THE POTASSIUM, SODIUM AND CESIUM CONTENT OF ANIMALS AND THE RELATIONSHIP OF COMPOSITION

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ABSTRACT

THE POTASSIUM, SODIUM AND CESIUM CONTENT OF ANIMALS AND THE RELATIONSHIP TO COMPOSITION

By Alan Henry Kirton

The potassium, sodium and cesium content of animals and their parts were determined and related to their composition. Live lambs and pigs were studied as well as 38 lb. samples of ground pork and lamb. The samples of ground pork and lamb were chosen to cover a wide range in chemical composition. The samples were uniform in size in order to make calibration of the scintillation counting system easier. Potassium-40 and cesium-137 were investigated as non-destructive methods for measuring animal composition. Flame photometry was used for measuring the sodium content and as an alternative method for measuring potassium content.

A comparison was made of four methods of extracting potassium and sodium from muscle samples for flame photometry. The methods included homogenization in 2% TCA, oven ashing, acid ashing or boiling in water followed by acidification of the solution. Results suggested that oven ashing was inaccurate as an extraction procedure. Extraction by homogenization in a 2% TCA solution was found to be reliable and readily adaptable to the equipment available, so it was adopted for use in this study.

Potassium-40 analyses showed that live lambs weighing an average of 88 lb. contained 0.18% potassium. Their carcasses averaged 48 lb. and contained 0.23% potassium. An average of 37 gm. of potassium was removed from the skin and wool by washing the lambs, although they had been shorn rather recently. Flame photometry and potassium-40 measurements were in essential agreement as to the potassium content of the fatty tissues and muscular tissues from lamb carcasses. Similar results were obtained for the ground pork and lamb. For lambs, bone contained approximately one

half and fatty tissue approximately one quarter as much potassium as the muscle tissue (0.30%).

Pigs averaging 198 lb. in live weight had 0.20% potassium in their empty bodies (G.I. contents excluded) and 0.21% potassium in their carcasses. The potassium content of the remaining body compartments was determined individually and the data are presented.

Where data were available for comparison, it was shown the composition of the animals, their carcasses and ground meat samples could be more accurately predicted from flame photometrically determined potassium than from potassium-40. The relationships between composition and potassium content were closer for pork than for lamb. In general, the standard errors of the regression equations for predicting composition from potassium content were too large to suggest that the method based on potassium-40 is likely to have any widespread application. Possible reasons for the magnitude of the standard errors have been fully discussed, as well as some possible non-destructive alternative methods for determining composition.

The sodium content of various tissues was also determined by flame photometry. In contrast to potassium, the levels of sodium in the carcass of the pig were higher than in the non-carcass compartments. Sodium was found to be less closely related to composition than potassium.

The cesium-137 levels in the lambs were found to agree with other data from North American sources. These levels were lower than some published from Scandanavia following nuclear weapons tests. The cesium content of the lambs was found to be unrelated to their composition.

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By

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INTRODUCTION

One of the most important deficiencies in research techniques available to medical and biological investigators, is an accurate, non-destructive method that could be used to measure the gross composition of the animal body. It is an obvious prerequisite that such a measurement should not result in the death of the subject. Such a method would have many applications in the animal industries as well as health related implications. A useful method could be utilized to predict composition either in physical terms (fatty tissue, muscle and bone) or in chemical terms (ether-extract, water, protein and ash).

If selection for leanness is to be successfully employed in a breeding program for meat animals, it is obvious that the method must enable one to recognize "meatiness" or muscling prior to mating. In many nutritional and physiological experiments, it would be desirable to measure the gross composition of the same experimental animals at the beginning and the end of an experiment in order to ascertain changes in composition. On a more practical level, there would be many advantages accruing from a method that would permit farmers to measure accurately when their meat animals were sufficiently fat for slaughter. It is, in fact, possible, that the day may come when farmers will be paid on the basis of the composition of the animals they market. The present assessment of fatness is commonly made by "eye" or "hand" and is frequently inaccurate and wasteful.

In the research laboratory, the only methods that can be used to obtain accurate body compositional data are direct analyses. In many laboratories, the expense, labor and physical difficulties involved in

direct analyses are sufficient to prevent such analyses from being used. This reduces the validity of the experimental work and indicates another of the areas in which a non-destructive method of measurement is urgently needed.

