

Body Water and Water Turnover Rate in Thoroughbred Horses in Training

G. J. JUDSON¹ and G. J. MOONEY²

1. Institute of Medical and Veterinary Science, Frome Road, Adelaide, S.A. 5000, Australia.
2. Hahndorf Veterinary Hospital, Princess Highway, Verdun, S.A. 5245, Australia.

Summary

Intravenous injections of tritiated water were used to determine total body water and water turnover rate in 28 Thoroughbred horses in training. It was found that the dilution of tritium in body water was well described by a two-component exponential decay curve. The first exponential component described a mixing process of tritium in body water, and the final exponential component represented the turnover of total body water.

Total body water and its turnover rate were not affected by stage of training. For the 28 horses examined, the values for total body water ranged from 67 to 78% of body weight, while the daily turnover rate of body water ranged between 15 and 45 litres. In horses, the mixing time of tritiated water in total body water is two to three hours. This contrasts with a mixing time of about six hours for sheep and cattle and of about one hour for man. However, the biological half-life of body water of eight to ten days is similar to that reported for man and ruminants.

Introduction

Tritium-labelled water has been used extensively as a tracer to determine the total body water and its turnover rate in mammals. However, only a few studies of the use of this tracer in horses have been reported (Julian *et al.*, 1956; Richmond *et al.*, 1962; Deavers *et al.*, 1973).

The present study was undertaken to characterize the dilution curve of tritiated water in body water of Thoroughbred horses and to determine whether the total body water and its turnover rate were affected by stage of training. It is a common belief among trainers and assisting veterinarians that training of horses is associated with a slight dehydration of the animal.

Materials and Methods

Studies were carried out on 28 clinically healthy Thoroughbred geldings comprising seven horses at each of four stages of training: horses within the first week of a two-month conditioning period (initial stage), horses at the end of the conditioning period but

without racing experience (barrier stage), horses which had competed in three races (early racing), and horses which had competed in six or more races (racing).

The horses were housed in loose-boxes and maintained on a diet consisting of wheat or oat chaff, oats, bran and lucerne hay. Water was available *ad libitum*.

Body water kinetics were estimated in 16 horses in autumn of 1978 and in a further 12 horses in autumn of 1979. In 1978, four horses at each stage of training were used, and in 1979, three horses at each stage of training were used to estimate body water kinetics. Horses were given light exercise about 3 h prior to commencing the estimate of body water kinetics.

Body water kinetics of the horses were estimated using a single injection of tritiated water (Radiochemical Centre, Amersham, England). A known dose of tritiated water of 1.4 to 1.9 mCi in 4 to 6 ml physiological saline was injected in the external jugular vein. Blood samples were collected from the contralateral jugular vein into lithium heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey, USA) at 1, 2, 4, 7, 23 and 31 h after injection of tritiated water in the first study and at 0.5, 1, 2, 4, 24 and 48 h after tritiated water injection in the second study.

Tritiated water was recovered from 1 to 2 ml plasma by vacuum sublimation. The samples were sublimated to dryness to avoid any possible isotope fractionation effects. Sublimated water was assayed for radioactivity by counting 0.5 ml in 5 ml Insta Gel (Packard Instrument Co. Inc., Illinois, USA). Aliquots of the tritiated water in saline, prepared for injection, were diluted 1 in 10^6 with water and assayed for tritium as described for plasma samples. Measurement of radioactivity was carried out using a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co. Ltd., USA), and the external standard was calibrated to correct for quenching.

The specific radioactivity/time curve of tritium in plasma water, following injection of tritiated water, was plotted on semilogarithmic co-ordinates. The dilution curve was resolved into exponential components by the conventional peeling process. These initial estimates of slope (m) and intercept (a) of the exponential components were used to calculate the line of best fit using the programme described by White *et al.* (1969).

The values were examined by analysis of variance and differences between means tested by Duncan's multiple range test (Duncan 1955).

