STUDIES ON THE ENERGY METABOLISM OF RUMINANTS

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for the degree of Doctor of Philosophy
in the Faculty of Science.

by

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CONTENTS

SYNOPSIS

Published evidence suggests that feeding standards currently in use, that are based on the total digestible nutrient, Scandinavian feed-unit or net energy (starch equivalent) systems of evaluating feed energy, are not a sound guide for the feeding of cattle or the organization of feed supplies. Errors inherent in total digestible nutrient and feed-unit standards are largely a reflection of the limitations of the two systems. In contrast the net energy system gives a thermodynamically sound measure of the efficiency of utilization of feed energy but determined net energy values for individual feeds show wide variation and at the present time there are insufficient data to provide a reliable standard.

In Section I of the thesis, an analysis has been made of sources of error in the determination of net energy values. Major errors are shown to arise in the determination of the net utilization of metabolizable energy, from variations in the efficiency with which cattle utilize feed energy and also from a magnification of technical and interpretational errors by the design of experiment used. The conclusion is reached that, to overcome variations due to the individuality of cattle used as test animals, net energy values should be determined on a much wider scale than hitherto. This would be possible if a simple method of determining energy exchange in cattle could be developed to replace the laborious and time-consuming calorimetric method. To this end, it has been proposed that fat storage should be estimated indirectly, from the gain in body weight less the protein and ash retentions determined by conventional balance methods and the water retention determined by some indirect means, a problem that has formed the basis of much of the experimental work reported in the thesis.

Analytical methods used in the course of the work are described in the first part of Section II. In the second part, the details of construction and of operation of a closed-circuit

respiration chamber for the determination of the energy exchange of the calf are given.

Indirect methods of estimating water retention in cattle are discussed in Section III, and it is suggested that a suitable method would be to predict water retention from body retentions of sodium and potassium. Analyses of muscle, liver, fat, brain tissue, serum, erythrocytes, posterior chamber fluid and pericardial fluid from cattle of widely differing ages, for water, sodium and potassium are reported, and it is shown that with the exception of brain tissue and serum, the water content of these tissues can be predicted from the equation:-

$$\underset{(g./100\ g.)}{\text{Water}} = 0.2922 \underset{(mg./100\ g.)}{\text{Na}} + 0.1471 \underset{(mg./100\ g.)}{\text{K}}$$

This equation is shown to apply also to cartilage but to underestimate the water content of skin and to overestimate grossly the water content of bone. Part of the sodium of bone is not associated with water but a means of correcting for sodium stored in this way is given. The prediction equation is shown to apply also to gut contents and to the foetus and uterine fluids, with the exception of abomasal contents and allantoic fluid.

In Section IV the empirical equations developed in Section III have been used to predict water retention in experiments with milk-fed calves and the results obtained have been compared with water retention determined simultaneously by methods based on respiration calorimetry. Though the overall agreement was good, over short periods marked discrepancies arose due to day-to-day variations in the excretion of water, sodium and potassium. The errors of prediction of water retention in these experiments suggested that for the determination of the energy exchange of the calf, the indirect method would be less satisfactory than the classical calorimetric method.

Similar experiments with sheep are described briefly in Section V but in many of them the indirect method grossly underestimated energy storage, due to a secretion of potassium through the skin of

sheep. Though the results could not, therefore, be used to assess the probable accuracy of the indirect method for the determination of energy exchange in cattle, they showed that difficulties would arise from the high potassium content of many cattle feeds if the method was applied to cattle. Under most circumstances, the intake of potassium by cattle would be so large that errors in the determination of potassium contents of feeds would in themselves be sufficient to prevent the accurate prediction of water retention, and therefore of energy storage, in cattle according to the scheme outlined.

INTRODUCTION

In the past 50 years there has been a dramatic increase in our knowledge of animal and human nutrition. At the end of the nineteenth and the beginning of the twentieth centuries nutrition was concerned solely with energy and nitrogen metabolism, whereas today, nutritional status is assessed by reference to a large number of essential food constituents. Yet, as Huffman and Duncan (1944) have pointed out, the most common dietary deficiency in farm stock under practical feeding conditions is one of energy, and it arises not only from local or regional shortages of feed but, to a greater extent than is generally realised, from a lack of knowledge of optimal levels of feeding (Blaxter, 1950). About half a century after the pioneering work of Kellner (1905) and Armsby (1917) on the development of scientific feeding standards for farm animals, there is still no fully accepted, or indeed acceptable, basis for the calculation of the energy requirements of cattle or of the ability of the various feeds to meet these requirements.

Energy feeding standards for cattle

The first outstanding progress in the evaluation of animal feeding-stuffs was made by Thaer in 1809 (see Thaer, 1809, 1810, 1812, 1837, 1880), when he introduced a system based partly on crude chemical analyses of feeds and partly on the results of feeding trials in which the value of feeds was assessed by reference to hay. With the progressive accumulation of knowledge of the chemical composition of feeds, his standard was subsequently modified by Boussingault (1839), by Liebig (1842), by Grouven (1858) and others until, in 1864, Wolff (1864) attempted to express feed-values solely in terms of the protein, fat, carbohydrate and fibre that a feed contains. Thus, the biological aspect of Thaer's system was lost.

Total digestible nutrient system During the course of the nineteenth century, it was gradually recognised that a part of the feed energy is excreted by an animal in the faeces and urine (see Henneberg, 1860) and Wolff in 1874 made the first attempt to express the nutritive requirements of the dairy cow in terms of digestible nutrients. Subsequently, many assessments have been made of the relative value of feeds in terms of total digestible nutrients, commonly referred to as T.D.N. (Atwater, 1874-5, 1890; Hills, 1900; Haecker, 1903; Hills, Jones and Benedict, 1910; Woll and Humphrey, 1910; Savage, 1912). The standard compiled by Morrison (see Morrison, 1949) is still widely used; in it the total digestible nutrients of feeds are calculated as the sum of the digestible protein, crude fibre and nitrogen-free extract and 2.25 times the digestible fat.

Net energy (starch equivalent) system The most significant development in the history of the evaluation of animal feeding-stuffs came, however, with the formulation of the net energy concept by Kühn and Kellner of Germany (Kellner, 1905, 1912, 1920; Kellner and Köhler, 1900) and, independently, by Armsby of the U.S.A. (Armsby, 1905, 1909, 1917). The concept takes account of all energy losses associated with the utilization of feed energy; namely losses in the faeces, urine and expired combustible gases and the direct heat losses arising from the ingestion, digestion and metabolism of a food. Thus, a thermodynamically sound measure of that part of the feed energy available for body maintenance, for muscular activity and for production is provided. According to the net energy system, the energy value of a food for beef production would be measured as the calorific equivalent of the fat and protein deposited in the body, and for milk production it would be measured as the calorific equivalent of the milk produced, after a correction had been made for any change in the fat or protein content of the body. Armsby chose as his basic unit the net calorie, whereas Kellner expressed his food values in terms of the value of pure

starch for fattening. The units are, however, fully interchangeable, 1 kg. of Starch Equivalent being equal to 2360 net Calories for fattening.

Scandinavian feed-unit system In the Scandinavian countries, a system of estimating the comparative value of feeds for milk production has developed which is similar in concept to Thaer's system of 'hay values' but uses as a standard a concentrate feed of barley or a barley-oat mixture. The early standards were based on extensive feeding experiments carried out by Fjord and his collaborators in Denmark (see Eskedal, 1954) and by Winkel, Svendsen and Hansson in Sweden (see Hansson, 1902) but these experiments were not subject to chemical control. Hansson (see Hansson, 1923) has since published a scientific standard for the feeding of dairy cows based on the feed-unit system and derived from an extensive series of carefully controlled feeding trials in which attention was given to the chemical composition of the feeds used and of the milk produced. In it, he expressed the milk producing values of feeds in terms of a unit of digestible starch and provided factors for the calculation of values on the basis of the chemical composition and digestibility of feeds.

Critique of the different systems of evaluating energy requirements of cattle and the energy contents of feeds

Many practical feeding standards based on one or other of the above systems have been published in the last 50 years but there is much uncertainty as to which, if any, of them provide a reliable guide for the feeding of cattle. In a critical review of feeding standards for cattle Blaxter (1950) has shown that the energy requirements for maintenance and milk production given in the primary standards differ very markedly. Expressed in terms of net energy for fattening, the extreme variation noted in maintenance requirement calculated according to different standards was of the order of 40% and similar discrepancies were found in the relative values of the various foodstuffs as assessed by the different

standards. Such differences make the use of any published standard suspect.

Of the three systems of evaluating the energy contents of feeds and energy requirements detailed above, the net energy (starch equivalent) system is theoretically the most satisfactory since it is the only one which takes into account all losses of energy associated with the utilization of feed energy. The most serious criticism of the system is that put forward by Forbes (1933) and by Mitchell (1934, 1937) that the net utilization of feed energy is only at a maximum when the feed forms part of a balanced ration. It is undoubtedly true that energy utilization is affected by gross nutritional deficiencies (Kleiber, 1945-6), though not invariably so (Blaxter and Rook, 1955), and possibly also by gross nutritional imbalance, but an analysis by Breirem (1944, 1953) of Kellner's original results indicates that variations in crude protein and crude fibre content which occur in practical rations are insufficient to influence significantly the utilization of feed energy.

In the development of a practical feeding standard, however, factors other than the theoretical correctness of the system on which it is based determine its usefulness. The object of a standard is that it should serve not only as a guide to the feeding of individual cattle but also in the organization of feed supplies on individual farms and on a national scale. Since the utilization of feed energy is known to vary markedly from one animal to another and also with one animal from time to time, it is critically important that any standard should be based on determinations made with a fairly large number of cattle under a variety of circumstances.

The technical difficulties associated with the determination of net energy and starch equivalent values with cattle by the classical method of respiration calorimetry have seriously limited the number of determinations on which a standard can be based (to the extent that most of the values reported in the literature are

the result of the early work by Kellner and Armsby) and consequently the mean values reported for the various feeds are of mixed significance. Kleiber, Regan and Mead (1945) have calculated that the coefficient of variation attached to the net energy value of starch, which formed the basis of Kellner's standard, was ± 11%, and Meigs (1925) has pointed out that in determining the net utilization of the metabolizable energy of a clover hay Armsby and Fries (1905, 1908) obtained values as widely scattered as 51, 78 and 93%.

Total digestible nutrient standards and Scandinavian feed-unit standards are therefore frequently used in preference to the net energy and starch equivalent standards, since they are based on the results of very extensive feeding trials and the mean values for the various feeds are known with a fair degree of certainty. There does not appear to be, however, a sufficiently general appreciation of the limitations of these standards. An analysis by Breirem (1944) of results obtained by Kellner has shown that the digestible nutrients of roughages are not used by an animal with the same efficiency as those contained in concentrate feeds and the total digestible nutrient system is thus not suitable for the comparison and evaluation of feeds of different types. This has been emphasized in practical experiments conducted by Moore and co-workers (Irvin, Shaw, Saarinen and Moore, 1951; Moore, Irvin and Shaw, 1953). A more convincing illustration of this point has been provided recently by Blaxter and Graham (1954). In the Scandinavian feed-unit system, changes in body substance are allowed for by reference to changes in body weight, a procedure which can give rise to considerable errors (see Møllgaard, 1929).

It seems likely therefore that all the feeding standards in current use are subject to quite sizeable errors, which cumulatively give rise to the large differences between standards indicated by Blaxter (1950). It is difficult to determine which of the available feeding standards is the most correct. A practical comparison of the various standards has been attempted by Yates, Boyd and Pettit (1942). In an analysis of numerous trials conducted

in Denmark, they interpreted the results on the basis of the intake of feed energy as measured by starch equivalent, total digestible nutrient and feed-unit standards. They concluded that the starch equivalents of feeds provide a more reliable basis for the rationing of livestock than do feed-units or digestible nutrient standards. Olsson (1951) has shown with working horses that the replacement of concentrates in the ration by hay according to net energy relationships maintained the weight of the animals, whereas replacement according to metabolizable energy relationships did not.

Determination of the net energy values of feeds

The foregoing discussion shows that current feeding standards, irrespective of the system on which they are based, are not a reliable guide to the feeding of cattle. It shows also that the errors present in total digestible nutrient and feed-unit standards are in the main attributable to the limitations of the systems on which they are based, whereas the limitations of the net energy and starch equivalent standards are to a much greater extent a reflection of the technical difficulties associated with the determination of the complete energy exchange of cattle by the classical method of respiration calorimetry. This suggests that a profitable approach to the development of a reliable feeding standard would be to seek a simpler method for the study of energy exchange to be used in the determination of the net energy values of feeds.

In this thesis a study has been made of the main sources of error in the determination of net energy values of feeds in energy balance studies with cattle. As a result, a new method of studying energy exchange has been developed and it has been compared with the classical method of studying energy exchange in experiments with calves and sheep.

SECTION I

AN ANALYSIS OF THE CONVENTIONAL METHOD OF DETERMINING NET ENERGY VALUES

The net energy principle

The following definitions are an essential introduction to a study of net energy values.

The gross energy of a feed is the heat liberated on combustion as determined by bomb calorimetry.

The metabolizable energy of a feed is the gross energy less the energy lost to the animal in the faeces, urine and expired combustible gases. It is not a term of distinct physiological significance since it includes the heat lost during fermentation in the gut and it does not include the energy of excretions into the alimentary tract, which is known as the 'metabolic faecal energy' (Blaxter and Mitchell, 1948).

The heat production of an animal can be measured by 'direct' or 'indirect' calorimetry. In 'direct' calorimetry, heat production is calculated from the heat loss determined by calorimetric methods. (This is not a practical method in the determination of net energy values and will not be referred to in the subsequent discussion.) In 'indirect' calorimetry, the heat production is calculated from oxygen consumption, carbon dioxide production and urinary nitrogen excretion, or from carbon and nitrogen balance data.

The energy balance is given by subtracting the heat production from the metabolizable energy intake, or it may be calculated from carbon and nitrogen balances.

The net energy value of a feed is defined as the increase in energy storage which occurs when a unit weight of the food is added to the production ration of a fattening animal. The relation between energy balance and feed intake is curvilinear and net energy values are determined only between the limits of maintenance and approximately $2\frac{1}{2}$ times maintenance, over which range the relationship is

approximately linear. Below the maintenance level of feeding, the heat losses associated with the utilization of feed energy are less than those observed above the maintenance level, whilst at levels of feeding above approximately $2\frac{1}{2}$ times maintenance, energy balance per unit of food declines due, among other things, to a decrease in digestibility.

The net energy value of a food may be calculated from the following equation:

$$\text{Net energy value} = K_A \times K_M \times \frac{G}{F} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where, $K_A = \dfrac{E_2 - E_1}{M_2 - M_1}$

and $K_M = \dfrac{M}{G}$

K_A = the net availability of the metabolizable energy, expressed as a %

K_M = the metabolizable energy as a % of the gross energy

G = the gross energy of the food (Cal.)

F = the food intake (kg.)

E = the energy balance (Cal.)

M = the metabolizable energy intake (Cal.)

Subscripts 1 and 2 refer to the levels of feeding, one at or about maintenance and the other at some higher level.

Errors attached to determined net energy values of feeds

The error attached to a net energy value of a feed determined according to the classical difference procedure can be estimated from an analysis of replicate determinations of net energy values reported in the literature. The analysis of variance technique has been used to give in Table 1 the standard deviation and coefficient of variation attached to determinations of net energy values reported by several authors. With the exception of the results of Forbes, Braman, Kriss and Swift (1933), the standard

TABLE 1

Errors attached to the net energy values of feeds

Authority	Number of feeds	Degrees of freedom within feeds	Standard deviation (Cal/kg.)	Coefficient of variation (%)	Remarks on method of calculating the net energy values
Forbes, Braman, Kriss and Swift (1933)	3	3	± 19	± 1.2	Maintenance and fasting levels compared; two animals.
Forbes, Braman, Kriss and Swift (1933)	2	2	± 111	± 5.2	Maintenance and one and one-half times maintenance; two animals.
Mitchell, Hamilton and Haines (1940)	3	9	± 442	± 37.6	Maintenance and less than one and one-half times maintenance; four steers in each group.
Mitchell and Hamilton (1941)	4	11	± 151	± 8.3	Maintenance and fasting levels compared; four steers in each group.
Forbes, Fries and Braman (1925)	2	4	± 104	± 10.9	Computed fasting value used as a base line.
Kleiber, Regan and Mead (1945)	1	7	± 141	± 7.9	Fasting and maintenance levels compared.
Ritzman and Benedict* (1938)	12	25	± 175	± 8.3	Fasting level and approximately maintenance to twice maintenance.
Pooled value**	27	61	± 221	-	

*From Table 3

**Computed from the summated sums of squares of deviations and degrees of freedom within feeds

deviation is consistently high. The mean value of ± 221 Cal./kg. dry matter is equivalent to ± 9.3 units of starch equivalent, i.e. approximately two-thirds of the estimates of the starch equivalent of a hay with a starch equivalent of 40 would lie between the values of 31 and 49.

An analysis of many determinations of metabolizable energy of feeds made by Ritzmann and Benedict (1938) is given in Table 2, and this shows that only a small error (coefficient of variation, ± 1.66%) is attached to a determined metabolizable energy of a feed. A similar analysis of determinations of the net energy of feeds made by the same authors (Ritzmann and Benedict, 1938) gave a much larger error (coefficient of variation, ± 8.33%). The high error attached to determined net energy values of feeds is, therefore, associated largely with the determination of the net availability of the metabolizable energy.

There are four factors which could contribute to the error: (a) analytical and instrumental errors; (b) errors of interpretation; (c) the magnification of technical and interpretational errors by the experimental design; (d) the biological variations of animals in the utilization of feed energy.

Analytical and instrumental errors These are usually small (see Mitchell, 1935) and it is unlikely that improvements in technique would reduce significantly the error attached to a net energy value.

Errors arising from the interpretation of the results of indirect calorimetry The computation of energy storage from carbon and nitrogen storage by use of the factors of Armsby (1917) has been shown by Blaxter and Rook (1953) to give rise to an error of the order of a few per cent. This error, however, would be systematic and would not be included in the estimates of variation given in Table 1.

The respiratory quotient method of computing energy storage makes the following assumptions:- (1) that the dissimilation of fat, protein and carbohydrate results in characteristic respiratory

TABLE 2

Analysis of variance of the metabolizable energy value of rations composed of single feeds (calculated from the data of Ritzmann and Benedict, 1938)

Source of variation	Degrees of freedom	Mean square	Variance ratio (e^{2z})
Between feeds	11	128,076	3.45*
Within feeds	25	3,711	
Total	36	-	

Coefficient of variation within feeds ± 1.66%

*Significant when P = 0.01

TABLE 3

Analysis of variance of the net energy values of rations composed of single feeds (calculated from the data of Ritzmann and Benedict, 1938)

Source of variation	Degrees of freedom	Mean square	Variance ratio (e^{2z})
Between feeds	11	255,884	8.37*
Within feeds	25	30,559	
Total	36		

Coefficient of variation within feeds ± 8.33%

*Significant when P = 0.01

quotients, and (2) that the Bliebtreu equation (Bliebtreu, 1901) gives a correct estimate of heat production when the non-protein respiratory quotient is greater than 1.0. In a metabolism experiment in which the non-protein respiratory quotient does not rise above 1.0, calculation of the heat production by this method is valid provided account is taken of the loss of intermediate metabolites and of urinary carbon dioxide. The absolute accuracy of the calculation is, however, determined by the values taken for the calorific value of body fat, protein and carbohydrate and for the respiratory quotients which arise during their dissimilation. Only small systematic errors would be incurred and these would not be included entirely in the estimates of variation in Table 1.