Reviews of the many non-destructive methods that have been or are currently being investigated have been compiled by Keys and Brozek (1953), Harrington (1958) and by Brozek and Henschel (1961). Many of the methods described appear promising, but at present, no one method appears to have the qualities of sufficient accuracy and ease of operation to be used on any widespread basis. A recent method which appears to have many advantages is based on the natural radioactivity from potassium-40, which is a normal component of all animal bodies. The method is easy to apply and causes a minimum of inconvenience to the subject being studied.

Experimental Objectives

As earlier reports did not establish the accuracy with which animal composition could be estimated from their potassium-40 content, it was decided to study the relationship between potassium content and actual composition along with the source and extent of the errors involved. It also appeared to be desirable to have an alternative to the potassium-40 method of estimating potassium. Thus flame photometry, which is a destructive technique, was also employed. Because it is a normal procedure in many laboratories studying potassium-40 to simultaneously measure the cesium-137 content (one of the products of nuclear weapon testing), these data were also obtained in one of the present experiments. As the literature suggests the possibility of potentially useful relationships between

sodium content and gross body composition, the samples that were prepared for potassium analyses by flame photometry were also analysed for sodium.

REVIEW OF LITERATURE

Potassium and Animal Composition

Theoretical Basis - Evidence has accumulated showing that potassium is found mainly in the intracellular fluid of animals, and is present as a relatively constant proportion of this fluid compartment for a given species (Manery, 1954; Conway, 1957; Wolstenholme and O'Connor, 1958; Robinson, 1960). The membranes of animal cells are capable of performing metabolic work, which establishes a concentration difference of ions on the two sides of the membrane (Guyton, 1956). Potassium is the main cation found in the intracellular fluid, and sodium is the main cation in the extracellular fluid.

The level of potassium present in a given species has been shown to be influenced by age and sex (Spray and Widdowson, 1950; Wolstenholme and O'Connor, 1958; Anderson and Langham, 1959; Allen *et al*., 1960). The potassium content increases quite rapidly early in life and then levels out and later slowly decreases. Anderson and Langham (1959) have shown that after 11 to 12 years of age, the potassium concentration in the bodies of human females is less than in males.

Anderson (1959) has stated, "Since the concentration of potassium in living cells is held constant by homeostatic processes, a determination of potassium content is equivalent to determination of cellular mass. There is no potassium in fat and very little in bone. ..... Applications to the meat industry are based on this proportionality between potassium and the mass of lean tissue."

These arguments present the rationale for expecting relationships between potassium content and composition. As most of the intracellular

fluid is present in the muscular tissue and organs of the body, it would be expected that the higher the proportion of muscular tissue in a sample, the higher the proportion of potassium. On the other hand, the higher the proportion of fatty tissue (containing little intracellular fluid in mature animals) the lower would be the proportion of potassium.

A review of the literature showed that Anderson's statement concerning the potassium content of fat and bone are incorrect (table 1), unless they are elaborated to read "chemical fat" and "crystalline bone". In this connection, some confusion of terms exists in the animal and medical literature. When a medical or animal research worker refers to fat and bone, he is most often referring to the fatty tissue and green bone (containing marrow and in some cases a little flesh), which can be dissected or separated from the animal body. It is well known that bone marrow contains potassium (Archdeacon et al., 1961), while there is evidence from direct analyses in both the rat (Bergstrom and Wallace, 1954) and man (Casey and Zimmermann, 1960) that bone contains an appreciable amount of potassium, which cannot be explained on the basis of intracellular and extracellular fluid (table 1). However, Blaxter and Rook (1956) were unable to detect potassium in sections of the metacarpal bones of cattle.

Data from the literature indicating the potassium content of muscle, fatty tissue and bone are presented in table 1. These data confirm that there is considerably more potassium in muscle than in fatty tissue or bone. Since bone forms a very much lower proportion of an eviscerated carcass than muscle, it would seem reasonable to expect a relationship between potassium content and composition. This relationship and the reciprocal relationship with fat would be expected to hold whether the composition is measured in terms of muscle, fatty tissue and bone or in

Table 1. Summary of data taken from the literature on the potassium content of various tissues.

