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Some problems in the use of antipyrine and *N*-acetyl-4-aminoantipyrine in the determination of body water in cattle

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An accurate in vivo estimation of the body water of animals would be of great value in many nutritional and physiological studies. In the ruminants a method for the separate measurement of body water of the tissues, tissue spaces and circulatory systems (empty body) and of the water of the gastro-intestinal tract (gut water) would be of additional value. One of the most promising methods for determining body water indirectly is the injection into the blood stream of a non-toxic compound that evenly and rapidly distributes itself throughout all body water, that is transformed in or excreted from all body tissues slowly and uniformly, and that can be conveniently and accurately estimated in blood plasma or other body tissues. Soberman, Brodie, Levy, Axelrod, Hollander & Steele (1949) proposed the use of antipyrine (AP) as such a compound for measuring body water indirectly in man and dogs, and later Brodie, Berger, Axelrod, Dunning, Porosowska & Steele (1951) proposed the use of *N*-acetyl-4-aminoantipyrine (NAAP), as this latter compound did not bind with plasma proteins to the same extent as AP. Kraybill, Hankins & Bitter (1951) adapted the AP method for use with cattle and found in a study with thirty animals of varying degrees of fatness that the value for body fat, calculated from body water determined by AP, agreed well with that calculated from specific-gravity measurements. Wellington, Reid, Bratzler & Miller (1956) reported that in twenty cattle determinations of body water by the AP technique and by toluene distillation of the body tissues after slaughter agreed closely.

On the other hand, White & MacDonald (1956), using cattle, and MacFarlane, Morris & Howard (1956), using sheep, reported unpredictable occurrences of impossible values when AP was used to measure body water. MacFadden & Richards (1956) and Swanson & Neathery (1956) found that the length of time that feed and water were withheld before the injection of AP had a significant effect on the estimated amount of body water. Garrett, Meyer & Lofgreen (1959) in an experiment with thirty-six steers did not find a significant correlation between body fat determined by the use of AP and that determined by specific-gravity measurements of the ninth–tenth–eleventh rib sections. In trials with sheep these authors reported also that the length of time feed and water were withheld before the injection of AP had a marked effect on the estimated body water. Rumen samples from these sheep showed that

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the concentration of AP in the rumen water varied between 75 and 162 % of the concentration in the serum water at the same time.

Reid, Balch, Head & Stroud (1957), in a study to compare the use of AP and NAAP for estimating body water in cattle, noted that these compounds were not found in the reticulo-ruminal water in the same concentration as in the blood water. The diffusion of AP into the reticulo-rumen was much more rapid than that of NAAP. This observation led the authors to propose that the simultaneous use of AP and NAAP might enable the water content of the whole body, the empty body and the gut to be determined, if means could be derived for making allowances in non-fistulated animals for the amounts of reference substances entering the gut.

An experiment has been carried out at this Institute to study the changes in body energy content of milking cows when given a diet at different levels of intake. Both AP and NAAP were used to measure changes in body composition. The experiment provided an opportunity to study further the simultaneous use of AP and NAAP as a measure of gut water, and the AP and NAAP contents in the water of the saliva, reticulo-rumen, faeces and milk. Additional cows and steers, slaughtered after the injection of AP and NAAP, were used to study the distribution of these compounds throughout the gut. Results of these studies are reported in this paper.

EXPERIMENTAL

Estimation of body water. Two lactating Friesian cows (A and D) each with a rumen fistula received, over a period of 17 weeks, a complete diet in amounts sufficient to cause daily body-weight gains of roughly 1 lb (overfed cows). The diet contained equal weights of hay and concentrates. Two similar cows (B and C) were given the same diet in amounts to result in daily body losses of roughly 1·3 lb (underfed cows). The underfed cows had previously been overfed, and the overfed cows had previously been underfed. Estimates of body water were made on these cows at intervals of about 3 weeks, by the simultaneous use of AP and NAAP.

In the estimation of body water 80–100 ml of an aqueous solution containing about 16 g AP and 10 g NAAP were injected into the jugular vein through a Polythene cannula. Blood samples withdrawn before the injection (blank sample) and at about 140, 190, 240, 290 and 340 min after the injection were analysed for AP and NAAP. Body water was estimated by extrapolating the blood-dilution curves (logarithms of concentrations plotted against time) to zero time (see Figs. 1 and 2) and using the equation

$$\text{Body water (l.)} = \frac{\text{Amount of AP or NAAP injected (mg)}}{\text{Concentration of AP or NAAP in serum water at zero time (mg/l.)}}.$$

Values obtained by this technique will be referred to as AP water space or NAAP water space.

During the first 9 weeks of the experiment water and food were available to the cows until about 1·5 h before the injection. Later this interval was increased to 4 h. No food or water was offered from this time until all blood samples had been collected.