Calculations of heat production involving the use of the Bliebtreu equation are, however, open to question since the equation is entirely empirical and is known to overestimate heat production in the steer on occasions by 500 to 1000 Cal. If, in a difference experiment, the higher plane of nutrition gave rise to an R.Q. greater than 1.0 and the lower did not, an interpretational error of this order could arise. Such occasions, however, would be of infrequent occurrence.

Variation arising from the experimental design The determination of a net energy value according to equation 1 (p. 8) requires estimates of the energy balance to be made at two levels of feeding, and the errors attached to the two estimates are attached finally to the net energy value. The experimental design, therefore, maximises analytical and instrumental errors and the errors of interpretation, and in an experiment in which the two planes of nutrition differ only slightly, these errors will assume an importance greater than has been suggested in the previous discussion. This is illustrated by the high errors attached to the results of Mitchell, Hamilton and Haines (1940) (see Table 1) for the net energy of glucose, in which the planes of nutrition differed by less than half the maintenance requirement.

Also, in the difference type of experiment used in the

determination of net energy values, variations in the maintenance requirements of an animal are included in the error attached to the net energy value. Ritzmann and Benedict (1938) have emphasized the wide variations in heat production which may occur over periods as short as 6 to 8 weeks and an experiment with cattle for the determination of a net energy value may cover a similar period. The variations observed by Ritzmann and Benedict were of the order of ± 2000 Cal./day, equivalent to approximately 20% of the basal heat production of a steer. It appears probable, therefore, that a large part of the estimate of variation recorded in Table 1 may be attributed to variations in maintenance requirement throughout the course of an experiment.

Variation arising from differences in the utilization of feed energy by animals Animals may differ in their utilization of feed energy in two ways: they may have different maintenance requirements for energy or they may differ in the efficiency with which they utilize feed energy. According to the conventional method of calculating net energy values, variations in maintenance requirements from animal to animal, as distinct from variation in the maintenance requirement of an individual animal throughout the experimental period, do not give rise to different values for a single feed. The efficiency with which an animal utilizes its feed energy is, however, included in the value; in fact, a net energy value is a measure not only of the available energy of a feed but of the efficiency with which an animal utilizes it. Variations in the efficiency of the converter are thought to be responsible for a large part of the variation observed in calorimetric studies with cattle. The errors attached to net energy values reported in the literature are confounded and it has not been possible to use them to give a precise estimate of variation between individuals in the efficiency of feed utilization but in experiments conducted by Forbes, Braman and Kriss (1930), with two steers, similar in size and breed, on three rations, the utilization of metabolizable energy by one steer was consistently

and significantly higher than in the other. The mean net efficiencies for the utilization of metabolizable energy were 56.6 and 59.5%. It can reasonably be assumed that considerably larger differences exist between cattle dissimilar in breed and function.

Methods of increasing the usefulness of net energy values

From the foregoing discussion it follows that the two factors largely responsible for the high errors attached to the net energy determinations recorded in Table 1 are (a) the use of experimental designs in which only small differences in nutritional plane are employed and (b) the variations due to biological differences between animals and within individual animals from time to time. Any attempt to increase the usefulness of net energy values must seek to minimize the effect of either or both of these factors.

The introduction of experimental designs in which large differences in nutritional plane are employed is always limited by the appetite of the animal and by the decrease in digestibility observed at high levels of feeding. To minimize the effect of the biological variation observed in animals by increasing the number of determinations on which a net energy value is based, would appear, therefore, to offer the greatest scope.

A new approach to the determination of the net energy values of feeds

In the past, replication has been limited by the technical difficulties associated with the determination of energy balances on farm animals and by the cost of building and running respiration chambers. Because of these technical difficulties, many determinations of net energy values have been based on respiration studies lasting only 12 or 24 hr. and in such short periods undue importance is attached to the activity of the animal. To facilitate replication, it is therefore necessary to dispense with respiration chamber technique. This would be desirable also from another point of view. The conditions within a respiration chamber are not

comparable with those of stall-feeding, since exercise is limited and the atmosphere of the chamber contains carbon dioxide, water vapour and other gases in quantities sufficient to be obnoxious, if not harmful.

Several possible indirect methods of studying energy exchange, that are independent of respiration chamber technique, have been reported in the literature. The determination of heat production from insensible loss of body weight has been considered by both Kriss (1930) and Mitchell and Hamilton (1936) but both groups of workers are of the opinion that the method could not be used with accuracy in the determination of energy balances in cattle. The insensible loss of body weight measures the differences between the losses from the body of water vapour and carbon dioxide and the uptake of oxygen, and for its interpretation a knowledge of the respiratory quotient is necessary. Kriss, and Mitchell and Hamilton, found that, under ordinary conditions of stall-feeding, the respiratory quotient is too variable to be assumed constant during the period of a determination.

Recently, a number of methods of estimating qualitative differences in body composition have been proposed by American workers. Keys, Brozek, Henschel, Mickelsen and Taylor (1950) used measurements of skinfold thickness and body proportions to assess the degree of fatness in humans, but such methods are of low accuracy. Lesser, Blumberg and Steele (1952) have introduced a novel scheme for the estimation of body fat based on the different solubilities of cyclopropane in the fatty and fleshy tissues of the body but this method is also of low accuracy. The schematic division of the body into its four major constituents - fat, protein, ash and water - as performed by Keys et al. (1950) and by McCance and Widdowson (1951) in qualitative studies with humans could, however, be adapted for use in quantitative studies of energy metabolism, since the principle of the method can be applied equally to the growth of an animal, as follows:-

$$G = F + P + W + A \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where,

G = total gain of body substance

F = gain of body fat

P = gain of body protein

W = gain of body water

A = gain of body ash

Fat storage can therefore be determined by difference if the increments of body substance, protein, ash and water can be measured. In the intact animal, the closest approximation to the gain in body substance is given by the gain in weight, and equation (2) would therefore have to be modified as follows:-

$$F = L - (P+W+A) - w - d \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

where,

L = gain in weight

w = change in water content of the gut

d = change in dry matter content of the gut

Protein and ash retention by an animal, which includes protein and ash retained within the gut, can be estimated with accuracy from the nitrogen and ash retentions. Changes in the dry matter content of the gut would therefore lead to only small errors amounting to a weight of fat equivalent to the weight of non-protein organic matter stored in or lost from the gut. The accuracy with which fat storage could be predicted from equation (3) would thus depend on the accuracy of the measurements of body weight and of the change in water content.

For the indirect method to provide a satisfactory alternative to the method of respiration calorimetry for the study of energy metabolism in cattle, it must permit the estimation of energy retention with a comparable accuracy. No fully satisfactory estimate of the error attached to a measurement of energy storage in cattle by the calorimetric method can be derived from the many published results of energy metabolism studies, since the errors of technique and interpretation are confounded with the variations in heat

production due to short term changes in the metabolism of the animal under study. Kriss (1925), however, has made numerous simultaneous estimations of heat production in cattle by the indirect C and N balance method and by the direct measurement of heat losses. The standard deviation of a series of 36 estimates of heat production in milking cattle was ± 388 Cal./24 hr. and there was no systematic difference between the estimates. This suggests that fat storage in cattle can be estimated by calorimetric methods to within ± 50 g./day. The relatively simple experimental technique required by the indirect method would make it possible to study energy storage over much longer periods than is usual in calorimetric studies. A suitable period would be of the order of 3 weeks, and fat storage would have to be estimated to within 21 x 50 g., i.e. approximately 1 kg.

Errors of this order could quite easily arise in the measurement of body weight gain by the equipment commonly used for measuring body weight of cattle, but the most modern weighbridges are capable of greater accuracy. If, however, it is assumed that the measurement of body weight gain is absolute, water retention in cattle over a 21-day period would have to be estimated to within ± 1 kg., which is equivalent to less than ± 0.5% of the body water of a mature steer.

The remainder of this thesis is concerned with the development of a method of estimating water retention that would have such a degree of accuracy that it could be used in the determination of energy storage of cattle by the indirect method.

SECTION II

EXPERIMENTAL METHODS

a) Methods of Analysis

The collection and sampling of material

Body tissues and fluids of cattle Samples of body tissues and fluids were obtained from cattle slaughtered in an abattoir. Extreme care was taken to avoid contamination of the samples and to prevent loss of moisture from them by evaporation.

Two samples of blood were taken from each animal immediately after death. One was allowed to clot to provide a sample of serum and the other was defibrinated by shaking with glass beads. Tissue samples were obtained immediately the animal was skinned and sub-samples of 100 - 500 g. were transferred quickly to tared stoppered bottles. The brain cavity was opened within 15 - 30 min. of death and samples of brain tissue were taken with the same precautions. Bone was sectioned with a meat saw before removing the skin, thus avoiding excessive contamination or loss of moisture from the sample. Samples of the fluids from the pericardial sac and the posterior chamber of the eye were also obtained. Large samples of the contents of the rumen, abomasum, small intestine and caecum were taken into stoppered vessels immediately the gut was removed from the animal.

The uteri of four pregnant cows were collected at the abattoir and brought to the laboratory for dissection. They contained foetuses of 3, 5, 7 and 8 months, as judged by the criteria of Hammond (1927). The foetuses of 3 and 5 months were macerated in a high speed homogeniser and samples of the homogenates were taken into stoppered bottles. Tissue samples were taken from the foetus of 7 months, following the procedure adopted for cattle. Samples of amniotic fluid and allantoic fluid were taken from three of the uteri. No attempt was made to analyse the walls of the hypertrophied uteri since contamination and loss of moisture were unavoidable.

A small number of tissue and fluid samples from two wethers were also taken, following the procedures described.

Foods, food refusals, urine and faeces The usual care was taken in the balance trials to ensure that the collection of materials was quantitative and that samples were representative.

Determination of water

Body tissues other than fat The samples in their original containers were dried at 100° to constant weight.

Fat Large samples of approximately 40 g. were dried at 40° in vacuo over $CaCl_2$.

Body fluids The moisture content was determined by the method of Golding (1934).

Foods, food refusals, milk, urine and faeces About 5 to 10 g. of material were dried at 100° to constant weight.

Determination of ash

From 5 to 10 g. of material were dried, charred over a low bunsen flame and then incinerated at 550° in a muffle furnace.

Determination of nitrogen

A sub-sample of fresh material containing up to 25 mg. of nitrogen was digested with concentrated sulphuric acid (20 ml.) and potassium sulphate (8 g.) with the addition of copper sulphate (2 g.) and selenium (0.2 g.) as catalysts. The ammonia was distilled into boric acid and titrated directly with standard acid, using the screened indicator methyl red - brom-cresol green.

Determination of carbon

Samples of fresh material containing not more than 0.1 g. carbon were combusted in a stream of oxygen and the gases were passed over heated copper oxide and a copper oxide - lead flux (see Pregl, 1924). The carbon dioxide was absorbed on solid caustic soda dispersed with pumice stone.

Determination of calorific value

Calorific values were determined in a Baird and Tatlock bomb calorimeter. The temperature change was measured with a thermistor thermometer*, with a range of 15° to 20° and reading to $\frac{1}{400}$°. The thermistor was calibrated against a long-stem mercury-in-glass thermometer, with an N.P.L. certificate, reading to $\frac{1}{20}$° over the range of 15° to 20°.

In the calculation of the calorific value, use was made of the Regnault-Pfaundler cooling correction and deductions were made for the heats of formation and solution of the higher oxides of nitrogen and sulphur. The water equivalent of the bomb was determined with benzoic acid (thermochemical grade, British Drug Houses, Ltd.).

With dried materials (hay, concentrates, etc.) samples were prepared for analysis by compressing 1 - 2 g. of material into a small pellet. The calorific contents of faeces and milk were determined on samples of the fresh material dried at 60° to minimize the loss of volatile organic constituents. The calorific content of calf urine was calculated from the carbon content, using a factor of 3.65 Cal./g. of carbon (Tomme and Taranenko, 1939), but for sheep urine a direct determination was made on 10 ml. of urine evaporated to dryness under vacuum over concentrated sulphuric acid.

Determination of potassium and sodium

The methods used for the determination of potassium and sodium were modifications of the sodium cobaltinitrite and zinc uranyl acetate procedures. Certain of the modifications were suggested by Dr. S. J. Rowland, of the National Institute for Research in Dairying, Reading, to whom the author is grateful.

Great care was taken in the preparation of all the apparatus used. Glassware was cleaned in chromic acid, washed free from acid and finally boiled in distilled water.

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*The author is indebted to Dr. W. R. Beakley of the Hannah Institute for the design of the instrument. Details of the technique used to obtain linear calibration have been published (Beakley, 1951).

Potassium With the exception of serum and other body fluids, a sample of material containing up to 200 mg. of potassium was dried and ashed at 550°. The ash was extracted with 5 ml. of N-HCl plus 25 ml. of distilled water and the solution filtered. Aliquots of 5 ml. were diluted with water to 20 ml. and the potassium was precipitated at 20° by the rapid addition, with stirring, of an equal volume of the sodium cobaltinitrite reagent of Kramer and Tisdall (1921), previously warmed to 20° and filtered. After 2 hr. the precipitate was filtered on a gooch crucible, washed with 70% ethanol, dried and weighed. Potassium in standard solutions was precipitated under identical conditions and a calibration curve obtained.

For serum and other body fluids the method of Barry and Rowland (1953) was adopted. The potassium was precipitated directly from 1 ml. quantities of material (Kramer and Tisdall, 1921) contained in a 15 ml. graduated centrifuge tube, by the rapid addition with mixing of 3 ml. of a more dilute sodium cobaltinitrite reagent (obtained by dilution of the reagent of Kramer and Tisdall (1921) with an equal volume of water). Precipitation was done at 20°. After 2 hr. the mixture was centrifuged, the supernatant liquid decanted and the precipitate washed successively with 5 ml. volumes of 35%, 70% and 70% ethanol (Eden, 1943). The precipitate was dissolved in 3 ml. distilled water, 1 ml. of a 1% solution of choline hydrochloride was added and the solutions mixed thoroughly. One ml. of a freshly prepared 2% potassium ferrocyanide solution was then added, the volume made to 6 ml. and the contents mixed. After centrifugation, to remove slight cloudiness due to denatured protein, the green colour was determined photometrically (Jacobs and Hoffman, 1931). A series of standards was included in each batch of determinations.

Sodium

Preparation of protein and phosphate free solutions With body tissues, feeding stuffs, faeces, urine and milk, a suitable quantity of material was ashed as for potassium. The ash solution was made alkaline to phenol-phthalein by the addition of solid calcium hydroxide and filtered. With serum and other body fluids, 2 ml. were

diluted with 10 ml. of water in a 25 ml. volumetric flask. Five ml. of a 15% solution of trichloroacetic acid was added dropwise with mixing, followed by 5 ml. of a saturated solution of lead acetate and one drop of caprylic alcohol. The solution was made to volume and filtered.

Determination of the sodium Aliquots of the above sera, containing at least 0.75 mg. of sodium, were concentrated to approximately 1 ml. and the sodium precipitated by the addition of 10 ml. of zinc uranyl acetate reagent (Peters and Van Slyke, 1932). The solution was stirred for 5 min. and allowed to stand for 1 hr. The precipitate was transferred quantitatively to a sintered glass crucible, washed first with 95% ethanol saturated with sodium zinc uranium acetate and then with ether, and finally it was dried by drawing air through it. It was weighed after desiccation for 1 hr. Blank corrections were determined for both procedures by extrapolation of a graph relating weight of precipitate to weight of sodium in a series of standard solutions.

Determination of chloride

The Volhard procedure described by Peters and Van Slyke (1932) was used.

Determination of the red cell weight of blood

Ten ml. (y g.) of defibrinated whole blood were centrifuged in a 15 ml. graduated centrifuge tube and the serum layer pipetted into a crucible. The cells were washed by centrifugation with three 5 ml. quantities of physiological saline, the washings were added to the serum, and after the last washing the volume of clear liquid remaining above the cell layer was recorded (x ml.). The dry matter contained in the serum plus washings and the dry matter content of the whole blood were determined.

The following calculations gave an estimate of the red cell weight.

1) weight of serum dry matter in y g. of whole blood (A) = dry matter in separated serum plus washings - weight of NaCl in (15-x) ml. of saline

2) weight of serum in y g. of whole blood (B) = $\frac{100\ A}{\%\ \text{dry matter of the serum}}$

3) red cell weight of blood = $\frac{y - B}{y}$

b) Respiration apparatus for use with calves

Energy balance studies with animals present technical difficulties quite distinct from those encountered in the determination of nitrogen or mineral balance, since insensible losses in the form of direct heat loss or of gaseous excretion products have to be measured. In the 'direct' method of studying energy exchange, the animal is housed in a calorimeter and the actual heat loss is determined directly. The cost of construction of an animal calorimeter for use with farm animals is in most instances prohibitive and 'indirect' methods, for which less costly apparatus is required, are usually preferred.

The heat production of an animal may be determined 'indirectly' by calculation from data on oxygen consumption, carbon dioxide production and urinary nitrogen excretion. If the energy intake in the food and the losses of energy in the faeces, urine and rumen gases are also determined, the complete energy exchange of the animal can be calculated. Alternatively, the energy balance may be calculated from C and N balance data, on the assumption that fat and protein of a constant composition are the only energy containing substances stored in the animal body. Though the assumptions made in the calculation of energy balance by either of the above methods are not strictly correct, the two methods give results in close agreement with one another and with those determined 'directly' (Atwater and Benedict, 1902; Kriss, 1925).

For serial 24 hr. determinations of energy exchange by either of the indirect methods, the experimental animal must be housed in a respiration chamber. The design of the apparatus may be based on one of two principles, that of Pettenkofer or that of Regnault-Reiset

(see Paechtner, 1931). In the Pettenkofer (open-circuit) type of apparatus, the animal is housed in a sealed chamber through which air is passed at a measured rate, and the increase in carbon dioxide content, and in certain apparatus the fall in oxygen content, is determined. Two major technical problems have to be over come in the construction of this type of respiration chamber; the volume of the outgoing air has to be measured accurately and a continuous sample of the effluent air has to be taken for analysis for carbon dioxide and oxygen content.

