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To the Graduate Council:

I am submitting herewith a thesis written by Milton White Neathery entitled "The Determination of Body Water in Cattle by the Use of Antipyrine." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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THE DETERMINATION OF BODY WATER IN CATTLE BY THE USE OF ANTIPYRINE

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

Milton White Neathery

December 1955

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CHAPTER I

INTRODUCTION

A satisfactory method for determining total body water in living animals has been sought by physiologists because of the great importance of water in the body. The presence of water is essential to the great mass of chemical reactions on which life depends. Water furnishes the single molecular element for the fabrication of tissue. It furnishes the means of transport of virtually all enzymes, substrates, antibodies, metabolites, gases, electrolytes, and other substances that must move to and from the cell.

The dairy cow is able to consume considerably large amounts of water because of her large gastrointestinal tract. The water level in the body is not constant. Some of the things that might alter this level are degree of fatness, stage of lactation, environmental temperature, and water intake. Certain diseases seem to cause a dehydrating effect on the animal, while others seem to cause edema. A knowledge of the total body water of the cow would help in the study of these conditions. The true weight of the animal under normal conditions could be determined. The degree of fatness could be estimated, since total body water has been found to be inversely proportional to total body fat. It would aid in the understanding of why the dressing percentage of some

cattle are higher than others. Since body water is an important element in the manufacture of milk, possibly variations in milk production could be more thoroughly understood if the total body water of the cow was known.

One of the latest methods employed in the determination of total body water in vivo is the antipyrine dilution method. Antipyrine has been used to measure total body water in humans, rabbits, dogs, monkeys, sheep, swine, and beef cattle. At the present time very little information, if any, is available on the determination of total body water of dairy cattle by the use of antipyrine.

The purpose of this study was to evaluate the antipyrine dilution method for determining total body water of identical twin dairy cattle and from these data estimate the per cent of total body fat.

CHAPTER II

REVIEW OF LITERATURE

Methods of Total Body Water Determination

The methods of total body water determination may be divided into three main categories. They are desiccation, body specific gravity measurements, and the dilution method.

Desiccation

One of the earliest studies of desiccation in man, as reported by Hardy and Drabkin (12), was that of Bischoff in 1863. He obtained a total water value equal to 58.5% of body weight. In 1945 Mitchell *et al.* (22) determined the total body water of a 35 year-old white man, whose death was due to an acute heart attack. The per cent of total body water was found to be 67.85 of total body weight.

Because of the necessity of sacrificing the animal in this method, the studies have been directed toward the use of laboratory animals. Studies of the relationship of total body water and chemically combined nitrogen content to fat content were conducted by Pace and Rathbun (28). They found that the water content and chemically combined nitrogen content of 50 guinea pigs in proportion to their body weight were constant when the body fat was considered. Water was found to constitute 72.4% of the fat-free body mass, and

exhibited a standard deviation of ± 2.11%. The authors postulated that the lean body mass was relatively constant in gross chemical composition, in which body fat may be considered to act as a diluent.

Murray (25) found the non-fatty portion of cattle, sheep, and swine to be fairly constant, and the per cent of total body water was inversely proportional to age and body fat.

Annegers (1) determined the total body water of rats and mice by the desiccation method. Data from 16 normal female albino rats and 14 normal female albino mice were analyzed by the method of multiple regression in which the independent variables were fat-free dry body weight and body fat, and the dependent variable was total body water. In the rats, total body water was found to be 25.8 g. There was a change in water content of 2.17 g. for each gram of fat-free-dried weight. In mice total body water was found to be 1.52 g. There was a change in body water of 2.63 g. for each gram of fat-free-dried weight. The changes in body water per gram of fat for the rats and mice were - 0.05 and - 0.12 g. respectively. These values were not statistically significant. It was surprising to note that no independent effect of body fat on total body water was demonstrated. This is contrary to the belief of other earlier investigators (16, 36, 25).

Specific Gravity

Total body water and total body fat have been determined from specific gravity measurements (2, 17, 21, 27, 31, 40).

This method in vivo requires complete cooperation from the subject being measured. The specific gravity value is the difference between body weight in the air and body weight under water at maximum expiration. The residual air in the lungs must be accounted for by using the nitrogen or helium displacement techniques. The complications of this procedure makes its use impractical for determining total body water in farm animals. However, a successful attempt has been made by Messinger and Steele (21) in determining total body water in human subjects. Total body water and total body fat were determined of nine normal individuals by the specific gravity and antipyrine dilution methods. Their results indicated a good agreement between the two methods used.

The results of Messinger and Steele were later confirmed in a similar experiment by Osserman et al. (27). Total body water and total body fat values as determined by specific gravity measurements were compared to total body water and total body fat values as determined by the antipyrine dilution method as described by Soberman et al. (36). The per cent of body fat was determined from specific gravity data by equation I.

$$\text{(I) Per cent body fat} = 100\left(\frac{5.548}{\text{S.G.}} - 5.044\right)$$

The per cent of body fat was determined from antipyrine data by equation II.

$$\text{(II) Per cent body fat} = 100\left(\frac{\text{body wt.} - \frac{\text{wt. of body water}}{0.718}}{\text{body weight}}\right)$$

$$= 100\left(\frac{71.8 - \%\ \text{body water}}{.718}\right)$$

The per cent of body water was determined from specific gravity data by equation III.

$$\text{(III) Per cent body water} = 100\left(4.340 - \frac{3.983}{\text{S.G.}}\right)$$

The results of their experiment with 81 men showed a good agreement, both in total body water and total body fat, between the two methods. Their results are shown in Table I.

The specific gravity method of determining total body water and total body fat has been demonstrated on eviscerated animals (31, 17), but this method requires the sacrificing of the animal.

Rathbun and Pace (31) determined the total body fat of 50 normal male and female guinea pigs. The guinea pigs had specific gravity readings ranging from 1.021 to 1.096. The results of the fat analysis ranged from 1.5 to 38.8% of the wet weight of the eviscerated carcass. When calculated as per cent of dry carcass weight the range was from 5.0% to 66.8%.

TABLE I

COMPARISON OF SPECIFIC GRAVITY AND ANTIPYRINE METHODS FOR DETERMINING TOTAL BODY WATER OF 81 HUMAN SUBJECTS[a]

Measurement	Mean	Range
1. Age (yrs.)	26.6	18-46
2. Body Wt. (Kg.)	75.3	53.6-102.7
3. Specific Gravity	1.068	1.002-1.100
4. Body Fat from S. G. (%)	15.0	0-38.4
5. Body Fat from antipyrine (%)	15.0	0-40.1
6. Body Water from S. G. (%)	61.0	44-72.0
7. Body Water from antipyrine (%)	61.0	43-72.9
8. Water in lean body mass	71.8	66.3-79.0
Standard error (% of No. 8)	± 3.8	
Standard deviation (%)	± 2.99	

[a]Source: E. F. Osserman, "In Vivo Measurement of Body Fat and Body Water in a Group of Normal Men," J. Appl. Physiol., 2: 633. 1950.

It was found that the per cent of fat of the whole body was equivalent to that of the eviscerated carcass over the entire range. It was postulated by the authors that fat is probably laid down proportionally in the viscera compared with the remainder of the body. The experiment also showed that as the specific gravity increased the fat content decreased. They derived the following equation for determining body fat from their specific gravity data:

$$\text{Per cent fat} = \frac{5.362}{\text{S.G.}} - 4.880 \quad .$$

Kraybill *et al.* (16) determined the total body water and total body fat of 24 head of yearling Hereford steers and heifers and six head of crossbred cows by the specific gravity and antipyrine methods. The percentage of fat (separable adipose tissue) and ether extract in the carcass was obtained indirectly by Hopper's (15) estimating equation from a physical and chemical analysis of the 9-10-11 rib portion of the wholesale rib cut. Carcass fat values obtained by physical and chemical analysis were compared with the body fat values obtained by the antipyrine and specific gravity methods.

Total body water was calculated from the body specific gravity. Specific gravity of the viscera, carcass, and whole animal was made by essentially the same procedure as reported by Rathbun and Pace (31). The equation developed by Pace and Rathbun (28) for calculation of water from whole animal specific gravity was applied.

$$\text{Percentage of water} = 100 \left(4.424 - \frac{4.061}{\text{S.G.}}\right)$$

The results obtained by the two methods agreed very well. Twenty-four Herefords had body water values ranging from 43.9 to 63.0% by the antipyrine method and values ranging from 43.1 to 63.3% by the specific gravity method.

On the basis of relative consistency of water content (73.2%) of the fat-free mass, the per cent body fat was calculated from body water data by means of the following equation:

$$\text{Per cent fat} = 100 - \frac{\text{Per cent of body water}}{0.732} .$$

It was determined that the mean values for fat determination by the two methods are not significantly different at the 5.0% level of significance. Osserman *et al.* (27) states that the standard error of estimate for calculating body fat from antipyrine data is $\pm$ 3.8%.

There was a close correlation between the per cent fat determined by the antipyrine method and the ether extract and separable fat values of the dressed carcass.

In 1953 Kraybill *et al.* (17) conducted a similar experiment with swine. The results again showed a good agreement between the two methods. The per cent body water of 24 hogs ranged from 36.7 to 64.5 as determined by the antipyrine method compared to 33.8 to 59.6 as calculated by the specific gravity method.

Dilution Method

Several chemical substances have been used to determine total body water. Sulfanilimide was proposed for the dog (29), but an earlier experiment by Sise (34) showed that it was not equally distributed in body tissues. Urea has been suggested by Painter for dogs (29) and for humans (18), but later rejected by Ralls (30) because of the variability in the formation of endogenous urea as well as unequal distribution. Thiourea has been tried with dogs (8) but rejected by Chesley in humans (7) and Williams and Kay (41) because of its unequal distribution. Potassium was also considered (3, 42) but was demonstrated to be distributed in the body potassium rather than in body water (10, 26).