Estimation of weight of gut contents and empty body-weights. Before the injection of

AP and NAAP, the contents of the reticulo-rumen of each cow were manually removed, sampled for dry-matter content, and returned. Total gut contents and gut water were calculated on the assumption that the amount in the reticulo-rumen made up 73 % of that in the total gut (Mäkelä, 1956; M. A. Carroll, unpublished). The cows were weighed on a balance having an accuracy of ± 2 lb before the injection of AP and NAAP, and again after the last bleeding. Empty body-weight was determined by subtracting the weight of total gut contents from the live weight.

Influence of ration on estimated body water content. After the experimental feeding period and as soon as pasture became available, one cow that had been overfed (A) and one that had been underfed (C) were put out to pasture while the other two cows (D and B) remained in the cowshed and were given hay. During the period from the completion of the experimental period and when pasture was available (7 weeks) the cows had been given hay with no concentrates and milking had ceased. A fortnight after the two cows had been put to pasture body-water estimations were made on the four cows with AP and NAAP. Samples of saliva, rumen contents and faeces also were collected before the injection and after the first and fourth bleeding, as described below. The cows were not permitted to consume food or water during a period of 4 h before the injection or during the period that blood samples were being collected.

Influence of water intake on estimated body-water content. In a further test two cows (A and D) received no water during a period of 4 h before the injection of AP and NAAP and two (B and C) received 170 lb each through the rumen fistula just before the injection. Again, no feed or water was offered during the period that blood samples were being collected. Body water had been estimated in these cows 1 week before when none had received feed or water during a period of 4 h before the injection of AP and NAAP.

Collection of samples from the gut of fistulated animals. On two occasions, when the cows had been injected with AP and NAAP, samples of reticulo-rumen contents, saliva and faeces were collected from each cow before the injection (blank samples) and immediately after the first and fourth blood samples were taken. Reticulo-rumen samples were obtained by manually removing the total contents through the fistula, mixing, sampling and returning the contents. Saliva samples were obtained by holding a small rubber bag over the cardiac orifice when the reticulo-rumen was partly empty and collecting the swallowed saliva (Bailey, 1958). Faeces samples were collected from the rectum.

On one occasion samples of reticulo-ruminal and abomasal contents were collected at hourly intervals commencing 2 h after the injection of AP and NAAP from a steer weighing 350 lb with a rumen fistula and a cannula in the first part of the duodenum. The samples collected from the duodenal cannula were essentially typical abomasal contents as judged by pH and are so described under 'Results'.

Collection of samples from the gut of slaughtered animals. One steer that weighed 1270 lb and four cows that averaged 1470 lb were slaughtered roughly 3 h after being injected with about 20 g AP and 14 g NAAP. Samples of blood and of the contents from the reticulo-rumen, omasum, abomasum and the small and large intestines were collected immediately after slaughter. Before slaughter the steer and one cow had been receiving a diet of hay and concentrates, another of the cows had been on kale and grass pasture and the remaining two cows had been receiving dehydrated grass. On the day of slaughter the animals' normal morning meal was withheld, the cow on pasture being brought from the field at 05.30 h. They were then transported to a local slaughter-house and immediately killed with a captive-bolt humane killer, pithed, bled, skinned and opened; the average time of slaughter was 08.30 h. The viscera were removed and sampled without delay.

Collection of milk samples. Milk samples were collected from the four cows (A, B, C and D) after the final bleeding, about 6·5 h after the injection, when body water was estimated at the 16th week of the experiment. The cows had been milked about 4 h before the injection of AP and NAAP. Their yields of milk from which the samples were collected were: A, 17 lb; B, 12 lb; C, 11 lb; and D, 22 lb.

Measurement of entry of AP and NAAP into and their absorption from the rumen. When samples of saliva and rumen contents, collected about 2·5 and 5 h after the injection, were analysed for their concentration of AP and NAAP it was noted that, although both markers entered the rumen in the saliva and in proportion to the concentrations in the blood, there was a much greater concentration of AP than NAAP in rumen contents (see p. 526). In order to know more fully how AP and NAAP reached and left the rumen, a fistulated cow was intravenously injected with 16 g AP and 10 g NAAP made up to 100 ml with water, and samples of blood, saliva and rumen contents were collected, the first 10 min after the injection. A solution (100 ml) of 16 g of AP and 10 g NAAP was added to the reticulo-rumen of another fistulated cow, the contents of the reticulo-rumen being first removed, mixed thoroughly with the solution and then returned to the rumen. Blood samples were collected 30, 90, 150 and 210 min after the AP and NAAP had been put into the rumen.