The most accurate method makes use of mercury air pumps (Møllgaard, 1929; Kleiber, 1935) but the equipment is too expensive for general use. Workers in Europe (Heinzl, 1944) and in Australia (Marston, 1948) have used wet gas meters for the measurement of the effluent air volume but such instruments are not available in this country. In an open-circuit respiration chamber for calves constructed by Dr. K. L. Blaxter (see Blaxter, Graham and Rook, 1954), the method of Benedict, Collins, Hendry and Johnson (1929), in which the out-going air is passed through a drying column before its volume is measured by an ordinary dry gas meter, was adopted. A continuous sample of effluent air was taken by means of a sampling device worked by the meter needle, which completed an electrical circuit at one point in each revolution. Though the apparatus was used in studies of the energy exchange of the calf (Blaxter, 1952), it was not an unqualified success. Prior to each run, considerable time had to be spent in the setting of the sampling device and the operation of the chamber was tedious, requiring constant attention. Quite apart from these technical difficulties, the most accurate results were obtained when the carbon dioxide content of the chamber air was maintained at 0.7 to 0.9%. The heat production of an animal within the chamber could not, therefore, be considered typical for animals maintained under more natural conditions.

In the development of an apparatus for use in the present studies, attention was turned to the possibility of constructing a Regnault-Reiset type of respiration chamber which would prove more

satisfactory in operation than the above Pettenkofer apparatus. The Regnault-Reiset principle is that of maintaining the experimental animal in a closed system from which the water vapour and carbon dioxide are removed continuously and in which the oxygen consumed is continuously replaced from a reservoir. The major technical difficulties to be overcome are the construction of an apparatus free from leaks and the removal of the large quantities of carbon dioxide and water vapour produced by a sheep or a calf.

A respiration chamber of this type, for use with calves, was successfully constructed, and it has proved suitable for serial 24 hr. determinations of energy exchange. Details of its construction will be given in full. A respiration chamber similar in construction but modified by Dr. K. L. Blaxter and Mr. N. McC. Graham for use with sheep (Blaxter, Graham and Rook, 1954) was used in the studies of energy balance with sheep.

Details of construction of the Regnault-Reiset type respiration chamber and ancillary apparatus

The apparatus is illustrated in Plate 1, and a sectional view, together with an index of parts, is given in Figure 1.

The respiration chamber The chamber (a) was constructed from 1/8 in. sheet iron strengthened at the edges with 1½ in. angle iron. The sheet iron was first attached to the angle iron with rivets, the heads of the rivets were then brazed and the angle iron was welded to the sheet metal. The oil-seal (b) was rivetted on to the body and was lined with red lead sealing compound. The placing of the seal at the top of the chamber, as opposed to the more usual position at the base, increased the rigidity of the structure and also facilitated the testing of the apparatus for leaks, since the body of the chamber could be filled completely with water.

The outlet and inlet pipes (c_1, c_2) of the circulation system, the oxygen inlet pipe (d), the urine outlet (e), the gas-sampling tap (f), the inlet for liquid diet (g) and the conduit piping for the electrical leads (h) were next fitted. The method of joining a

PLATE 1. The Regnault-Reiset type of respiration chamber and ancillary apparatus

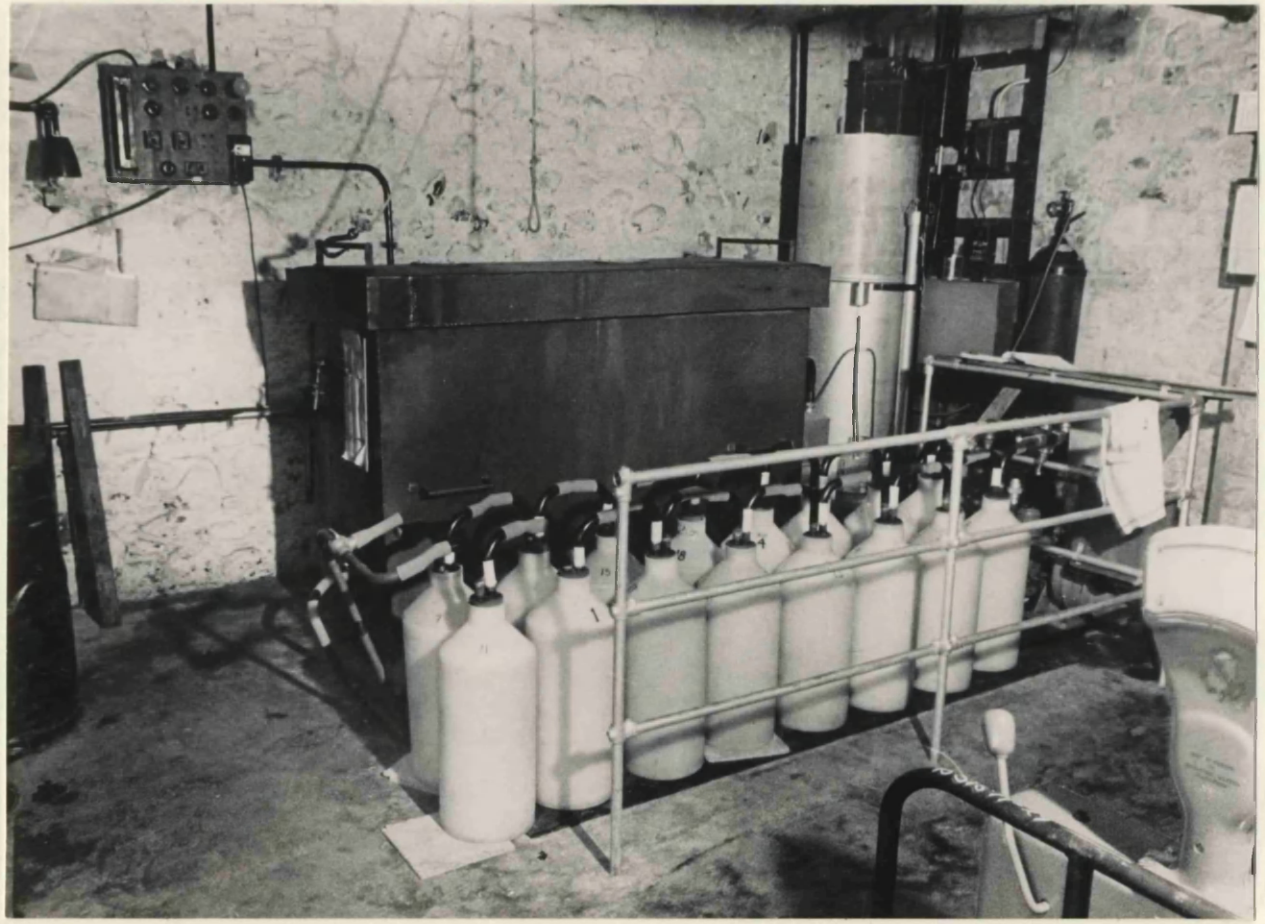
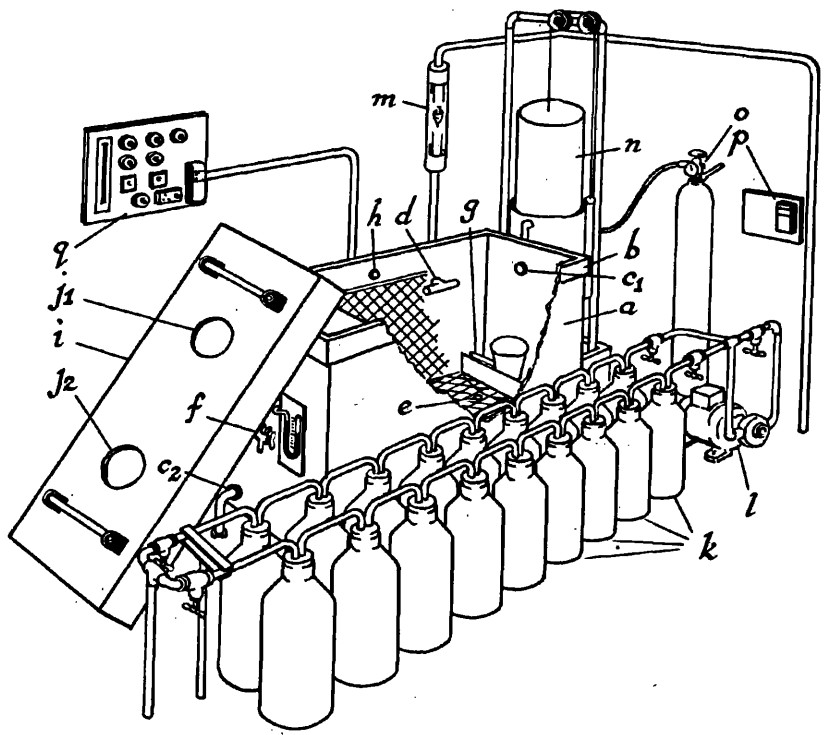


FIGURE 1. Sectional view of the Regnault-Reiset type of respiration chamber and ancillary apparatus: a, body of chamber; b, oil seal; c 1, 2, outlet and inlet pipes of circulation system; d, oxygen inlet; e, urine outlet; f, gas sampling tap; g, inlet for liquid diet; h, electrical leads; i, lid of chamber; j_1, 2, inspection windows; k, absorption system; l, rotary air compressor; m, direct reading flow gauge; n, spirometer; o, oxygen cylinder; p, compressor switchboard; q, chamber switchboard.



pipe to the chamber was as follows: the end of the pipe was threaded, a brass flange was brazed on to the pipe beyond the thread, a rubber washer was placed next to the flange and the pipe was fitted into position through a hole in the chamber. A second washer and flange were placed on the pipe from the inside of the chamber and the pipe was held in position by a nut screwed up tightly against the second flange. The conduit pipe was of a U-shape and after the electrical leads were inserted it was sealed by filling with molten wax. The feed funnel was connected to the feed pipe through a glass U-tube which was sealed by filling it with water. The urine outlet seal consisted of a straight pipe leading from the base of the chamber into a small nickel cup, from the top of which a pipe led off into a collection vessel.

The lid of the chamber (i) was constructed in a manner similar to that of the body of the chamber. The inspection windows (j_1, j_2) fitted into the lid were made from two layers of perspex sealed to either side of the sheet iron with sealing compound and fixed in position with nuts and bolts. The heads of the bolts were sealed by painting with a solution of perspex in acetone.

All seams and joints in the apparatus were covered with red lead paint. The inside of the chamber was painted white.

The animal cage The cage is illustrated in Plate 2 and the details of its construction are given in Figure 2. The framework of $\frac{3}{4}$ in. angle iron was covered with galvanized sheeting and large mesh wire netting. The heating element of the thermostating circuit was held in mica guides immediately above the circulation fan at the head of the cage. The thermostat, sensitive to changes of 0.2°, was attached to the body of the chamber at the end opposite to the heater. The temperature inside the chamber could be varied between about 2° above ambient air temperature and 30°, and the two fans and baffle arrangement gave a satisfactory temperature distribution, in spite of the fact that the urine pan tended to cut the chamber into two temperature zones.

PLATE 2. The animal cage used in conjunction with the Regnault-Reiset type of respiration chamber

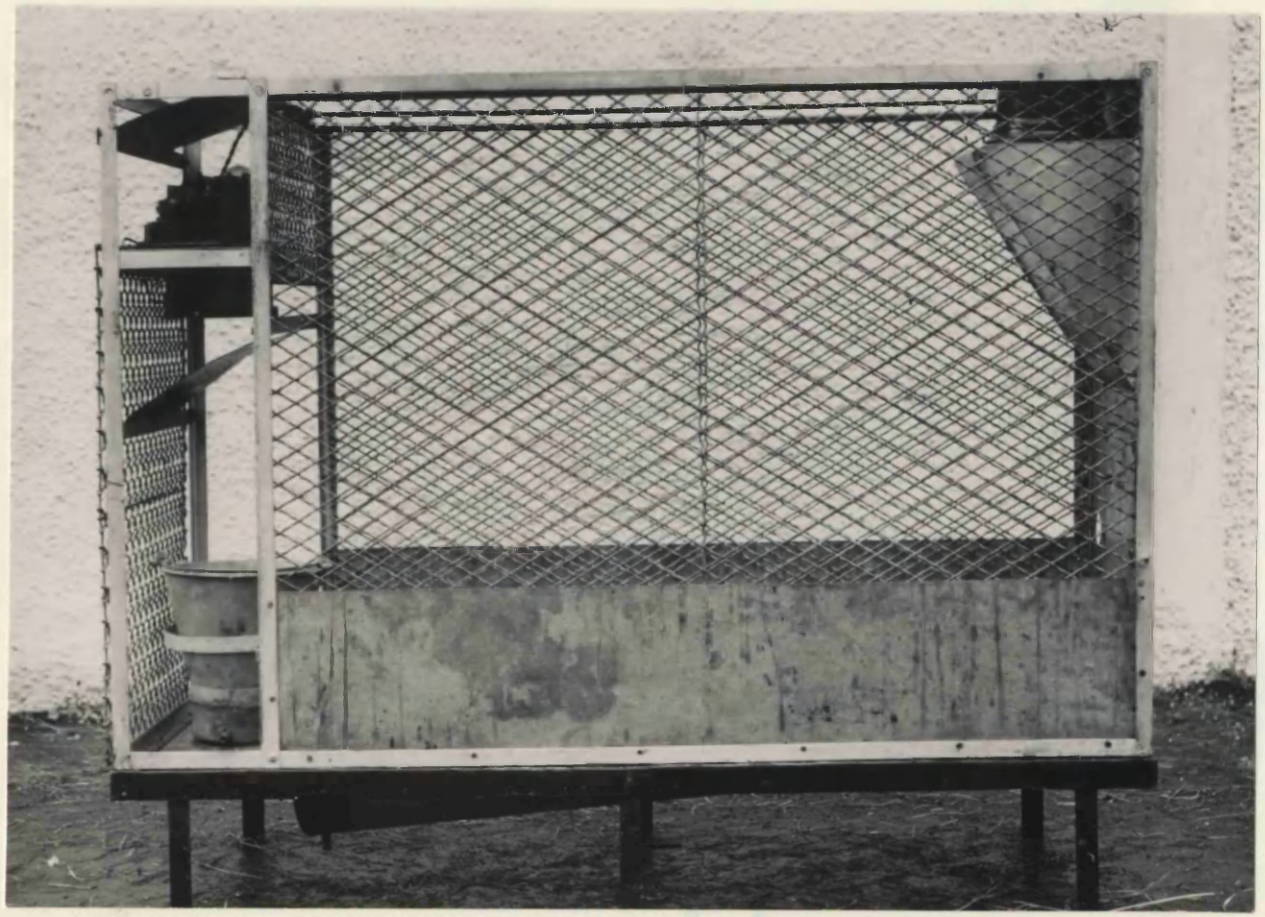
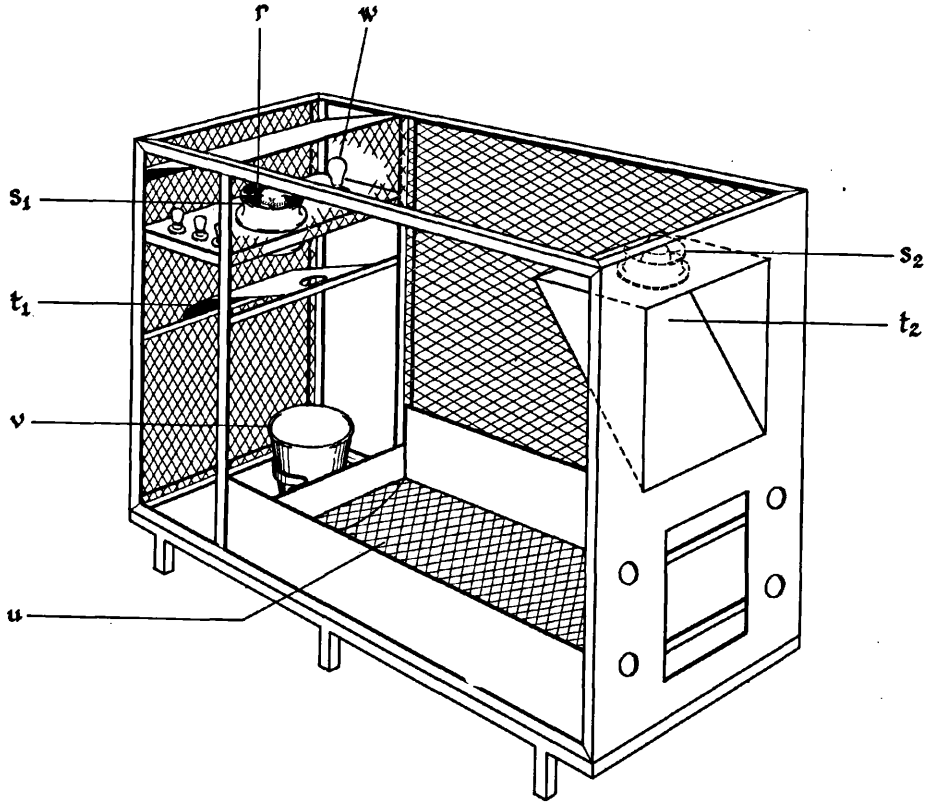


FIGURE 2. Sectional view of the animal cage used in conjunction with the Regnault-Reiset type of respiration chamber: r, heating element in air-stream of fan; s_1, 2, air circulating fans; t_1, 2, air baffle plates; u, urine collection pan; v, feed bucket; w, light.



The feeding device consisted of a bucket connected by tubing through the base to the feed inlet pipe and was fixed below the heating element on a level with the floor of the animal cage.

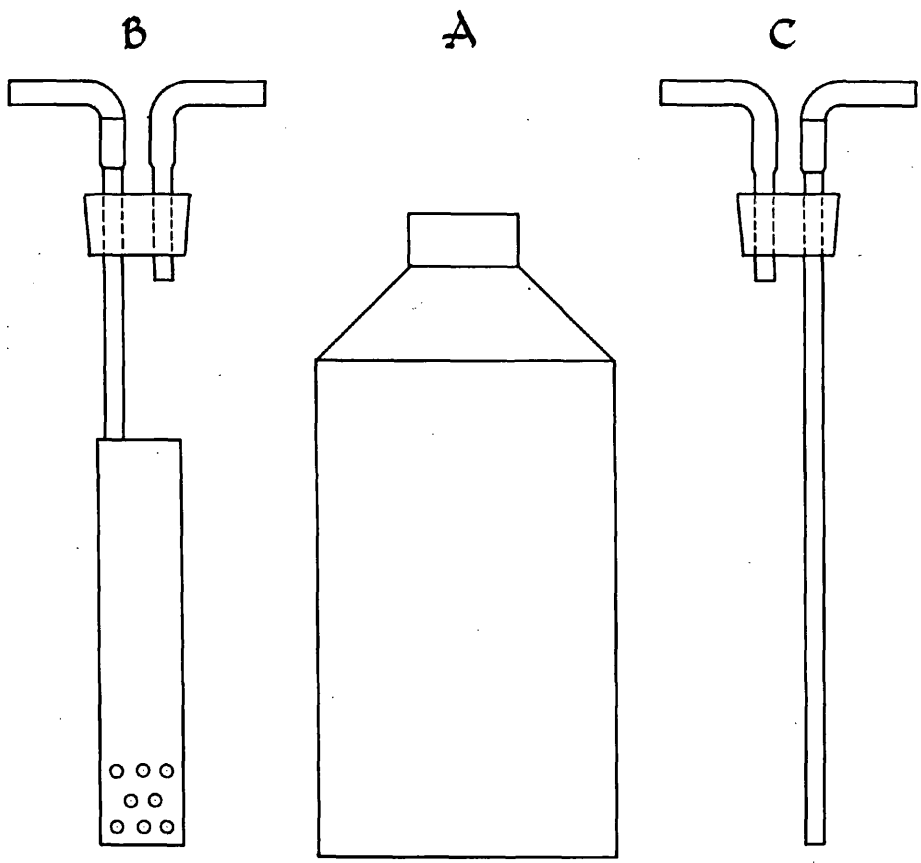
The absorption system The absorption system (k in Figure 1) was designed to take two absorption trains and the air flow could be directed through either as desired. All permanent joints in the system were sealed with hemp and gas fitters compound or were brazed. The connections between bottles were made with 1 in. fibre-walled rubber tubing.