Tissue	Comment	Species or meat type	Potassium level (ppm)[a]	Method	Reference
Bone	K in excess of what could be accounted for by intracellular and extracellular fluid	Rat	1017	Flame	Bergstrom and Wallace (1954)
Bone	Dry marrow-free rib	Human	587	Flame	Casey and Zimmermann (1960)
Bone marrow		Rabbit	1500 - 2800	Flame	Archdeacon *et al*. (1961)
Muscle		Cattle	3070 - 4440	Gravimetric	Blaxter and Rook (1956)
Fat	Kidney	Cattle	0 - 200	Gravimetric	Blaxter and Rook (1956)
Bone	Metacarpal	Cattle	0	Gravimetric	Blaxter and Rook (1956)
Muscle	Tenderloin	Calf	2800	K-40	Green *et al*. (1961)
Fat	Body	Calf	300	K-40	Green *et al*. (1961)
Muscle	Various	Cattle	3800 - 4700	K-40	Van Dilla *et al*. (1961)
Muscle	1-mo. calf	Calf	4500	K-40	Van Dilla *et al*. (1961)
Muscle	1-yr. steer	Steer	3000	K-40	Van Dilla *et al*. (1961)
Muscle	2-yr. steer	Steer	2800	K-40	Van Dilla *et al*. (1961)
Bone	Pooled	Cattle	Trace	K-40	Van Dilla *et al*. (1961)
Muscle	Methodology paper	Beef	4211	Flame	Berman (1960)
Muscle	Trimmed	Beef	2620 - 5270	Flame	Toscani and Buniak (1947)
Muscle		Veal	2670 - 3540	Flame	Toscani and Buniak (1947)
Lean	Separable	Beef	3650	K-40	Kulwich *et al*. (1961)
Lean	Separable, fat-free basis	Beef	3750	K-40	Kulwich *et al*. (1961)
Muscle	Rib-eye	Beef	2900 - 3400	Emission spectrograph	Mitteldorf and Landon (1952)
Muscle	Adult	Pig	3715 - 4190	-	Dickerson and Widdowson (1960)
Muscle	Tenderloin	Pig	2600	K-40	Green *et al*. (1961)
Fat	Body	Pig	100	K-40	Green *et al*. (1961)
Muscle	Tenderloin (boar)	Pig	3800	K-40	Green and Robinson (1962)
Lean	Separable (ham)	Pig	3130	K-40	Pfau *et al*. (1961)
Lean	Separable (same sample)	Pig	3010	Flame	Pfau *et al*. (1961)
Fat	Separable	Pig	445	K-40	Pfau *et al*. (1961)
Fat	Separable (same samples)	Pig	403	Flame	Pfau *et al*. (1961)
Bone	Separable	Pig	1290	K-40	Pfau *et al*. (1961)
Bone	Separable (same samples)	Pig	1100	Flame	Pfau *et al*. (1961)
Muscle		Sheep	2980	Gravimetric	Blaxter and Rook (1956)
Fat	Fatty tissue	Sheep	0 - 1030	Gravimetric	Blaxter and Rook (1956)
Muscle	Biceps femoris, fat-free blood-free basis	Sheep	4254, 4460	Flame	Mounib and Evans (1960)
Muscle		Lamb	2040 - 2950	Flame	Toscani and Buniak (1947)
Muscle		Sheep	2700, 3100	Flame	Harris *et al*. (1952)

terms of protein, water, ether-extract and ash. To be of practical use, such relationships would have to be very close or have advantages over methods currently employed for measuring these animal components. The possibility of non-destructively measuring composition by means of a low level gamma ray counter capable of measuring potassium-40 activity appear to offer such an advantage.

Measurement of Potassium Content from the Radioactivity of Potassium-40 - Suttle and Libby (1955) have reported that potassium-40 comprises 0.0119 percent of the natural potassium isotopes and has a half-life of about 1.25×10^9 years. Potassium-40 emits 10 beta particles for every gamma ray. The natural mixture of potassium isotopes emits 2.96 gamma rays per second per gram with an energy of 1.45 Mev.

Kulwich et al. (1960a) stated that, "Potassium from different sources does not vary by more than ± 0.5% in its potassium-40 content (Vinogradov, 1957), so that a determination of the potassium-40 content of a biological sample should provide an excellent index of the total amount of potassium present. Potassium-40 is the principal naturally radioactive isotope present in all organisms. The data of Vinogradov (1957) indicates that, in the case of humans, there is 7 times as much radioactivity emitted by potassium-40 as there is by the next most prevalent naturally radioactive isotope, carbon-14."

