The measurement of total body water in living pigs by deuterium oxide dilution and its relation to body composition

By R. A. HOUSEMAN, I. McDONALD AND K. PENNIE

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. Deuterium oxide was used to estimate body water in twenty-four pigs of widely differing body composition and of average weight 83·9 kg.

2. After infusion of the isotope, blood samples were collected every 30 min for 4 h. The resulting plasma was purified by a heat-distillation procedure, after which it was analysed for D_2O by infrared spectroscopy.

3. Approximately 24 h after infusion of the D_2O each pig was killed, and its composition determined both by chemical analysis and physical dissection.

4. Equilibration of D_2O in the body was found to be complete within 2 h of injection of the tracer.

5. The mean D_2O space was found to be 8·6% greater than the mean empty body water space, but only 2·2% greater than the total body water space.

6. Empty body water and total body water were estimated from the regression lines with residual standard deviations of 2·7 and 1·9% respectively. Similarly, the residual standard deviations of the regressions involving the other fat-free components were 6·3% for dissectible lean, 3·2% for fat-free mass, and 5·6% for crude protein.

7. The residual standard deviations of the regressions in which the weights of dissectible fat and total body lipid were predicted were 6·0 and 6·7% respectively.

The naturally occurring non-radioactive isotope of water, deuterium oxide (D_2O) has been frequently used to estimate body water in several species because it is considered, as is tritium, to be an ideal test solute for measuring body water.

It has been used extensively in human subjects (Schloerb, Friis-Hansen, Edelman, Solomon & Moore, 1950; Edelman, Olney, James, Brooks & Moore, 1952; Bradbury, 1961; Hytten, Thomson & Taggart, 1966), but in farm animals there have been very few investigations in which D_2O has been used. This is presumably because of the high cost and because specialized equipment is required to measure D_2O concentrations.

D_2O has been used for estimating body fat in pregnant Blackface ewes (Foot & Greenhalgh, 1970). This study is of particular interest because a heat distillation technique (Turner, Neely & Hardy, 1960) was used for purifying the samples, which were then analysed by infrared spectroscopy (Stevens & Thurston, 1954). These are relatively simple procedures compared with the vacuum distillation technique (Schloerb, Friis-Hansen, Edelman, Sheldon & Moore, 1951) for purifying the samples and the falling drop method for determining the D_2O concentrations (Hytten, Taggart, Billewicz & Jason, 1962).

Groves & Wood (1965) working with suckling pigs used D_2O for estimating changes in total body water (TBW). The relationship between D_2O and TBW was fairly close ($r = 0{\cdot}8185$), but it was found that D_2O slightly underestimated TBW. The

Table 1. *Composition of the diets*

	135 g crude protein/kg air-dry diet	220 g crude protein/kg air-dry diet
Components (g/kg):		
Ground barley	920	620
Weatings	40	190
White fish meal	20	96
Soya bean	20	94
Supplements (g/kg main ingredients):		
Limestone	6·7	—
Dicalcium phosphate	10·0	—
Salt	1·0	—
Vitamin–mineral mixture*	2·2	2·2
Cyanocobalamin (mg)	0·1	—

* Isaac Spencer and Co. (Aberdeen) Ltd; Parkhill No. 2, providing per kg complete diet:

Vitamin A	660 μg	Zinc	100 mg
Cholecalciferol	15 μg	Magnesium	10 mg
Riboflavin	2 mg	Manganese	30 mg
Cyanocobalamin	5 μg	Iron	60 mg
Calcium pantothenate	10 mg	Cobalt	0·9 mg
Nicotinic acid	10 mg	Iodine (stabilized)	1·0 mg
Copper	200 mg		

authors concluded that this was possibly due to the slow equilibration of D_2O with the water in the bladder.

D_2O dilution studies have not been undertaken in older pigs, and the object of the experiment to be described here was to make estimates of TBW in 90 kg pigs, which differed widely in fatness, and to relate these to various measurements of body composition made by subsequent carcass analysis.

EXPERIMENTAL

Animals and diets

Twenty-four Large White × (Large White × Landrace) pigs were used which were obtained from the Institute's hysterectomy-derived pig herd. The group consisted of twelve castrated males and twelve females which had been reared at different rates from 25 to 90 kg. The treatments were combinations of increasing daily feed intake (70–140 g/kg$^{0·75}$) with two dietary protein concentrations (135 and 220 g crude protein/kg) and were intended to promote large differences in body fatness. The compositions of the diets are given in Table 1.