Each absorption train consisted of nine 20-litre capacity bottles manufactured from polythene by a welding process. The absorption bottle and the two types of head for use with calcium chloride and liquid potash are shown in Figure 3. The first four bottles in each train were charged with 8 to 10 kg. granulated calcium chloride (about 1 in. mesh) for the absorption of water vapour. The next two bottles contained approximately 5 litres of strong (50° baumé) potash for the absorption of carbon dioxide and were followed by a further three bottles filled with calcium chloride to absorb moisture carried over from the liquid potash.

Air was circulated through the absorption train by means of a rotary compressor (1) capable of delivering 500 cu. ft. of air per min. at 13 lb./sq. in. pressure, and the circulation rate was controlled by a by-pass system fitted with a steam valve. The rate of flow was at first measured with a dry gas meter but this was later replaced by a direct reading flow gauge (m).

The quantity of absorbents given were arrived at by trial and error and have proved sufficient for the absorption of the water vapour and carbon dioxide produced by a calf weighing up to 150 lb. and consuming 8 litres of cows' whole milk daily, provided a standardized system for the renewal of the absorbents was adhered to. The requisites for the satisfactory absorption of the gases were that the carbon dioxide level in the chamber did not rise above 0.3% and that the gain in weight of the last bottle of each calcium

FIGURE 3. Absorption bottle for use with the Regnault-Reiset type of apparatus: A, 20 litre capacity absorption bottle of welded polythene; B, absorption head for use with liquid potash; C, absorption head for use with solid anhydrous calcium chloride.



chloride series was not more than 30 g. As soon as the last bottle gained as much as 30 g., the first bottle of the series was recharged with absorbent, the other bottles were each moved up one place in the train and the recharged bottle placed at the rear. At the end of each 24-hr. run the first of the two potash bottles was renewed and the bottles were interchanged.

In the apparatus for sheep, methane was allowed to accumulate within the chamber and the quantity produced was estimated by analysis of the chamber air.

Oxygen supply Oxygen was stored over water in a large volume spirometer (n), the construction of which has been described by Blaxter and Howells (1951). The oxygen lead passed through a water bubble valve, which responded to a negative pressure of approximately 1 cm. of water and ensured that the gas was completely saturated with water vapour.

The gas analysis apparatus A change in the composition of the chamber air due to changes in barometric pressure or atmospheric temperature, or due to incomplete absorption of carbon dioxide would lead to errors in the estimation of carbon dioxide production and oxygen consumption and it is therefore necessary to determine the composition of the chamber air at the beginning and end of each experimental period. The gas analysis apparatus used in the present studies was of the Haldane type, consisting of potash and pyrogallol absorption pipettes controlled by a single two-way stopcock (Carpenter, Lee and Finnerty, 1930) with a compensating burette system. The novel feature of the apparatus was an automatic levelling device consisting of a levelling burette which could be shut off from the measuring burette by a stopcock, when a fine adjustment of the potash and pyrogallol levels was given by a screw clip attachment. Opening the levelling burette alternately to room air and to a constant head of negative pressure gave a continuous movement of the mercury in the bulb of the measuring burette. This system, though slow in operation, excluded the possibility of accidents, in the form of

mercury being drawn into the absorption burettes or of absorbents being drawn into the measuring burette, which are apt to occur occasionally with a hand levelling device.

Testing of the apparatus for leaks In a closed circuit respiration chamber a leak in the apparatus has a direct effect on the inflow of oxygen from the spirometer, and it is particularly important that a leak should be detected readily and that where necessary its magnitude should be measured. During construction, the apparatus was tested for leaks at each stage by filling the body of the chamber and the lid with water. The polythene absorption bottles were tested by holding in water under pressure. In the assembled apparatus, leaks were detected by running the chamber for 24 hr. periods, during which time any leaks that might exist gave rise to a change in pressure within the chamber. (Corrections were made for change of temperature or atmospheric pressure.) In the course of experimental periods, the development of a leak was shown by a change in air composition and its extent was calculated from the change in nitrogen content of the chamber.

Procedure for the determination of the energy exchange of the calf

The heating element and the fans were switched on half-an-hour before the start of a run to allow the chamber to attain working temperature. Meanwhile, the spirometer was filled with oxygen and the absorption bottles charged and weighed. The calf was harnessed for the separate collection of faeces and urine and then weighed. It was fitted with a faeces collection bag, the lid of the chamber was removed and the calf was lifted into the chamber by means of a sack hoist mounted in the roof. The lid of the chamber was replaced and after temperature equilibrium was regained, a sample of chamber air was taken for analysis, the system immediately closed and the time recorded. The reading of the spirometer and the spirometer temperature were noted and the lead from the spirometer bell to the chamber was opened. The compressor was started and

the speed of circulation was adjusted to 4 cu. ft./min.

The oxygen supply was renewed as required by temporarily isolating the chamber from the spirometer and refilling it from a cylinder of oxygen (o). The calf was fed at the usual intervals and the urine was collected throughout the run. The barometric pressure was recorded daily.

At the end of 24 hr. the chamber air was again sampled for analysis and the volume of oxygen consumed was recorded. The air stream was then switched to the second series of absorption bottles which had in the meantime been charged and weighed. The first series of bottles was weighed and reassembled in the train. At the end of 48 hr. a similar procedure was adopted but the calf was also removed from the chamber for weighing and the collection of faeces.

A similar routine was adopted for sheep, but the animal was removed from the chamber for weighing and removal of faeces at the end of each 24 hr. run, at which time the days rations were placed in the feed hoppers.

The calculation and interpretation of results

The protocols for a typical 48 hr. period are set out in Tables 4, 5, 6 and 7. The energy balance was calculated by two procedures to give a constant check on the accuracy of the results.

In method A (Table 7), the factors for the carbon and calorie content of fat and protein were those given by Blaxter and Rook (1953). These factors were derived from analyses of samples of muscle, liver, brain and fatty tissue of cattle in a normal state of nutrition by extrapolating to zero and to 16% N content the lines relating calorific value or carbon content to nitrogen content. In this way the errors inherent in the use of Armsby's (1917) factors for pure protein and fat were considerably reduced, since account was taken of the non-nitrogenous material which is deposited in muscle tissue along with muscle protein.

In method B (Table 7), the heat production was calculated from

TABLE 4

An example of the respiration chamber record sheets
Calf Respiration Chamber Experiments

Experiment: Magnesium deficiency
Duration: 12.20, 14.10.52 to 12.12, 15.10.52
Calf No.: 214
Run No.: 5
Day: 1

Feed

1 4,017.0 - 1,004.0 = 3,013.0
2 4,058.5 - 1,004.0 = 3,054.5

Wt. given 6,067.5 g.
Volume = 6,000 ml.

Urine

6,854.5
1,943.0

Weight 4,911.5 g.

Volume = 4,935 ml.

Faeces Not collected

Body weight

	lb.	oz.
Final	-	-
Initial	110	5

Barometric pressure

Initial	750
Final	758

H_2O loss from chamber

Bottle No.	(1)	(2)	
14	12,386.0	12,817.0	= 431.0
16	11,363.5	11,682.0	= 318.5
15	11,301.0	11,463.0	= 162.0
5	11,009.0	11,026.0	= 17.0
			928.5 g.

CO_2 production

Bottle No.	(1)	(2)	
4	12,043.0	11,963.0	= - 80.0
18	12,203.0	12,596.0	= 393.0
6	12,092.0	12,198.0	= 106.0
17	14,373.0	14,710.0	= 337.0
2	11,622.0	11,628.0	= 6.0
			762.0 g.

O_2 consumption

Time	Temperature	Spirometer reading	Consumption
12.20	10.0	96.20	
7.30	10.0	26.05	70.15
7.30	10.0	95.80	
12.00	9.5	52.95	42.85
12.00	9.5	95.25	
6.05	9.0	28.85	66.40
6.05	9.0	96.35	
12.12	10.0	44.65	51.70
			231.10

TABLE 4 (CONTD.)

An example of the respiration chamber record sheets
Calf Respiration Chamber Experiments

Experiment: Magnesium deficiency
Duration: 12.35, 15.10.52 to 12.26, 16.10.52
Calf No.: 214
Run No.: 5
Day: 2

Feed
1. 4,103.0 - 1,004.0 = 3,099.5
2. 4,082.0 - 1,004.0 = 3,079.0

Weight given 6,178.5 g.

Volume = 6,000 ml.

Urine
7,528.0
1,946.5

Weight 5,582.0 g.

Volume = 5,586 ml.

Faeces
447.0
296.0

Weight = 151.0 g.

Body weight	lb.	oz.
Final	111	1½
Initial	-	-

Barometric pressure
Initial 758
Final 758

H_2O loss from chamber

Bottle No.	(1)	(2)	
1	15,192.0	15,543.0	= 351.0
11	12,882.5	13.235.0	= 352.5
8	13,364.5	13,546.0	= 181.5
5	11,026.0	11,047.0	= 21.0
			906.0 g.

CO_2 production

Bottle No.	(1)	(2)	
12	12,461.0	12.419.0	= - 42.0
13	12,061.0	12.345.0	= 284.0
9	12,130.0	12,499.0	= 369.0
7	12,277.0	12,433.0	= 156.0
10	12,052.0	12,072.0	= 20.0
			787.0 g.

O_2 consumption

Time	Temperature	Spirometer reading	Consumption
12.35	10.0	96.30	
6.40	9.5	38.20	58.10
6.40	9.5	91.80	
12.15	9.5	37.80	54.00
12.15	9.5	95.40	
5.50	8.5	44.25	51.15
6.01	8.5	96.60	
12.26	10.0	43.50	53.10
			216.35

TABLE 5

Analysis of the gas within the respiration chamber

Run	Day	Gas	Initial (%)	Final (%)	Correction to be applied to crude figures for oxygen consumption and carbon dioxide production (litres)*
5	1	CO_2	0.18	0.11	- 1.08
		O_2	20.60	21.93	- 20.56
	2	CO_2	0.19	0.16	- 0.46
		O_2	20.71	20.07	+ 9.84

*Total volume of chamber air = 1,540 litres

TABLE 6

Calculation of the carbon dioxide production and the oxygen consumption, corrected to a 24 hr. basis

Run	Day	CO_2 (g)	Spirometer Reading	Spirometer Temperature	Mean Barometric Pressure	O_2 factor*	Crude values (litres)		Corrected 24 hr. value (litres)	
							CO_2	O_2	CO_2	O_2
5	1	762.0	231.1	9.5	754	2.266	385.4	523.7	386.5	505.9
	2	787.0	216.4	9.5	758	2.278	398.1	492.9	400.1	505.9

*Factor for the conversion of the spirometer reading to litres of oxygen at N.T.P.

TABLE 7

Calculation of protein, fat and energy storage

METHOD A

PROTEIN STORAGE

Run	Days	Diet			Urine			Faeces			Nitrogen		Protein balance (N x 6.25) (g.)	Carbon stored as protein (protein x 0.5134) (g.)
		Volume (ml.)	Nitrogen content (g./100 ml.)	Nitrogen intake (g.)	Volume (ml.)	Nitrogen content (g./100 ml.)	Urine nitrogen (g.)	Weight (g.)	Nitrogen content (%)	Faecal nitrogen (g.)	Excretion (g.)	Balance		
5	1 and 2	12,000	0.538	64.56	10,521	0.376	39.56	151	1.124	1.70	42.59	21.97	137.31	70.29

CARBON AND FAT STORAGE

Run	Days	Diet			Urine			Faeces			Carbon			Carbon stored as fat (g.)	Fat storage (g.)
		Weight (g.)	Carbon content (g./100 ml.)	Carbon intake (g.)	Volume (ml.)	Carbon content (g./100 ml.)	Urine carbon (g.)	Weight (g.)	Carbon content (%)	Faecal carbon (g.)	Respiratory (g.)	Total excretion (g.)	Balance (g.)		
5	1 and 2	12,360	5.28	652.2	10,521	0.34	36.2	151	10.50	15.9	424.13	478.68	173.56	103.27	138.8

CALCULATION OF ENERGY BALANCE

Energy stored as protein = 137.3 x 5.322 = 730.8 Cal.
Energy stored as fat = 138.8 x 9.367 = 1,300.1 Cal.

Total energy storage 2,030.9 Cal.

METHOD B

CALCULATION OF THE HEAT PRODUCTION FROM THE NON-PROTEIN RESPIRATORY QUOTIENT

Run	Day	Urine nitrogen (g.)	Protein			Non-protein			Cal./litre of O_2	Non-protein Cal.	Total Cal.
			CO_2 (litres)	O_2	Cal.	CO_2 (litres)	O_2	R.Q.			
5	1	19.78	94.2	116.9	524.2	292.3	389.0	0.751	4.740	1844.0	2368.2
	2	19.78	94.2	116.9	524.2	306.0	389.0	0.787	4.785	1861.2	2385.3

CALCULATION OF ENERGY BALANCE

Run	Days	Diet			Urine		Faeces			Heat production (Cal.)	Energy balance (Cal.)
		Weight (g.)	Cal./100 g.	Cal. intake	Carbon (g.)	Cal.	Weight (g.)	Cal./100 g.	Faecal Cal.		
5	1 and 2	12,360	61.34	7,581.6	36.19	361.9	151	120.6	182.1	4,753.5	2.284.1

CALCULATION OF FAT STORAGE

Total energy stored = 2,284.1 Cal.
Energy stored as protein = 730.8 Cal.

Energy stored as fat = 1,553.3 Cal.

the non-protein respiratory quotient, using the tables of Zuntz and Schumberg (1901). The oxygen consumption and carbon dioxide production resulting from protein metabolism were calculated from the urinary nitrogen excretion by assuming that 5.91 litres of oxygen were consumed and 4.76 litres of carbon dioxide were produced for each g. of nitrogen excreted in the urine. The heat produced by the metabolism of protein was assumed to be 26.5 Cal./g. of nitrogen.

The accuracy of a determination of heat production in the closed circuit apparatus

The accuracy with which the heat production of a calf can be determined in the apparatus is indicated by the analysis of forty duplicate determinations of heat production of calves given in Table 8. Over 50% of the duplicates differed by less than 2% and the occasional values which differ by as much as 10% were to be expected in an animal as active as the calf.

TABLE 8

Percentage differences between duplicate determinations of heat production over 48 hr. periods in forty experiments with calves

Difference between duplicates (%)	Number of experiments	Percentage frequency
0 - 2	21	52.5
2 - 4	9	22.5
4 - 6	5	12.5
6 - 8	3	7.5
8 - 10	1	2.5
10 - 12	1	2.5

SECTION III

ESTIMATION OF WATER STORAGE IN THE ANIMAL

The method proposed in Section I for the indirect determination of energy balance depends for its success on the development of a simple yet accurate method of estimating water storage by the animal. The classical method of determining water storage is based on a balance of intake and output, and involves the estimation of respiratory water loss and water produced in the body as a result of metabolic processes. These can only be determined accurately by respiration chamber technique, and estimation of water by the classical procedure would not lead to a simpler method of studying energy exchange. The estimation of metabolic water and respiratory water loss from insensible loss of body weight is essentially the same problem as the estimation of heat production from insensible loss (see p. 13) and would, therefore, suffer from the same limitations.

There are, however, a number of indirect methods of studying changes in water content of the animal body and they can be divided into two major groups: those in which measurements of total body water are made at the beginning and end of the experimental period, and those in which only the change in water content is measured. Methods of the first type offer the simpler technique since they dispense with the continuous collection of faeces and urine required in a balance study. The method chosen, however, must comply with the limits of accuracy stated in Section I, namely that water storage over a 21-day period must be estimated to within $\pm$ 1.0 kg. To attain this accuracy with a steer weighing 500 kg. and having a body water content of 325 kg. and storing 0.25 kg. water per day, total body water content would have to be estimated with an accuracy of $\pm 0.3/\sqrt{2}$, or $\pm$ 0.2%, whereas the change in water content would have to be estimated with an accuracy of only $\pm$ 20%.

The determination of total body water from the specific gravity of the body presents many technical difficulties, whether the method of under-water weighing (Behnke, Feen and Welham, 1942; Brozek, 1946) or of body volume determination (Kohlrausch, 1929-30) is used. Moreover, corrections have to be made for the air present in the lungs, and in the ruminant account would have to be taken of the gases present in the digestive tract. Such an approach, therefore, could not readily be applied to cattle.

An alternative method of estimating body water is based on the distribution of water soluble compounds within the body fluids. A known quantity of a water soluble material, which ideally is metabolized and excreted slowly, and which distributes itself evenly in the water of the body, is injected into the blood stream of the animal. After allowing time for an equilibrium to be established, its concentration in the serum water is measured. The total body water is calculated from the dilution, after making corrections for the metabolism and excretion of the compound during the period of equilibration. Many substances have been tried, e.g. urea (Painter, 1940); thiourea (Jongbloed and Noyons, 1938); deuterium (London and Rittenburg, 1950); tritium (Pace, Kline, Schachmann and Harfenist, 1947); antipyrine (Soberman, Brodie, Levy, Axelrod, Hollander and Steele, 1949) and its derivatives (Brodie, Berger, Axelrod, Dunning, Porosowska and Steele, 1951). Of these, antipyrine and its derivatives have proved most satisfactory. Antipyrine is a non-toxic compound which is distributed quickly and evenly throughout the body water. Its metabolism shows a marked species difference and, though it has proved satisfactory for determinations in man (Soberman et al., 1949), a rapid rate of metabolism in the dog and other animals leads to less satisfactory results (Soberman, 1950). When the method was applied to cattle in connection with the present work, the rate of metabolism observed was intermediate between that found in man and in the dog, and the

accuracy of the method, as judged by replicate determinations on a single animal, was low. These results have been confirmed by the work of Kraybill, Harkins and Bitter (1951). Soberman (1949) has compared the antipyrine dilution technique with a direct desiccation procedure for determining the body water of the rabbit and the error of the method was about ± 3%. Thus, though the method could be of value in studying changes in the body composition of cattle over long periods, it has not the accuracy required in the present study.