Anderson (1958) described how the danger of counting the gamma radioactivity from cesium-137, one of the products of nuclear weapon testing, can be minimized by limiting the range of the gamma spectrum from which counts are recorded. As the gamma rays from cesium-137 have an energy of 0.66 Mev. it is possible to simultaneously record the activity from this source and the activity of potassium-40 (1.45 Mev.) on separate

channel settings of the detection apparatus, with a small spill over of counts from the other channel. The natural radioactivity of radium and thorium contribute many more counts to the lower energy channel than does potassium, and the main fission products in fallout such as ruthenium-103 and rhodium-106 will be counted almost exclusively in the lower channel (Anderson, personal communication).

It can therefore be seen that the measurement of the penetrating, high energy gamma rays of potassium-40 furnish a non-destructive method of measuring the potassium content of biological material. The first determinations of body potassium by means of its natural radioactivity were reported by Sievert (1951, 1956) and by Burch and Spiers (1953), using large high-pressure ionization chambers. Although the early work was mainly directed toward detection of small amounts of the other gamma emitters, Sievert noted a change in potassium content with age and differences between the sexes. He explained the differences in terms of body composition. Burch and Spiers (1953) estimated the potassium content of 13 human subjects to be 0.21% of body weight.

Measurement of Potassium Content by Flame Photometry - Flame photometry offered an alternative but destructive method of measuring the potassium content of animal tissues. A very thorough review of the field of flame photometry has been given in a recent book by Dean (1960). Since different methods of preparing the samples for flame photometry are reported in detail later in this thesis, the pertinent literature will be reviewed and discussed at that time.

Relationships between Potassium Content and Composition - Cheek and West (1955) investigated the potassium content of 30 rats using flame

photometry. They plotted a regression equation which showed that lean body mass was related linearly to total body potassium. Woodward et al. (1956) demonstrated a linear relationship between the gamma activity and lean body weight of 13 human subjects. However, no error of estimate was given. Kulwich et al. (1958) reported a highly significant correlation between the gamma activity per pound and the percent of fat-free lean from two pairs of hams at various stages of subdivision (the radioactivity was attributed to potassium-40). The gamma activity was also negatively correlated with percent fat. Zobrisky et al. (1959) reported that the potassium-40 content of live hogs may possibly be useful as a rapid, non-destructive index for determining meatiness. Up to 1959 the results available showed that significant relationships occurred, but did not give any indication of the accuracy for predicting composition.

In 1960 (a), Kulwich et al. used a destructive procedure to measure the beta radioactivity (from potassium-40) of 34 meat samples taken from pork hams. The standard errors for the regression equation using the beta radioactivity to predict the percentage of the chemical components and their corresponding correlations were as follows: ether-extract -- 2.2% ($r = -.99$); protein -- 0.64% ($r = 0.99$); moisture -- 1.7% ($r = 0.99$). Later Kulwich et al. (1961a) related the potassium-40 gamma activity of 34 hams to their separable components which were expressed on both a weight and a percentage basis. Potassium-40 (net count per minute per pound of ham) was significantly correlated with percent separable lean (0.87) and percent separable fat (-.86) in the hams. The standard error of the regression equation for predicting percent separable lean was 2.5 in the hams studied, which had a range of 47-68% in separable lean.

Kulwich et al. (1961b) carried out a similar experiment with 16 beef rounds. The potassium-40 content was correlated with percent separable lean (0.80), percent separable fat (-.87) and percent separable bone (0.06). The standard error of the regression equation for predicting lean from gamma activity was 2.1%. A group of German workers (Pfau et al., 1961) have shown a close relationship between the potassium-40 content and the amount of lean in hams (r = 0.90).

Research has also been undertaken on human subjects, relating potassium content to composition. In order to make comparisons, the composition had to be determined non-destructively by some indirect method. Allen et al. (1960) demonstrated a linear relationship between the potassium content and M_3 (residual mass of body after removal of bone mineral, fat and water from gross body mass) for a group of subjects, but no estimate of the accuracy of prediction was given. M_3 was estimated from body water determinations.