Comparison of intravenous and intramuscular injection. In most of the studies reported in the literature AP and NAAP were injected into the blood through a hypodermic needle. In the preliminary phases of this work when a needle was used it was noted that occasionally when an animal struggled during an injection the needle came out of or passed through the vein and some of the solution was injected intramuscularly or subcutaneously. Garrett *et al.* (1959) made a similar observation. To determine the effect on the resulting dilution curves and hence on the estimated body-water content, one cow was injected intramuscularly in the neck with 80 ml of an aqueous solution containing 16 g AP and 10 g NAAP. Blood samples were collected at 135, 180, 230, 280 and 330 min after the injection.

Methods of chemical analysis. All samples of blood, saliva, rumen and abomasal contents, and of milk were analysed for AP and NAAP by the precipitation method, and samples of the contents from the omasum and intestines and tissue samples by the extraction method of Brodie, Axelrod, Soberman & Levy (1949) and Brodie & Axelrod (1950), incorporating the changes suggested by Dumont (1955) for AP. Samples of saliva and rumen and abomasal contents were filtered through fine-mesh cotton gauze before the analyses. In all instances, with the exception of the slaughtered animals, blank samples collected before the injection of AP and NAAP were used to correct the optical-density readings on the samples containing AP and NAAP.

RESULTS

Body water estimated by AP and NAAP

The estimates of body-water content obtained with AP and NAAP (AP and NAAP water spaces) are shown in Table 1. No values were obtained for cow A in the 14th week of the experiment, because some of the AP and NAAP solution was spilled during the injection. Similarly, no values were obtained for cows A and B in the 15th week because the cows drank water just before the injection. They were injected

Table 1. *Influence of level of feeding on water content of body and gut estimated with antipyrine (AP) or* N-*acetyl-4-aminoantipyrine (NAAP)*

Cow	Weeks after beginning of experiment*	Body-weight (lb)	Body water content (%) by AP	Body water content (%) by NAAP	Difference in body water content (AP – NAAP) (lb)	Gut water content† (lb)
A (overfed)	0	1082	58·5	47·8	116	184
	2	1118	59·3	44·2	170	186
	6	1134	51·2	44·4	78	192
	9	1149	57·0	45·3	134	195
	16	1156	61·8	46·8	174	157
	17	1144	62·5	47·5	162	147
D (overfed)	0	1068	61·6	47·5	151	183
	2	1106	57·9	46·6	124	186
	6	1112	59·0	47·1	132	184
	9	1126	58·2	43·3	168	192
	14	1140	64·5	47·8	190	151
	16	1164	67·7	46·4	248	182
	17	1163	65·5	47·3	211	158
B (underfed)	0	1159	55·4	42·2	152	193
	2	1103	55·8	46·4	103	171
	6	1090	58·3	46·8	126	180
	9	1070	57·6	42·2	164	186
	14	986	55·5	50·5	49	120
	15	983	54·9	51·1	37	115
	16	967	57·3	49·2	78	121
C (underfed)	0	1178	63·0	49·5	159	177
	2	1124	54·0	42·0	135	169
	6	1086	68·1	45·7	244	157
	9	1068	60·3	45·1	162	158
	14	994	60·1	50·8	93	105
	15	1008	61·7	50·7	111	109
	16	992	61·6	48·8	127	113
Mean					141	162

* Water and food were withheld for 1·5 h before injections of AP or NAAP after 0, 2, 6 and 9 weeks, and for 4 h thereafter.

† Calculated from reticulo-rumen emptyings before injection of AP or NAAP on the assumption that 73 % of the gut water was in the reticulo-rumen (Mäkelä, 1956; M. A. Carroll, unpublished).

1 week later. The correction suggested by Reid *et al.* (1957) for amounts entering the gut was not applied to the values for body water space estimated by AP and NAAP. Our findings confirm the observation of Reid *et al.* (1957) and Reid, Balch & Glascock (1958) that the water space of the animals was greater when estimated by AP than by

NAAP. The mean difference was 87% of that calculated, from the contents of the reticulo-rumen, to be in the gut. Reid *et al.* (1957) reported that the difference between mean values for the body water space estimated by AP and NAAP was 81% of the calculated contents of the whole gut and, when corrected for the amount of these reference substances entering the gut, the differences between the mean water spaces by the two substances approached the value for the amount of water calculated to be in the gut. In this earlier work it was assumed that the concentration of AP and NAAP in the total gut water was the same as that in the reticulo-rumen. Results presented below indicate that this assumption was not correct.

As the experiment progressed the changes in the percentage of body water of the cows determined by AP and NAAP were not gradual, as one would expect with over-fed or underfed cows, but erratic at times (Table 1). However, during the period from the 14th to the 17th week after the beginning of the experiment, when food and water were withheld for 4 h before the injection of AP and NAAP, body-weight changes of the cows were small and the repeatability of body-water measurements by AP was ±2·8% and by NAAP ±1·8%. It is likely that a part of these variations was due to real changes in body water. Blaxter & Rook (1953) have reported that the repeatability of values for percentage of body water estimated with AP in cattle on a constant diet was ±5%.