The first dilution substance to be used to determine total body water in vivo was deuterium oxide. Hevesy and Hoffer (13) in a short note described the use of deuterium oxide for the measurement in man in 1934. Since that time this technique has been used for measurement of total body water in rats (20), and in guinea pigs (9), and in humans (12, 21, 23, 32, 36).

Antipyrine has been used to determine total body water in humans (21, 27, 33, 36, 38), in swine (17), in rabbits, dogs, and monkeys (35), in sheep (14), and in beef cattle (16).

For measurement of total body water, a substance should possess the following characteristics:

(1) Even and rapid distribution throughout the body water.

(2) Non-toxic in required doses.

(3) Slow transformation and slow excretion from the body.

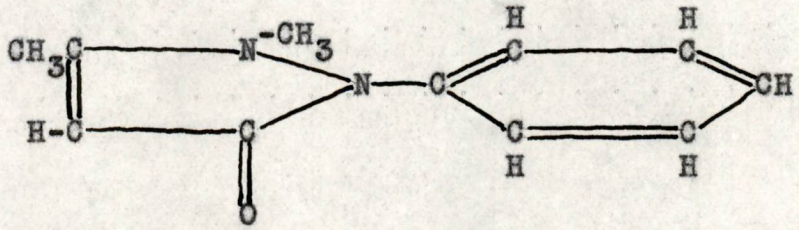
(4) Accurate and convenient estimation of its concentration in the plasma.

Antipyrine, deuterium oxide, tritium oxide, N-acetyl, 4-amino-antipyrine are chemical substances which meet these requirements to a satisfactory degree, and agree closely when used to determine total body water (36, 37).

Antipyrine has long been noted for its antipyretic properties, but it was not until Brodie et al. (5) devised a technique for its estimation in biological materials that it has become a noted analgenic agent for determining total body water.

The following physical and chemical properties of antipyrine have been listed by Greenberg (11).

(1) 1, 5-dimethyl-2-phenyl, 3-pyrazolone.



(2) It is made from reacting phenylhydrazine with acetoacetate, forming phenylmethylpyrazolon. This compound is then methylated by suitable means.

(3) It occurs as colorless crystals or crystalline powder.

(4) It is colorless, has a slight bitter taste, and is neutral to litmus.

(5) It has a molecular weight of 188.22.

(6) Its melting point is between 111° and 114°C. and boils at 319°C.

(7) At a temperature of 25°C. about 100 g. of antipyrine is soluble in 100 cc. of water or chloroform, 70 g. in 100 cc. of alcohol, and 23 g. in 100 cc. of ether.

(8) It becomes colored with ferric chloride, nitrous acid, and sweet spirits of nitre.

The antipyrine dilution technique is based on the fact that it is completely mixed throughout the total body water of the animal and that a quantitative analysis can be made from the plasma and other biological fluids of the body. In order to understand this principle, one must know the fate of antipyrine in the body.

Fate of Antipyrine in the Body

Absorption and Excretion

According to the bibliographic review of antipyrine made by Greenberg (11), children as well as young and old adults excrete the drug in the urine about the same time

after injection (between 45 minutes and one hour). The amount injected did not influence the time it appeared in the urine. According to Greenberg, the speed of appearance of antipyrine in the urine depends on the mode of administration. In rabbits antipyrine appeared in the urine 5 minutes after intravenous injection, 10 to 15 minutes after subcutaneous, 15 minutes after rectal, and 20 minutes after oral administration. In man antipyrine appeared in the urine 15 to 20 minutes after oral administration and 25 to 30 minutes after rectal administration.

Brodie and Axelrod (4) gave two human subjects 1.2 g. of antipyrine daily in divided doses for five days. Urine and stool collections were made during the last 72 hours of administration. Less than 0.1% of the amounts given were recovered in the stools, suggesting essentially complete absorption from the gastrointestinal tract. The drug had previously been shown to be stable when incubated for 24 hours with stool suspension at 37.0°C. The urinary excretion in this as well as single dose experiments accounted for only 5.0% of the total dose. Thus most of the antipyrine underwent transformation in the body.

Soberman et al. (36) studied the excretion of antipyrine in eight human subjects after the intravenous injection of 1.0 g. of the compound. They found that only 0.3 to 0.6% of the amount injected was excreted in four hours following the injection.

Transformation of Antipyrine

To calculate the total body water by this method, one must know the concentration of antipyrine in the plasma at the time of injection. This is accomplished by determining the concentration of blood plasma samples taken from the animals at different intervals after injection. These concentrations are plotted on semi-logarithmic paper and by extrapolating the straight portion of the time-concentration curve back to zero time, the concentration at the time of injection can be obtained. The rate at which antipyrine is metabolized in the body depends upon the animal injected and the size of the animal.

Brodie and Axelrod (4) found that when 300 to 600 mg. of antipyrine were administered orally to human subjects in the post-absorptive state, absorption was rapid with peak plasma levels being reached in one to two hours. Plasma levels declined slowly with measurable amounts of the drug persisting 24 hours beyond the time of its administration. The rate of metabolism was examined in subjects to whom 1.0 g. of antipyrine was given intravenously. The drug disappeared from the plasma at rates varying from 1.0 to 12.0% per hour. This rate is in good agreement with that obtained by Scribante et al. (33). They found that the rate of metabolism of antipyrine in nine human subjects, five males and four females, ranged from 4.4 to 7.3% with an average of 6.1%.

One of the difficulties of determining the concentration of antipyrine at the time of injection of animals, other than humans, is that the drug is metabolized at a faster rate than it is in human subjects.

Soberman (35) found the transformation of antipyrine in the dog to be 30.0%, and he postulated that this method for determining total body water might not be practical for the dog. The transformation after even distribution in five monkeys, four dogs, and four rabbits was found to be 30.0% per hour and varied from 20.0 to 50.0%. However, in a given animal the rate was constant over a period of seven hours. The transformation in swine has been found to be 36.0% per hour (17) and in beef cattle to be 20.0% per hour (16).

Brodie and Axelrod (4) found that 30.0-40.0% of the antipyrine injected in man was excreted in the urine in the conjugated form of 4-hydroxyantipyrine. Four-hydroxyantipyrine could not be detected in the plasma. When 1.0 g. of 4-hydroxyantipyrine was administered orally to two subjects, 92.0, 94.0, and 95.0% of the administered dose was recovered in the urine collected over a 24-hour period. Their results indicated that 4-hydroxyantipyrine derived in the body from antipyrine is converted almost quantitatively into a conjugated form and excreted as such. They concluded from their work that about 5.0% of the antipyrine is excreted unchanged and the remainder, about 60.0%, is transformed through an unknown route, perhaps involving ring cleavage.

Distribution of Antipyrine Throughout Body Tissues

In order for antipyrine to mix equally and thoroughly throughout the body water, it must not be selectively stored in large amounts by any organs or tissues of the body.

Brodie and Axelrod (4) found the concentration of antipyrine to be nearly the same in plasma water, and tissue water, of whole blood, cerebrospinal fluid, brain, muscle, heart, lungs, kidneys, liver, and spleen in man after the intravenous injection of 1.5 g. Soberman (35) obtained similar results with dogs, rabbits, and monkeys, although there were slightly higher concentrations in the liver and kidneys. Soberman et al. (36) studied the distribution of antipyrine in representative tissues of two dogs that had received antipyrine intravenously. The tissues of one dog were examined one and one-half hours and those of the other dog two and three-fourths hours after injection. The antipyrine concentration in the water of the tissues was found to be nearly the same as that in plasma water, with the exception of a somewhat lower concentration in the kidney. The results of their experiment are shown in Table II.

Soberman et al. (36) also studied the distribution of antipyrine in man. The tissues of two patients who had received antipyrine orally for several days prior to death were analyzed post-mortem. Each had received the last dose of antipyrine about five hours before death. The tissue

TABLE II

DISTRIBUTION OF ANTIPYRINE IN WATER OF DOG TISSUES[a]

Tissues	Antipyrine in Tissue Water		Ratio, Tissue H_2O Antipyrine / Plasma H_2O Antipyrine	
	Dog 1 (mg./l.)	Dog 2 (mg./l.)	Dog 1	Dog 2
Plasma	29.2	54.2		
Heart	31.1	56.8	1.07	1.04
Muscle 1	30.6	51.0	1.05	0.94
Muscle 2	30.7	54.6	1.05	1.00
Spleen	30.1		1.03	
Kidney	32.5	61.3	1.12	1.12
Liver	30.4	60.0	1.04	1.10
Lung	24.3	47.1	0.83	0.86
Brain	28.9		0.99	
Cerebrospinal Fluid	25.7	53.8	0.88	0.99
Average			1.01	1.01
Adipose Tissue, abdominal	26.3		0.90	
Adipose Tissue, groin	26.9		0.92	
Adipose Tissue, perirenal	28.9		0.99	

[a]Source: Robert J. Soberman, "The Use of Antipyrine in the Measurement of Total Body Water in Man," J. Bio. Chem., 179: 31. 1949.

samples were removed and homogenized within four hours post-mortem. A sample of blood was not taken from the first patient. Again the results, as shown in Table III, show a relatively equal distribution of antipyrine in plasma water and the water of various tissues examined, except for a lower concentration in the lungs and a higher concentration in the liver.

The author states that since most of the ratios of transudate to plasma were less than 1.0, suggested either incomplete distribution or some binding on plasma protein. The extent to which antipyrine is bound to non-diffusible constituents of plasma was determined by dialysis against isotonic phosphate of pH 7.4 at 37.0°C. The results indicated there was a binding of about 10.0%.

According to the review of antipyrine by Greenberg (11), the drug was found in all organs except the spleen and in all the body fluids (saliva, gastric and intestinal juices, bile, milk, sweat, lumbar fluids, amnionic fluid, as well as urine) when injected intravenously into rabbits. Two hours after administration the greatest amount of antipyrine was found in the liver, lungs, kidneys, and muscles; after four hours the greatest amount was found in the gastrointestinal tissues and the liver; after six hours antipyrine was found mainly in the liver, stomach, and only a little in the blood.