A new approach to the problem of estimating fat content of the living human being has been claimed by Forbes et al. (1961). They related the potassium-40 activity of children and young adults to their skinfold thickness measurements and weight:height ratio, both of which were regarded as indices of fatness. Forbes et al. reported that the correlations between the potassium-40 measurement and average skinfold thickness was 0.80 for the males, and with weight/height was 0.56 (males only). The data were plotted but no regression equations were presented to show the accuracy of prediction. In ensuing publications, (Anderson and Langham, 1961; Forbes and Hursh, 1961) the validity of the claim to have developed a new method for a non-destructive measurement of fat content was discussed.

It would appear in the light of the literature reviewed that the claim is not completely justified.

Sodium and Animal Composition

Theoretical Basis - There is some evidence to suggest that the sodium content of animals might be related to their composition. It is fairly well known that sodium ions form a relatively constant proportion of the extracellular fluid (Keys and Brozek, 1953; Manery, 1954), in fact, the dilution volume of radioactive sodium is often used as a measure of the extracellular fluid space. The sodium dilution volume is regarded as a measure of exchangable sodium. Exchangable sodium differs from total body sodium because of a sizable pool of slowly exchangable bone sodium (Edelman, 1945a, b; Bergstrom and Wallace, 1954; Forbes and Perley, 1951; Casey and Zimmerman, 1960).

Edelman (1961) explains the distribution of sodium in the body in the following words: "The distribution of body sodium is unusual because of the complex nature of bone sodium. There appear to be three distinct phases of bone sodium: (a) free extracellular sodium (presumably all exchangable), (b) exchangable sodium adsorbed to the surface of bone crystals, and (c) nonexchangable bone sodium in the crystalline structure. ..... These estimates indicate that total exchangable sodium represents about 70% of total body sodium."

Exchangable sodium in human beings varies little between sexes and with age when expressed on a per unit weight basis (Edelman, 1961). In healthy animals, the extracellular water comprises a relatively constant part of total body water, which is known to be related to body composition (Keys and Brozek, 1953). Thus, it would seem reasonable to investigate the relationship between sodium content and composition.

Relationship of Sodium Content to Composition - Edelman (1961) has stated, "Accordingly, one might expect a close dependence of plasma sodium concentration on the amounts of sodium, potassium and water in the body. This, in fact, is precisely what has been found." However, Edelman did not relate exchangable sodium to body water, which would have been the interesting relationship from the point of view of the present experiment. Blaxter and Rook (1956) present an equation from which the water content of cattle tissue may be estimated from the sodium and potassium content. The above experiments suggest that it could be of interest to relate the composition of tissue or of entire animals to their sodium content.

Cesium-137 and Animal Composition.

Cesium-137 was discovered in the late 1940's, and it is well known that this isotope is produced by fission of uranium-235 in atomic energy reactors (Thoraeus, 1961). Of greater concern is the fact that this isotope is one of the longer lived by-products of nuclear weapons testing (Langham and Anderson, 1959; Langham, 1961), with a half-life of approximately 30 years.

Theoretical Basis - In animals, cesium-137 is found in the same tissue sites as potassium (Langham, 1961). The level in animal tissues is being followed with interest in many countries, because of the potential danger of radiation damage to man and other animals if the concentration should become too high (Langham, 1961). The cesium-137 level gives an indication of the amount of radioactive fallout. Because the tissue distribution of cesium-137 is similar to that of potassium, it might be expected that there would be a relationship between cesium-137 content and composition.

Relationship of Cesium-137 Content to Composition - Kulwich et al. (1961a) reported a correlation of 0.47 between the weight of lean in pork ham and the cesium-137 content. Pfau et al. (1961) noted a correlation of 0.78 between the percent lean in hams and their cesium content. In both cases, these correlations were lower than the equivalent correlation coefficients for potassium which were 0.96 and 0.90, respectively. Kulwich et al. (1961b) have reported similar results for beef rounds. In this case, the correlation between the cesium-137 content of the round and the weight of lean was 0.81, which was lower than the equivalent potassium-40 correlation (r = 0.98).

DEVELOPMENT OF FLAME PHOTOMETRY PROCEDURES

MATERIALS AND METHODS

Apparatus

A Beckman Model D.U. Spectrophotometer with Model 9200 flame attachment was used. The flame attachment was fitted with an oxygen-hydrogen burner and its operation has been described in Beckman Instrument Instruction Manual 334-A.