Housing and management

The pigs were weaned at 6 weeks of age and creep feed was available for a further 2 weeks. At 8 weeks the pigs were given a standard diet containing 170 g crude protein/kg. At 25 kg live weight each pig was transferred to a piggery with a controlled environment. Here they were housed individually and fed twice daily. Increments of feed intake were made for each pig every 7 d after they had been weighed. The pigs were weighed at the same time each week in order to minimize the variation in the

intestinal contents. When each pig approached 90 kg live weight it was given a standard intake of feed for 7 d in order to reduce variation in intestinal contents.

Management of the pigs during the in vivo study

Immediately before each pig was confined to a metabolism cage it was weighed. It was then deprived of food and water for about 12 h before the administration of D_2O.

In order to obtain frequent and uncontaminated blood samples the venous catheterization method described by Anderson & Elsley (1969) was used.

Injection of D_2O into the blood-stream

D_2O which had a minimum purity of 99·7% (Koch–Light Laboratories) was infused into the blood-stream of each pig as a saline solution (9 g NaCl/l) using a Watson–Marlow peristaltic pump. The dose rate was about 1 g D_2O/kg body-weight. The rate of infusion was about 12 ml/min. Immediately before and after infusion, the D_2O container was weighed to the nearest 0·005 g and heparinized saline solution (100 i.u. sodium heparin + 9 mg NaCl/ml) was pumped into the system.

Sampling of body fluids

In a preliminary trial it was found that equilibration of D_2O in the body was virtually complete within 2 h of injection of the tracer. In the present experiment, therefore, blood samples were collected, every 30 min, in heparinized containers (Beckton Dickinson Ltd, Rutherford, New Jersey, USA) from each pig up to 4 h after injection. Each blood sample was immediately centrifuged at 1000 *g* for 15 min and the resulting plasma decanted into air-tight vials and stored at 1° to await analysis.

Urine and faeces voided during the equilibration period were not collected because it had been found in preliminary trials that these were difficult to purify and analyse.

Estimation of D_2O in body fluids

The estimation of D_2O in biological fluids involves (*a*) the purification of the sample without isotopic fractionation, (*b*) the estimation of D_2O in the resulting D_2O–water mixture.

In this experiment the plasma samples were purified by means of a heat-distillation method (Turner *et al.* 1960). D_2O was estimated in the purified samples by means of infrared spectroscopy (Pye Unicam SP200). The basis of this method is that the absorption of infrared radiation at a wavelength of 4000 nm by a distilled-water–D_2O mixture is compared with that of a distilled-water reference solution. A series of standards was constituted in the range 0·5–2·5 ml D_2O/l, and each unknown was compared against two standards of higher and lower concentrations. The concentration of D_2O in each unknown was calculated from the expression used by Foot & Greenhalgh (1970).

Slaughter of the animals and subsequent processing

Each pig was killed approximately 28 h after the D_2O infusion. It was suspended by the hind legs and weighed on a steelyard to the nearest 20 g. The major blood

vessels of the neck were cut and each carcass was bled as fully as possible, and the weight of blood was recorded.

The carcass was then cut down the mid-ventral line and the entire alimentary tract removed, weighed, emptied and reweighed. The intestinal contents were homogenized and sampled for the estimation of dry matter.

After the hair had been removed by electric clippers, the carcass was divided into three parts, so that both physical dissection and chemical analysis could be facilitated. The three carcass divisions were designated middle, left side and right side.

The middle of the carcass included the head, backbone, blood, flare fat, internal organs and the entire alimentary tract. The left side of the carcass was stored horizontally in a doubly-sealed polyethylene bag for 16 h at 1°, after which it was physically dissected into skin + subcutaneous fat, lean and bone.

The right side of the carcass was jointed into pieces weighing approximately 7 kg. These were stored at −25° in doubly-sealed polyethylene bags, as were the other portions of the carcass. After 5 d they were allowed to thaw and each component was removed from the polyethylene bag, weighed and then minced. The mince was mechanically mixed and then sampled. About 1 kg of the homogeneous mince from each of the three carcass divisions was used for chemical analysis.

Estimation of D_2O space

Estimates of the D_2O space of each pig were made from the equilibrium plasma D_2O concentration. The equilibrium plasma D_2O concentration was calculated from the mean of three plasma values obtained between 2 and 3 h after injection of the isotope. The equation was

$$D_2O \text{ space (g)} = \frac{\text{weight of solution injected (g)} \times D_2O \text{ concentration in solution (g/g)}}{\text{equilibrium concentration in body fluid (ml/ml)}}.$$

RESULTS

Body composition of the pigs

Table 2 shows the mean values for each of the measurements that were made and the extent to which these measurements varied over the twenty-four animals. The animals were all approximately of bacon weight but varied considerably in body composition. Despite the precautions that had been taken, there was substantial variability in gut fill, intestinal water, and bladder water. Apart from these, the greatest coefficients of variation were those relating to lipid content. The composition of the fat-free material was relatively constant.