Indirect methods for the determination of water balance are based on the assumption that the osmotic pressure of body fluids is constant. The osmotic pressure is contributed to mainly by the cations Na^+ and K^+ and the anions Cl^- and $-HCO_3^-$, and their storage throughout a balance period should reflect the storage of water. In experimental studies of the starvation of epileptic humans, Gamble, Ross and Tisdall (1923) used the urinary excretion of sodium and potassium to estimate the losses of extra- and intracellular water. In their calculations oedema fluid containing 148.0 mg.-equiv. of Na^+ and 2.5 mg.-equiv. of K^+ per litre of water, and muscle water containing 48.0 mg.-equiv. of Na^+ and 112.5 mg.-equiv. of K^+ per litre of water were considered typical of extra- and intracellular fluids. Harrison, Darrow and Yannet (1936) calculated the change in extracellular fluid volume from the chloride balance, assuming that the chloride ion was confined to the extracellular spaces. They were then able to measure the change in intracellular volume from the balance of any other ion for which the distribution between the extra- and intracellular fluids was known.

From the results given in these two papers, it is not possible to determine how closely electrolyte balance is correlated with water balance. It is clear that the correlation cannot be high at all times for an animal under normal management, since at certain intervals large quantities of water may be consumed without an equivalent intake of electrolytes. If, however, the animal is in the same state of equilibrium at the beginning and at the end of

the balance period, even if the total body water is not accounted for entirely by body electrolytes, water balance should be measured accurately by electrolyte balance. It was therefore decided to test the accuracy with which changes in the water content of the body could be calculated from changes in electrolyte content.

The cations Na^+ and K^+ together account for over 90% of the cations of body fluids and, under most conditions, bear a constant relationship to other cations such as Ca^{++} and Mg^{++}. The problem, therefore, was resolved into the estimation of water retention from sodium and potassium retention, and the first phase of this study, i.e. the determination of the relationship between the sodium, potassium and water contents of body tissues and fluids, is described in this section. It is usual in experimental studies of water metabolism to distinguish between water contained in the extra- and intracellular spaces, since there are marked differences in the composition of the two phases. In in vivo studies, however, this distinction is difficult to demonstrate exactly (cf. Lavietes, Bourdillon and Klinghoffer, 1936; Gilligan and Altschule, 1939) and in the present work no attempt has been made to relate the water storage to specific changes in the extra- and intracellular fluids.

Experimental

Tissues and body fluids of cattle, ranging in age from 1 week to 5 years, were analysed for sodium, potassium and water content. The analyses of muscle, pericardial fluid, posterior chamber fluid, blood serum, erythrocytes, fatty tissue, brain and liver tissue were first studied to develop a regression equation relating sodium and potassium content to water content. The accuracy with which the equation estimated the water content of other tissues was then determined.

Results

Muscle accounts for over 50% of the total body water and the

TABLE 9

The contents of water, Na and K in the muscle of cattle of different ages

Animal	Age (weeks)	Sample No.	Composition of muscle					
			Water %	Na (mg./100 g.)	K (mg./100 g.)	Na (mg.-equiv./ litre water)	K (mg.-equiv./ litre water)	Total Na + K (mg.-equiv./ litre water)
A	1	1	78.4	94	351	52	114	166
		2	78.8	104	330	57	107	164
B	1	1	78.1	71	406	39	133	172
		2	77.8	73	421	41	139	180
C	15	1	78.2	61	446	34	146	180
		2	78.2	51	441	28	134	162
D	15	1	77.9	57	424	32	139	171
		2	77.9	55	458	25	150	175
E	15	1	78.4	46	425	25	139	164
		2	78.4	50	448	28	146	174
F	40	1	77.0	56	434	32	144	176
		2	77.3	55	421	31	139	170
G	About 300	1	75.0	81	335	47	114	161
		2	69.5	91	278	57	102	159
H	About 300	1	76.7	58	367	33	122	155
		2	74.9	67	380	39	130	169
			Mean value			37.5	131.1	168.6
			Standard deviation			± 10.66	± 28.6	± 7.5
			Coefficient of variation %			± 28.4	± 21.8	± 4.4

accuracy with which water retention may be predicted from sodium and potassium retention must depend largely on the constancy of the relationship between water, sodium and potassium in muscle. Analyses were made of sixteen samples of muscle from eight animals and the results are given in Table 9. The contents of sodium and potassium expressed in mg.-equiv./litre of water showed large variations, ± 28.4% for sodium and ± 21.8% for potassium, whereas the equimolecular sum of sodium plus potassium had a coefficient of variation of only ± 4.4%. Muscle contains both extra- and intracellular fluids and the proportions vary considerably from sample to sample. Since extracellular fluid is rich in sodium and intracellular fluid rich in potassium, the closer relationship between the equimolecular sum of sodium plus potassium and water content is to be expected. To relate water content directly to the equimolecular sum of sodium and potassium may not, however, be the most satisfactory procedure. These two ions are not the only dations present in body fluids and the water associated with one gram-equivalent of sodium may not be the same as that associated with one gram-equivalent of potassium. Part of the sodium or potassium may also be bound to protein or other cell constituents. The figures for muscle showed a slight increase in sodium plus potassium content with increasing potassium content, but it was not statistically significant. With this single tissue, however, the variation in potassium content was small.

A much wider variation was obtained by reference to the analyses of other tissues given in Tables 10 and 11. For each tissue, the equimolecular sum of sodium and potassium was more closely related to water content than was either the sodium or the potassium content. The mean values for the equimolecular sum of sodium and potassium for the different tissues, however, varied from 143.2 ± 28.8 for fat to 184 ± 5.3 for brain and the variations could not be explained solely by changes in the proportions of sodium to potassium, but the values for the individual tissues require detailed comment.

Body fluids (Table 10) The equimolecular sum of sodium

TABLE 10

The water, Na and K contents of the blood, blood cells, serum and certain "protein-free" fluids of cattle

Animal	Age (weeks)	Composition of tissues					
		Water (%)	Na (mg./100 g.)	K (mg./100 g.)	Na (mg.-equiv./ litre water)	K (mg.-equiv./ litre water)	Total Na + K (mg.-equiv./ litre water)
				Defibrinated whole blood			
A	1	83.7	214	165	111	50	161
B	1	79.6	192	186	105	60	165
C	15	80.1	279	36	152	11	163
D	15	80.7	274	51	148	16	164
E	15	85.1	291	15	149	7	156
F	40	79.9	255	48	139	15	154
G	About 300	83.6	292	41	152	13	165
H	About 300	83.2	292	45	153	14	167
O	About 200	78.1	279	71	155	23	178
					Mean and standard deviation		163.7 ± 6.7
				Cells			
A	1	65.3	0*	441	0	173	173
B	1	65.1	27	370	18	145	163
C	15	66.6	207	55	135	21	156
D	15	66.3	190	92	124	36	160
E	15	68.3	197	10	125	4	129
F	40	61.1	174	74	124	31	155
G	About 300	71.7	162	69	111	28	139
H	About 300	72.5	201	64	120	23	143
O	About 200	78.1	186	129	125	51	176
					Mean and standard deviation		154.8 ± 15.7
				Serum			
A	1	93.7	346	17	161	5	166
B	1	92.9	343	17	160	5	165
C	15	91.1	337	21	161	6	167
D	15	92.4	343	17	162	5	167
E	15	92.3	332	17	155	5	160
F	40	92.5	341	20	160	6	166
G	About 300	89.9	359	25	174	6	180
H	About 300	90.0	350	33	169	9	178
O	About 200	90.4	365	18	175	5	180
					Mean and standard deviation		169.9 ± 7.4
				Pericardial fluid			
A	1	97.7	344	14	153	4	157
F	40	96.3	329	18	149	5	154
G	About 300	97.5	342	21	153	6	159
H	About 300	97.6	333	19	148	5	153
					Mean and standard deviation		155.7 ± 2.75
				Posterior chamber fluid			
A	1	98.8	325	20	143	5	148
B	1	97.7	310	16	138	4	142
C	15	98.9	329	13	145	3	148
D	15	98.9	330	23	145	9	154
E	15	98.9	314	33	138	8	146
F	40	98.8	327	17	144	4	148
G	About 300	98.8	345	22	152	6	158
H	About 300	99.9	329	19	145	5	150
					Mean and standard deviation		149.2 ± 1.45

*The method of calculation (see p. 20) in this instance resulted in a negative value for the Na. This was ignored in the ensuing calculations.

TABLE 11

The water, Na and K contents of brain and liver tissue and of fat depôts (perinephric fat) of cattle

Animal	Age (weeks)	Composition of tissues					
		Water (%)	Na (mg./100 g.)	K (mg./100 g.)	Na (mg.-equiv./ litre water)	K (mg.-equiv./ litre water)	Total Na + K (mg.-equiv./ litre water)
				Liver			
A	1	74.5	71	298	42	102	144
B	1	75.3	84	276	49	94	143
C	15	72.4	92	342	55	121	176
D	15	72.5	70	337	42	119	161
E	15	74.6	80	360	47	123	170
F	40	73.6	72	325	43	113	156
G	About 300	70.2	75	346	47	126	173
H	About 300	72.3	66	372	40	132	172
					Mean and standard deviation		161.0 ± 4.1
				Brain			
A	1	81.7	140	341	74	107	191
B	1	81.2	145	317	78	100	178
C	15	80.7	143	335	77	106	183
D	15	81.2	147	322	78	102	180
E	15	81.5	145	322	77	101	178
F	40	79.5	122	370	67	119	186
G	About 300	77.9	143	335	80	110	190
H	About 300	77.9	136	340	76	112	188
					Mean and standard deviation		184.2 ± 5.25
				Perinephric fat			
A	1	28.6	62	11	94	10	104
B	1	40.7	99	11	106	7	113
C	15	19.7	74	8	162	10	172
D	15	33.5	104	10	135	8	143
E	15	46.3	139	20	131	11	142
F	40	13.5	38	3	121	6	127
G	About 300	3.9	13	0	186	0	186
H	About 300	7.1	27	0	165	0	185
I	About 300	7.2	22	0	132	0	132
J	About 300	6.5	19	0	128	0	128
					Mean and standard deviation		143.2 ± 28.8

plus potassium per litre of water was consistently higher in serum than in either posterior chamber fluid or pericardial fluid. The difference was due entirely to a higher sodium content since the values for potassium were always comparable.

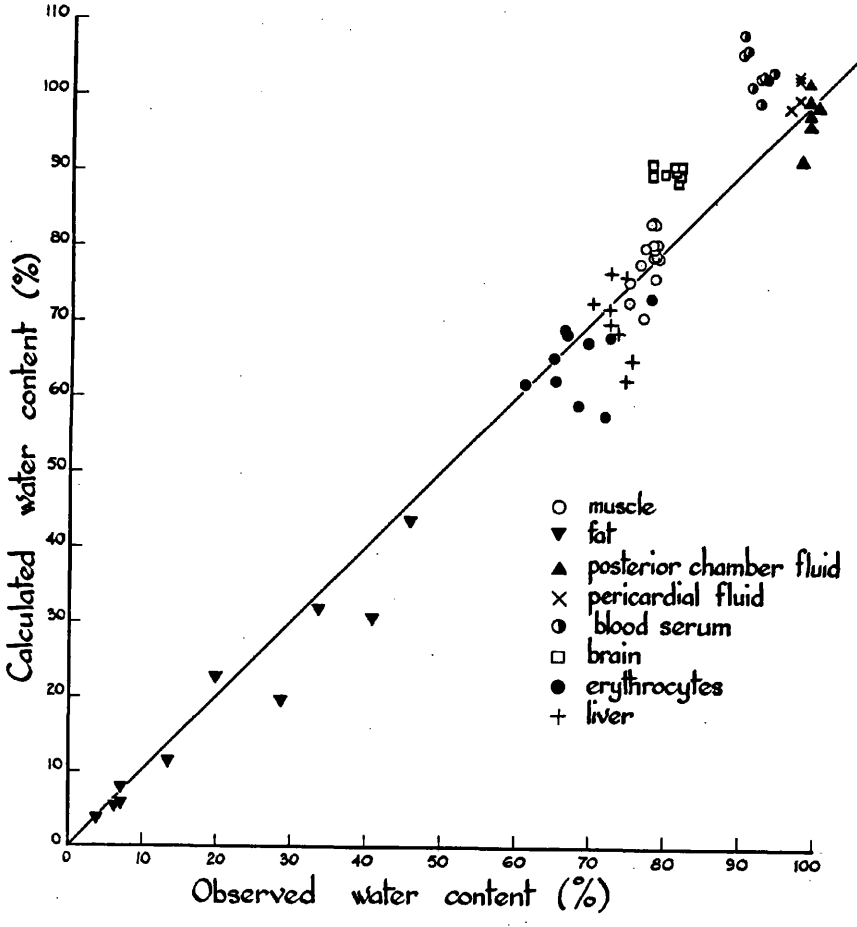
Erythrocytes (Table 10) The red blood cells showed a marked variation in composition. Cells from animals that were 1 week old contained little sodium whereas in animals that were 15 weeks old or more the cells were high in sodium and low in potassium. The blood cells of the week-old calf would be largely the result of foetal erythropoiesis, and it would appear that erythrocytes formed in the foetus have a composition quite distinct from those produced during extra-uterine life. The large error attached to the mean sodium plus potassium content may have been due in part to the method of calculation (see p. 20) which gives a summation of errors but there was also a positive correlation between the sodium plus potassium and the potassium content.

Fat (Table 11) The very high standard deviation attached to the mean equimolecular sum for the samples of fatty tissues is probably due to inaccuracies in the analytical procedures. The accuracy of a water determination on fat by direct desiccation is not high, and since the contents of sodium and potassium were low in most of the samples it was not possible to determine them with accuracy. The samples of fatty tissues from young animals contained a high proportion of muscle tissue and that accounted for their high water content.

Liver (Table 11) The values for the samples of liver tissue were remarkably constant apart from the two values for the 1 week old calves which had an electrolyte content significantly lower than that observed in the tissues of the mature animals.

Brain (Table 11) The total mg.-equiv. of sodium and potassium per litre of brain water were significantly higher than that observed in any of the other tissues which have been analysed. This cannot be explained entirely by the high ratio of potassium to

FIGURE 4. The relationship between the directly determined water content of body tissues and fluids and the water content calculated from their sodium and potassium contents.



sodium and, as will be shown later, it must be concluded that part of the sodium of brain tissue is present in non-ionic form.

The empirical relationship between the sodium, potassium and water of the body

Tabulation of the mean values for the sodium plus potassium content of the water contained in the different tissues in order of increasing potassium content (Table 12) shows that, apart from the values for brain and serum, an increase in the mg-equiv. of $Na^+ + K^+$/litre of water is associated with an increase in the concentration of potassium relative to sodium. An equation of the type:-

$$\underset{\text{(g./100 g. tissue)}}{\text{Water}} = a + b_1 \underset{\text{(mg./100 g. tissue)}}{Na} + b_2 \underset{\text{(mg./100 g. tissue)}}{K}$$

was therefore fitted, by the method of least squares, to the results for tissues and fluids other than brain and serum, and the following equation was obtained:-

$$\underset{\text{(g./100 g.)}}{\text{Water}} = 3.702 + 0.2794 \underset{\text{(mg./100 g.)}}{Na} + 0.1395 \underset{\text{(mg./100 g.)}}{K} \quad \ldots\ (4)$$

The regression was very highly significant but an equation containing a constant term would not be suitable for the prediction of water storage in balance experiments. Since the error attached to the intercept was $\pm$ 4.55, the intercept of $\pm$ 3.702 was not significantly different from zero and a further analysis of the values, in which the intercept was deliberately placed at zero, gave:-

$$\underset{\text{(g./100 g.)}}{\text{Water}} = 0.2922 \underset{\text{(mg./100 g.)}}{Na} + 0.1471 \underset{\text{(mg./100 g.)}}{K} \quad \ldots\ (5)$$

The errors attached to the two regression coefficients, obtained by analysis of variance technique, were:-

Regression coefficient for Na, 0.2922 ± 0.00391

Regression coefficient for K, 0.1471 ± 0.00266

The standard deviation of the residuals was $\pm$ 4.6 g. of water, a

TABLE 12

The mean Na + K content of the water contained in tissues and fluids in relation to their content of K

Tissue	No. of samples	Total Na + K (mg.-equiv./ litre water)	K (mg.-equiv./ litre water)
Pericardial fluid	4	156	5
Fatty tissue	10	143	5
Serum	9	170	6
Posterior chamber fluid	8	149	6
Blood cells	9	155	57
Brain	8	184	107
Liver	8	161	116
Muscle	16	169	131

TABLE 13

The accuracy of prediction of the water content of tissues from their Na and K contents

Tissue	Observed water content (%)	Over- (+) or under- (-) estimation of water content	
		g./100 g. tissue	% of observed water content
Posterior chamber fluid	98.8	- 0.5	- 0.5
Pericardial fluid	97.3	+ 3.8	+ 3.9
Muscle	77.0	+ 0.7	+ 0.9
Liver	73.2	- 2.1	- 2.9
Red blood cells	68.3	- 3.1	- 4.5
Fat (juvenile)	30.4	- 3.8	- 12.5
Fat (adult)	6.2	- 0.1	- 1.2
Serum	91.7	+ 12.5	+ 13.6
Brain	80.2	+ 10.1	+ 12.6

TABLE 14

The effect of age on the accuracy of prediction of the water content of cattle from their Na and K contents

Animal	Age (weeks)	Over- (+) or under- (-) estimation of water content	
		g./100 g. tissue	% of observed water content
A	1	- 2.11	- 2.8
B	1	- 3.38	- 4.7
C	15	+ 1.85	+ 2.7
D	15	+ 0.98	+ 1.4
E	15	- 2.18	- 2.9
F	40	- 0.10	- 0.1
G	About 300	+ 0.72	+ 1.1
H	About 300	- 0.68	- 0.9
		Mean value	- 0.8

value slightly higher than that obtained for equation (4).

It can be calculated from equation (5) that 149 mg.-equiv. of Na^+ and 174 mg.-equiv. of K^+ are associated in the body with a litre of water. The cationic concentration which would give rise to an osmotic pressure equal to that of body fluids is 148 mg.-equiv./ litre of water, a value close to that obtained for sodium. The value observed for potassium is, however, considerably higher and it must be concluded that at least 15% of the potassium is in combination with cell constituents and exerts a negligible osmotic pressure. Stone and Shapiro (1948) have shown that 25% of the potassium of brain and muscle is non-diffusible.

The differences between the water contents of the individual tissues calculated from equation (5) and those determined directly (Table 13, Figure 4) were significant only with brain and serum. In the tissues to which the equation applies, the Na : Cl ratio (when the concentration of the ions are expressed in mg.-equiv.) is 1.0 : 0.8, whereas in brain the ratio is 1.0 : 0.66. (Similar data are not available for serum, since it was not separated under conditions which excluded the chloride shift.) It must be assumed, therefore, that part of the sodium of brain and possibly of serum is not freely diffusible.

