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The use of tritiated water, 4-aminoantipyrene and *N*-acetyl-4-aminoantipyrene for the measurement of body water in living pigs

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1. Body water content was estimated in sixteen pigs at each of three weights 27, 55 and 90 kg with tritium, 4-aminoantipyrene (4-AA) and *N*-acetyl-4-aminoantipyrene (NAAP). The values obtained were compared with chemical analysis of the whole carcass. 2. The tritium method estimated total body water content accurately at 27 kg live weight but overestimated it by approximately 10% at both 55 and 90 kg live weight. 3. Antipyrene was eliminated from the pig at a rate of 55%/h and could not be used for body water estimations. 4. Measurements with NAAP on nine pigs tended slightly to underestimate total body water content. Estimates with 4-AA showed no association with the values obtained by chemical analysis.

In many nutritional studies on the effect of diet on the performance of pigs, growth rate and food conversion are the criteria used, but changes in body composition are of importance, since it has been shown that changes can occur while growth rate remains unaltered (Jones, Hepburn, Cadenhead & Boyne, 1962; Bayley, 1963).

Recently Elsley & McDonald (1964) have shown that changes in the plane of nutrition, that is mainly in the energy intake, of pigs bring about changes in the rate of fat deposition while the proportions of bone and muscle remain constant when measurements are expressed on the basis of the fat-free weight. The fat and water components of the body are closely and negatively correlated (Hörnicke, 1959; Kay, 1963), and therefore estimates of body water can be used to provide estimates of fat.

The use of the dilution technique for the measurement of body water has been investigated in many species and various solutes have been tried. The most common solutes are tritiated water (^3H_2O) (Bradley, Davidsson, MacIntyre & Rapoport, 1956; Siri, 1956; Done & Payne, 1957; Reid, Balch & Glascock, 1958), deuterium oxide (Moore, 1946) and antipyrene (Kraybill, Hankins & Bitter, 1951; Herrold & Sapirstein, 1952; Clawson, Sheffy & Reid, 1955; Dumont, 1955). Most of the work using these solutes has been done with small laboratory animals or ruminants, and in most of it body water estimates using two different solutes have been compared without reference to body composition determined at autopsy; for larger animals the estimates have not been compared with water values obtained by desiccation (Steele, Berger, Dunning & Brodie, 1950; Hurst, Schem & Vogel, 1952; Scribante, Maurice & Favarger, 1952; Faller, Petty, Last, Pascele & Bond, 1955; Shumway, 1959–60; Panaretto, 1960–1). Reid *et al.* (1958) compared estimates of body water in live rabbits measured by tritium, antipyrene and *N*-acetyl-4-aminoantipyrene (NAAP) dilutions. They reported that the tritium estimate of body water was 0·5% higher, and the

antipyrene estimate was 0·2% higher, than the value obtained by desiccation, and NAAP overestimated the water content of the body minus the water of the gut contents by 1·9%.

The work now described was undertaken to compare body water in pigs measured with ^3H_2O, antipyrene, 4-aminoantipyrene (4-AA) and NAAP with body water determined by chemical analysis of the entire carcass.

EXPERIMENTAL

Animals and treatment. Three groups of Large White pigs of approximately 27, 55 and 90 kg mean live weight were used. The 27 kg group contained eleven unrelated pigs which were allocated at random to two diets. The 55 and 90 kg groups contained eight pairs of male litter-mate pigs. One diet provided 19% crude protein and 65% total digestible nutrients (TDN) (diet C), and the other 16% crude protein and 79% TDN (diet D) and it was hoped that these diets would produce differences in body composition. The diets were given according to the scale suggested by Lucas & Calder (1955).

Dose rates of the reference substances. Estimations of body water were made on the 27 kg group with ^3H_2O and 4-AA, on the 55 kg group with ^3H_2O only and on the 90 kg group with ^3H_2O and NAAP (in nine pigs). A known weight of a solution containing about 1 g of either 4-AA or NAAP in 10 ml distilled water, or the same volume of ^3H_2O having an activity of 0·65 mc tritium, was injected into an ear vein. The delivery of the syringe was checked by weighing before and after injection.