TABLE III

DISTRIBUTION OF ANTIPYRINE IN WATER OF HUMAN TISSUES[a]

Tissues	Sub. 1 (mg./l.)	Sub. 2 (mg./l.)	Ratio, Tissue H_2O Antipyrine / Plasma H_2O Antipyrine		
			Sub. 1	Sub. 1	Sub. 2
Plasma		78.7			
Heart	88.0	78.0	0.99		
Kidney	87.0	78.5	1.00		
Liver	103.0	93.0	1.18		
Muscle 1	90.0	65.5	0.83	1.04	1.06
Muscle 2	94.0	72.0	0.92		
Lung	88.0	70.5	0.90		
Skin	95.0	74.0	0.94		
Pericardial Fluid		75.1	0.95		

[a]Source: Robert J. Soberman, "The Use of Antipyrine in the Measurement of Total Body Water in Man," J. Bio. Chem., 179: 31. 1949.

Toxicity

The dilution substance used should not be toxic to the animal in the required dose of determining total body water.

Soberman et al. (36) reported that no untoward effects were observed in 200 patients given 1.0 g. of antipyrine intravenously. Specifically, there was no methemoglobinemia or depression of white blood cell count.

Soberman (35) found antipyrine to be non-toxic to 30 dogs, 10 rabbits, and 8 monkeys given 75.0 mg. of the drug per kilo body weight intravenously, with the exception that five dogs exhibited excessive salivation and one dog vomited immediately following a rapid injection.

Significance of Body Water

Experimental evidence seems to show antipyrine to meet the qualifications of a good dilution substance as pointed out by Soberman et al. (36).

Steele et al. (38) determined the total body water of 51 normal males and 31 normal female human subjects by the antipyrine method. The males ranged from 24 to 96 years with an average of 51, and the females from 21 to 94 years with an average age of 45. The group consisted of laboratory technicians, medical students, physicians, and ambulatory patients of a chronic disease hospital who were free from diseases which lead to edema or abnormal accumulation

of body fluids. In the men, water ranged from 40.0 to 68.0% of body weight and averaged 53.0%, while in women, the proportion of water ranged from 30.0 to 53.0% and averaged 45.0%. The females showed a lower percentage of body water and a greater degree of obesity.

They studied the reproducibility of four successive measurements of three subjects from 48 to 72 hours apart. It was found to be $\pm$ 8.0%.

Using the same procedure, Osserman et al. (27) found the total body water of 81 men, between the ages of 18 to 46, to be an average of 61.0% with a range of 43.0 to 72.9%.

Scribante et al. (33) determined the total body water of nine humans, five males, and four females. The males averaged 53.6% body water and the females averaged 46.2%.

The possibility that antipyrine might be used to determine total body water of cattle was studied by Kraybill et al. (16). Twenty-four head of yearling Hereford steers and heifers and six head of crossbred cows and heifers were used in the study. The two groups of Hereford cattle were divided equally by sex and lines of breeding and were comparable in age and weight. The six head of crossbreds varied in age from 36 to 204 months and in weight from 760 to 1220 lbs. This variation provided a wide range in composition with respect to the proportions of muscle, fat, and bone.

A wide range in level of fatness was made possible by maintaining group I of the Hereford cattle on a high plane of nutrition and group II on a lower plane. Group I cattle were fed cracked corn (fullfed), mixed hay, and cottonseed oil meal. Group II cattle were fed one-half the amount of grain given to group I. Variation in composition was also provided by selecting a range in slaughter weights from 500 to 1050 lbs. for Hereford steers and heifers.

To obtain a constant weight prior to injection, the animals were fasted 24 hours and water was eliminated ten hours preceding the body water measurement.

Their results showed the total body water values to range from 43.9 to 63.0% of the total body weight.

Kraybill et al. (17) also determined the total body water of swine. The per cent of total body water of 24 hogs ranged from 36.7 to 64.5%. They concluded that the antipyrine method was less accurate for swine than for cattle because of a faster rate of transformation of the drug in swine. The transformation rate for swine was 36.0% per hour. They previously had found the transformation in cattle to be 20.0% per hour.

Swanson (39) studied the possibility of large shifts in the water content of dairy cows during and after the administration of a thyroactive supplement to the ration by the antipyrine dilution method. Three identical twin cows

received 15 g. of Protamone daily for 70 days. Two other cows which were comparable in body weight were used as controls. One treated cow did not have a control. Body weight, heart girth, and paunch girth measurements were determined weekly for the treated and control groups during the Protamone-feeding period and after the withdrawal of it from the ration. The control group gradually increased in weight throughout the observation period. The treated group lost an average of 70 to 75 lb. and girth reduction of 3.5 to 4.5 in. during the first six weeks. A slight increase in weight and girth began, but after five more weeks when withdrawal of the supplement began, the treated cows still averaged 54 lb. below their pretreatment weight and 3.5 in. below their pretreatment girth. At the same time the nontreated cows had gained 39 lb. and 0.8 in. The loss of 3.5 in. in paunch girth during the early weeks of treatment would represent about 7.5 gal. of gastrointestinal fill or more than 60 lb. of weight compared to 54 lb. average actual loss. The 5.5 in. increase in girth after withdrawal could represent about 89 lb. of weight, whereas the actual average increase in the treated cows at this time was 95 lb.

Total body water was determined of both groups during the thyroactive feeding period and after the withdrawal of it. The treated group had an average of 69.0% body water and the control cows had an average of 71.0% body water

during the treated period. The treated group had an average of 64.0% body water after the withdrawal of Protamone compared to 63.0% for the controls. The differences between the controls and treated cows either during or after treatment were not significant. The total body water data failed to show that thyroactive supplement feeding caused a dehydrating effect or that part of the rapid weight gain after treatment was due to restoration of normal water content.

Experimental evidence seems to show that the antipyrine dilution method is an inexpensive and simple method for determining total body water in live animals. The main concept of this method is that antipyrine, when injected into the animal, is completely mixed with the total body water and is not selectively stored by any organ or tissue of the body. Experiments show that there are slightly higher concentrations of antipyrine in the liver than in the plasma. This is understandable in view of the fact that the liver is a detoxifying organ and it regulates the movement of certain elements into the blood stream. The metabolism of antipyrine was found to be 6.1% per hour by humans, 30.0% by dogs, 36.0% by swine, and 20.0% by beef cattle. Because of the slow transformation of antipyrine in human subjects, an accurate concentration of the drug can be determined at the time of injection. For this reason the method would seem more practical for determining total body water in humans than for dogs, swine, or

beef cattle; however, in these animals the rate of metabolism has been found to be relatively constant for five hours after injection.

Total body water values as determined by the body specific gravity and the antipyrine dilution method show a close correlation between the two methods.

CHAPTER III

EXPERIMENTAL PROCEDURE

Animals Used and Treatment

Thirteen pairs of identical twin dairy cattle and one single twin cow from the University of Tennessee experimental dairy herd were the experimental subjects. They ranged in weight from 440 to 1170 pounds and in age from 11.5 to 57.8 months. This wide range in weight and age afforded a wide variation in the physical composition of the cattle. Some of the pairs of twins were on uniform treatments. Others were being treated differently. These subjects thus gave information regarding the repeatability of body water determination and its significance under different conditions. Four twins were receiving 100 mg. of l-thyroxin daily. Thirteen pairs of twins of different ages were fasted for sixteen hours and water was withheld twelve hours prior to injection. Four twin heifers and three twin cows were maintained on a high plane of nutrition while their respective mates were maintained on a normal plane of nutrition.

For all the determinations, the animals were tied away from water and feed during the injection and sampling period. They were weighed before injection and again after the sampling period. The average weight was used in the calculation of

total body water. Height and heart girth measurements were determined on the subjects the day of injection.

Injection and Sampling Procedure

Doses of 15 to 25 g. of antipyrine were accurately weighed into prescription bottles. The antipyrine was dissolved in about 100 ml. of sterile distilled water. The amount of dose given was about 2 g. per 100 lb. body weight in order to maintain reliable levels of antipyrine for the five hours collection period. To insure that the complete dose was delivered into the blood stream, it was taken up in a 100 ml. syringe and injected into the jugular vein by means of a polyethylene catheter. The catheter was rinsed with a heparinized saline solution after injection and before withdrawal from the vein. The time of injection was carefully noted.

A control sample of blood was taken prior to injection. Additional samples were taken at 2.5, 3.0, 3.5, 4.0, and 4.5-hours after injection. The blood samples were taken from the jugular vein on the opposite side of the neck from which the injection was made. The sample tubes contained 0.05 cc. of a 1:10,000 solution of heparin and about 30 ml. of blood was taken for each sample. The samples were placed in a refrigerator immediately after collection.

The blood samples were centrifuged at 2200 r.p.m. for 15 minutes and the plasma was withdrawn from the centrifuged

samples by aspiration, put in stoppered tubes, and stored in the refrigerator. The plasma was obtained from the blood samples on the day of injection and the antipyrine determinations were made the following day.

Determination of Antipyrine Concentration in Plasma

Antipyrine concentrations were determined by the precipitation procedure as described by Brodie et al. (5). Two milliliters of plasma, 2 ml. of distilled water, and 2 ml. of zinc sulfate reagent were added to a 50 ml. Erlenmeyer flask. Two milliliters of 0.75 N sodium hydroxide were added to the flask drop by drop. The flask was swirled continually during the addition of sodium hydroxide and for one-half minute afterward. The precipitated mixture was then transferred to 12 ml. centrifuge tubes and centrifuged for 15 minutes at 3000 r.p.m. Three milliliters of the supernatant fluid of each sample was transferred to corex cuvettes. Five-hundredths milliliters of 4 N sulfuric acid was added to each sample and mixed by inverting the cells. The optical densities of the samples were measured in a Beckman quartz spectrophotometer at a wave length of 350 millimicrons before and after the addition of 0.1 ml. of a freshly prepared 0.2% sodium nitrite solution. The sodium nitrite solution was prepared fresh for each set of determinations to insure correct maximum density reading of the samples. Distilled water similarly treated was used for a zero setting of the spectrophotometer.