Results

It was found that the dilution of the tritium in body water of horses was well described by a two-component exponential decay curve of the form:

$$SR_t = a_1 e^{-m_1 t} + a_2 e^{-m_2 t} \quad (1)$$

where SR_t = specific radioactivity of plasma water ($\mu\text{Ci}/\text{litre water}$) at elapsed time, t ; a_1 = zero time intercept ($\mu\text{Ci}/\text{litre water}$) of the component, i ; m_1 = rate constant of the component, i (h^{-1}); t = time in hours (h).

The initial component ($a_1 e^{-m_1 t}$) described an equilibration of tritium in water throughout the body, and the final component ($a_2 e^{-m_2 t}$), which was apparent about two to three hours after injection of tritiated water, represented the gradual excretion of radioactivity from the body. From equation 1 the following features of the body water compartments were determined:

$$\text{accessible water pool (litres)} = \frac{d}{a_1 + a_2} \quad (2)$$

$$\text{total water pool, } V \text{ (litres)} = \frac{d}{a_2} \quad (3)$$

$$\text{total output of water (litres/h)} = V m_2 \quad (4)$$

$$\text{biological half-time of total water pool (} h \text{)} = \frac{0.693}{m_2} \quad (5)$$

where d is the dose (μCi) of tritiated water injected into horses, a_1 and a_2 are the zero-time intercepts of the initial and final components of the specific radioactivity dilution curve, respectively, m_2 is the rate constant of the final component and 0.693 is the antilogarithm of $\log_e 2$.

Table 1 gives the mean values of body weight and of water pool sizes and water kinetics for horses at each of the four stages of training. These mean values, apart from those of accessible pool size, were not affected by stage of training ($p > 0.05$). The estimated mean accessible pool size of horses at the racing stage was significantly lower ($p < 0.05$) than that observed at other stages of training.

Discussion

The initial volume of distribution of tritiated water, the accessible water pool, was variable, with mean values ranging from 42 to 58% of body weight. By its size, the accessible pool must include, in addition to the water in the extracellular pool, water in the intracellular pool or gastrointestinal tract. Estimates of the extracellular fluid pool in horses are of the order of 18 to 26% of body weight (Kohn *et al.*, 1978; Thornton 1978; Carlson *et al.*, 1979). The variability in the size of the accessible water pool (see Table 1) may in part reflect technique, since only two or three blood samples were taken over the first two hours after injection of tritiated water to define the initial dilution of tritium in body water. The smaller accessible pool in horses in early racing, compared to horses at other stages of training, is difficult to explain, since this pool is not a clearly identifiable anatomical compartment and that total body water was not altered by stage of training.

The equilibration or mixing time of tritiated water in total body water of horses of two or three hours agrees with the mixing times observed by Deavers *et al.* (1973) who used this tracer in ponies. In contrast, the mixing time of tritiated water is about one hour in man (Prentice *et al.*, 1952) and five to six hours in sheep (Till and Downes, 1962) and cattle (Shumway *et al.*, 1956). The slow equilibration of tritium in body water of ruminants was due to the relatively large proportion of body water contained in the rumen (Till and Downes, 1962). The delay in the mixing time of tritium in horses is also probably due to the large volume of fluid in the large intestine. Argenzio *et al.* (1974) reported that the water content in the large intestine was equivalent to as much as 10% of the body weight of ponies.

TABLE 1. Water compartments and water kinetics.*

Stage of training	Body weight (kg)	Accessible water pool		Total water pool		Total output (litres/day)	Half-time (days)
		(litres)	(% body weight)	(litres)	(% body weight)		
Initial	507 ± 22	295 ± 29	58 ± 5.8	359 ± 20	71 ± 2.6	33 ± 6.5	7.7 ± 1.5
Barrier	504 ± 23	268 ± 41	53 ± 6.7	351 ± 16	70 ± 1.6	28 ± 7.2	9.2 ± 3.2
Early racing	478 ± 31	199 ± 59**	42 ± 12.0**	337 ± 21	70 ± 4.1	26 ± 6.6	9.7 ± 3.5
Racing	494 ± 53	271 ± 67	54 ± 9.6	341 ± 33	69 ± 2.0	29 ± 7.5	8.7 ± 2.1

* Mean values ± SD for 7 horses.