Administration of solute and collection of blood. The reference solute was administered through an ear vein, and all blood samples were collected from the tail. A small piece of the tail was cut off and the blood collected in a heparinized centrifuge tube or round-bottomed flask. To stop bleeding, a plastic finger stall was placed over the tail end and, to collect subsequent samples, the stall and blood clot were removed.

Samples of blood for determination of tritium were collected in a round-bottomed flask and distilled with benzene in a Dean & Stark (Quickfit & Quartz Ltd, Stone, Staffs.) apparatus. The distillate (water and benzene) was collected in a stoppered test tube and allowed to stand for at least 3 h to permit small droplets of benzene to rise to the surface. Of the blood water 0·5 ml was mixed with 5 ml of a solvent and phosphor mixture containing 2,5-diphenyloxazole (PPO) and 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP) dissolved in a mixture of 1,4-dioxane, ethyl alcohol and toluene. The tritium activity of the samples was counted in a liquid scintillation counter at $+5°$ and an extra-high tension of 1750 V. Preliminary experiments had shown that the volume of phosphor, the counting temperature and the extra-high tension quoted above gave maximum counting efficiency.

Antipyrene was determined by the method of Brodie, Axelrod, Soberman & Levy (1949) as modified by Kumar (1957). Determination of 4-AA was by the method of Huckabee (1956) and the method for NAAP was essentially that of Brodie, Berger, Axelrod, Dunning, Porosowska & Steele (1951) except that the same volumes of plasma and standard were used and the two run concurrently.

Procedure for carcass analysis. Each pig was killed, bled, and weighed before and after bleeding. The difference between these weights gave a check on the weight of blood drained. The body of the pig was chilled at 1° in a large sealed Polythene bag for a minimum period of 16 h so as to minimize water losses by evaporation when the abdominal cavity was opened.

The head was removed by cutting just behind the ears at right angles to the trunk. The abdominal wall was opened along the median line and the cut continued by sawing through the sternum. Separation of the ventral surface was completed by cutting through the ischial arch at the symphysis pubis and symphysis pelvis. The diaphragm was cut off close to the abdominal wall. The rectum was dissected out, and by cutting around the anus the alimentary tract was removed in one piece. The heart, lungs, kidney, spleen and trachea were dissected from connective tissue and other parts of the digestive tract. These parts were weighed and stored in Polythene bags.

The digestive tract was opened and the contents were weighed, mixed and sampled for dry-matter determination. The remaining tissue was placed in a Polythene bag and also weighed. Differences in the weights of the contents of the alimentary tract measured by the two methods were assumed to be due to loss of moisture. The carcass was hung up by the hind legs and separated into halves by sawing down the middle of the vertebral column.

Each side was dissected into five standard joints, which for the 90 kg pig weighed about 6 kg each. The front leg was removed by cutting around the scapula, and the rear leg by cutting between the head of the femur and acetabulum and at right angles to the skin surface. The portion remaining was cut posterior to the last thoracic vertebra and between the seventh and eighth thoracic vertebras, the cut always made parallel to the ribs. Each joint was placed in a Polythene bag and weighed before being placed in a cabinet at −20° for at least 36 h.

Table 1. *Moisture content of six pig carcass samples after vacuum drying as determined by oven drying at* 102°

Sample	Mean residual moisture (%)
Head	2·10
Front leg	2·47
Fore ribs	1·67
Mid ribs	1·38
Loin	1·93
Rear leg	2·48
Gut	6·18
Viscera	5·19

While the joints were still frozen they were ground in an Ancol bone grinder (Union Food Machinery Equipment Ltd, London) to give a pulverized material which was then minced mechanically and subsampled.

The dry-matter content of each joint was determined by vacuum drying of duplicate 150 g samples for 24 h and then making a correction for residual moisture by using the values given in Table 1. The dried material was passed through a small

hand mincer and stored in an airtight jar until analysed. All the samples were analysed for crude protein (total nitrogen × 6·25), ether-extractable matter, ash and water by the methods given by the Association of Official Agricultural Chemists (1960).

