing between 150 and 250 g were used. These were given 10 μc of tritiated sodium chloride solution (0·9 g sodium chloride in 100 ml. tritiated water) either intraperitoneally, or intravenously. The intravenous injection was given through a polythene cannula inserted into the jugular vein 18 hr previously. Following the administration of the tritiated water the animals were left for 2 hr and then given 5 ml. of water orally. All urine passed up to 2½ hr after injection of the tritiated sodium chloride solution was rejected, but the first 3·5 ml. of urine passed after 2½ hr was collected. (Preliminary experiments had shown that after 2½ hr tritiated water given by the above routes had reached equilibrium in the rat.) The rat was then weighed and killed, and the urine processed as stated above. After counting, the virtual tritium space at approximately 2½ hr was calculated.

As it was desirable to compare this space with a direct determination of total body water, the corpse, after mincing, was placed in an oven kept at $100 \pm 2°$ C and dried to constant weight; care being taken to trap the tritiated water vapour.

Cat. Young adult cats of both sexes were used (wt. 1·0–2·25 kg). They were starved during the night and the following morning tritiated sodium chloride solution (50 μc/kg) was administered intraperitoneally in a volume of not more than 2 ml. After 2½ hr the animals were anaesthetized with pentobarbitone sodium and a sample of blood was withdrawn. From two of the cats a further blood sample was withdrawn at 3 hr, to check whether any marked change had occurred between 2½ and 3 hr in the tritium concentration of the plasma. Plasma solids were determined by drying six samples of plasma at 100° C to constant weight.

Guinea-pig. Young adults were used of weight ranging between 300 and 420 g and of both sexes. An intraperitoneal dose of tritiated sodium chloride solution (50 μc/kg) was given and a procedure was used similar to that employed for the determination of cat body water.

Rabbits of both sexes (wt. 1·6–2·3 kg) were used. An intraperitoneal dose of tritiated sodium chloride solution (50 μc/kg) was given and a procedure similar to that for the determination of cat body water was used except that the animals were not anaesthetized and blood samples were taken from the ear.

Human beings. Healthy male students, aged between 21 and 24 yr and weighing between 47 and 64 kg, each drank a glass of water (approx. 500 ml.) containing 3 mc of tritiated water 1 hr after breakfast. Urine samples were collected just before the experiment started and at quarter- or half-hourly intervals for up to 5 hr afterwards. Recovery determinations were performed on the samples of urine taken before the experiment started and the figures obtained were used to correct for tritium content in the urine subsequently collected. (This assumed that the quenching factor remained constant throughout the experiment.)

RESULTS

Figure 1 shows that a straight-line relationship exists between tritium concentration in urine and recorded count up to a concentration of tritium in urine of 500 nc/ml. Similar experiments show a straight-line relationship for tritium in water over the same range and also for tritium in plasma up to 100 nc/ml. (this being the maximum concentration of tritium in plasma investigated). Each sample was counted for 1000 sec. It was found that an individual count at a concentration of 40 nc/ml. was subject to an experimental error of $\pm 2\%$ (S.E. of the mean of 8 counts). This was the lowest tritium concentration met with in practice. The S.E. was slightly less at higher tritium concentrations.

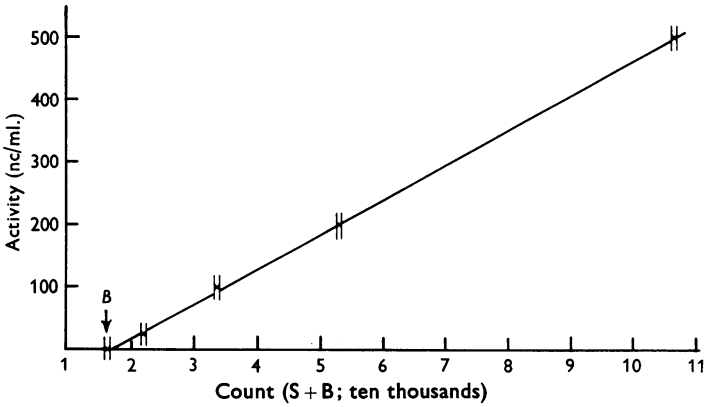

Fig. 1. Relationship between concentration of tritium in urine and the mean count observed for 1000 sec. Horizontal distance between parallel lines represents S.E. of 10 such counts. In all figures *S* equals true count of specimen, *B* equals background count.

Table 1 shows the percentage recovery of tritium from various body fluids in several species. In any of the species investigated a higher percentage recovery is observed from plasma than from the urine.

Table 2 compares the virtual tritium space of the rat with direct measurement of total body water obtained by a desiccation procedure. It can be seen that the virtual tritium space estimated after both intravenous and intraperitoneal injection of tritium is approximately 4% higher than the total body water obtained by desiccation.