Influence of diet on body-water estimations

When grazing began, the two cows going on to pasture and the two cows continuing on hay had similar mean body-weights (1055 and 1046 lb, respectively) and were similar in average body condition as judged by visual appraisal. Seven weeks earlier the mean body-weights of cows receiving these diets were 1054 and 1058 lb, their empty body-weights were 902 and 892 lb, their empty body-water spaces estimated by AP (AP water space less the gut water) were 58 and 58% and by NAAP (NAAP water space) were 56 and 57%, respectively. After the two cows had been on pasture for 2 weeks their mean body-weight was 1098 lb and the two cows remaining on hay averaged 1050 lb. The mean empty body-weights with pasture and hay were 967 and 828 lb, respectively, the empty body-water spaces were 67 and 54%, respectively, estimated with AP and 57 and 55%, respectively, with NAAP. The two cows on pasture contained on average 70 lb less reticulo-rumen contents than the two cows given hay (99 and 169 lb, respectively).

Influence of large water intakes on body-water estimations

The blood-dilution curves for the two cows that received 170 lb of water just before the injection of AP and NAAP were not linear. The logarithms of the concentrations of AP and NAAP at the fourth and fifth bleeding were above a straight line drawn through the logarithms of the concentrations at the first, second and third bleeding (Figs. 1, 2). This departure from linearity was more marked with AP than with NAAP. Stated in another way, the hourly rate of disappearance of AP from the blood was 23% from the first to the third bleeding and 18% from the third to the fifth bleeding. The corresponding rates of disappearance for NAAP were 16 and 14%. The cause of the departure from linearity is not known but it may have been an abnormally rapid onward passage of digesta and water from the reticulo-rumen and a subsequent absorption of water, and with it the marker substances, from the lower gut. From

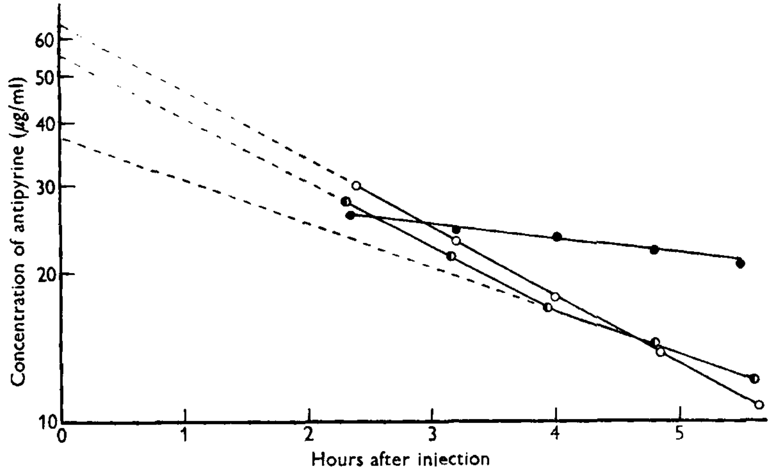

Fig. 1. Representative blood-dilution curves for antipyrine showing the method of estimating body water by extrapolation to zero time (broken lines); values are given for normal intravenous injection (○—○), intravenous injection after the cow had received 170 lb water (◐—◐) and intramuscular injection (●—●).

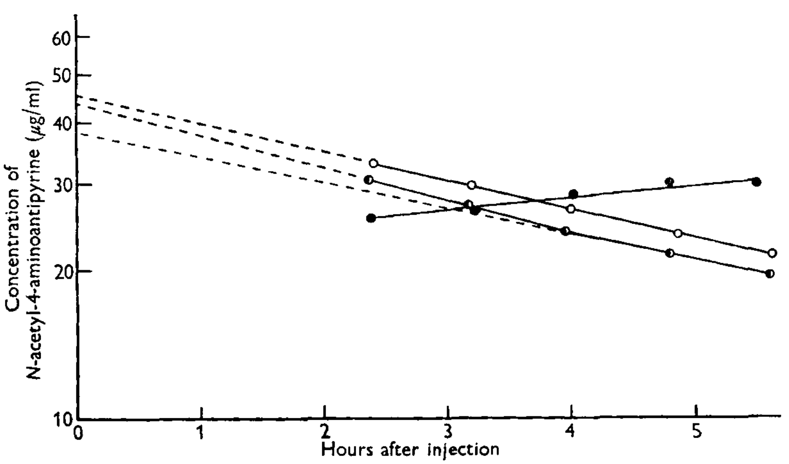

Fig. 2. Representative blood-dilution curves for *N*-acetyl-4-aminoantipyrine showing the method of estimating body water by extrapolation to zero time (broken lines); values are given for normal intravenous injection (○—○), intravenous injection after the cow had received 170 lb water (◐—◐) and intramuscular injection (●—●).

results presented below it seems possible that 4 h after the injection of AP the concentration of AP in the rumen water was greater than in the blood. Any AP passing into the blood at this time would tend to counteract the loss of AP from the blood due to metabolism. If this assumption is correct it would explain why, in the rumen contents, with the lesser amounts of NAAP in comparison to AP the departure from linearity of the dilution curve for NAAP was less than that for AP.