Equilibrium time of the reference substance (solute) within body water. Tritium attains a stable equilibrium in blood 2 h after intravenous injection; the activity then remains constant for about a further 4 h (Fig. 1). To confirm that equilibration had been

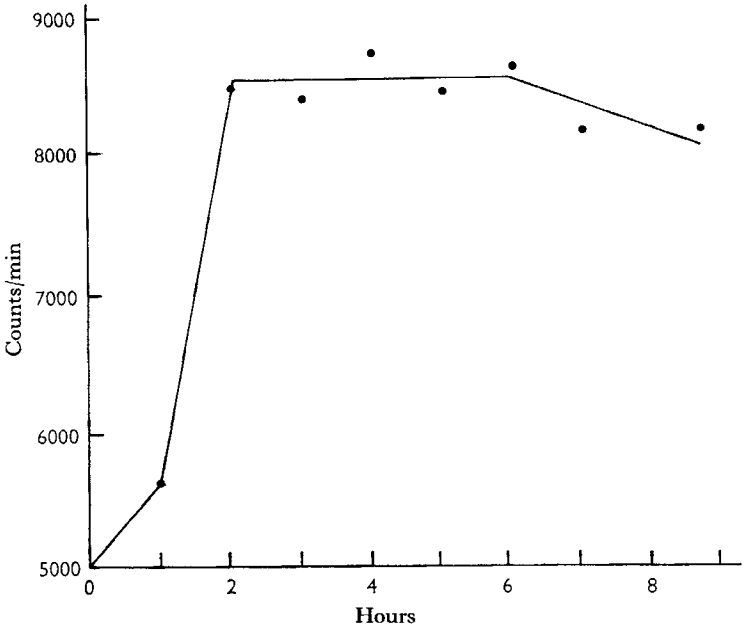

Fig. 1. Relationship in pigs between activity of tritium in blood water and time after intraperitoneal injection.

Table 2. *Activity (counts/min) of water samples recovered from muscle tissue and various organs of pigs of 90 kg live weight killed 6 h after an injection of* ^3H_2O

Pig no.	Liver	Kidney	Lungs	Heart	Muscle	Gut contents	Blood
1	4700	4700	5000	4700	—	4700	4400
2	6400	6300	6100	6100	—	6400	6000
3	8700	8700	8000	7800	8100	7900	8000
4	6300	6200	5800	5700	5600	6200	6100
5	3300	3200	3400	3300	3400	3100	3100

Values were corrected for background counts of 600/min and for a resolving time of 340 μsec during which the instrument is insensitive.

achieved between tissues, five pigs were killed 6 h after an injection of ^3H_2O and the activities of water samples from liver, kidney, heart, lung, muscle and alimentary tract were determined (Table 2).

Unlike ^3H_2O, antipyrene does not attain a uniform concentration in the plasma after equilibrium within the body water because it is gradually eliminated from the body

and there is a decline in the plasma concentration. The rate of elimination in pigs, as shown in Fig. 2, was found to be approximately 55%/h. This confirms Dumont's (1955) finding. He also queried whether antipyrene attained an even distribution throughout the water of the various tissues in the pig. Consequently antipyrene was not a suitable solute for our experiment.

The rate of elimination of 4-AA varied from 7 to 22%/h and the value for NAAP was about 10%/h.