It can be seen from Table 14 that no significant effects of age or individuality of the animal on the accuracy of prediction of the water content of tissues from their sodium and potassium content were observed.

The accuracy of prediction of the water content of bone, cartilage and skin from their contents of sodium and potassium

Approximately 30% of the total body sodium is present in the skeletal tissues (Davies, Kornberg and Wilson, 1952) and the values given in the literature for the sodium content of bone are, in relation to the water content, several times higher than those found in other sodium-rich tissues. In contrast, the sodium

TABLE 15

The water, Na and K contents of the skin, cartilage and bone of cattle

Animal*	Composition			Water content calculated from equation (5)	Calculated water as a % of that determined
	Water (%)	Na (mg./100 g.)	K (mg./100 g.)		
			Skin		
S	66.0	208	19	63.6	96.4
S	70.1	222	15	67.1	95.7
T	64.1	191	9	57.1	89.1
T	66.7	203	16	61.7	92.5
			Cartilage		
S	71.9	230	6	68.1	94.7
T	69.5	226	5	66.7	96.0
V	65.3	235	29	73.0	111.8
			Bone		
S	17.8	467	0	766.9	1141
S	29.6	403	0	398.0	592
S	23.6	454	0	362.3	836
T	32.4	376	0	336.4	505
T	29.2	393	0	393.2	585
T	24.8	343	0	404.0	601

*All these animals were about 3 years old

content of cartilage (Silber, 1933) is only slightly higher than that of other soft tissues. Water storage predicted by means of equation (5) would be in error if there was a release or storage of sodium in the skeleton during the period of a balance experiment. A further error in the use of the equation would arise if the superficial moisture on the hair and skin of an animal was not estimated accurately by the sodium and potassium balance technique. Samples of bone, cartilage and skin analysed for sodium, potassium and water content gave the results presented in Table 15. The accuracy with which equation (5) predicts the water content of the tissues is indicated in the last column of the table.

The results for cartilage are quite comparable with those of other soft tissues. The high sodium content of bone, however, is confirmed but, as shown in Table 16, the sodium in excess of that accounted for by the water content is closely correlated with both the ash and calcium contents. Excess sodium stored in bone throughout a balance period could therefore be calculated from the ash or calcium storage. Statistical analysis of the results showed that the excess sodium was more closely related to ash content than to calcium content and the following equation relating the excess sodium of bone to ash content was obtained.

$$\underset{(mg.)}{\text{Excess Na}} = 10.34 \times \underset{(g.)}{\text{Ash}} - 68.7 \quad \ldots\ldots\ldots\ldots (6)$$

The intercept, however, was not significant and placing it deliberately at zero, gave:-

$$\underset{(mg.)}{\text{Excess Na}} = \underset{(g.)}{\text{Ash}} \times 8.606 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Since only 80% of the total body ash is stored in bone, the excess sodium retained throughout a balance period would be given by:-

$$\underset{(mg.)}{\text{Excess Na}} = \underset{(g.)}{\text{Ash}} \times 6.88 \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

TABLE 16

The 'bound' Na in bone in relation to the Ca and ash content

Animal	'Bound' Na (mg./100 g.)	Ca (%)	Ash (%)
S	25.8	11.77	30.35
S	26.5	12.47	31.79
S	29.3	14.12	36.45
T	30.1	13.39	34.62
T	37.2	16.41	42.46
T	40.6	17.25	44.50

In the four samples of skin analysed, the water content was consistently underestimated by equation (5). If this discrepancy was due to superficial moisture of the skin and hair, the size of the discrepancy would vary from time to time, depending on atmospheric and other conditions, and over a short term experiment this could lead to a considerable error in the estimation of water content.

The accuracy of predicting the amount of water in the contents of the alimentary tract from their sodium and potassium contents

The contents of the alimentary tract are in a constant state of flux, receiving large quantities of food and water at irregular intervals and being mixed continuously with secretions into the gut, and are the parts of the body which would be least expected to adhere to the relationship expressed in equation (5). Table 17 summarizes the analytical results for the contents of the four major compartments of the alimentary tract of three adult animals, one of which was starved for 5 hr. before slaughter.

Only with the contents of the abomasum did the water content calculated from equation (5) differ grossly from that determined directly. The gastric secretions contain a high concentration of hydrogen ions and consequently a low content of sodium and potassium. Generally, the error attached to an estimate of water content by equation (5) was higher for gut contents than for other body tissues. With animal R, however, the estimates were higher than those for the unstarved animals and showed an accuracy comparable to that for other tissues.

The accuracy of prediction of the water of foetal tissues and uterine fluids from their sodium and potassium contents

During pregnancy, particularly in the later stages, very large quantities of water are stored by the animal in the foetus and surrounding uterine fluids. The analytical results for these materials, and the accuracy with which the water contained in them

TABLE 17

The amounts of water and concentrations of sodium and potassium in the gut contents of adult cattle

Organ	Animal*	Composition			Calculated water content (%)	Calculated water as a % of that determined
		Water (%)	Na (mg./100 g.)	K (mg./100 g.)		
Rumen	P	95.1	301	24	91.5	95.8
	Q	96.1	293	33	90.3	94.0
	R	85.0	261	136	96.2	113.2
Abomasum	P	92.5	146	555	50.7	54.8
	Q	78.1	115	81	45.5	58.3
	R	91.7	157	97	60.1	65.5
Small intestine	P	90.3	197	79	68.5	75.9
	Q	92.6	251	75	84.3	91.0
	R	92.8	299	94	101.1	108.9
Caecum	P	87.3	196	85	69.7	79.8
	Q	84.3	171	133	69.5	82.4
	R	90.7	228	156	89.5	98.7

*Hay and water were available to P and Q immediately before slaughter, but R had been starved for 5 hr. before it was killed

TABLE 18

The water and electrolyte contents of foetal tissues and fluids

Material	Age (months)	Composition			Water content calculated from equation (5) (%)	Calculated water as a % of that determined
		Water (%)	Na (mg./100 g.)	K (mg./100 g.)		
Whole foetus	3	91.2	233	160	91.5	100.3
	5	89.0	202	185	86.1	97.8
Foetal tissue						
Muscle	7	85.7	141	244	77.0	89.8
Liver	7	82.2	136	229	73.3	89.2
Brain	7	90.1	188	231	88.8	98.6
Amniotic fluid	3	98.9	307	66	98.4	99.5
	5	98.9	286	64	92.9	93.9
	8	98.8	341	14	101.7	102.9
Allantoic fluid	3	98.5	173	38	56.1	57.0
	5	97.6	72	124	39.2	40.2
	8	96.2	152	67	54.3	56.4

is predicted by equation (5), are given in Table 18.

With the 7 months old foetus, estimates of the water content of the muscle and liver were low but the calculated water content of the brain gave a correct estimate, which was higher by 9% than the values for liver and muscle. This suggests that the sodium, potassium and water relationships in foetal tissues differ from those in the tissues of mature animals, but that the preferential retention of sodium by brain tissue occurs also in foetal life. The correct estimates of the whole foetuses of 3 and 5 months were due apparently to the brain water accounting for a large part of the total water.

The water content of amniotic fluid was predicted accurately from the sodium and potassium contents, but a discrepancy of the order of 50% was observed with allantoic fluid, which is known to contain large quantities of urea and other non-polar metabolic end products.

Discussion

The empirical relationship expressed in equation (5) applies to most of the tissues and fluids of the animal body. The standard error of estimate of the water content of muscle, fatty tissue, liver, the red blood cells, cartilage and the fluids of the pericardial sac and the posterior chamber of the eye was $\pm$ 2%. Within the limits of this error, no significant differences in the relationship between the sodium, potassium and water contents of these tissues were observed, nor was any effect of the age or individuality of the animal noted. If, however, the variation represented by a standard deviation of $\pm$ 2% arose from variations in the relationship between sodium, potassium and water from time to time and from animal to animal, it would be sufficient to invalidate the use of equation (5) for the prediction of water storage, since the limit of error given on p. 30 is $\pm$ 0.2% of the total body water. If on the other hand, it was due to errors in the analysis of the materials it would be of less consequence. That

this was the source of error is suggested by the results in Table 14, which show that the deviations for individual tissues were greater than those for the individual animals.

The methods used for the determination of water content are subject to small systematic errors, since drying to constant weight at 100° causes the loss of volatile organic constituents and oxidative changes in the lipid fractions. These errors are systematic but will compensate to some extent for one another and only with the fatty tissues of young animals would the water content be expected to be in error by more than ± 1%. In the determination of sodium and potassium, duplicate analyses agreed to within ± 1% in tissues in which the elements were present in significant amounts, but greater absolute errors may have been incurred. Where these elements were present in low concentration, the accuracy was not so high. It is therefore conceivable that errors in the analytical methods could give rise to a standard error of estimate of ± 2%.

Of the various tissues in which the water content was not predicted accurately by equation (5), the water present in brain, serum and skin is only a small proportion of the total body water and the deviations observed would give rise to an error of less than 1% of the total body water. Moreover, these errors would be systematic and would not affect markedly the accuracy of a predicted water balance unless changes in the water of any of these individual tissues was much greater than those in the rest of the body. The sodium bound in the mineral salt of bone, however, accounts for 20 to 30% of the body sodium and predicted water storage would be in considerable error if corrections were not made for storage or loss of sodium in bone. Corrections of the sodium balance by means of equation (8), which relates the bound sodium to ash stored, prior to the use of equation (5), would lead to more accurate results.

The water contained in the gut of an animal is approximately

15% of the total body water. Furthermore, the erratic body weight changes noted in animals is due almost entirely to sporadic changes in the 'fill' of the animal, and the accuracy of an estimate of water storage would depend largely on the accuracy with which a change in water of the gut could be estimated from equation (5). The water of the abomasal contents amounts to only a small percentage of the total water of the gut and its underestimation would give rise to a systematic error of only ± 0.2%. The smaller discrepancies observed for the other gut contents would also produce systematic errors of less than ± 1% of the total body water. The effect of starving animal 'R' (Table 17) on the accuracy of prediction of the water content of the gut emphasized, however, the importance of ensuring that an experimental animal is under comparable conditions at the beginning and at the end of a balance period. Any error incurred by a change in equilibrium would no longer be systematic but would be a direct error on the estimated water storage.

The greatest inaccuracies in the use of equation (5) for estimating water storage would arise during the last few months of pregnancy. During that period allantoic fluid may be stored at the rate of 2.4 kg. in a month, and the water balance could be in error by as much as - 1.2 kg. due to the underestimation of water stored in the allantoic fluid. The method would, therefore, be of limited value during the later stages of pregnancy, but there remains the possibility of correcting the water balance for the storage of allantoic fluid by calculation from the figures given by Hammond (1927).

It is difficult to assess the final accuracy with which the total body water of an animal could be calculated from the body sodium and potassium but, with the exception of allantoic fluid, the final errors incurred by the discrepancies noted in the various tissues should not exceed ± 2%. It is, however, doubtful whether

water balance could be estimated in a metabolic experiment with anything like the same accuracy.

SECTION IV

USE OF THE INDIRECT METHOD FOR ESTIMATING STORAGE OF WATER AND FAT IN THE CALF

In the preceding section, equations relating body water, sodium, potassium and ash have been derived which provide a satisfactory basis for the indirect estimation of total body water. Experiments are now reported in which these equations were used to estimate water retention in animals from sodium, potassium and ash retentions, and the accuracy of the procedure has been assessed by reference to simultaneous estimates of water retention by methods involving respiration calorimetry. At the same time, fat storage estimated as the difference between the body weight gain and the retentions of ash, protein and water (calculated from sodium, potassium and ash retentions) was compared with fat storage calculated from retentions of carbon and nitrogen.

Calves were used as experimental animals since during growth they are capable of storing large quantities of water relative to their body weight, and by varying the food intake a wide range in water retention can readily be obtained. If it proved possible to estimate water retention in calves over a wide range of actual retentions, a more critical test would be provided than would be possible with mature cattle.

Plan of experiment

Calves were fed various amounts of cows' whole milk or of an artificial milk diet prepared by the method of Clark (1927), and serial determinations of the intake and excretion of nitrogen, ash, water, sodium and potassium and of fat storage and body weight gain were made over periods of 6 to 16 days. Details of the calves used and of their experimental treatment are given in Table 19.

The calves were purchased when a few days old and on arrival at the Institute were harnessed for the separate collection of

TABLE 19

Treatment of experimental animals

Calf No.	Run	Diet	Level of feeding (litres/day)	Length of experimental period (days)
167	1	whole milk	4½	8
168	1		4½	8
169	1		4½	8
188	1		3½	6
188	2		7	16
188	3		2*	8
219	1	artificial milk	4	10
220	1		6	8

*In addition this calf was fed 2 litres of water daily

faeces and urine and confined individually to metal-lined metabolism cages in a house maintained at 55 - 60°F. They were reared on cows' whole milk until required for experimental purposes and were fed at intervals of approximately 12 hr., half the daily diet being given at each feed.

A preliminary period of at least 4 days on a constant food intake preceded each experiment. During the course of an experiment the calves were confined to a respiration chamber and the water vapour and carbon dioxide production and oxygen consumption were determined over successive 24-hr. periods. Faeces and urine were collected quantitatively and analyses of feed and excreta were made to permit the calculation of the retentions of protein, water, ash, sodium and potassium and the storage of fat, as described in detail in Section II. All collection periods were of 48 hr. duration and were started at approximately 5 hr. after the morning feed. The animals were weighed to the nearest half ounce at the beginning and end of each collection period.

Results

Comparison of water retention estimated by the 'classical' method with that estimated by 'difference' from the gain in body weight

The water retained by an animal may be determined by the technique of respiration calorimetry in two different ways. In the so-called 'classical' method, water retention is determined as the difference between the intake of water and the excretion in the urine and faeces and through the lungs and skin. With the intake, however, has to be included what is termed 'metabolic' water, which arises in the dissimilation in the body of fat, protein and carbohydrate. This has been calculated from the catabolized nutrients, as estimated from data on carbon dioxide production, oxygen consumption and urinary nitrogen excretion (see Møllgaard, 1929), by applying the following factors:

On oxidation in the body: 100 g. protein produce 41 g. water
100 g. fat produce 107 g. water
100 g. carbohydrate produce 60 g. water

These figures were obtained by Magnus-Levy (1907) and, strictly, they refer only to humans given a 'mixed' diet; their applicability to cattle has been assumed by workers in animal nutrition (cf. Brody, 1945). Details of the calculation of water retention in calves by the classical method are given in Table 20, and it is clear that an accurate estimate of metabolic water is required, since it is of the same order as the water retention. It is therefore possible that a considerable systematic error has resulted from the use of the above factors to calculate the metabolic water.

The absolute error of an estimate of water retention by the classical method would include also errors in the estimation of water intake and excretion. These could be quite large since, as will be seen in Table 20, the water intake and excretion is many times greater than the water retention. In the instances quoted in Table 20, a systematic error of 1% in the estimation of the water content of the diet or of the urine would produce an error of the order of 350 g. in the estimated water retention. An error of 1% is certainly possible in the gravimetric estimation of the water content of urine by drying to constant weight at 100°, due to the loss of urea and other volatile organic constituents.

The second method of estimating water retention, which for convenience will be referred to as the 'difference' method, is based on the fact that the change in weight of an animal is made up of changes in the body content of fat, protein, ash and water; it is assumed that changes in the carbohydrate content of the animal body are small. Since fat and protein storage can be calculated with accuracy from C and N retention, and ash retention can be determined directly, it is possible to make an estimate of water retention by subtracting values for fat, protein and ash retention from the observed gain in body weight. Here, one would expect the main source of error to be in the determination of body weight gain. In the present series of experiments, body weight

TABLE 20

Calculation of water storage in calves by the classical method (8-day periods)

Calf No.	Total intake of water (g.)	Metabolic water (g.)	Water excretion (g.)			Water retention (g.)
			Urine	Faeces	Lungs and Skin	
167	35,272	2,187	28,042	235	5,456	3,726
168	35,316	2,250	28,055	304	5,730	3,477
169	35,449	2,298	26,980	144	7,427	3,196

TABLE 21

Storage of water, fat, protein and ash by calves
(8-day periods)

(The values not in brackets are in grams. The values in brackets express each retention as a percentage of the body weight gain)

Calf No.	Water retention	Protein storage	Fat storage	Ash retention	Body weight gain
167	2,869 (67.5)	724 (17.0)	510 (12.0)	147 (3.4)	4,250
168	3,163 (85.2)	340 (9.1)	114 (3.0)	93 (2.5)	3,710
169	3,049 (71.2)	759 (17.7)	350 (8.1)	122 (2.8)	4,280

was recorded to the nearest half ounce but it is difficult to weigh an active calf with such accuracy. It is probable that the error of estimation was of the order of ± 50 g., giving rise to an error in the estimated water retention of about ± 70 g. This error would of course be independent of the length of the experimental period and of the rate of water retention. Errors would also arise in estimating the C, N and ash retentions, and in calculating fat and protein storage from C and N retentions, but such errors would not prove critical since, as shown in Table 21, storage of fat, protein and ash in the calf accounts for only a small part of any gain that occurs in body weight.

A series of estimates of water retention in calves by the two methods is given in Table 22 and the results are compared graphically in Figure 5. The continuous line in the figure is the line for complete agreement. Details of the calculation of water retention by the two methods are set out in Table 23.

With the exception of the results for Calf 167, estimates of water retention were made over successive 2-day periods and for the twenty-three 2-day comparisons, water retention by the classical method was on average 62.3 ± 36.0 g./day higher than the estimated retention by the difference method, a value approaching statistical significance at the 5% level ($0.1 > P > 0.05$). In spite of the known limitations of the two methods as outlined above, this discrepancy seemed unduly large. In two of the experiments (Calf 167; Calf 188, Run 1), difficulties were encountered in the collection of urine due to a blockage of the urine outlet pipe by hair from the body of the calf, causing a loss of urine into the bottom of the respiration chamber. Though at the time it was considered that no overall loss of urine had occurred, the large discrepancies between estimates of water retention by the two methods in these two experiments suggested later that some urine might possibly have been lost. A check on any loss of urine was made by comparing the measured gain in weight with the difference in weight between materials entering and leaving the respiration

TABLE 22

Comparison of water retention in calves determined by the classical method and the water retention determined by 'difference' from the gain in body weight

Calf	Run	Days	Water intake (g.)	Water retention		Water retention by classical method less water retention obtained by difference (g.)
				By classical method (g.)	By difference (g.)	
167	1	1 and 2	8,790	945	1,499	275
		3 and 4	8,941	829		
		5 and 6	8,645	1,171	1,344	608
		7 and 8	8,896	781		
		1 - 8	35,272	3,726	2,843	883
168	1	1 and 2	8,805	438	1,363	- 925
		3 and 4	8,862	1,263	438	825
		5 and 6	8,842	740	456	284
		7 and 8	8,807	1,036	906	130
		1 - 8	35,316	3,477	3,163	314
169	1	1 and 2	8,861	602	701	- 99
		3 and 4	8,802	1,181	948	233
		5 and 6	8,931	925	875	50
		7 and 8	8,855	488	526	- 38
		1 - 8	35,449	3,196	3,050	146
188	1	1 and 2	7,115	795	688	107
		3 and 4	7,088	380	47	333
		5 and 6	7,098	1,222	996	226
		1 - 6	21,301	2,397	1,731	666
188	2	1 and 2	13,427	1,601	1,537	64
		3 and 4	13,447	836	837	- 1
		5 and 6	13,434	1,191	897	294
		7 and 8	13,823	976	941	35
		1 - 8	54,131	4,604	4,212	392
188	2	9 and 10	13,635	1,137	877	260
		11 and 12	12,102	319	139	180
		13 and 14	13,239	1,841	1,766	75
		15 and 16	13,230	792	322	470
		9 - 16	52,206	4,089	3,104	985
188	3	1 and 2	7,629	307	205	102
		3 and 4	7,545	230	74	156
		5 and 6	7,578	- 88	241	- 329
		7 and 8	7,597	310	- 123	433
		1 - 8	30,349	759	397	362
		Totals		22,248	18,500	3,748
		Mean 2-day values		824.0	685.2	138.8

FIGURE 5. The comparison of 2-day estimates of water retention made by the classical and 'difference' methods.

