Rate of Body-Water Distribution studied with Triple Labelled Water

THE rate of equilibration of extra- and intracellular water is determined by the passage of the water through the cell membrane. The transfer of water through cell membranes can proceed by various mechanisms. The simplest mechanism is diffusion of water molecules as a whole. Another mechanism is a biological carrier system, such as exists, for example, in the tubular mechanism of the kidney; and another route of transfer is ionic migration of protons and hydroxyl ions independently through the cell membrane.

Our results show that, within the experimental accuracy, no difference could be found in the rates of distribution of the three isotopes and their respective volumes of distribution (Fig. 1). (The standard deviations for the determination of oxygen-18, deuterium and tritium were 1, 2 and 2 per cent respectively.) The percentage of body water in our measurements is in full agreement with values obtained with deuterium as a tracer[2]. The small differences in fluid volumes of distribution could be explained either by the different rates of exchange between these isotopes and body constituents on one hand and the different routes of excretion on the other. Different results were obtained, however, in deeply anæsthetized animals.

Table 1. VOLUME OF DISTRIBUTION OF LABELLED WATER IN RABBITS

Rabbit No.	Time after injection (min.)	Isotopic composition of blood samples: Oxygen-18 (per cent)	Deuterium (per cent)	Tritium (μc.)	Volume of distribution from isotope dilution* Oxygen-18 (ml.)	Deuterium (ml.)	Tritium (ml.)
U2	2·5	0·267	0·168	$2·5 \times 10^{-2}$	1,260	1,235	1,278
U2	10	0·260	0·151	$2·25 \times 10^{-2}$	1,415	1,392	1,420
U2	29	0·254	0·140	$2·07 \times 10^{-2}$	1,560	1,517	1,542
U2	140	0·250	0·129	$1·98 \times 10^{-2}$	1,670	1,665	1,615
A–3	4	0·274	0·235	$1·40 \times 10^{-1}$	1,320	1,295	1,250
A–3	40	0·262	0·210	$1·15 \times 10^{-1}$	1,570	1,545	1,525

* *U2* was injected with 5 ml. of water containing: oxygen-18, 18·6 per cent; deuterium, 37·6 per cent; and tritium, 6·4 μc./ml. The isotopic composition of the blood was found to be before injection: oxygen-18, 0·195 per cent; deuterium, 0·016 per cent; tritium, zero activity. *A*–3 was injected with 5 ml. of water containing: oxygen-18, 18·4 per cent; deuterium, 40·3 per cent; and tritium, 33·3 μc./ml. This rabbit had been used in previous experiments so that its body water (blood) was slightly isotopically enriched at the time of injection, namely oxygen-18, 0·20 per cent; deuterium, 0·08 per cent; tritium, $5·4 \times 10^{-3}$ μc./ml.

In order to elucidate the mechanism, water triply labelled with oxygen-18, deuterium and tritium was used. The aqueous system injected into rabbits contained therefore primarily $H^{16}OH$, $H^{16}OD$, $H^{18}OH$ and $H^{18}OD$ in equilibrium. The experiments, which were carried out on rabbits, were aimed at comparing the rates of transfer of the three isotopes from the blood to the intracellular volume and at the determination of the total volume of their distribution.

In our experiments, water containing 16–20 per cent of oxygen-18, 35–45 per cent of deuterium and 5–50 μc. of tritium per ml. was injected into the rabbits intravenously. Blood samples were withdrawn at intervals, centrifuged and the water distilled on a vacuum line at room temperature. The isotopic composition of oxygen was determined by decomposition of the water by alkaline hypobromite solution over cobaltic oxide in a sealed ampoule[1], the oxygen gas being analysed on a *CEC*21–401 isotope-ratio mass spectrometer. Two other portions of the water were totally reduced to hydrogen by zinc powder in sealed ampoules at 400° C., and one of these was analysed by a hydrogen/deuterium isotope-ratio mass spectrometer; the other hydrogen sample was introduced into a Geiger counter for activity determination. Some typical results are shown in Table 1.

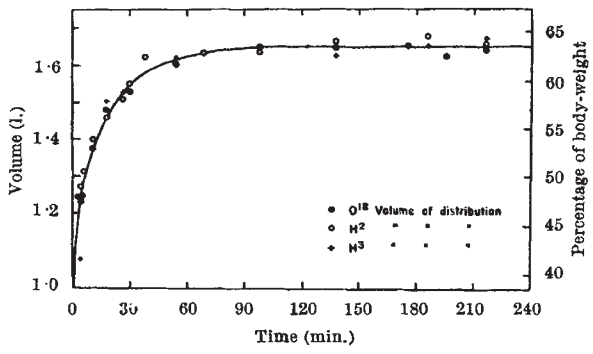

Fig. 1. The rate of distribution of water labelled with oxygen-18, deuterium and tritium as a function of time

The biological half-lives for oxygen-18, deuterium and tritium in rabbits were found to be 80, 220 and 130 hr. respectively (with a standard deviation of 10 per cent). The shorter biological half-life of oxygen-18 may be due to the additional route of excretion by expiration. The difference between tritium and deuterium may indicate a proton-transfer mechanism in the excretion process in which an isotope effect is involved.

As no appreciable difference could be detected between the rates of distribution of the three isotopes, it may be concluded that under normal conditions the most probable mechanism for water transfer through the cell membrane involves water molecules as such, and does not involve the breaking of the hydrogen–oxygen bond or prior ionization.

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[1] Anbar, M., *Int. J. App. Rad. and Isotopes* (in the press).
[2] Edelman, S., *Amer. J. Physiol.*, **171**, 279 (1952). Edelman, S., *et al.*, *Surg. Gyn. Obst.*, **95**, 1 (1952).

Radioprotective Properties of Cystamine, Cysteamine and Cysteine when tested with Chick Fibroblasts *in vitro*

IT is well known that a number of SH-containing compounds afford a certain amount of radioprotection to many organisms[1,2]. However, little information is available about the effects on cells *in vitro*. The present report deals with a series of experiments on the effects of cystamine, cysteamine and cysteine when added to cultures of chick fibroblasts shortly before irradiation.