In order to obtain the maximum reading, the optical densities were read at 5, 10, 15, and 20 minutes after the addition of sodium nitrite. In most cases the maximum reading was obtained at 15 minutes; however, it was noticed as the room temperature in the laboratory increased, the maximum reading was obtained in ten and sometimes five minutes. It is possible that the increased temperature accelerated the reaction of sodium nitrite and antipyrine. The corrected reading of each sample was obtained by adding the density reading of the control sample to the preliminary reading of the antipyrine sample and subtracting this value from the maximum density reading of each sample.

Standard Solutions

A stock solution of 100 micrograms of antipyrine per milliliter of 0.07 N sulfuric acid was prepared. From this stock solution, concentrations of 5, 10, and 15 micrograms per milliliter of 0.07 N sulfuric acid were prepared. A standard curve was determined by placing 3 ml. of each of the three concentrations into corex cuvettes and reading the optical densities before and after the addition of 0.2% sodium nitrite solution. Seven-hundredths normal sulfuric acid similarly treated was used for the zero setting. Since the antipyrine concentrations of the standard solutions remained constant, it was not necessary to run a standard with each set of antipyrine determinations. The antipyrine

concentration of each sample was determined by taking the corrected optical density values and reading the corresponding concentrations direct from the standard curve.

Brodie et al. (5) found antipyrine to be bound to plasma protein in the range of 10.0%. The binding of antipyrine to this extent would constitute a large error in the calculation of antipyrine concentrations. To check the extent of binding, or the recovery of antipyrine from blood plasma, 1.0 cc. of a 100 microgram concentration of an antipyrine standard solution was added to 5.0 cc. of a control plasma sample. The optical densities were read in duplicate on the control samples and plasma samples plus antipyrine. The 5.0 cc. control plasma sample gave a small value of 2.1 micrograms per milliliter of plasma. The plasma plus 1.0 cc. of the 100 micrograms per milliliter standard gave a total concentration of 102.1 micrograms or 17.0 micrograms per milliliter. The samples that had a known concentration of antipyrine added to the plasma before precipitation had a concentration of 17.4 micrograms per milliliter or a recovery of 102.3%. This essentially suggests that under these conditions added antipyrine was not bound by the plasma protein, and that recovery of it by the precipitation method was accurate.

Calculation of Per Cent Total Body Water

For the calculation of the per cent total body water, the plasma water, concentration of antipyrine in the plasma

at the time of injection, and the weight of the animal must be known.

Plasma water was determined by drying samples of plasma to a constant weight in a vacuum oven. Aluminum dishes were numbered and placed into the oven and dried at 100°C. for one hour. After drying, they were placed in a desiccator and allowed to cool to room temperature. The dishes were weighed and 1.0 cc. of plasma was added and quickly weighed to the nearest milligram. The samples were allowed to evaporate overnight before being placed into the vacuum oven. The samples were dried for two hours, allowed to cool to room temperature, and then re-weighed. The grams of water per milliliter of plasma were calculated by subtracting the weight of the solid from the weight of the plasma.

The concentration of antipyrine per milliliter of plasma at the time of injection was determined by plotting the values of each sample on semilogarithmic paper and extrapolating the straight portion of the time-concentration curve back to zero time. When the observed values did not form a straight line, the average regression line was determined by applying the least squares formula for the concentration of each sample.

$$Y = Na + b\leq X$$

$$XY = a\leq X + b\leq X^2$$

X = the time intervals used

Y = logarithim of the concentration of each sample

b = slope of regression line

a = point where line crosses zero time.

The time-concentration curve showed that the injected dose of antipyrine was completely mixed with the body water at approximately two and one-half hours after injection.

The plasma water antipyrine was calculated by equation I.

(I) Plasma water antipyrine (micrograms/g. water) =

$$\frac{\text{Antipyrine concentration at zero time}}{\text{Grams of water per milliliter of plasma}}$$

Total body water was calculated by equation II.

(II) Total body water (Kg.) =

$$\frac{\text{Plasma water antipyrine}}{\text{Amount of drug injected (g.)}}$$

The per cent of total body water was calculated by equation III.

(III) Per cent total body water =

$$\frac{\text{Total body water (Kg.)}}{\text{Average weight of cow (Kg.)}}$$

CHAPTER IV

RESULTS

Several factors have been found to cause the percentage of total body water to vary in the animal body. In this study total body water determinations were made of identical twin dairy cattle which were handled under different conditions. The effects of age, body fat, fasting, the feeding of thyroxin, and the metabolism rate of antipyrine upon body water content were some of the conditions studied.

The Effects of Age of Cows Upon Body Water Content

Twenty-two total body water determinations were made of identical twins of different ages. The twins ranged in age from 11.0 to 57.8 months. Twins showing evidence of excess fat were excluded from the study. The twins exhibited a range in total body water from 35.6 to 74.8% of their average body weight. The results as indicated in Table IV show rather large variations among the whole group, and variations between cattle of the same age. The correlation of age to per cent total body water was $r = 0.0102$, which was not statistically significant at the 5.0% level of probability. The data thus presented failed to show an inverse relationship between total body water and age, as was found in earlier experiments by Murray (25). Murray, however, determined the total body water of oxen by the desiccation method.

TABLE IV

THE RELATIONSHIP OF AGE TO TOTAL BODY WATER OF IDENTICAL TWIN DAIRY CATTLE

No. of Cow	Age (mo.)	Total Body Water (%)
3'	11.5	60.7
14	16.3	52.0
2	20.4	57.4
29	22.2	74.8
27	32.5	59.4
28	32.5	55.7
9	35.7	64.2
10	35.7	68.3
7	41.4	61.8
8	41.4	69.7
4	41.4	66.6
3	41.4	63.1
487	51.6	65.6
488	51.6	55.1
16	54.6	61.6
17	54.6	56.6
20	56.4	64.6
21	56.4	58.2
22	57.3	35.6
23	57.3	58.5
18	57.8	48.5
19	57.8	56.0

The Relationship of Body Water to Body Fat

Four pairs of identical twin heifers and three pairs of mature, identical twin cows were used for this study. Because of their identical inheritance, one could assume that the physical composition, other than fat, such as bone and muscle would be the same for each pair of twins. Height and girth measurements were determined on the day of injection to see if any difference in fatness could be detected by this method. Twins 1 and 2, 3' and 4', 13 and 14, and 29 and 30 were used in the heifer group. Twins 3 and 4, 16 and 17, and 487 and 488 were used in the cow group. In order to produce a difference in fatness between twins of each pair, twins 1, 4', 13, 30, 3, 17, and 488 were maintained on a high plane of nutrition while their respective mates were maintained on a normal plane of nutrition. The fat heifer group received large amounts of grain and were limited to the amount of hay consumed. The normal heifer group had received grain up to one year of age but thereafter no grain was fed and hay was fed ad libitum. The values of TDN consumed daily, 30 days prior to injection, and the weight, height, and heart girth measurements of the heifers taken on the day of injection are shown in Table V. The minimum requirements of Morrison's Standard (24) were used in the calculation of TDN. These heifers were young, and weekly height and heart girth measurements showed they were still growing, so the increase in

TABLE V

BODY WEIGHTS, HEIGHTS, HEART GIRTH MEASUREMENTS, AND FEEDING LEVELS OF TWIN HEIFERS USED TO COMPARE TOTAL BODY WATER VALUES TO DEGREE OF FATNESS

No. of Heifer	Description of Animal	TDN, % of Morrison's Standard (%)	Body Weights (lbs.)	Height at Withers (in.)	Heart Girth (in.)
1	Fat	107.0	869	48.5	69.0
2	Normal	87.9	659	46.5	60.5
4'	Fat	143.2	532	41.0	55.0
3'	Normal	103.0	440	40.3	50.5
13	Fat	121.0	618	44.8	60.0
14	Normal	71.6	480	43.6	54.0
30	Fat	112.0	712	45.3	64.5
29	Normal	100.0	495	43.9	55.5
Average	Fat	120.8	682	44.9	62.1
	Normal	90.6	518	43.5	55.0
Average Difference		30.2	164	1.4	7.1

weight could not be attributed altogether to an increase in fatness. For this reason, three pairs of mature twin cows were used to measure the effect of body fat on total body water not complicated by growth. Twins 17, 3, and 488 were fed up to 10 lb. of grain daily for 78, 71, and 17 days respectively, prior to total body water determinations, in order to produce a difference in fatness over their control mates. The body weights, heights, and heart girth measurements of the fat cows and their normal controls are shown in Table VI.