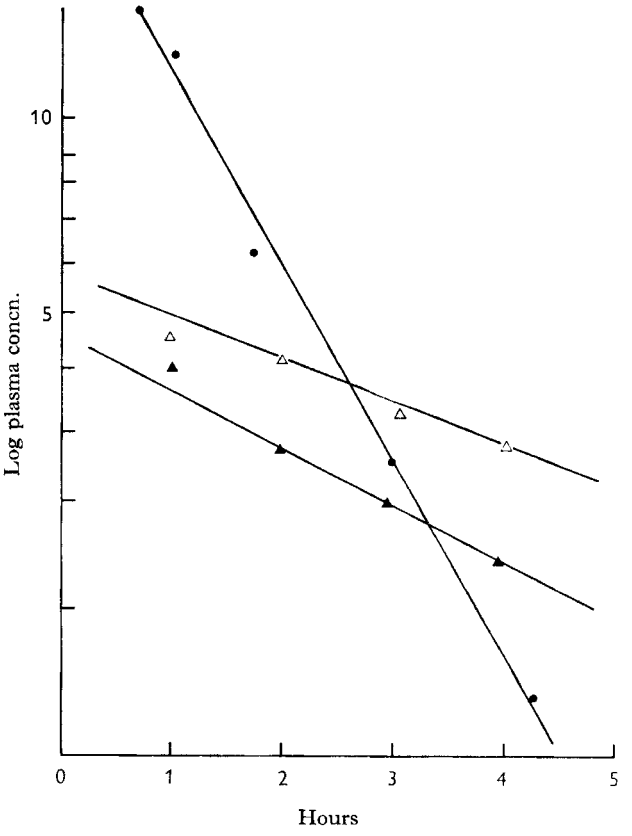

Fig. 2. Rates of elimination of antipyrene, 4-aminoantipyrene and *N*-acetyl-4-aminoantipyrene in pigs. ●, antipyrene (μg/ml); △, NAAP (μg/10 ml); ▲, 4-AA (μg/ml).

Calculation of body water from dilution of the solute. When ^3H_2O was the solute, blood samples were taken 2, 3 and 4 h after injection and the mean blood activity was used to calculate body water according to the equation:

$$\text{Body water (ml)} = \frac{\text{dose injected (counts/min for } ^3H_2O)}{\text{equilibrium activity/ml plasma water}}.$$

With 4-AA and NAAP the plasma concentrations determined at 2, 3, 4 and 5 h were plotted on a logarithmic scale. The concentration of solute used in the calculation was determined by extrapolating back to zero time the line joining the concentrations in

plasma water. This should give the concentration provided none of the solute had been eliminated or elimination had proceeded at constant rate (Brodie *et al.* 1949). The body water was then calculated as follows:

$$\text{Body water (ml)} = \frac{\text{dose injected (mg) of 4-AA or NAAP}}{\text{concentration at zero time (mg/ml)}}.$$

RESULTS

Chemical analysis of each side of the pigs weighing 27 kg indicated that doubling the values obtained for the right side gave values very close to those obtained by analysis of the complete carcass. Consequently only single sides of the pigs weighing 55 and 90 kg were analysed.

Table 3. *Estimate of chemical composition obtained from the half carcass of sixteen pigs of 27 kg live weight expressed as a percentage of the value obtained from the whole carcass*

Constituent	Mean	SE of mean	SD
Ash	99·8	±0·47	±1·9
Water	100·2	±0·31	±1·2
Protein	100·4	±0·49	±2·0
Fat	99·2	±0·72	±2·9

Table 4. *Individual estimates of body water (kg) using ^3H_2O, 4-AA and NAAP and the corresponding values obtained by chemical analysis for pigs weighing 27, 55 and 90 kg*

27 kg live weight				55 kg live weight			90 kg live weight			
Diet	^3H_2O	4-AA	Chemical analysis	Diet	^3H_2O	Chemical analysis	Diet	^3H_2O	NAAP	Chemical analysis
A	14·9	21·0	14·7	C	37·3	29·5	C	52·1	—	46·8
A	16·3	16·3	15·9	D	37·8	32·0	D	55·1	—	45·0
B	13·0	11·1	12·9	C	36·5	30·1	C	54·8	—	44·2
A	16·3	18·2	15·7	D	37·0	32·2	D	51·0	—	44·6
B	12·3	15·3	12·4	C	31·9	29·0	C	57·6	—	44·8
B	14·5	15·3	14·0	D	36·7	31·0	D	56·4	50·3	49·2
B	13·6	18·0	13·5	C	35·0	31·0	C	48·6	45·3	47·3
B	15·9	17·3	16·0	D	39·2	31·5	C	54·3	40·9	45·9
A	16·4	14·2	16·9	C	32·2	28·9	D	57·7	—	51·1
A	16·4	15·3	16·2	D	36·8	32·7	C	55·1	50·8	50·4
A	17·2	18·9	16·6	D	40·7	32·8	D	62·0	51·7	51·8
				D	41·0	33·9	D	—	45·2	43·4
				C	34·6	29·0	C	51·1	45·7	46·2
				D	34·2	30·3	D	—	48·0	48·8
				C	37·5	31·1	C	57·9	48·0	52·4
				D	39·3	33·6				