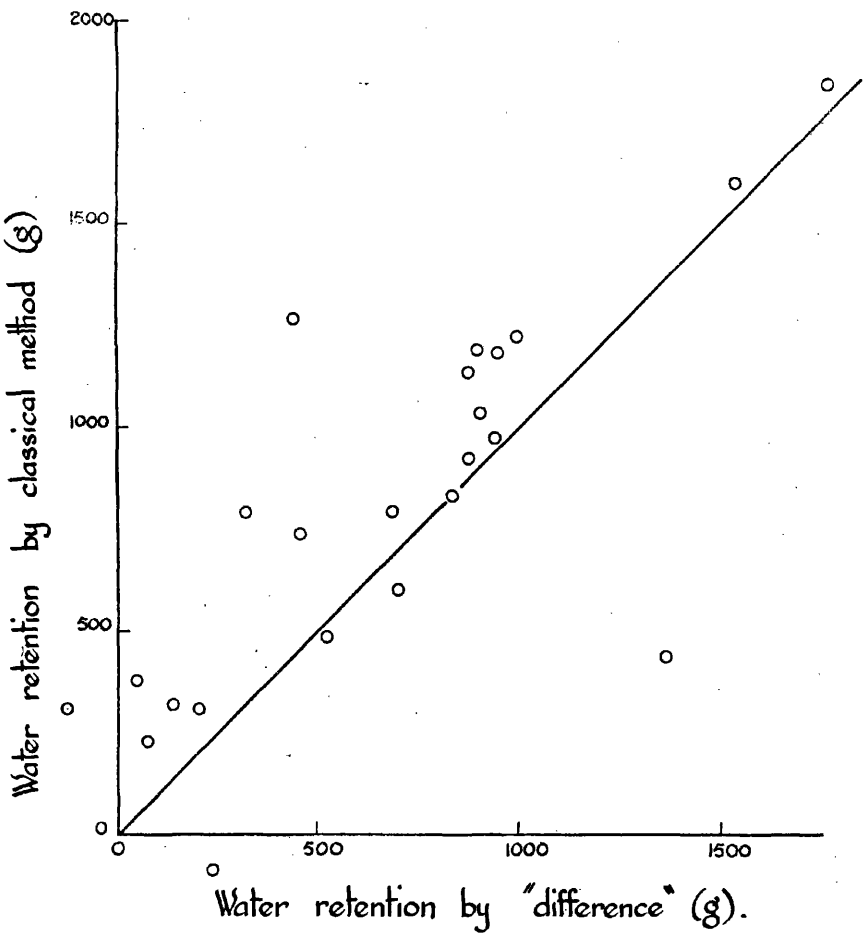


TABLE 23

The computation of water retention in calves 168 and 169 over a period of 8 days

(all values are expressed in g. water/8 days)

		Calf 168	Calf 169
(a) Classical method			
Intake of water in milk and washings	(a)	35,316	35,449
Metabolic water	(b)	2,250	2,298
Water in faeces	(c)	304	144
Water in urine	(d)	28,055	26,980
Water vapour expired	(e)	5,730	7,427
Water retention (a-b-c-d-e)		3,477	3,196
(b) Difference method			
Change in body weight	(a)	3,710	4,250
Fat retention	(b)	114	350
Protein retention	(c)	340	728
Ash retention	(d)	93	122
Water retention (a-b-c-d)		3,163	3,050

TABLE 24

The method of checking the quantitative collection of excreta in a respiration trial by comparing the observed gain in weight with the weight gain computed from the weights of materials taken in and excreted by the calf

(Results for calf 196 over 4-day periods)

	Period 1	Period 2	Period 3
Intake		(weights in g./day)	
Feed	3,010	3,004	5,082
Water	400	400	400
Oxygen	620	644	609
Total (A)	4,030	4,048	6,091
Output			
Urine	2,541	2,350	3,581
Faeces	68	113	391
Water vapour	899	880	1,107
Carbon dioxide	660	593	718
Hair and skin debris	7	10	6
Total (B)	4,175	3,946	5,803
Computed gain in weight (A-B)	- 145	102	288
Measured gain in weight	- 151	85	312
Discrepancy	+ 6	+ 17	- 24

chamber. The calculations involved are set out in Table 24 for three satisfactory experiments, in which the two values were in close agreement. For the two experiments in question (those with Calf 167 and Calf 188, Run 1), the computed gain in weight was considerably larger than the measured gain in weight and the results in these two experiments have been rejected on technical grounds.

The remaining five experiments, providing a total of twenty 2-day comparisons, gave a mean estimate of the daily water retention by the classical method which was 55.0 $\pm$ 40.2 g. higher than that by the difference method. Although this difference was not statistically significant ($0.20 > P > 0.10$), it was considerable and served to emphasize the difficulties involved in the determination of water retention by calorimetric methods. Attempts to account for this difference by a specific error in technique, other than the unavoidable experimental errors already mentioned, were not successful. The possibility that water vapour was not collected quantitatively by the absorption train, due to moisture condensing on the walls of the respiration chamber, was considered but did not appear to be a major source of error. The greatest difference between the temperature of the chamber air and the room temperature recorded during the experiments was 5°, with the chamber air at a temperature of 15°. To obtain condensation on the chamber walls, the air within the body of the respiration chamber would have to have been at least 80% saturated with water vapour. With the flow rate of chamber air of 4 cu. ft./min., the highest water vapour production recorded of about 1,000 g./day would produce only 10% saturation.

The mean of estimates of water retention by the two methods has therefore been taken as the best estimate of water retention, and will be referred to subsequently as the 'observed' water retention. The validity of taking the mean of the values obtained for Calf 188, Run 2b, may be questioned, since over the 8-day period the estimates of water retention by the two methods differed

TABLE 25

The error attached to the mean of estimates of water retention by the classical and difference methods, calculated from the results given in Table 22, excluding those for calves 167 and 188 (Run 1)

Number of simultaneous 2-day estimates of water retention by the classical and difference methods	=	20
Variance of the differences between the estimates and their mean	=	612,648
Standard deviation of the mean from the two estimates (2-day values)	=	$\pm$ 179.6 g. H_2O
		or $\pm$ 90 g. per day

by almost 1 kg. The omission of the results for this calf would not, however, alter the conclusions drawn later as to the accuracy of prediction of water retention by the indirect method. In two experiments, with Calves 219 and 220, water retention was estimated by the difference method only and these values have been used as a true measure of water retention.

The error attached to the observed water retention was estimated from the variance of the differences between the estimates by the classical and difference methods and their mean. The results are given in Table 25. The two methods of estimation can be regarded statistically as independent. Though the error of ± 90 g./day may appear large, it is slightly less than ± 2% of the mean daily water intake of 5 kg.

The accuracy of prediction of water retention by the indirect method

In all the experiments reported, water retention was estimated from sodium and potassium retention by means of equations (5) and (8). The calculations involved are set out in Table 26. In Table 27, the indirect estimates of water retention have been compared with the observed water retention in seven satisfactory experiments. The 2-day and cumulative 8-day values have also been presented graphically in Figure 6. For the twenty-nine 2-day observations, the two sets of values showed a mean difference of only 7.2 g./ 2-day period. In view of the fact that the mean water intake by the calves during these experiments was of the order of 5 kg./day, this overall agreement is good and suggests that there is no systematic over- or under-estimation of water retention by the indirect method.

Over 2-day periods, however, the predicted water retention deviated considerably from the observed water retention. The standard deviations for experimental periods of 2, 4, 6 and 8 days' duration have been calculated and are given in Table 28 and for periods of four days and longer, the value was roughly constant at ± 300 g. (The improved accuracy of prediction of water

TABLE 26

The calculation of predicted water retention from the measured retentions of sodium and potassium

(Results for calf 220, run 1, day 1)

Balance results

	Intake	Excretion in Urine	Excretion in Faeces	Retention
Ash (g.)	47.18	28.26	3.58	17.34
Sodium (g.)	6.79	5.98	0.01	0.80
Potassium (g.)	4.95	3.72	0.02	1.21

Prediction of water retention

Sodium retention = 0.80 g. .. .. (a)
Ash retention = 17.34 g. .. .. (b)
Sodium retained in bone = ash retention (g.) x 6.88 x 0.001 = 0.12 g. (c)
Sodium retained associated with body water (a - c) = 0.68 g. .. (d)
Water retained in body with sodium = (d) x 0.2922 x 1000 = 199 g. (e)
Potassium retention = 1.21 g. .. .. (f)
Water retained in body with potassium = (f) x 0.1471 x 1000 = 178 g. (g)
Predicted water retention = (e + g) = 377 g.

TABLE 27

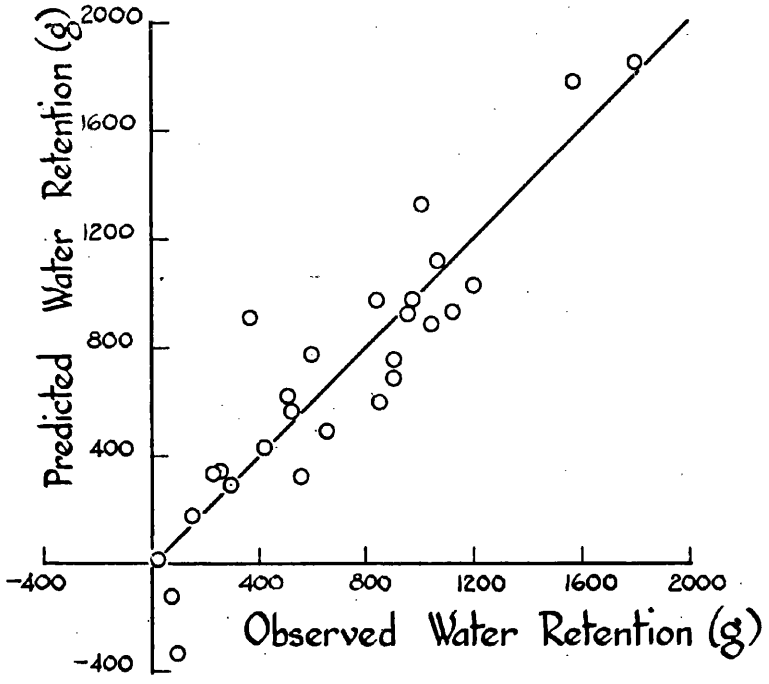
Comparison of predicted water retention with observed water retention in calves

Calf	Run	Days	Water intake (g.)	Observed water retention (g.)	Predicted water retention (g.)	Predicted water retention less observed water retention (g.)
168	1	1 and 2	8,805	901	684	- 217
		3 and 4	8,862	851	592	- 259
		5 and 6	8,842	598	778	180
		7 and 8	8,807	971	979	8
		1 - 8	35,316	3,321	3,033	- 288
169	1	1 and 2	8,861	652	489	- 163
		3 and 4	8,802	1,065	1,116	51
		5 and 6	8,931	900	754	- 146
		7 and 8	8,855	507	619	112
		1 - 8	35,449	3,124	2,978	- 146
188	2	1 and 2	13,427	1,569	1,781	212
		3 and 4	13,447	837	971	134
		5 and 6	13,434	1,044	885	- 159
		7 and 8	13,823	959	925	- 34
		1 - 8	54,131	4,409	4,562	153
188	2	9 and 10	13,635	1,007	1,332	325
		11 and 12	12,102	229	336	107
		13 and 14	13,239	1,804	1,854	50
		15 and 16	13,230	557	318	- 239
		9 - 16	52,296	3,597	3,840	243
188	3	1 and 2	7,629	256	341	85
		3 and 4	7,545	152	181	29
		5 and 6	7,578	77	- 118	- 195
		7 and 8	7,597	94	- 336	- 430
		1 - 8	30,349	579	68	- 511
219	1	1 and 2		1,122	932	- 190
		3 and 4		422	433	11
		5 and 6		523	463	- 60
		7 and 8		1,201	1,038	- 163
		9 and 10		367	906	539
		1 - 10		3,635	3,772	137
220	1	1 and 2		- 1,654	- 644	1,010*
		3 and 4		1,697	897	- 800*
		5 and 6		294	293	- 1
		7 and 8		24	17	- 7
		1 - 8		361	563	202
		Totals		19,026	18,816	2,275
		Mean 2-day values		656.1	648.8	- 7.24
		Standard deviation				± 202.3

*Calf restricted in movement during this period and part of the diet given on day 2 was not consumed until day 3. These values, therefore, have not been included in the calculation of the standard deviation.

FIGURE 6. The accuracy of prediction of water retention in calves from the measured retentions of sodium and potassium.

(a) 2-day periods



(b) Periods of 8 or 10 days

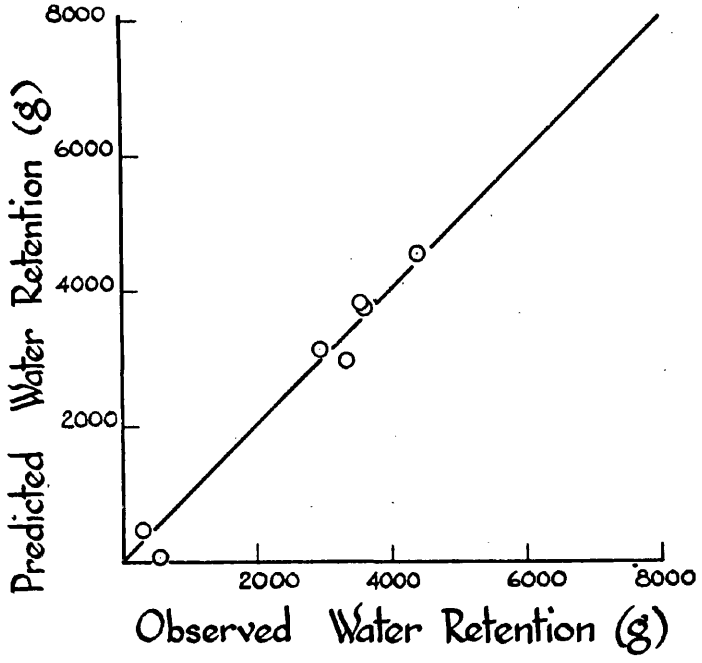


TABLE 28

The discrepancy between the observed water retention and water retention predicted from sodium, potassium and ash retention

(Calculations made from the results given in Table 27)

Period of observation (days)	Number of observations	Variance of estimates by the two methods	Standard deviation of estimates (g. H_2O)	Standard deviation (g. H_2O/2 days)
2	27	1,063,648	± 202.3	± 202.3
4	14	91,114	± 301.8	± 150.9
6	7	88,139	± 296.9	± 99.0
8	7	84,569	± 290.0	± 72.7

retention with increasing period length may be seen from Figure 6.) If, then, it is assumed that the error attached to the predicted water retention is invariant with period length, the error attached to the predicted water retention in an experimental period of 21 days' duration would be about ± 14 g./day.

The accuracy of estimates of energy retention by the indirect method

In the calculation of energy retention by the indirect method, the errors arising in the prediction of water storage produce an equivalent error, on a weight basis, in the predicted fat storage. In the calf, where fat and protein storage account for only a small part and water for a fairly large part of any gain in weight, the errors in the predicted water retention become more critical in the calculation of energy storage. This would not normally be the case with adult cattle, in which water storage accounts for a much smaller part of any gain in weight.

Estimates of energy storage calculated from the protein storage (N retention x 6.25) and the predicted fat storage (obtained by subtracting from the body weight gain the measured retentions of protein and ash and the predicted water retention), are compared in Table 29 with estimates of energy storage by the classical C and N balance technique. The mean values for the two methods of calculation differed by only 289 Cal./2-day period but the estimates over individual 2-day periods showed considerable discrepancies. As shown in Table 30, the standard deviation of estimates by the two methods was roughly constant at ± 3,000 to ± 3,700 Cal. for experimental periods of from 4 to 8 days' duration. This figure includes any error in the estimation of energy retention by the C and N balance technique but in these experiments the accuracy of such estimates was not assessed. In later work with sheep, however, Blaxter and Graham (1955) found that under similar conditions the standard deviation of estimates of energy retention by the C and N balance technique from those by the calorie balance technique was ± 57 Cal./24 hr. and that there was

TABLE 29

Energy retention estimated from body weight change and the retentions of Na, K, ash and N (indirect method) compared with energy retention determined by the C and N balance method (calorimetric method)

Calf	Run	Days	Energy storage (Cal.)		
			Calorimetric method	Indirect method	Difference
168	1	1 and 2	1,259	7,619	6,360
		3 and 4	36	- 1,406	- 1,442
		5 and 6	891	- 2,125	- 3,016
		7 and 8	2,691	8	- 683
		1 - 8	2,877	4,096	1,219
169	1	1 and 2	1,872	3,858	1,986
		3 and 4	2,136	562	- 1,574
		5 and 6	1,534	2,668	- 134
		7 and 8	1,610	739	- 871
		1 - 8	7,152	7,827	675
188	2	1 and 2	3,682	1,397	- 2,285
		3 and 4	2,869	1,614	- 1,255
		5 and 6	2,775	2,888	113
		7 and 8	3,208	3,358	150
		1 - 8	12,534	9,257	- 3,277
188	2	9 and 10	2,703	- 1,559	- 4,262
		11 and 12	1,108	1,136	28
		13 and 14	3,724	2,900	- 824
		15 and 16	3,007	1,171	- 1,836
		9 - 16	10,542	3,648	- 6,894
188	3	1 and 2	- 3,172	- 4,446	- 1,274
		3 and 4	- 2,697	- 3,699	- 1,002
		5 and 6	- 2,098	1,264	3,362
		7 and 8	- 2,034	- 38	1,996
		1 - 8	-10,001	- 6,919	3,082
219	1	1 and 2	2,492	4,272	1,780
		3 and 4	2,872	2,769	- 103
		5 and 6	2,440	3,002	562
		7 and 8	2,466	3,993	1,527
		9 and 10	2,387	- 2,662	- 5,049
		1 - 10	12,657	11,374	- 1,283
220	1	1 and 2	- 664	- 10,125	- 9,461
		3 and 4	1,549	9,042	7,493
		5 and 6	464	473	9
		7 and 8	808	874	66
		1 - 8	2,157	264	- 1,893
	Totals		37,918	29,547	-8,371
	Mean 2-day values		1,307.5	1,018.9	-288.6

TABLE 30

The discrepancies between estimates of energy retention by the classical C and N balance technique and by the indirect method, calculated from the results given in Table 29

Period of observation (days)	Number of observations	Variance of estimates by the two methods	Standard deviation of estimates by the two methods (Cal.)
2	27	138,842,357	± 2,311
4	14	119,630,602	± 3,034
6	7	53,365,611	± 2,893
8	7	82,308,840	± 3,706

no systematic difference between the two. If similar errors arose in the present work, the error of prediction of energy storage would not be less than ± 3,000 g. Even assuming that this error would not be increased with increasing length of experimental period, energy retention could not be predicted with an error of less than ± 150 Cal./day in a trial of 21 days' duration.