The 768 mμ wavelength setting was used for potassium determinations, and the 589 mμ setting was used for sodium. The burner was operated at a pressure of 6 lb. per square inch of hydrogen and 13 lb. per square inch of oxygen. This gave the optimum burner conditions according to the manufacturers recommendations. The photometer was operated on phototube position 1 (with filter at in position) for potassium and phototube 2 (filter out position) for sodium. A slit width of 0.01 - 0.03 was used for sodium and 0.15 - 0.30 was used for potassium. A sensitivity setting of 5 was used on the power supply unit, while the selector switch was set at position 0.1.

Sample Preparation

Before flame photometry can be used to measure the concentration of an element in a sample, a method must be employed to extract the element and get it into a solution that is suitable for atomization. Such a solution must not contain any substance that will clog the very fine atomizer burner tube.

Several methods have been successfully employed to extract potassium from animal tissues and fluids (Dean, 1961), and several methodology studies have been made in this area. Grove _et al._ (1961) compared wet and dry ashing with particular reference to the temperature at which samples may be ashed without getting losses. It was found that the maximum temperature at which animal tissues may be dry-ashed for a 24 hour ashing period without loss of sodium and potassium is 550°C for 1 - 5 gm. samples. Stone and Shapiro (1948) boiled muscular tissue under reflux and compared the potassium content of the supernatant liquid with the potassium remaining in the tissues. They reported that the same concentration of potassium was found in the boiled tissues as in the supernatant liquid. Siebert _et al._ (1951) compared dry ashing of tissue with boiling under reflux, as methods of extracting the potassium from the tissue, and found that the two methods gave results that were in agreement to within about 4%. Mounib and Evans (1957) compared homogenization in 2% trichloracetic acid (TCA), boiling under reflux, and boiling under reflux plus acidification as methods of extracting sodium and potassium from tissue samples. Homogenization in 2% TCA gave results which appeared to be reliable, and in the case of the boiled tissue samples, it appeared that acidification was necessary to get complete ion release from some tissues.

The method of homogenization in 2% TCA, which was subsequently adopted by Mounib and Evans (1960), was suited to the equipment available in this laboratory. However, it seemed advisable before using this method on a routine basis, to compare it with another method. The TCA method was finally accepted as the routine method for potassium and sodium extraction from the tissue, so it is described in detail.

A 2% TCA solution (w/v) was made by dissolving 40 gm. of TCA in 2000 ml. of de-ionized distilled water. Between 1.5 and 3.5 gm. of previously ground tissue was accurately weighed and transferred by washing into an aluminum blender. The sample was homogenized for 5 - 10 minutes with 150 ml. of 2% TCA (measured with pipettes). The solution was then transferred to a 250 ml. Erlenmeyer, which was stoppered and stored overnight in a 38°C cooler. The length of storage following homogenization (0 - 48 hours) was found to be without effect on the results. The stored samples were filtered through Whatman No. 40 filter paper into polyethylene storage bottles, and 5 ml. of this solution was made up to 15 ml. in a test tube with 2% TCA solution. The test tube was capped with "Parafilm" and shaken to get complete mixing.

In addition to the method adopted and outlined above three other extraction procedures were compared. The first of these involved oven ashing at less than 550°C for 24 hours in porcelain crucibles, after a preliminary rough ether-extraction (diethyl ether), if the samples were very fat. The ash was then dissolved in a few drops of concentrated HCl and diluted with 150 ml. of 2% TCA solution.

The second method of extraction utilized wet ashing with nitric and perchloric acids after a preliminary rough ether-extraction. The procedure outlined by Benne and Lindon (1960) was followed after some minor modifications. After digestion was complete and the liquid evaporated, the sample was dissolved in 150 ml. of 2% TCA solution. The last of the three methods involved refluxing the sample for 30 minutes in 150 ml. of de-ionized distilled water, followed by acidification with a few drops of HNO_3 as recommended by Moumib and Evans (1957).

Following extraction the procedure for all methods was the same. Five ml. of the extracted sample was diluted to 15 ml. in a test tube

with a 2% TCA solution. The test tubes were always covered with "Parafilm" and shaken to ensure complete mixing. Blank solutions were prepared and all samples were diluted using a 2% TCA solution. The photometer sample beakers were washed with the solution that they were to contain before being filled for a series of readings.