The results of this study are shown in Table VII. The normal heifer group exhibited a range in total body water of 52.0% to 74.8% with a mean value of 61.2%. The fat heifer group exhibited a range in total body water of 52.6 to 65.4% with a mean value of 59.5%. The mean difference of 1.7% was not statistically significant at the 5.0% level of probability. The normal cow group exhibited a range in total body water of 63.5 to 73.1% with a mean value of 66.4%. The fat cow group exhibited a range in total body water of 53.0 to 65.5% with a mean value of 59.8%. The mean difference of 6.6% between the fat and normal group was statistically significant at the 2.0% level of probability. The combined groups of normal heifers and cows showed a mean total body water of 64.7% as compared to 59.7% for the fat heifer and cow group. The mean difference of 5.0% was statistically significant

TABLE VI

BODY WEIGHTS, HEIGHTS, AND HEART GIRTH MEASUREMENTS OF TWIN COWS USED TO COMPARE TOTAL BODY WATER TO DEGREE OF FATNESS

No. of Cow	Description of Animal	Body Weight (lbs.)	Height at Withers (in.)	Heart Girth (in.)
488	Fat	1054	48.5	70.0
487	Normal	960	48.1	68.0
17	Fat	1239	50.1	76.0
16	Normal	1157	50.4	75.0
3	Fat	1113	49.5	72.0
4	Normal	1039	49.3	71.0
Average	Fat	1135	49.4	72.7
	Normal	1039	49.3	71.0
Average Difference		96	0.1	1.7

TABLE VII

THE EFFECT OF FATTENING OF IDENTICAL TWIN CATTLE UPON TOTAL BODY WATER

Normal Group		Fat Group	
No. of Cow	Body Water (%)	No. of Cow	Body Water (%)
3'	60.7	4'	65.4
2	57.4	1	64.4
2	--	1	59.5
14	52.0	13	52.6
29	74.8	30	55.5
487	65.6	488	55.1[a]
487	69.3	488	53.0
487	73.1	488	62.9[b]
4	63.6	3	65.5
4	66.6	3	63.1
4	65.8	3	57.2
4	63.6	3	65.5[a]
16	63.5	17	56.8[a]
Average	64.7	Average	59.7

[a]Values for twins after fattening period.

[b]Value for twin after calving.

at the 2.0% level of probability. As shown in Table VII, twin No. 3 of the fat cow group actually showed a higher percentage of total body water than did her control after the fattening period. The value for No. 3 was also higher than that determined for her before the fattening period. It will also be noted that twin No. 488 of the fat cow group showed an increase in total body water after calving. This increased percentage of water was accompanied by a loss of weight.

Body Water in Normal State and Fasted

The effect of fasting on total body water was studied on eight pairs of identical twin cattle of different ages. If the gastrointestinal fill of cattle consisted of a higher percentage of water than the rest of the body weight, a significant drop in total body water after fasting should occur. If it were possible for cattle to reach a constant weight during fasting, then any difference in total body water would reflect a greater significance regarding the fatness of the animals. In this study, the twins were fasted for 16 hours and water was withheld 12 hours prior to injection. It was found that this period of fasting was sufficient for the animals to reach a relatively constant weight. Normal body water values were determined prior to the fasting period.

The results, as shown in Table VIII, indicate that seven of the sixteen animals actually showed an increase

TABLE VIII

THE EFFECT OF FASTING ON TOTAL BODY WATER OF IDENTICAL TWIN DAIRY CATTLE

No. of Cow	Normal Group				Fasted Group				Interval Between Determinations
	Average Weight (lb.)	Weight Lost[a] (lb.)	Body Water (%)	Body Water (lb.)	Average Weight (lb.)	Weight Lost[a] (lb.)	Body Water (%)	Body Water (lb.)	(Days)
3'	440	12	60.7	267	425	10	62.3	265	28
4'	526	20	65.4	344	533	10	68.2	363	28
13	618	16	52.6	325	694	4	49.2	341	123
14	480	16	52.0	250	473	5	58.2	275	123
29	496	19	74.8	371	493	18	48.1	237	35
30	712	20	55.5	395	726	16	33.5	243	35
27	973	50	55.1	536	905	30	60.3	546	15
28	960	48	51.0	490	930	28	60.9	566	7
9	806	30	64.2	519	765	14	62.1	475	7
10	835	26	68.3	570	798	12	62.2	496	7
7	1094	32	61.9	677	1055	26	55.5	585	7
8	989	34	69.8	689	944	28	66.2	625	7
3	954	24	57.2	546	928	12	57.6	534	7
4	860	26	65.8	566	861	22	59.7	514	7
16	1123	42	61.6	692	1081	30	63.2	683	8
17	1171	30	56.6	663	1142	28	55.4	633	8
Av.	815	28	60.6	494	797	18	57.9	461	28
Av.[b]	845	29	60.3	510	824	19	59.8	492	27

[a]Weight lost during the injection and sampling period.

[b]Average excluding Twins No. 29 and 30.

in total body water, while nine animals showed a decrease in body water after fasting. The group exhibited a range in total body water of 51.0 to 74.8% with a mean value of 60.8% before fasting, as compared to a range of 33.5 to 68.2% and a mean value of 57.6% after fasting. The average drop of 3.2% in total body water after fasting was not statistically significant at the 5.0% level of probability.

Effects of Hyperthyroidism Upon Body Water

One important effect of feeding thyroxin to dairy cattle is a loss in body weight during the feeding period. After the withdrawal of thyroxin from the ration, body weight increases rapidly and in some cases even to a higher weight than the treated cow's normal control. Some investigators have hypothesized that this loss in weight might be due to a dehydrating effect during the treatment period and a restoration of normal body water after the withdrawal of the thyroactive material.

To study this effect of thyroxin, three pairs of identical twin cows and one single twin cow which served as her own control were used. One twin of each pair had received 100.0 mg. of l-thyroxin daily prior to injection of antipyrine. Higher pulse rates, respiration rates, and rectal temperatures showed that the treated cows were in a state of hyperthyroidism, as compared to their normal controls. Normal body water values were determined of twins 6 and 23 before

receiving thyroxin. Body water values were also determined of them approximately one month later during the thyroxin-feeding period. Normal body water values were determined for twins 8 and 9 approximately one month after the withdrawal of thyroxin from the ration. It was assumed that at this time the functioning of the thyroid gland had returned to normal.

The results of this study are shown in Table IX. Twins 9 and 23 showed an increase in percentage of total body water and twins 6 and 8 showed a decrease in percentage of total body water during the thyroxin-feeding period. Obviously the calculated percentage of water for No. 9 is impossible. This high value was probably due to a change in the rate of metabolism, giving a low concentration of antipyrine in the plasma at the time of injection. Twins 7 and 10 of the normal group showed a decline in per cent body water between the two injection periods. This decline was probably due to the fact that these cows were further advanced in lactation and getting fatter. The data thus presented definitely does not support the hypothesis that thyroxin-feeding causes a dehydrating effect on the treated cows, and a restoration of normal body water after the withdrawal of thyroxin from the ration. These results are in accord with those found by Swanson (39) in a previous experiment.

TABLE IX

THE EFFECT OF FEEDING THYROXIN ON THE TOTAL BODY WATER OF IDENTICAL TWIN DAIRY CATTLE

Normal Group		Treated Cows Not Receiving Thyroxin		Normal Group		Period During Thyroxin Feeding	
No. of Cow	Body Water (%)	No. of Cow	Body Water (%)	No. of Cow	Body Water (%)	No. of Cow	Body Water (%)
10	68.3	9	64.2[a]	10	53.8	9	106.7
7	61.9	8	69.7[a]	7	47.6	8	51.2
22	35.6	23	58.5[b]	22	21.7	23	61.5
--	--	6	60.8[b]	--	--	6	48.9

[a]Normal values obtained one month after withdrawal of thyroxin.

[b]Normal values obtained before feeding thyroxin.

Blood Level of Antipyrine

The injected dose of about 2 g. of antipyrine per cwt. was found to be sufficient for obtaining reliable plasma concentrations for 4.5 hours. As shown in Figure 1, the time required for complete mixing of antipyrine with the total body water of the cow was 2.5 hours after injection. The blood level of antipyrine as shown in Figure 1, was typical of most of the determinations made. Had the injected dose not been thoroughly mixed, a higher concentration at the 2.5 hours interval would be shown above the normal regression line of antipyrine.

To determine how long after injection that antipyrine could be detected in the plasma, blood samples were taken from one pair of twins at 2.5, 3.0, 3.5, 4.0, 4.5, 5.5, 6.5, and 7.5 hour intervals after the injection of 25 g. of antipyrine. The results of one of the twins, as shown in Figure 2, indicate that detectible amounts of antipyrine were found in the plasma sample taken 7.5 hours after injection; however, the metabolism rate of antipyrine was irregular after 4.0 hours. The results of the other twin was similar to the one shown in Figure 2. Such irregularities could seriously alter the calculated body water percentages, unless enough samples are taken to establish the true linearity of antipyrine metabolism for each cow.