Discussion

The experiments reported here were planned to provide an estimate of the accuracy with which water retention can be predicted from sodium and potassium retention by means of the equations developed in Section III. It was thought at the time that the classical method of estimation of water retention, which involved direct measurements of water intake and excretion, would provide an accurate estimate against which the accuracy of predicted values could be assessed. With the start of the work, however, it became clear that under all circumstances the water intake and excretion of the calf is many times greater than the water retained within the body and that relatively small systematic errors in the estimation of water intake and excretion would lead to serious error in the estimated water retention. To provide a measure of such errors, a further method of estimating water retention dependent upon calorimetric observations but involving measurements independent of those used in the classical method was devised, and water retention was calculated by both methods in most of the experiments. Agreement between the two methods was not good and though the mean of estimates by the two methods was used as the best available measure of water retention, the error attached to this mean value was such that the assessment of the accuracy of the prediction of water retention by the indirect method cannot be considered unequivocal.

The overall agreement obtained between the water retention calculated by means of the prediction equation and the observed water retention was extremely good. In seven experiments, the

predicted water retention amounted in all to 18,816 g. compared with a value for the observed water retention of 19,026 g., the total exchange of water in these experiments being 260 kg. Thus, the theoretical basis of the prediction equation, that the retention of water within the animal body occurs concomitantly with the retention of sodium and potassium, is sound. Moreover, it appears that the empirical relationship between body sodium, potassium and ash and body water established by analysis of individual body tissues and fluids, applies equally to increments of body substance. The data were not extensive enough to indicate whether the accuracy of prediction of water retention varied significantly from calf to calf but no significant differences between animals with respect to the relationship between water content and sodium and potassium content of body tissues and fluids were observed in Section III.

These results were obtained with calves receiving only liquid diets but the prediction equation was derived from results obtained with both calves and mature cattle, and it seems unlikely that there are gross overall differences between the undeveloped and the mature ruminant with respect to body water, sodium and potassium relationships. This work with calves suggests therefore that the equations developed in the previous section would provide a reasonable basis for the prediction of water retention in mature cattle under natural conditions of feed supply. Under conditions which depart markedly from normal feeding practice, such as the feeding of experimental rations deficient in sodium and potassium, or in circumstances in which the acid-base equilibrium or the tissue hydration of the body was disturbed, this might not be so, but in trials designed to provide a measure of the energy value of feeds care would normally be taken to ensure that the general nutrition of the experimental animals was adequate.

Although this excellent overall agreement between the predicted and observed water retention was obtained, in short term comparisons over peridds of 2 to 8 days with individual calves, quite marked discrepancies were observed. The magnitude of these

discrepancies appeared to be independent of the length of period and of the total water retention. As discussed previously, the measured water retention includes with the water stored within the animal body any sporadic change in the amount of water present in the gut contents and in the urine retained in the bladder. Though the water present in the gut and in the urine is invariably associated with sodium and potassium, there is not the strict physiological relationship between them that there is in the body tissues and fluids. Sporadic changes in the water, sodium and potassium contents of the urine and the materials present in the gut would therefore lead to short term errors in the estimation of water retention by means of the prediction equation and could account for the observed discrepancies.

In the experiments reported here, these short term discrepancies gave rise to a roughly constant error in the predicted water retention of ± 300 g. over experimental periods of 2 to 8 days, and by assuming that the error was independent of period length it was calculated that water retention would be estimated to within ± 14 g./day in a trial of 21 days' duration. This would mean that in the calculation of energy storage by the indirect method, errors resulting solely from the use of the prediction equation to estimate water retention could amount to ± 130 Cal./day. By means of respiration calorimetry, using either the C and N balance or the calorie balance techniques, it is possible to estimate the energy exchange of a sheep, which is comparable in body size to the calf, to within ± 57 Cal./day over a period of 4 days. The indirect method then cannot be considered to provide a satisfactory alternative to the technique of respiration calorimetry for the study of the energy exchange of calves. It would of course be possible to reduce this estimated error of ± 130 Cal./day for the indirect method either by continuing the experimental trials over longer periods or by making estimates of the energy exchange on a single ration with a number of animals, but to do so would be to lose much of the advantage, in terms of labour and facility, of

the indirect method. Alternatively, an attempt could be made to reduce the error by emptying the bladder of urine at the beginning and end of each experimental period, either by catheterizing the animal or by conditioning it to void its urine on the application of a stimulus.

Errors similar in origin to the above would arise also in the prediction of water retention in mature cattle; indeed, the greater capacity of the alimentary tract resulting from the development of the rumen would be expected to increase such errors due to sporadic changes in fill. It would, however, be wrong to assess the probable accuracy of prediction of water retention in the adult animal solely on the basis of the present results obtained with the calf.

SECTION V

A NOTE ON THE USE OF THE INDIRECT METHOD FOR THE DETERMINATION OF FAT STORAGE IN MATURE RUMINANTS

The results of the preceding section have shown that, in experimental trials of relatively short duration, the major source of error in the prediction of water retention results from day-to-day variations in the patterns of excretion of water, sodium and potassium. Such variations are likely to be influenced by the rate of turnover of these metabolites and the results obtained with calves cannot be considered to apply in detail to mature cattle since the marked difference in ration and the development of the rumen must modify considerably the patterns of water, sodium and potassium metabolism.

It has not been possible to conduct critical tests of the indirect method with steers or lactating cows, since facilities for direct energy balance studies with large farm animals were not available. The respiration chamber designed for work with calves has, however, been suitably modified for use with sheep by Dr. K. L. Blaxter and Mr. N. McC. Graham (Blaxter, Graham and Rook, 1954) and, in collaboration with these workers, the indirect method was compared with the classical method of studying energy exchange in a number of trials with sheep. It was first shown that the relationship between body water, sodium and potassium established in cattle applied equally to the tissues and fluids of the sheep. The accuracy of prediction of water content from the sodium and potassium contents of some samples of tissues and fluids taken from sheep is given in Table 31.

The results of energy trials with sheep fed a ration consisting solely of dried grass are given in Table 32. In the single run carried out with sheep 1, which was given a level of feed approximately equal to the maintenance requirement of the animal, the predicted energy storage calculated over a 6-day period differed from that obtained by the C and N balance method by

TABLE 31

The water, sodium and potassium contents of tissues and fluids of the sheep

Sheep	Material	Composition			Predicted water storage (g.)	Over- (+) or under- (-) estimation (%)
		Water %	Na (mg./100 g.)	K (mg./100 g.)		
A	Muscle	71.2	74	300	65.9	- 7.5
		63.3	73	296	64.9	+ 2.5
B	"	69.5	74	300	65.8	- 5.3
		68.2	73	296	64.9	- 4.8
A	Liver	69.2	81	289	66.2	- 4.3
B	"	69.0	82	324	71.8	+ 4.2
A	Posterior chamber fluid	98.8	28	336	101.8	+ 3.0
				Mean underestimation of water content		- 1.74%*
A	Perinephric fat	25.2	51	103	30.1	+ 19.4
B	"	6.0	16	0	4.7	- 21.7
A	Erythrocytes	64.8	224	56	73.6	+ 13.6
A	Serum	91.3	341	48	106.7	+ 16.9
B	"	90.6	359	64	114.3	+ 26.2
A	Brain	77.6	149	333	92.5	+ 19.2
B	"	78.1	148	323	90.6	+ 16.0

*This value does not differ significantly from zero ($P > 0.05$)

152 Cal./day, a value of the same order as that obtained with calves. With experiments with sheep 2 and 3, however, which covered a wide range in the level of feeding, much larger errors were found, especially at the higher levels of feeding. A check on the accuracy of the various analytical methods in use, in particular the methods of estimation of sodium and potassium in the dried grass feeds which contained a high ratio of potassium to sodium, did not reveal any gross error. Yet, in the results for sheep 2 and 3, and particularly in the trials in which the sheep were maintained at a high level of feeding, the retention of potassium in relation to the retention of sodium seemed unduly high when compared with the results obtained previously with calves. Eventually, a search of the literature revealed that potassium is secreted in fairly large quantities through the skin of the sheep as the major inorganic constituent of a material known as suint, a normal component of the fleece (Freney, 1934). Analyses of several samples of fleece taken from sheep confined under conditions which precluded the possibility of contamination, have confirmed the very high potassium content of fleece (values of up to 6% have been obtained), as compared with the results reported for analyses of the skin of cattle, in which species there is no evidence of a skin secretion of sodium or potassium. By the measurement of fleece growth over limited areas of newly shorn sheep and analysis of certain protected areas for potassium content, the calculated secretions of potassium through the skin of three sheep were 425, 555 and 360 mg. of potassium/day. Daly and Carter (1955) have reported secretions of suint in fleece of up to 3 g./day, equivalent to approximately 700 mg. potassium/day, and have shown that suint production is roughly proportional to fleece growth rate, which in turn is dependent upon plane of nutrition. Skin losses of potassium of this order would give rise to very large errors in the prediction of energy storage by the indirect method; a skin loss of 700 mg. potassium/day would produce an error of the order of 1,000 Cal. in

TABLE 32

Estimates of energy storage in sheep by the indirect and the C and N balance methods

Sheep	Feed intake (g./day)	K retention (g./day) (mean for 8 days)*	Na retention (g./day) (mean for 8 days)*	Energy storage (Cal./day)	
				Indirect method (mean for 8 days)*	C and N balance method (mean for 4 days)*
1	750	0.81	- 0.03	- 61	91
2	500	0.88	0.40	- 2,277	84
	750	0.64	0.16	- 1,110	13
	1,000	1.80	0.65	- 1,340	395
	1,250	0.24	0.38	- 2,321	434
	1,500	1.30	0.68	- 1,984	625
3	500	± 0.00	- 0.14	- 823	- 353
	750	0.47	0.26	- 1,219	-61
	750	0.07	0.17	- 1,647	227
	1,000	0.55	0.45	- 522	- 8
	1,250	1.67	0.45	- 1,999	517
	1,500	2.02	1.00	- 2,943	633
	1,750	2.94	0.97	- 3,498	905
	2,000	2.09	1.54	- 4,934	1,045

*For sheep 1, the values given are the mean for 6 days only

the predicted energy storage, and losses of this type are thought to account for the large errors observed in Table 32. Since methods of determining the skin secretion of potassium by sheep are not likely to be of sufficient accuracy to allow estimates of body storage of potassium to be made with precision, the indirect method does not offer an alternative approach to the estimation of energy storage in sheep, an animal so commonly and conveniently used for net energy studies on feeds. Likewise, they could not be used to assess the probable value of the indirect method for the determination of the energy exchange of cattle.

This work with the sheep served a very useful purpose, however, in that it pointed to a critically important difference between the milk-fed calf and the mature ruminant. In the calf fed solely on cows' whole milk, the diet would not normally provide more than 12 g. potassium and 4 g. sodium daily. According to equation (5), the physiological equivalent of this amount of sodium and potassium is roughly 3 litres of water and a systematic error of 0.5% in the estimation of sodium and potassium would not lead to an error greater than 15 g. in the predicted water retention. In contrast, in the work with the sheep, that do not differ markedly in body size from the calves, the daily intake was much higher and varied from 10 to 50 g. of potassium and from 2 to 10 g. of sodium and an error of 0.5% in their estimation could give an error of 50 g. in the predicted water retention.

Relatively high intakes of sodium and potassium are characteristic of rations normally fed to sheep and cattle. In a series of balance experiments conducted by Forbes, Schulz, Hunt, Winter and Remler (1922) with milking cows giving up to 5 gallons of milk daily, the potassium intake varied from 93 to 151 g. and the sodium intake from 13 to 27 g. In studies by Ward, Blosser and Adams (1951), daily intakes of up to 384 g. of potassium and 21 g. of sodium were recorded. Thus in cattle limitations in the methods of analysis of materials for sodium and potassium would be sufficient in themselves to prevent an accurate estimation of

water retention and consequently of energy storage by this sodium and potassium method. The chemical methods currently used to estimate sodium and potassium in cattle feeds are likely to be subject to systematic errors of at least 1%; such errors in the measurement of a daily intake by cattle of only 100 g. potassium/day would give an error in the predicted water retention of 147 g./day, or about 3 kg. in a 21-day period. Alternative methods of analysis based on flame photometric and spectrographic techniques would give rise to even larger errors.

At the present time, it is this technical problem of determining with accuracy the retention of sodium and potassium by an animal fed a ration containing a high content of these elements, especially of potassium, which discourages the further study of the estimation of water retention in cattle from sodium and potassium retention and the use of the predicted water retention in the indirect estimation of energy storage. It is a matter for speculation whether the newer techniques in analysis, for example those involving the use of radio-active isotopes, may in the future offer an error of only $\pm$ 0.1% or less, which would raise again the possibility of estimating energy storage in cattle indirectly according to the approach outlined in this thesis.

SUMMARY

1. Systems of evaluating the energy contents of feeds and the energy requirements of cattle have been briefly reviewed. Evidence is quoted which indicates that feeding standards and tables of feed values currently in use, that are based on the total digestible nutrient system, or on the Scandinavian feed-unit or the net energy (starch equivalent) systems of evaluating feed energy, are not reliable guides to the feeding of cattle. The net energy system is theoretically the most sound but very large errors are attached to published net energy and starch equivalent values of feeds.

2. An analysis has been made of sources of error in the determination of net energy values. The analysis has shown that the errors arise largely in the measurement of the net efficiency of utilization of metabolizable energy, and that they are caused in part by wide biological variations that exist between animals and within individual animals from time to time. It was apparent also that technical and interpretational errors tend to be magnified by the design of experiment used. It was concluded that the determination of net energy values on a wider scale than has been possible hitherto would reduce considerably the error due to the variability of the test animals; this has not been attempted previously because of the need to determine the total energy exchange of cattle by means of respiration calorimetry, an expensive, laborious and time-consuming technique.

3. Alternative methods of determining energy exchange in cattle were considered, and it was concluded that a method might be devised which would be based on the indirect estimation of fat storage from the gain in body weight less the body retentions of protein, ash and water, water retention itself being determined by some indirect method. This possibility was therefore investigated, and it was concluded that the calculation of water retention from

body retentions of sodium and potassium might be suitable for the indirect estimation of fat storage in cattle.

4. Analyses of tissues and fluids from cattle of widely differing ages were made, and from the results it was shown that with the exception of blood serum, brain, skin and bone, the water contents of the tissues and fluids could be predicted from the equation:-

$$\underset{(g./100\ g.)}{\text{Water}} = 0.2922\ \underset{(mg./100\ g.)}{Na} + 0.1471\ \underset{(mg./100\ g.)}{K}$$

Differences in the relationship between the water and the sodium and potassium contents of the tissues and fluids of cattle aged from less than 1 week to more than 5 years were not statistically significant.

The equation predicted satisfactorily the water contents of materials from the digestive tract except for the contents of the abomasum. It also predicted the water contents of whole foetuses and of amniotic fluid but not of allantoic fluid.

5. It was concluded that despite the anomalous behaviour of brain, serum, skin, bone, abomasal contents and allantoic fluid, this empirical equation relating the amounts of water, sodium and potassium in individual tissues of the body, should permit an accurate prediction of the total body water content of cattle from the total body content of sodium and potassium, provided a correction was made for the 'bound' sodium of bone, the only exceptions being animals in the later stages of pregnancy.

6. Full details have been given of the construction and operation of a closed-circuit respiration chamber (Regnault-Reiset type) for use with calves in this work.

7. A series of respiration studies were made with calves to test the validity of the conclusion referred to in paragraph 5. In these studies the calves were fed cows' whole milk or an artificial milk, and simultaneous determinations of sodium, potassium, nitrogen, carbon, ash, water and energy retention were made.

8. In five satisfactory experiments, estimates of water retention from two independent sets of data, both dependent on respiration chamber technique for their determination, differed by 55 ± 40.2 g. water/day. This difference was not statistically significant. Taking the mean of estimates of water retention by these two methods as the true water retention, the error of prediction of water retention from sodium, potassium and ash retention was roughly constant at ± 300 g. water over experimental periods of from 2 to 8 days. Assuming that this error of prediction would not be increased over longer experimental periods, it should be possible over a 21-day period to predict water retention in a calf with an error of only ± 14 g./day.

9. In the same series of experiments energy retention was calculated from nitrogen retention and from the fat storage estimated indirectly from the gain in body weight less the protein, ash and predicted water retentions. It was found not to differ significantly from the energy retention determined calorimetrically by the carbon and nitrogen balance technique. Over experimental periods of 4 or 8 days, the standard deviation of estimates of energy retention by the two methods varied from ± 3000 to ± 3700 Cal. Assuming that the error of estimation would not be increased over longer experimental periods, in a trial lasting 21 days the predicted energy retention would be in error by ± 150 Cal./day. In a respiration trial lasting only 4 days, energy retention in a sheep, an animal similar in size to the calf, can be estimated by calorimetric methods with an error of only ± 56 Cal./day.

10. A series of calorimetric trials with sheep, similar to the trials made with calves have been described. With the sheep, however, losses of potassium through the skin in a substance known as 'suint' prevented the accurate determination of potassium retention and it was concluded that the indirect method of estimating water retention from sodium and potassium retentions could not, therefore, be applied to sheep.

11. It has been pointed out in the thesis that if the

indirect method of estimating water retention were to be applied to cattle on normal diets as distinct from calves on milk diets, difficulties would arise from the high potassium content that is typical of most cattle feeds. Since these high potassium contents could result in potassium intakes of up to 300 g./day, analytical methods with an error of 0.1% or less would be necessary for measurements of the potassium intake if the procedure was to have the accuracy required for the prediction of water retention from sodium, potassium and ash retentions.

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