It is obvious from Fig. 1 that the AP water space determined from the first, second and third bleeding would be appreciably less than that estimated from the third, fourth and fifth bleeding. The difference between the two NAAP water spaces would be less than that obtained with AP (Fig. 2). When, for the purposes of comparison, it was assumed, as suggested by Reid *et al.* (1957), that AP water space approximated to total body water and NAAP water space to empty-body water, the average empty-body water contents of the two cows estimated from the first, second and third bleedings were 40 and 60 % when estimated by AP and NAAP, respectively, and from the third, fourth and fifth bleedings they were 60 and 62 % respectively. (Empty-body water estimated by AP = AP water space less gut water.) The mean empty-body water contents of these two cows 1 week before when food and water were withheld for 4 h before the injection were 55 and 56 % when estimated by AP and NAAP, respectively.

Of the 250 lb water in the reticulo-rumen before the injection of AP and NAAP (80 lb initially present and 170 lb added) only 112 lb were present when the rumen was emptied 6 h later. During this period about 60 lb water were excreted in the urine and faeces. If it can be assumed that 73 % of the gut water was in the reticulo-rumen 6 h after adding the water, then 70 lb of excess water must have been held in the blood and tissues. The assumed value (73 %) may be too high but it seems most unlikely that more than 70 lb of additional water could be in the contents of the lower gut and bladder, and it appears certain that some increase had occurred in the water content of the blood and tissues.

Concentration of AP and NAAP in the saliva and gut contents

The concentrations of AP and NAAP in the gut contents and saliva of cattle at various times after the injection of AP and NAAP are given in Tables 2–5. In general, the water in the contents from the mid-section of the small intestines showed the greatest concentration of NAAP (Table 2). There was considerable variability in concentration of AP and NAAP between different sections of the gut in the same animal, and between samples taken from the same part of the gut in different animals (Tables 2–4). Some of these differences seemed to be associated with diet (see p. 524).

The concentrations of AP and NAAP in the water of the reticulo-rumen relative to that in the blood increased with time after the injection (Tables 3–5) (the absolute concentrations did not increase in all instances), whereas their relative and absolute concentrations in the abomasum decreased with time after injection (Table 5). The latter observation agrees with the findings of Reid *et al.* (1958) with rabbits.

About 10 % of the AP and 7 % of the NAAP injected were found in the gut 2·5–3 h after the injection (Tables 2 and 4). Of these amounts about 80 % of the AP and 60 % of the NAAP were found in the reticulo-rumen and omasum, the remainder being in the abomasum and intestine.

Effect of diet on the concentration of AP and NAAP in the blood and gut contents

Among the animals slaughtered 3 h after the intravenous injection of AP and NAAP the concentrations of these compounds in the blood were lower in the cows given pasture or dehydrated grass than in those given hay and concentrates (Table 2). Similar values were obtained when the two fistulated cows on pasture and the two fistulated cows given hay were sampled hourly from 2·5 h after the injection of AP and NAAP. The hourly rate of disappearance of these two compounds from the blood of these cows is stated on p. 531.

Table 2. *Concentrations (μg/ml) of antipyrine (AP) and* N-*acetyl-4-aminoantipyrine (NAAP) in the water of the blood and of the contents of the gut after intravenous injection into cattle* slaughtered about 3 h afterwards*

Site	AP					NAAP				
	Steer 1	Cow 2	Cow 3	Cow 4	Cow 5	Steer 1	Cow 2	Cow 3	Cow 4	Cow 5
Blood	28	48	12	19	12	35	47	31	34	32
Rumen	23	12	18	24	15	5	2	9	9	6
Omasum	22	22	28	30	29	4	6	14	15	22
Abomasum	48	86	26	67	32	21	39	23	29	26
Intestine										
Small: anterior	49	77	33	44	37	27	47	31	50	27
middle	36	51	—	32	35	52	65	—	75	67
Large	22	35	24	29	26	22	35	25	27	23

* Steer 1 and cow 2 had been given hay with concentrate, cow 3 had been pastured on kale and grass, and cows 4 and 5 had been given dehydrated grass.