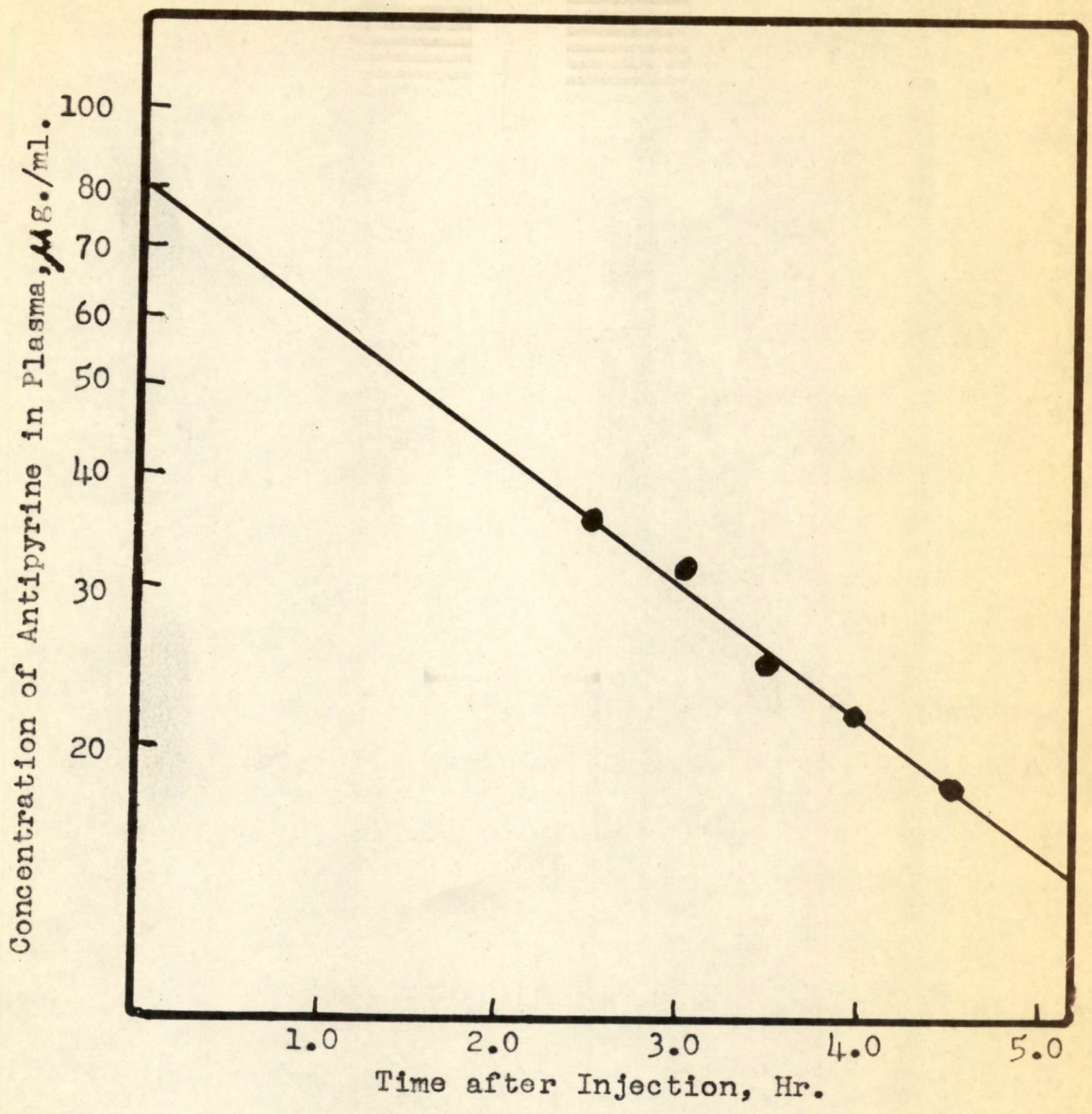


Figure 1. The disappearance rate of antipyrine from blood plasma of a cow after the injection of a 15 g. dose.

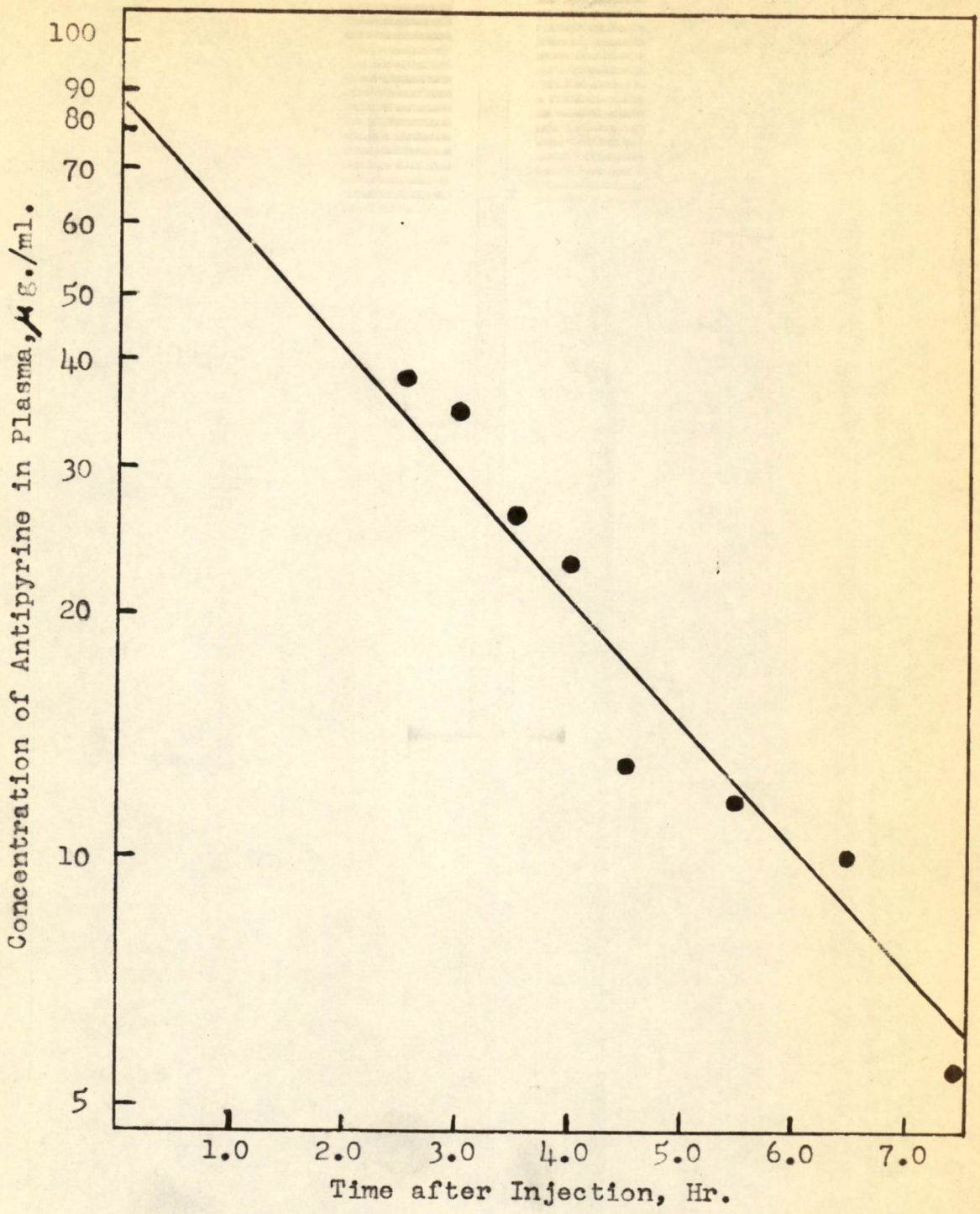


Figure 2. The concentration of antipyrine over a 7.5 hr. period in plasma of one cow after the injection of a 25 g. dose.

Rate of Metabolism of Antipyrine

The reliability of the antipyrine dilution method depends mainly on being able to estimate the concentration of antipyrine in the plasma at the time of injection. To accomplish this, the injected dose of antipyrine must be metabolized slowly in the body and at a constant rate. The metabolism rate of antipyrine was determined for each of the 65 determinations of total body water made in this study. The range in metabolic rates was from 16.9 to 60.1% per hour, with a mean rate of 33.0% per hour. For the same group, there was a range in total body water of 21.6 to 83.5%, with a mean rate of 59.7%. The results of this study are shown in Figure 3. It was suspected that there was some correlation of metabolic rate to the per cent total body water. Statistical analysis of these data showed that the correlation coefficient was $r = 0.437$, which was highly significant at the 1.0% level of probability.

The correlation of metabolic rate and total body water was determined for each of the normal-fat group, normal-thyroxin-fed group, and the normal-fasted group. It was suspected that the metabolic rate of the twins might be affected by these adverse conditions.

The correlation of metabolic rates to per cent total body water of the normal and fat group is shown in Table X. The normal group showed a mean metabolic rate of 33.5%

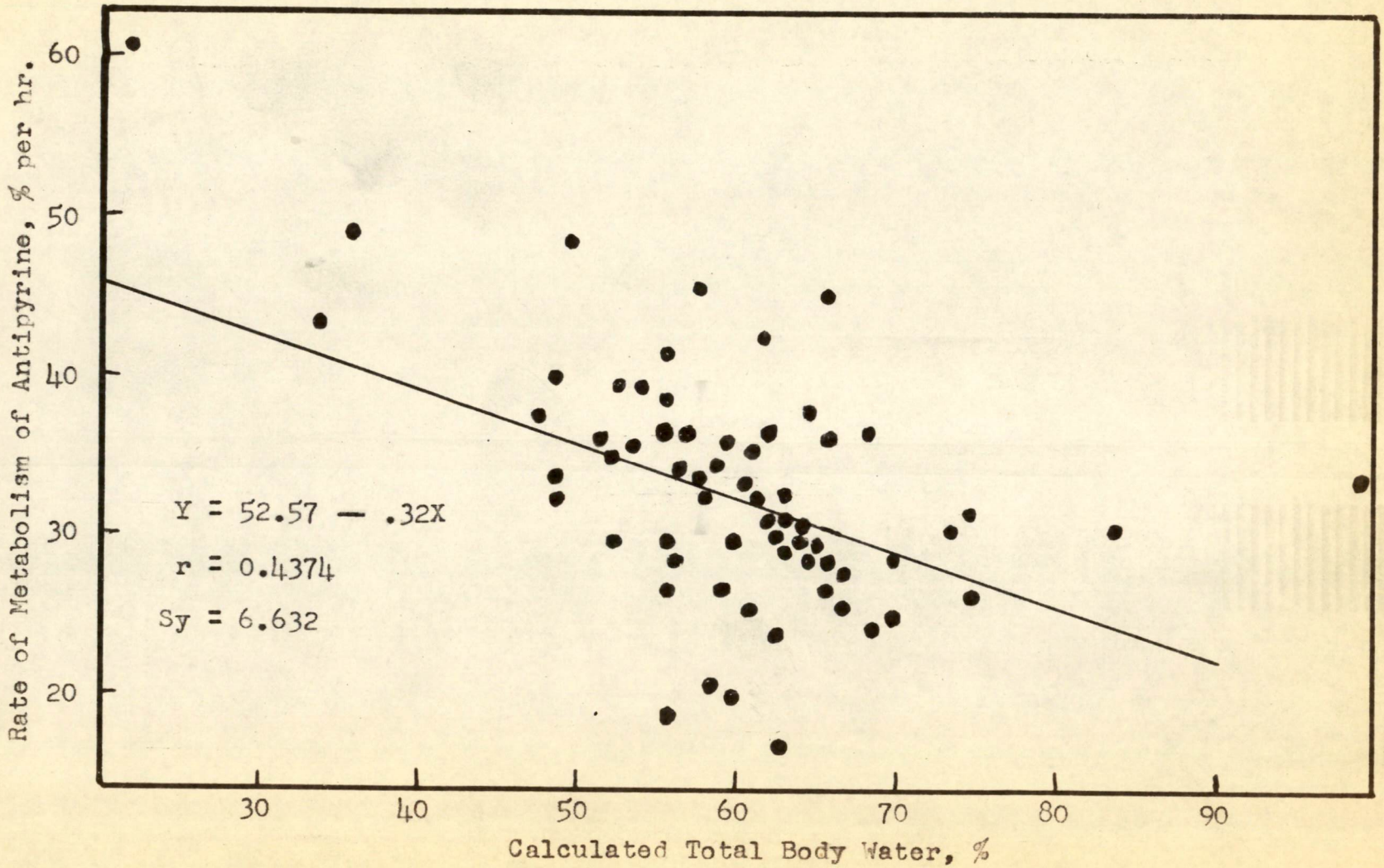


Figure 3. The correlation of rate of antipyrine metabolism to percentage of total body water, 65 determinations.