On dissection it was found that the mean difference in weight between the two sides of the carcass was 0·48 kg, the maximum being 1·80 kg. The losses which occurred during the dissection of the left-hand sides were small (mean value 1·6%, standard deviation 1·0% of the side weight) and these were assumed to be entirely of water. These losses were accounted for in the calculation of body water by subtracting the weight of dry matter from the weight of the entire body minus the weights of intestinal and bladder contents (i.e. weight of empty body water (EBW) = empty body-weight − weight of dry matter).

Table 2. *Body composition of the pigs*

(Mean values and standard deviations)

Measurement	Mean value	SD	SD as percentage of mean
From physical dissection (n = 24):			
Live wt (kg)	83·9	5·1	6·1
Gut fill (kg)	2·7	0·74	27·4
Water in bladder (kg)	0·5	0·9	180
Empty body-wt (kg)	80·6	4·6	5·7
Dissectible fat (kg)	17·7	3·4	19·2
Dissectible fat-free mass (kg)	61·8	4·8	7·8
Dissectible lean (kg)	34·6	3·5	10·1
From chemical analysis (n = 24):			
Total body water (kg)	47·6	4·8	10·1
Empty body water (EBW) (kg)	44·9	4·3	9·6
Water in intestinal tract (kg)	2·2	0·7	32·7
Total lipid (kg)	21·2	4·7	22·2
Fat-free mass (FFM) (kg)	59·4	5·8	9·8
Fat-free dry matter (FFDM) (kg)	14·5	1·6	11·0
Crude protein (CP) (kg)	12·0	1·3	10·8
Ash (kg)	2·5	0·3	12·0
Lipid (g/kg empty body-wt)	263	56	21·3
Ratio EBW:FFM	0·756	0·0096	1·3
CP:FFM	0·201	0·0073	3·6
CP:FFDM	0·826	0·012	1·5
Ash:FFDM	0·173	0·0062	3·6
From deuterium oxide determinations (n = 24):			
D_2O space (kg)	48·7	4·8	9·8

Table 3. *Regression equations for estimating, in pigs, empty body water, total body water, and other components of the body, other than fat, from D_2O space*

Body component (kg)	Regression equation on D_2O space (kg): Coefficient	Intercept	RSD	RSD as percentage of mean
Empty body water	0·869	+2·6	1·21	2·7
Total body water	0·989	−0·5	0·89	1·9
Dissectible fat-free mass	0·955	+15·4	1·67	2·7
Dissectible lean	0·596	+0·6	2·17	6·3
Fat-free mass	1·149	+3·5	1·88	3·2
Fat-free dry matter	0·280	+0·9	0·89	6·1
Crude protein	0·230	+0·7	0·67	5·6

RSD = residual standard deviation.

The dissectible fat consisted of subcutaneous fat and was therefore less than the chemically defined fat which also included intermuscular, intramuscular and intraperitoneal fat. The dissectible fat-free mass was correspondingly heavier than the chemical fat-free mass, but the two were highly correlated (r = 0·974).

Estimation of body water by D_2O

From the values in Table 2 it can be seen that the mean D_2O space was 8·5% greater than the mean weight of EBW but only 2·2% in excess of that of the TBW. Allowance could be made for these biases by using the regression equations given

Table 4. *Regression equations for estimating, in pigs, the fat content of the body from D_2O space and live weight jointly*

Body component (kg)	Regression equation on D_2O space (kg) and live wt (kg)				
	Regression coefficients				
	On D_2O space	On live wt	Intercept	RSD	RSD as percentage of mean
Dissectible fat	−0·846	+0·714	−1·0	1·07	6·0
Total lipid	−1·216	+0·904	+4·8	1·42	6·7

RSD = residual standard deviation.

in Table 3 to estimate EBW and TBW from D_2O space. TBW is estimated more precisely than is EBW (residual standard deviations 0·9 and 1·2 kg respectively), which tends to substantiate the findings of a previous study (Houseman, unpublished results) that D_2O concentration had equilibrated in the fluids of the intestinal tract and bladder as well as in the body fluids.

Estimation of weights of body components from D_2O space

Because of the relative constancy of composition of the fat-free body mass it is possible to use regression equations based on D_2O space to estimate not only water, but also the other components of the body except fat. Table 3 shows equations derived for this purpose from the present results. If account is taken of live weight, fat can also be estimated, in effect by difference. Table 4 gives multiple regressions of fat content on D_2O space and live weight. The precisions of the estimates from all the regression equations are indicated in absolute terms by the residual standard deviations and as percentages by the corresponding coefficients of variation. Comparisons between these and the standard deviations in Table 2 show how much of the total variation in each measured component of body-weight is associated with variation in D_2O space (or D_2O space taken jointly with live weight).