Table 3. *Variability in the concentration (μg/ml) of antipyrine (AP) and* N-*acetyl-4-aminoantipyrine (NAAP) in the water of blood, saliva and faeces, and of the contents of the rumen, sampled about 2·5 and 5 h after intravenous injection into milking cows* receiving a diet of hay and concentrates*

Sample	AP				NAAP			
	Cow A	Cow B	Cow C	Cow D	Cow A	Cow B	Cow C	Cow D
				2·5 h after injection				
Blood	15	26	25	18	22	32	28	25
Saliva	11	21	20	13	15	20	17	13
Rumen	14	21	22	18	4	6	6	4
Faeces	0	0	0	0	0	0	0	0
				5 h after injection				
Blood	5	15	13	7	14	21	19	15
Saliva	5	12	10	6	9	13	11	10
Rumen	16	21	20	12	8	7	8	6
Faeces	0	0	23	13	12	13	4	5

* Cows A and D were overfed and weighed on average 1138 lb; cows B and C were underfed and weighed on average 1069 lb.

The concentrations of AP and NAAP in the reticulo-ruminal water did not differ significantly between the cows given hay and those on pasture or dehydrated grass

(Tables 2–4). The animals given hay and concentrates had a higher concentration of AP and NAAP in the abomasal and intestinal water than the cows on pasture or dehydrated grass (Table 2). However, there was considerable variability and the differences were not consistent. These observations indicate that the more rapid rate of disappearance of AP and NAAP from the blood of animals on pasture as compared to those given hay cannot be accounted for entirely by increased entry into the gut.

Table 4. *Concentrations (µg/ml) of antipyrine (AP) and* N-*acetyl-4-aminoantipyrine (NAAP) in the water of blood, saliva, faeces and rumen contents after intravenous injection into cows receiving diets of hay or pasture*

	AP				NAAP			
	Hay		Pasture		Hay		Pasture	
Sample	Cow B	Cow D	Cow A	Cow C	Cow B	Cow D	Cow A	Cow C
	2·5 h after injection							
Blood	40	33	15	19	40	31	21	24
Saliva	29	26	10	13	28	21	15	15
Rumen	18	18	16	17	2	2	4	3
	5 h after injection							
Blood	24	21	5	8	30	24	13	15
Saliva	18	15	3	5	20	20	7	10
Rumen	21	21	12	13	4	2	6	6
Faeces	9	15	5	5	3	3	4	4

Table 5. *Concentrations (µg/ml) of antipyrine (AP) and* N-*acetyl-4-aminoantipyrine (NAAP) in the water of the blood, rumen contents and abomasum contents of a 350 lb steer at various intervals after intravenous injection*

Time after injection (min)	AP			NAAP		
	Blood	Rumen	Abomasum	Blood	Rumen	Abomasum
124	119	46	279	131	3	92
188	85	52	148	117	14	88
243	64	60	107	106	15	72
306	46	53	71	95	24	59
364	34	47	50	86	26	56

Concentration of AP and NAAP in milk

The concentration of AP and NAAP in milk 6 h after intravenous injection was about 80 and 97 %, respectively, of the amounts present in the blood at the time of milking. The cows had been last milked 3·5 h before the injection. The milk from the two cows that produced about 12 lb of milk at the milking contained a higher concentration of AP and NAAP (9·5 and 18·3 µg/ml, respectively) than that from the two cows that averaged 19 lb milk (3·6 and 15·8 µg/ml, respectively). The milk from the four cows contained on average 0·3 % of the AP and 1·1 % of the NAAP injected. In a previous experiment, not reported in this paper, the amounts of AP and NAAP recovered in the milk amounted to only 0·02 and 0·3 %, respectively, of that injected.

Entry of AP and NAAP into the reticulo-rumen and their absorption therefrom

During the period from 10 to 345 min after the intravenous injection of AP and NAAP, their concentrations in the saliva remained at about 80 % of those in the blood. The concentrations of AP and NAAP in the reticulo-ruminal water 20 min after the injection were 27 and 4 %, respectively, of those found in the blood water at the same time. On the basis of the mean saliva flow in the fasting cow reported by Bailey (1958), the weight of water in the reticulo-rumen, and the concentration of AP in the saliva, it is clear that the saliva alone cannot account for all of the AP found in the rumen contents 20 min after the injection. In this and other experiments reported here it was not possible to account for more than 30 % of the AP found in the reticulo-rumen as having entered in the saliva. It must be concluded that AP reaches the rumen by routes other than the saliva, presumably by direct passage through the rumen wall. Also, it must be concluded that most of this passage into the rumen takes place during the first few minutes after the injection as the concentration in the rumen water was almost as great at 20 min as at 160 min after the injection (17·8 and 19·5 μg AP/ml, respectively). It is possible to account for all of the NAAP found in the rumen as having entered in the saliva. Blood samples collected 10 and 20 min after the intravenous injection of AP and NAAP contained only 30 % more of these two compounds than expected from the dilution curves when extrapolated to the same times.