TABLE X

COMPARISON OF METABOLISM RATES OF ANTIPYRINE OF FAT AND NORMAL GROUPS

Normal			Fat		
No. of cow	Metabolism Rate (%/hr.)	Body Water (%)	No. of cow	Metabolism Rate (%/hr.)	Body Water (%)
4'	45.4	65.4	3'	27.5	60.7
14	29.7	52.0	13	39.7	52.6
2	45.3	57.4	1	38.0	64.4
29	26.2	74.8	30	41.5	55.5
4	28.1	65.8	3	33.9	57.2
487	30.3	73.1	488	33.9	62.9
16	29.6	63.5	17	34.1	56.8
Average	33.5	64.5	Average	35.5	58.5

compared to 35.5% for the fat group. The correlation coefficients were r = .3251 and r = .3186 for the normal and fat groups respectively. Both correlation coefficients were not statistically significant at the 5.0% level of probability.

The results of the normal-thyroxin-fed group are shown in Table XI. The normal group showed a mean metabolic rate of 43.3% and 45.9% total body water as compared to a mean metabolic rate of 37.9% and 66.9% total body water for the thyroxin-fed group. The results indicate that the feeding of thyroxin did not increase the metabolic rate of the treated cows over their normal controls, nor did it cause a dehydrating effect of the cows.

The results of the normal-fasted group are shown in Table XII. Eight pairs of identical twins showed a mean metabolic rate of 33.1% per hour and a mean total body water of 60.8% before fasting as compared to a mean metabolic rate of 31.8% per hour and a mean total body water value of 57.6% after fasting. The correlation coefficient of the group before fasting was r = .5629 as compared to the correlation coefficient of r = .5729 after fasting. Both correlation coefficients were statistically significant at the 2.0% level of probability.

The Relation Between Body Measurements and Body Water

The relationship of height and heart girth measurements to per cent total body water of all mature cows was studied.

TABLE XI

COMPARISON OF METABOLISM RATES OF ANTIPYRINE OF NORMAL AND THYROXIN-FED CATTLE

Normal			Thyroxin-fed		
No. of cow	Metabolism Rate (%/hr.)	Body Water (%)	No. of cow	Metabolism Rate (%/hr.)	Body Water (%)
10	39.3	53.8	9	33.8	106.0
7	37.5	47.6	8	35.1	51.2
22	60.1	21.6	23	42.7	61.5
6	36.5	60.8	6	40.0	48.9
Average	43.3	45.9	Average	37.9	66.9

TABLE XII

COMPARISON OF METABOLISM RATES OF ANTIPYRINE OF CATTLE BEFORE AND AFTER FASTING

No. of cow	Before Fasting		After Fasting	
	Metabolism Rate (%/hr.)	Body Water (%)	Metabolism Rate (%/hr.)	Body Water (%)
3'	27.5	60.7	30.0	62.3
4'	45.4	65.4	36.9	68.2
13	39.7	52.6	48.9	49.2
14	29.7	52.0	26.8	58.2
29	26.2	74.8	33.4	48.1
30	41.5	55.5	43.3	33.5
27	38.6	55.1	35.7	60.3
28	35.2	51.0	32.2	60.9
9	28.5	64.2	23.3	62.1
10	24.0	68.3	16.9	62.2
7	30.6	61.9	26.7	55.5
8	28.4	69.7	25.2	66.2
3	33.9	57.2	32.6	57.6
4	28.1	65.8	29.8	59.7
16	36.6	61.6	30.7	63.2
17	36.5	56.6	36.4	55.4
Average	33.1	60.8	31.8	57.6

Height and heart girth measurements for cows of different breeds and weights as compiled by Brody (6) were used as normal values. The ratio of height to heart girth of the twins was compared to the height-heart girth ratio of the normal values. Brody stated that the chest girth is one of the linearly measured complexes most closely correlated with changes in body weight; and height at withers, on the other hand, is one of the measurements least correlated with changes in body weight. After cows reach their maximum growth, then any additional gain in weight could be estimated by heart girth measurements. In this study, all animals whose ratio exceeded that of the normal ratio were considered to be fatter than normal. The deviation in the observed ratios from the normal ones were divided into four ranges. They were -.01 to -.059, -.06 to -.10, +.01 to +.059, and +.06 to +.10. Four cows representing eight total body water determinations fell into the range of +.06 to +.10, which was the fattest range. This group had a mean total body water value of 56.6%. Six cows representing eight total body water determinations fell into the range of +.01 to +.059. This group had a mean body water value of 63.1%. Eleven twins representing 23 total body water determinations fell into the range of -.01 to -.059. This group had a mean total body water value of 60.7%. Five twins representing eight total body water determinations fell into the range of -.06 to -.10, or the least fat group. This group had a mean total body water value of 60.7%.

Although the group denoted fattest by measurements was also fattest according to body water, the results show no definite correlation of height and heart girth measurements to total body water over the whole range of observations. Several animals fell into different categories of the height-heart girth rates at different times of total body water determinations.

CHAPTER V

DISCUSSION

A total of 65 total body water determinations have been made of identical twin dairy cattle. An evaluation of the antipyrine dilution method was made of cattle which were handled under different conditions. Such conditions under consideration were the effect of age, fatness, fasting, and the feeding of thyroxin. Although the number of cattle in each group was small, it was believed that the use of identical twins as normal controls would increase the significance of comparisons with low numbers.

The Effect of Age of Cows Upon Body Water Content

The results of this study, as shown in Table IV, indicated no significant inverse relationship between age and per cent total body water such as had been reported by Murray (25). Steele et al. (38) found no inverse relationship of age and total body water of 82 human subjects of different ages. They explain their results by stating that the effect of fatness is probably greater on total body water than age. MacFadden and Richards (19) recently determined the per cent of total body water of Holstein dairy calves, which had received 50.0 mg. of aureomycin daily to 16 weeks of age. Body water determinations were made at 1, 4, 8, 12, and 16 weeks of age. Their results showed that total body water

increased rapidly for both groups after eight weeks of age, with the increase slightly more for the treated group. These results suggested that the increase in total body water after eight weeks of age was due to rumen development, because prior to eight weeks the rumen is relatively small. Ruminant animals, such as cattle, have the capacity for holding more water than non-ruminants. The size of the rumen increases with age, so it is possible for a large cow to hold more water in relation to her weight than a young heifer. Another possible explanation is the low fattening tendency of aged dairy cows, due to temperament and lactation.

Relationship of Body Water to Body Fat

Several methods have been devised to estimate the fatness of animals and obesity in humans. Heart girth measurements, skinfold thickness, visual inspection, specific gravity, and chemical analysis of the carcass are some methods that have been used. Several investigators have suggested the use of total body water data as a method of calculating the percentage of body fat since body water and body fat bear an inverse relationship (16, 36, 25). The equation devised by Pace and Rathbun (28) ($\%\ \text{Fat} = 100 - \frac{\%\ \text{Body Water}}{0.732}$) could be used for determining the percentage of body fat, since the percentage of water in the lean-body-mass is relatively constant at 73.2%. Osserman *et al.* (27) found that

the standard deviation of body fat determined from the antipyrine dilution method was ± 3.8%.

In this study, an evaluation of the antipyrine dilution method was made of four twin heifers and three mature twin cows which were maintained on a higher plane of nutrition than their normal controls. Visual inspection, weights, heights, and heart girth measurements, as shown in Tables V and VI, indicated that the twins maintained on the higher plane of nutrition were fatter than their normal controls.

In the heifer group, the mean total body water value of the fat heifers was only 1.7% less than for their normal controls, although they appeared to be considerably fatter. The small difference was not statistically significant at the 5.0% level of probability. In applying the formula of Pace and Rathbun (28), the fat heifer group would show a mean percentage of body fat of 19.0% as compared to 16.7% body fat of the normal controls. Even though visual inspection and heart girth measurements indicated that there was a considerable difference in fatness between the two groups, the difference in per cent body fat as calculated by the Pace and Rathbun equation was only 2.3% body fat. The fat heifers were growing at a more rapid rate than their normal controls, so possibly the results of the fat heifers were complicated by growth.

In the mature cow group, the fat twins showed 6.6% less total body water than their normal controls. This difference was statistically significant at the 2.0% level of probability. The calculated per cent of body fat for the fat group was 19.3% as compared to 12.3% for the normal controls. As shown in Table VI, there was very little difference in height between twins of the same pair. Since height is a good measurement for estimating growth, this small difference would show that the body water determinations were not complicated by growth.

Although several investigators (16, 17, 25, 36) have found total body water to be inversely proportional to total body fat of different animals; the results of this study indicate that more research is needed to study the effect of fatness of growing heifers, or the effect of rate of growth upon the fat: water ratio in the body.