Table 3 compares the values for virtual tritium space (total body water) in a number of mammalian species.

Figure 2 shows the concentration of tritium in the urine with time (up to 5 hr) in three different subjects following the oral administration of tritiated water. In all ten human beings equilibrium was obtained by the third hour after administration.

TABLE 1. Recovery of tritium from urine and plasma (taking tritium in water as 100%; mean ± S.E.)

Fluid	Recovery (%)
Water	100 (10)*
Rat urine	95·6 ± 0·4 (10)
Rat plasma	99·4 ± 0·5 (10)
Cat plasma	100 ± 0·6 (10)
Rabbit plasma	97·6 ± 1·5 (9)
Human plasma	97·6 ± 1·0 (11)
Human urine	95·2 ± 0·8 (10)
Guinea-pig plasma	97·0 ± 0·5 (10)

* Figures in parentheses indicate the number of samples used in the determination.

TABLE 2. Comparison of virtual tritium space and direct measurement of total body water by desiccation in the rat (mean ± S.E.)

Route	Total body water (%)		
	Direct	Virtual tritium space	Difference
Intravenous	63·9 ± 1·0 (10)*	68·1 ± 1·2 (10)	4·2
Intraperitoneal	66·5 ± 0·8 (7)	70·8 ± 1·3 (2)	4·3

* Figures in parentheses indicate the number of animals used.

TABLE 3. Virtual tritium space (total body water) in mammals (%; mean ± S.E.)

Species	Route	Virtual tritium space
Rat	Intravenous	68·1 ± 1·2 (10)*
Cat	Intraperitoneal	70·6 ± 0·9 (8)
Guinea-pig	Intraperitoneal	75·2 ± 0·9 (18)
Rabbit	Intraperitoneal	65·5 ± 2·3 (7)
Man	Oral	70·8 ± 1·1 (10)

* Figures in parentheses represent number of animals used.

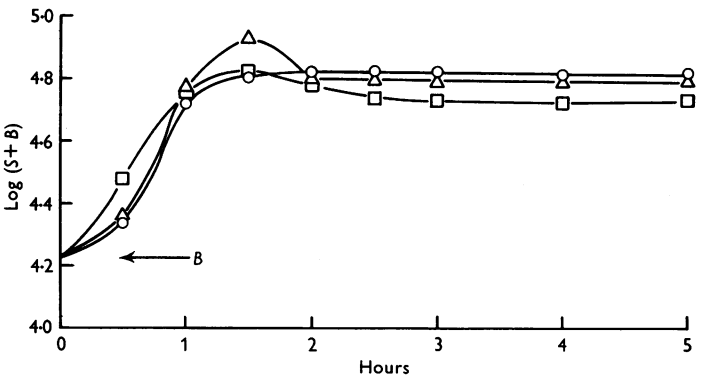

Fig. 2. The relationship between concentration of tritium in the urine and time over a period of 5 hr. Human subjects given a single dose of 3 mc tritiated water at time zero. Specimens were counted for 1000 sec.

Figure 3 shows the daily urinary concentration of tritium over a period of 10 days in two subjects. It can be seen that the biological half-life for tritium for these two subjects was approximately 6 days and 11 days, respectively. The mean value for ten subjects was $7{\cdot}5 \pm 0{\cdot}6$ days (mean $\pm$ S.E.), with a range of 5–11 days. The daily maximum temperature recorded during this period ranged from 79 to 93° F (26–34° C) and the minimum temperature from 69 to 75° F (20·6–23·8° C). The relative humidity at 7.00 hr ranged from 96 to 100 % and at 16.00 hr from 62 to 83 %.

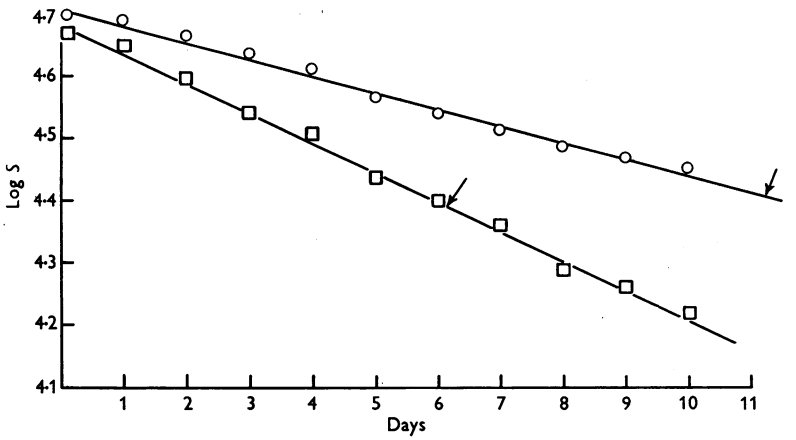

Fig. 3. Daily urinary tritium concentration in two human subjects following a single oral dose of 3 mc tritiated water. Specimens were counted for 1000 sec. Arrows show biological half-life.