When AP and NAAP were introduced into the rumen in amounts sufficient for a concentration of 350 μg AP/ml and 200 μg NAAP/ml the increase in their concentration in the blood was slow. The concentrations of AP in the blood water were 4, 11, 19 and 19 μg/ml 30, 90, 150 and 210 min after AP was introduced into the rumen. The corresponding concentrations of NAAP were 1, 2, 3 and 4 μg/ml. The slow passage of AP and NAAP from the reticulo-rumen into the blood suggested that these compounds were not absorbed through the rumen wall but passed down the gut with the digesta and were absorbed from the omasum, abomasum or intestines, notwithstanding the high concentrations of AP and NAAP observed in the lower gut. Also, since AP from the rumen enters the blood stream at a faster rate than NAAP, the build-up of AP in the rumen after intravenous injection must be due to AP entering the rumen in amounts considerably greater than those of NAAP.

Effect of incomplete intravenous injection of AP and NAAP

Blood-dilution curves for AP and NAAP injected intramuscularly instead of intravenously were essentially linear (Figs. 1, 2). The concentration of AP in blood was less at 325 min than at 140 min after the injection and the concentration of NAAP was greater at 325 min than at 140 min after the injection. This finding indicates that the absorption of AP from the tissues was at a slower rate than metabolism or excretion, whereas NAAP was absorbed from the tissues at a greater rate than it was excreted.

DISCUSSION

The object in seeking a method for estimating body water in vivo has been to enable body fat and body energy to be calculated by means of the well-known inverse relationship between body water and body fat. The assumption that body water makes up 73·2 % of the fat-free empty body as used by Kraybill *et al.* (1951) or that the relationship can be expressed as the equations proposed by Reid, Wellington & Dunn (1955) may not be applicable in all instances. Brody (1945) mentions that there is considerable evidence that the muscles and skin can store excess water temporarily when the water intake exceeds immediate requirements, and Passmore, Strong & Ritchie (1959) have shown that when obese patients begin dieting there may be rapid losses from the tissues of extracellular and intracellular water and thereafter intracellular water may continue to decrease while extracellular water may increase. Strauss (1957) in reviewing body-water changes in man and other animals has reported marked changes in body-tissue water due to administered water, deprivation of water and changes in environmental temperature. Results of experiments reported in our paper indicate that changes in body-weight of cows given extra water or put on pasture could not be accounted for entirely by changes in gut fill, and suggest a temporary storage of water in the body tissues. Such storage may account for some of the differences in percentage body water reported by MacFadden & Richards (1956), Swanson & Neathery (1956) and Garrett *et al.* (1959) when food and water were withheld for different lengths of time before the injection of AP. The work reported by these authors and our findings emphasize the need for more research on water metabolism in the ruminant animal to decide how long water must be withheld to attain equilibrium between water and body tissues without hydration or dehydration. This would seem to be a fundamental need before body composition can be confidently calculated from body-water estimations. Information is needed also on the possible changes that take place in the distribution of body water when cattle are turned to pasture.

Even if this question is resolved, it will still be necessary to know whether AP or NAAP estimate total or empty-body water with sufficient accuracy for most requirements and what problems are associated with their use. Blaxter & Rook (1956) discussed the biological and analytical errors involved in calculating body energy from body-water measurements. They reported that the error of estimating body water by AP was ±5 %, an error that was too large in their opinion for estimating body energy content. An error of ±5 % in estimating body water in an animal of average body condition (20 % fat) will result in errors of about 15 and 11 % in estimating body fat and body energy contents, respectively. In the experiments reported in this paper it was not possible to calculate the error in estimating body water because body compositions were not determined.

If it can be assumed on the basis of empty-body weights and estimated body water contents recorded 7 weeks previously, and also on whole body-weights recorded when the cows were turned to pasture, that the two groups of cows were of similar empty-body weights and water content when grazing began, then it seems likely that most of the difference in empty-body weight between the two cows on pasture and the two given hay was body water and not body tissue. Part of this difference could also have been due to an altered distribution of water in the reticulo-rumen and other sections of the gut for cows on pasture as compared to cows given hay. Unpublished results (M. A. Carroll) indicate that such alterations were not significant.

If the change in average empty-body weights was mainly in water then AP overestimated the change by about 60 lb and NAAP underestimated the change by about 40 lb. The rates of disappearance of AP and NAAP from the blood of the two cows given hay were 22 and 13 % per h, respectively. The reason for the increased rate of metabolism or excretion, or both, by the cows on pasture as compared to those given hay or the reason for the overestimation by AP and underestimation by NAAP of the gain in water are not known.

One of the important problems in the use of AP and NAAP in estimating body water in ruminants is that these two substances are not distributed in the gut water in the same concentration as in the blood water. Reid *et al.* (1957) proposed that it might be possible to derive factors that would correct for the amounts entering the gut. Because of the marked difference in the concentration of AP and NAAP in the water of the different sections of the gut, the variability between cows and within cows at different times and the effect of diet, it is unlikely that a uniform correction factor would increase the precision of the method appreciably.