Diet A contained 10% crude protein + 67% TDN; diet B contained 16% crude protein + 68% TDN; diet C contained 19% crude protein + 65% TDN; diet D contained 16% crude protein + 79% TDN.

The estimates of body water were compared with values determined by chemical analysis. The values are presented in Table 4. There was close agreement between the ^3H_2O estimate and the value obtained by chemical analysis for pigs weighing 27 kg; the mean difference between the body water determined by drying and by ^3H_2O

dilution was $+0{\cdot}75\% \pm 1{\cdot}12\%$. The estimates using 4-AA were very variable and were not significantly correlated with the values obtained by chemical analysis.

Although for the pigs weighing 55 kg each individual estimate using tritium tended to be higher than the values determined by drying (mean difference = $10 \pm 3\%$) there was, nevertheless, a significant correlation between the two sets. The regression equation was:

$$\text{Total body water} = 26{\cdot}3 + 0{\cdot}48 \times {}^3H_2O \text{ estimate; RSD} = \pm 1{\cdot}6.$$

In the 90 kg group no significant relationship was found, and the ^3H_2O estimate tended to be 10% higher than the value obtained by chemical analysis. There was close agreement between the body water content estimated by NAAP dilution and the value obtained by carcass analysis.

DISCUSSION

Two features emerged from the results for the three weights of pig. These were the precision of the estimates and the extent to which the ^3H_2O estimate of body water overestimated the value obtained by chemical analysis.

For each weight of pig the same activity of ^3H_2O was used for measuring the body water, namely 0·65 mc. The precision of the estimates was greatest for the group of smallest weight, and for the 90 kg group no significant relationship was observed between predicted and determined values. If, however, the dose rate had been increased in proportion to the live weight of the groups, then the accuracy of the estimates for the heavier weights might have been improved. This would have meant injecting about 1·2 mc into the 55 kg group and 2·0 mc into the 90 kg group for accuracy comparable to that of the 27 kg group.

Body water estimates from ^3H_2O dilution were found to be too high by 0·75, 10·8 and 8·6% at 27, 55 and 90 kg live weights respectively. An overestimate in body water is to be expected owing to the exchange that takes place between tritium and labile hydrogen in the body; this overestimate is usually of the order of 0·5–2·0% (Prentice, Siri, Berlin, Hyde, Parsons, Joiner & Lawrence, 1952; Reid *et al.* 1958).

In order to investigate the magnitude of this effect in the pigs of 90 kg live weight, vacuum-dried samples of liver, kidney and muscle were oxidized in a stream of dry oxygen, the vapours were passed over heated copper oxide, and any water formed was collected in a U tube surrounded by dry ice. The activity of a known volume of this water was counted and the activity per unit volume calculated. If the activity of the plasma at equilibrium is taken as 100, then the value for water recovered from dry liver was 21, from kidney 27 and from muscle 27.

Prentice *et al.* (1952) found that no detectable exchange took place in fat, whereas in liver, kidney and plasma solids the rate of exchange was consistently higher than in muscle and gastro-intestinal tract. On the assumption that a pig contains 25% total fat, 65% carcass and 10% viscera, the exchange values found and given earlier were used to calculate that an elevation in body water in the region of 5% was to be expected.