Body Water in Normal State and Fasted

When normal total body water values were determined for some of the twins, it was noticed that they lost a considerable amount of weight during the injection period. Although all of this weight loss was not due to water loss, it was believed the amount lost might influence the significance of the calculation of per cent total body water. It was also noticed that identical twins of approximately the same weight

showed considerable variation in per cent total body water. This could have been due to one twin of the pair increasing her body fill by consuming a large amount of water before the injection period.

To minimize the losses of weight during the sampling period and to see if these losses would reduce the variation in the calculated percentage of body water, both twins of each pair were fasted from 16 to 24 hours and water was withheld for 12 hours prior to the injection period. There was less weight lost during the sampling period of the fasted cows than there was for the non-fasted cows (Table VIII). Fasting did not decrease the differences in percentage of total body water between twins of a pair for all pairs tested, even though the weight lost during the sampling period was less for the fasted group than for normal group. The results of all trials, as shown in Table VIII, show that the average body weight lost due to fasting was 18 lb., whereas the average body water lost due to fasting was 33 lb. Thus, more pounds of water were lost than could be accounted for by total weight loss. The values for twins 29 and 30 were unreasonably low for both the normal and fasted periods, possibly because of some abnormality in their metabolism of antipyrine. In order to make an accurate evaluation of the effect of fasting on body water content, the values for these twins should be omitted. After the omission of these values,

the twins lost an average of 21 lb. of body weight due to fasting, and the loss of water due to fasting was 16.7 lb. These results indicate that about 80.0% of the weight lost in fasting was due to loss of body water. Since this exceeded the average concentration of total body water in the normal cows, the effect of fasting upon total body water was only a slight reduction in percentage from 60.3% to 59.8%. The average drop in per cent water due to fasting was not significant. Therefore, fasting was not necessary for obtaining reliable estimates of the body water content of cows.

As shown in Table VIII, the body water content of one pair of twins of the fasted group was determined 123 days after normal body water values were obtained. During this length of time other factors, such as age, degree of fatness, or insensible loss of water due to differences in environmental temperature, could have altered the percentage of body water besides the effect of fasting.

The Effects of Hyperthyroidism Upon Body Water

The results, as shown in Table IX, show that two twins increased in total body water during the thyroxin-feeding period and two twins showed a decrease in total body water during the thyroxin-feeding period. The value for No. 9 during the thyroxin-feeding period was unreasonably high. A very low concentration of antipyrine in the plasma at the time of injection was calculated for this cow. This low

concentration of antipyrine could possibly be due to slow or non-linear transformation in the body or antipyrine bound to plasma protein to an extent that it could not be detected by the precipitation procedure. The pulse rates, respiration rates, and rectal temperatures of the treated cows were higher than the controls, indicating that they had a higher metabolic rate than their normal controls. The value for No. 22 of the normal group was unreasonably low. Even though this cow did not receive thyroxin, she metabolized the injected dose of antipyrine faster than any of the other twins (60.1% per hr.). Possibly other factors are involved in the transformation of antipyrine in the body besides the metabolic rate of the cow. Although the normal cows were slightly heavier than the treated cows, it was believed that this difference was insignificant in affecting total body water content since it had been shown previously that most of this weight difference was due to gastrointestinal fill (39). Twins 6 and 8 of the treated group showed a lower percentage of body water during the thyroxin-feeding period. These two cows had also lost weight during the treatment period. The change in body weight due to thyroxin-feeding was probably due more to a loss of fill rather than a loss of total body water. These results agree very well with those reported by Swanson (39).

Blood Level of Antipyrine

The blood concentration of antipyrine does not remain constant. It is metabolized or transformed in the body at a rate depending on the subject injected. Previous experiments in humans and dogs have shown that the concentration of antipyrine per milliliter of water in the tissues is the same as in blood. To determine the true metabolism rate of antipyrine, several samples of blood must be taken after injection and the dosage of antipyrine should be at a sufficient level to obtain a reliable value in the plasma at these different intervals of withdrawal.

In this study, 2 g. of antipyrine per cwt. were found to be an adequate dose for obtaining reliable plasma concentrations for 4.5 hours. The results, as shown in Figure 1, indicate that the injected dose was completely mixed with the total body water in 2.5 hours. This was the length of time that Kraybill *et al.* (16) found antipyrine to be mixed in beef cattle. Kraybill, however, used 0.7 to 1.1 g. of antipyrine per cwt. as the injected dose. Soberman (35) found that even distribution was achieved 2 hours after the injection of 75 mg. of antipyrine per Kg. weight in dog, monkeys, and rabbits. He also found that the smaller the animals in a group the faster the rate of antipyrine metabolism and the faster even distribution was achieved. If this were true of dairy cattle, one would expect the time for complete mixing of antipyrine

with total body water to be less than for beef cattle, since dairy cattle usually have higher metabolic rates than beef cattle.

Measurable amounts of antipyrine were obtained in the plasma samples of two cows 7.5 hours after the injection of 25.0 g. The results, as shown in Figure 2, indicate that the metabolism rate of antipyrine was irregular after four hours. Although measurable amounts were obtained 7.5 hours after injected, it is questionable if these values are reliable in determining the true linearity of the regression of antipyrine in the body. The remaining antipyrine in the blood after 4.5 hours might be bound to a certain extent so average values cannot be determined accurately from these samples.

Rate of Metabolism of Antipyrine

The correlation coefficient ($r = 0.437$) of metabolism rate to per cent total body water of 65 determinations in this study was highly significant at the 1.0% level of probability. The mean rate for these determinations was 33.0% per hour. This is higher than the rates reported for humans, 6.1% (4), for beef cattle, 20.0% (16), and for dogs, 30.0% (35), and lower than the rate reported for swine, 36.0% (17). Soberman (35), who determined the metabolism rate of antipyrine by dogs, postulated that the antipyrine dilution method might not be practical for the dog because of the fast transformation of antipyrine in the body. These results suggest

that the method would be more practical on animals which metabolize the injected dose of antipyrine slowly in the body. In this study, some of the twins metabolized antipyrine at the same rate but showed different concentrations in the plasma at the time of injection. Some of the twins metabolized antipyrine at different rates, but they showed the same concentration in the plasma at the time of injection. This resulted in differences in body water due to differences in weight rather than differences in metabolism rates of antipyrine. Evidently, there are other factors involved, such as temporary storage in the liver and rate of excretion, which might alter the concentration of antipyrine in the plasma besides the metabolism rate.

There does not appear to be a direct relationship between oxygen metabolism and antipyrine metabolism of different animals. Dogs which have higher basal metabolic rates than dairy cattle, metabolize antipyrine at a slower rate. Dairy cattle usually have higher basal metabolic rates than beef cattle, and the results obtained from dairy cattle in this study show they metabolize antipyrine at a higher rate. Man has a higher basal metabolic rate than dairy cattle, although the metabolism of antipyrine in the body is considerably lower. Swine have a lower basal metabolic rate than man, although antipyrine metabolism is considerably higher. More research is needed to determine the factors affecting

the disappearance rate of antipyrine in plasma of dairy cattle.

Relationship Between Body Measurements and Body Water

It was believed that height and heart girth measurements might be good criteria for estimating body fat. In this study, body fat demonstrated a significant inverse relationship to total body water of mature cows. The degree of fatness of the twins determined by measurement was obtained from the deviation from the "normal" measurements as compiled by Brody (6). Total body water determinations were made on some twins more than once over a ten-month period. These twins fell into different levels of fatness as determined by measurements at different times of total body water determination. Although the twins found to be fatter by body measurement also were found to be fatter by body water measurement, several cows shown to be less fat by body measurement, demonstrated low percentages of body water. The variability of the height-heart girth ratio of any one cow as compared to height-heart girth ratio of the normal cow as derived from the average of several cows, might be so great that this method of estimating total body water on the basis of fatness would not be practical for all cows. Cattle of low height, but normal heart girth, may be considered most fat by body measurement, although they may be less fat as determined by

total body water. Possibly if height and heart girth measurements were taken from one cow on several successive months, accompanied by successive total body water measurements, then a significant correlation between the two methods might be shown.

CHAPTER VI

SUMMARY

Total body water determinations have been made of identical twin dairy cattle by the antipyrine dilution method. The effect of age, fatness, fasting, thyroxin feeding, and metabolism rate of antipyrine on per cent of total body water were studied. The relation between body measurements and body water was also studied.

Twenty-two total body water determinations were made of identical twins ranging in age from 11.0 to 57.8 months. The average total body water was 59.7% of their average body weight. The correlation of age to per cent total body water in this study was r = 0.010, which was not statistically significant at the 5.0% level of probability.

Total body water was found to be inversely proportional to body fat of mature cows. The fattened cows averaged 59.8% total body water compared to 66.4% for the normal controls. This difference, 6.6%, was highly significant at the 2.0% level of probability. Under the conditions of this experiment, body fat showed very little effect on body water of growing heifers. The fat heifers contained 1.7% less body water than their normal controls. This difference was not significant at the 5.0% level of probability.

Under the conditions of this study, fasting did not reduce the variability of the calculated percentages of body water that occurred in the normal state. The weight lost by the fasted cattle averaged 80.0% water. Fasting produced a slight reduction of total body water from 60.3% to 59.8%. This small difference was not significant.

The feeding of thyroxin failed to show an independent effect on total body water of four treated twins as compared to their control mates. Two cows increased in total body water, and two cows decreased in total body water during the thyroxin feeding period.

Two grams of antipyrine per cwt. were found to be an adequate dose for obtaining reliable plasma concentrations for 4.5 hours. Even distribution of antipyrine throughout the total body water was accomplished in 2.5 hours.

The average metabolic rate of antipyrine was 33.0% per hour. The correlation of metabolic rate of antipyrine to total body water was $r = 0.437$ for 65 determinations. This correlation was highly significant at the 1.0% level of probability.

Some cows estimated to be fatter than normal by body measurements were also estimated to be fatter from total body water values. The estimates from measurements were affected greater by the conformation of the cows, while those from total body water were not.

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