** Mean value less ($p < 0.05$) than mean values obtained at other stages of training.

Total body water in horses was not affected by stage of training (Table 1). The mean and range in values of the total body water for the 28 horses were 70 and 67 to 78% of body weight, respectively. These values are slightly higher than the average values of 64% and 55% obtained by Julian *et al.* (1956) for hot-blood horses and heavy draft horses, respectively, but agrees with findings of Deavers *et al.* (1973) of 68% in small ponies. The biological half-time of total body water in horses in the present study of 7 to 10 days is similar to the times observed by Richmond *et al.* (1962) for man and horses.

The daily loss of water was similar for horses at different stages of training, with means varying from 26 to 33 litres. These values are similar to those of Tasker (1967) who reported that horses with a mean body weight of 439 kg lost about 27 litres water: 4.9 litres/day in urine and 14.0 litres/day in faeces, and an estimated insensible water loss of approximately 8.5 litres/day. The daily water requirements of horses, however, depend on a number of factors including work load and meteorological conditions (Hinton 1978). In the present study, the horses were not worked during the period of estimation of total body water, and the meteorological conditions were similar for the two years during which the study was conducted. Although total body water or water turnover rate were not altered by stage of training in the present study, this does not exclude the possibility that such changes may occur in horses in training during the higher summer temperatures or in horses regarded by trainers as no longer competing to their potential.

Acknowledgements

We thank Dr J V Nolan, University of New England, for providing computer facilities for the analysis of the tritium dilution curves, and we are grateful to the Australian Equine Research Foundation for financial support.

References

- Argenzio, R. A., Lowe, J. E., Pickard, D. W. and Stevens, C. E. (1974) Digesta passage and water exchange in the equine large intestine. *Am. J. Physiol.* **226**, 1035–1042.
- Carlson, G. P., Harrold, D. and Rumbaugh, G. E. (1979). Volume dilution of sodium thiocyanate as a measure of extracellular fluid volume in the horse. *Am. J. Vet. Res.* **40**, 587–589.
- Deavers, S., Rosborough, J. P., Garner, H. E., Huggins, R. A. and Amend, J. F. (1973). Blood volumes and total body water in the domestic pony. *J. Appl. Physiol.* **34**, 341–343.
- Duncan, D. B. (1955). Multiple range and multiple 'F' tests. *Biometrics* **11**, 1–42.
- Hinton, M. (1978). On the watering of horses: a review. *Equine Vet. J.* **10**, 27–31.
- Julian, L. M., Lawrence, J. H., Berlin, N. I. and Hyde, G. M. (1956). Blood volume, body water and body fat of the horse. *J. Appl. Physiol.* **8**, 651–653.
- Kohn, C. W., Muir, W. W. and Sams, R. (1978). Plasma volume and extracellular fluid volume in horses at rest and following exercise. *J. Am. Vet. Med. Ass.* **39**, 871–874.
- Prentice, T. C., Siri, W., Berlin, N. I., Hyde, G. M., Parsons, R. J., Joiner, E. E. and Lawrence, J. H. (1952). Studies of total body water with tritium. *J. Clin. Invest.* **31**, 412–418.

- Richmond, C. R., Langham, W. H. and Trujillo, T. T. (1962). Comparative metabolism of tritiated water by mammals. *J. Cell. Comp. Physiol.* **59**, 45-53.
- Shumway, R. P., Trujillo, T. T., Bennett, J. A., Matthews, D. J. and Asplund, R. O. (1956). Fat determination in live steers using tritium injections. *Am. Soc. Anim. Prod. West. Sec.* **7**, 24,1-24,6.
- Tasker, J. B. (1967). Fluid and electrolyte studies in the horse III: Intake and output of water, sodium and potassium in normal horses. *Cornell Vet.* **57**, 649-657.
- Thornton, J. R. (1978). The measurement of extracellular fluid volume (radiosulphate space) in horses. *Br. Vet. J.* **134**, 283-285.
- Till, A. R. and Downes, A. M. (1962). The measurement of total body water in the sheep. *Aust. J. Agric. Res.* **13**, 335-342.
- White, R. G., Steel, J. W., Leng, R. A. and Luick, J. R. (1969). Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. *Biochem. J.* **114**, 203-214.