DISCUSSION

A number of substances have been used for determining total body water. Theoretically the best estimate would be obtained from a substance which quickly becomes dispersed in the total body water, is capable of easy and accurate estimation in urine or plasma, is not metabolized and behaves physiologically like normal water. It should also have no harmful effects on body tissues.

Whilst sulphonamides, antipyrine and *N*-acetyl-antipyrine are metabolized, deuterium oxide and tritium oxide do not suffer from this disadvantage. They are also relatively non-toxic (Brues, Stroud & Rietz, 1952) and would appear to fulfil most of the requirements of an ideal substance for total body water estimations. Prentice, Siri, Berlin, Hyde, Parsons, Joiner & Lawrence (1952) suggest that the body is unable to differentiate between heavy and normal water; but there is some evidence that this may not apply in all situations (Glascock & Dunscombe, 1954; Bowen, 1960). Deuterium oxide has the advantage over tritium oxide that

it is non-radioactive, but the disadvantages that it is difficult to estimate routinely and is expensive. Estimation of a total body water in man with deuterium costs, at present prices, approximately £5; with tritium, the cost is about 1*s*.

Tritium, however, in spite of its radioactivity, is safe to handle and of low toxicity, since it only emits β particles (Brues *et al.* 1952). Nevertheless, until recently it also has been difficult to assay. Previous methods have involved converting to gas and used ionization chambers (Pace *et al.* 1947; Prentice *et al.* 1952; Fallot *et al.* 1957), internal Geiger counters (Wing & Johnston, 1955) and proportional gas-phase counters (Robinson, 1951). But none of these methods is very suitable for rapid and accurate routine analysis of large numbers of samples.

Tritium suffers from the disadvantage that it will exchange with hydrogen other than that present in water. Schloerb, Friis Hansen, Edelman, Solomon & Moore (1950) estimate the labile hydrogen pool to be between 0·5 and 2 % of body weight, and Pace *et al.* (1947) noticed that the virtual tritium space compared with total body water values obtained in rabbits by desiccation procedure was 2–3 % higher. Our own estimations make this pool approximately 4 % of body weight, which is more in keeping with the value quoted by Elkinton & Danowski (1955).

Accuracy of count is dependent on the careful control of several experimental variables. Like Stitch (1959), we also have noted the marked quenching effect of acetone. The Ekco N612 counter uses a small counting pot. We have noticed up to a 5 % variation in count, using the same tritiated solution in different pots. Each pot was therefore calibrated as regards transmission with respect to one standard pot.

Langham *et al.* (1956) used a trichloroacetic precipitation procedure for human plasma before estimation of tritium. We have compared a trichloroacetic procedure with the one presented in this paper. In our experiments, using eight different plasmas, not only had it no advantage but it reduced the sensitivity of the procedure by about one-third without increasing the accuracy of the recovery.

Our results for total body water are generally slightly higher than those obtained by other workers. Pinson & Anderson (1951), using tritium, reported that the total body water in the rat ranged from 57 to 72 % of the body weight. Flexner, Gellhorn & Merrell (1942), using deuterium, reported mean guinea-pig body water as 65 % of the body weight.

Our figures for virtual tritium space in guinea-pigs are approximately 8 % higher. This may be due to the fact that our animals weighed less than those of Flexner *et al.* (1942) and hence probably had a lower fat content. Another possible reason is that though all the animals appeared normal, at post-mortem a small quantity of clear ascitic fluid was seen.

Our results for total body water in the rabbit are somewhat lower than those of Soberman (1949), who determined antipyrene space. Using deuterium, Schloerb *et al.* (1950) reported that the mean total body water in human beings was 61·8 % of the body weight. Fallot *et al.* (1957), using tritium, reported the value for total body water as 61 ± 4·5 % body weight. Our mean results are higher. The fact that our subjects lived in the tropics may account for some of these differences. Under tropical conditions the biological half-life for tritium was 7·5 ± 0·6 days (mean ± S.E.). This compares with figures of 10 and 9·3 days for the half-life of deuterium (Hevesy & Hofer, 1934; Schloerb *et al.* 1950) and 8–9 days for tritium reported by workers in other latitudes (Fallot *et al.* 1957). Further work is progressing on the effect of climate on water turnover.

SUMMARY

1. A method has been described for the rapid estimation of tritium. The recovery of tritium in certain body fluids by this method has been ascertained and the virtual tritium space has been estimated in a number of mammalian species.

2. In the rat this space was approximately 4 % higher than total body water estimated by a desiccation procedure.

3. The mean biological half-life for tritium in adult male students under tropical conditions was 7½ days.

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