In calculating the body water from ^3H_2O dilution, the mean activity in the blood was taken as being indicative of the equilibrium concentration. The activity of water obtained from four out of five samples of fresh tissue was found to be higher than that

Table 5. *Comparison of estimates of body water content of pigs injected with ^3H_2O based on mean activities of tissue and blood*

Pig no.	Mean tissue activity (counts/min)	Mean blood activity (counts/min)	Body water content (%) calculated from tissue activity	Body water content (%) calculated from blood activity
1	4760	4400	62·6	67·4
2	2660	6000	77·9	81·4
3	8200	8000	62·9	64·1
4	5967	6100	73·4	71·2
5	3283	3100	66·7	71·5

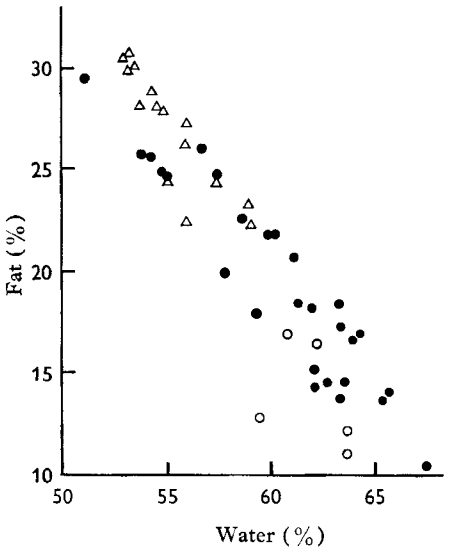

Fig. 3

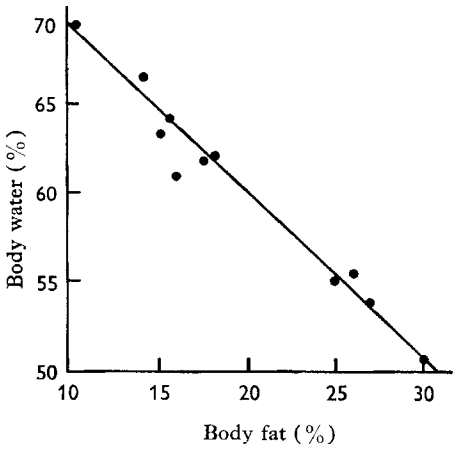

Fig. 4

Fig. 3. Relationship between ether-extractable fat and total water content of pig carcasses. ○, 27 kg live weight; ●, 55 kg live weight; △, 90 kg live weight.

Fig. 4. Relationship between body water and body fat contents in pigs of 27 kg live weight.

of the blood, and using the mean blood activity would therefore give a higher estimate of body water than using the mean activity of the various tissue samples. This is illustrated in Table 5. The values in Table 5 suggested that body water estimates might be increased by as much as 7% when the difference in blood activity was used for calculating the body water. The overall effect of the lower activity of tritium in plasma water and of exchange with non-aqueous tissue could be to overestimate body water by as much as 12%.

The mean content of fat in the pig is given by the formula:

$$\text{Fat}\,(\%) = \left(1 - \frac{w}{w^1}\right) 100,$$

where w = total body water as estimated by tritium-labelled water, and w^1 = the percentage of water in the fat-free body, varying from 75 to 78% according to the weight of pig.

Fig. 3 shows the relationship between contents of total ether-extractable fat and water for pigs of various live weights. The pigs at 27 kg live weight at any water content contained less fat than those at 55 or 90 kg live weight.

A high inverse correlation was noted between the content of ether-extractable fat and the estimate by tritiated water of body water at 27 kg live weight (Fig. 4). The relationship was similar for both treatment groups. At 55 kg live weight the relationship between content of ether-extractable fat and the body water estimate was not so precise owing to the variability of the body water estimates, and it was not possible to relate the body water estimate with content of ether-extractable fat for the group of pigs weighing 90 kg.

Although only a small number of animals was used for the NAAP estimations the underestimation of body water noted is in agreement with work on other species by Reid *et al.* (1958) and Reid, Balch & Head (1957). These workers suggest that NAAP does not penetrate water in the alimentary tract in appreciable amounts until after the 5th h and consequently can be used to estimate the water content of the body minus that of the gut.

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