It may be questioned, however, whether correction factors are necessary if it could be assumed that the amount of AP or NAAP entering the gut at any one time was proportional to that in the blood and that none was reabsorbed into the blood. That is, all AP or NAAP entering the gut would be considered as true excretions from the body and hence would not introduce an error in estimating body water from the blood-dilution curve. The rapid entry of AP into the reticulo-rumen and its slow but appreciable re-entry into the blood appear to disqualify AP for this estimate, and our study has shown the difficulties which prevent use of any constant factor to correct for the entry of AP into the gut. With NAAP the assumptions seem to be valid, in that all of the NAAP found in the reticulo-rumen apparently entered in the saliva, the amount in the saliva was in proportion to that in the blood, and very little of it was reabsorbed into the blood during the period when samples were being collected. The rate of entry into or absorption from the abomasum or intestines of NAAP was not studied. However, the gradual decrease in concentration of NAAP in the abomasum with time (Table 5) and the fact that the concentrations in the large intestine were less than in the abomasum or intestines indicated that there was a reabsorption. The error that this reabsorption would cause in estimating body water would depend on the amount of the NAAP injected that entered these sections of the gut, the rate at which it was reabsorbed and the length of time from when it entered until it was reabsorbed from the gut. Since only 2·5 % of the NAAP injected was found in the abomasum and intestines, and since its concentration decreased roughly in proportion to the decrease in concentration in the blood, there is some reason for optimism for the use of NAAP, uncorrected for the amount entering the gut, to estimate empty-body water in ruminants, if it is proved that NAAP becomes uniformly distributed throughout the water of the empty body. Dumont (1958) has questioned whether AP and NAAP are distributed in all body tissues in proportion to their water content and whether these tissues lose them at the same rate as blood.

Another problem in the use of body-water estimates for determining body composition in ruminant animals is that of determining empty-body weight or gut fill or both. With fistulated animals this is possible. Reid *et al.* (1957) suggested that it might be possible to determine gut water in non-fistulated animals as the difference between AP and NAAP water spaces. In our experiments, although the mean difference corresponded to 87% of the water estimated, by weighing the water in the reticulo-rumen, to be in the gut, the variation was too great to make the method practical. Although live weight approaches empty-body weight in monogastric animals deprived of food and water for extended periods of time, it is not so with ruminants. Mäkelä (1956) has shown that restricted food intake does not proportionally lower the weight of the reticulo-rumen contents, but the contents become more fluid.

Before markers can be relied on for the determination of water spaces in the living ruminant means must be devised for reducing the errors arising from uneven distribution of the markers, particularly in the gut water. These errors are undoubtedly reduced if the animal is deprived of food and water for some hours before the injection. It seems unlikely that there is some optimum period of deprivation after which the errors cancel out under varying conditions, and even if it were so a period of more than about 6 h is hardly feasible in experiments with milking cows.

This study has emphasized also the need for complete intravenous injection of the AP and NAAP solutions if the best estimates of body water are to be obtained. It had been assumed at this Institute that an incomplete intravenous injection could be detected by the non-linearity of the blood-dilution curve. In most instances this is true, and a non-linear curve should be viewed with suspicion. However, since a complete intramuscular injection resulted in blood-dilution curves that were essentially linear, it is possible that incomplete intravenous injection could go undetected if linearity of the dilution curve were used as a criterion. The use of a cannula of plastic or similar material through which to inject the solution, as suggested by Garrett *et al.* (1959), reduces the number of incomplete intravenous injections.

SUMMARY

1. The use of injected antipyrine (AP) and *N*-acetyl-4-aminoantipyrine (NAAP) as marker substances in the determination of body water content has been investigated in experiments with four cows with rumen fistulas and with four cows and a steer that were slaughtered.

2. Observations included the accuracy of repeated determinations of, and the effect of diet on, the estimates of body water content, the effect of large water intakes before injection, the concentration of the markers in saliva and milk, the rate of entry of the markers into the various parts of the gut and the rate of their reabsorption from the reticulo-rumen.

3. Estimates of the body water content made with AP were larger than those made with NAAP.

4. The results obtained with AP suggest that this marker cannot be relied upon invariably to give accurate values for the water content of either the total or the empty body; these observations were, however, not checked by analysis of slaughtered animals.

5. The results with NAAP suggest that this marker may offer a more reliable means of determining the water content of the empty body, provided the empty-body weight can be estimated, as for example in animals with large rumen fistulas.

6. It seems unlikely that differences between the estimates with AP and NAAP can at present be used for reliable indication of the water in the gut contents.

7. During these experiments it was observed that failure to achieve complete intravenous injection of the markers does not necessarily lead to non-linear curves for the subsequent concentration (log scale) of the markers in the blood.

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