DISCUSSION

In planning this investigation into the relationships between D_2O space and the body components of pigs of about bacon weight it was decided to use a range of diets and levels of feeding in order to ensure that the weights of the body components were sufficiently variable to provide a good test of the relationships. The success of the treatments in achieving this may be indicated by quoting the range of fat-free mass (from 50·2 to 69·9 kg) and the range of age of the pigs at slaughter (from 153 to 290 d). The mean values obtained on each treatment are of no interest in themselves, but the fattest animals were, as expected, those on high intakes of the low-protein diet. The castrated pigs were on average slightly fatter than the gilts but the difference was not statistically significant. The derivation of relationships between D_2O space and weights of body components from such diverse animals does not detract from their applicability to more uniform populations. The residual standard deviations given here would remain valid as estimates of the goodness of fit of the relationships.

Study of the ratios given in Table 2 based on the chemical analyses of the carcasses

shows that, whereas the ratio of total lipid to empty body-weight was extremely variable (coefficient of variation 21·3%), the ratios not involving fat were remarkably constant in view of the nutritional extremes to which the animals had been subjected. In particular, there was a coefficient of variation of only 1·3% for the ratio of EBW to fat-free mass and there was no evidence that this ratio depended on age, on degree of fatness, on fat-free weight itself, or on the sex of the animal. The mean value of 75·6% for the water content of the fat-free material agrees closely with values obtained in previous studies (Kraybill, Goode, Robertson & Sloane, 1953; Clawson, Sheffy & Reid, 1955; Gnaedinger, Pearson, Reineke & Hix, 1963; Reid, Bensadoun, Bull, Burton, Gleeson, Han, Joo, Johnson, McManus, Paladines, Stroud, Tyrrell, Van Niekerk & Wellington, 1968).

The relative constancy of body composition when fat is excluded means that measurements of EBW could be used to derive estimates of other components. For example, fat-free mass (y, kg) could be estimated from EBW (x, kg) with a residual coefficient of variation of 1·2% by the regression equation, $y = 1{\cdot}33x - 0{\cdot}350$, based on the present results. More important estimates of the other components could be derived from any measurement, such as D_2O space, which was itself an estimator of EBW. As can be seen from the residual coefficients of variation in Table 3, these estimates are less precise than the more direct estimates of EBW from D_2O space or of (for example) fat-free mass from EBW.

Although weight of total body lipid is much more variable than that of crude protein in relation to body water, it can be estimated from D_2O space with almost the same precision (coefficients of variation respectively 6·7 and 5·6% – Tables 4 and 3), provided that account is taken of live weight. In effect the estimate of total lipid is obtained by difference.

The D_2O dilution technique described here was found to estimate TBW more precisely than EBW, the coefficients of variation being 1·9 and 2·7% respectively. Further investigation showed that the partial correlation coefficient between D_2O space and bladder water at slaughter (at constant EBW and gut water) was 0·82, which is highly significant ($P < 0{\cdot}001$), but the corresponding correlation with gut water was not significant. This correlation of 0·82 does not arise from any direct relationship and must therefore indicate a high correlation between the weights of bladder water at slaughter and at the time when the D_2O measurement was made. The values of bladder water at slaughter had a very skewed distribution, seventeen being between zero and 0·43 kg and the remaining seven between 0·95 and 2·95 kg. The residual coefficient of variation of EBW could be reduced from 2·7 to 1·7% either by excluding the latter seven pigs from the analysis or by using weight of bladder water at slaughter as a second independent variable in the regression analysis. We conclude that the variation in weight of water in the bladder had an important effect in limiting the precision of estimation of EBW. It is possible that variation in weight of water in the gut may have had a similar effect but that this was not detected because of a low correlation between the weights of gut water at slaughter and at the time of measurement of D_2O dilution.

The D_2O space in this study was found on average to be 2·2% greater than the

measured TBW. This difference would tend to support the suggestions of Krogh & Ussing (1936), Ussing (1938) and Smith, Trace & Barbour (1936) that deuterium ions may also exchange with the non-aqueous hydrogen ions of proteins. In a more recent study, Reid, Balch & Glascock (1958) found that, for tritiated water, exchange with non-aqueous hydrogen ions resulted in an error of 0·5–2% in the estimation of body water in rabbits. The difference could alternatively arise from inadequacies in the sampling and analytical techniques. Problems of sampling are particularly magnified in this kind of experiment in which the ratio of carcass weight to analytical sample weight can be of the order of 30000 to 1.

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