Standard Solutions

For the first two experiments which involved both muscular and fatty tissue, a liter of primary standard containing 1000 parts per million (ppm) potassium and 200 ppm sodium was prepared as suggested by Dean (1960). Analytical grade KCl was used as the source of potassium (K) and the same grade NaCl as a source of sodium (Na). De-ionized distilled water was used for making up the standard. The solution was then stored in a polyethylene bottle.

Fifteen ml. of the primary standard was then made up to 500 ml. (volumetric flask) with 2% TCA solution. This gave a concentration of 30 ppm potassium and 6 ppm sodium. Then 100 ml., 75 ml., 50 ml., 30 ml. and 10 ml. of this solution was pipetted into polyethylene bottles (4 ounce capacity) and made up to 100 ml. with 2% TCA solution by pipette. This gave the range of 30, 22.5, 15, 9, 3 and 0 (blank TCA solution) ppm of potassium and 6, 4.5, 3, 1.8, 0.6 and 0 ppm sodium. These points were used in plotting the standard curve.

In experiment III, it was found that some of the body components of the pigs being analyzed had a higher Na:K ratio than was suitable for analysis using the original primary standard of 1:5. Thus, a new primary standard containing 1000 ppm potassium and 1000 ppm sodium was prepared and was diluted to give the desired strength for the standard curve.

Samples

In order to compare methods of extraction of potassium and sodium, a homogeneous sample of ground pork was utilized. A series of analyses was made on the pork after it had been extracted by both TCA homogenization and the oven ashing methods. When the two methods of extraction failed to agree, a recovery trial was carried out by running 5 ml. of the primary standard containing 1000 ppm potassium and 200 ppm sodium completely through the two extraction methods. The potassium and sodium contents were computed on the basis that they were present in 1 ml. of the original solution (i.e. containing 5000 ppm potassium and 1000 ppm sodium).

Using the TCA extraction method, it was possible that organic material could cause erroneous results by altering emission intensity (Dean, 1960). Thus, it was decided to use an acid-ashing procedure, which would destroy any organic material. A fourth accepted method, involving boiling of the sample was also used for comparative purposes. Ground beef and another ground pork sample were used for comparing all four methods of extraction.

Calculations

A standard curve was prepared by plotting percent transmittency against known concentrations of potassium and sodium. The potassium and sodium content of the tissues was computed from the concentration of these elements, which were estimated from the standard solution prepared for flame photometry. This concentration was multiplied by a dilution factor and finally corrected for the blank reading. The dilution factor was $\frac{150 + X}{X} \times 3$, where X is the weight of the tissue sample analyzed in grams, and the factor of 3 allows for the dilution of 5 ml. of the homogenized, filtered sample to 15 ml.

RESULTS AND DISCUSSION

The results of various methods of extraction upon potassium and sodium analyses are shown in table 2. On the tissue samples, the TCA homogenization extraction procedure resulted in higher potassium estimations and lower sodium estimations than the oven ashing procedure. The lower standard deviation of the TCA method showed that it was more reproducible than oven ashing.

As these two extraction procedures gave different estimates, it seemed to be desirable to run known quantities of potassium and sodium completely through the procedures to see if any loss or gain of the elements occurred due to faulty methodology. The results suggested that the TCA extraction was more accurate for potassium and that an apparent loss of sodium occurred in the ashing procedure. The recovered elemental levels were opposite to what would have been predicted from the tissue analyses. No explanation for this is known.

There were several possible reasons for the different results observed on the tissue samples. A loss of potassium might have occurred in oven ashing if the oven controls were inaccurate. Grove et al. (1961) have noted that the maximum temperature at which animal tissue may be dry-ashed for a 24 hour ashing period without loss of sodium and potassium is 550°C. However, this would not explain the larger sodium content observed in the oven-ashed samples. Another possible explanation was that an exchange of sodium and potassium occurred between the ash and porcelain crucible surface at high temperatures. A third possibility was the enhancement of the emission intensity from the TCA extracted samples due to the possible presence of organic components in the solution (Dean, 1960).

Table 2. A comparison of different methods of extraction of potassium and sodium.

Element	Sample	TCA homogenization		Oven ashed		Boiled + HNO_3		Acid ashed	
		n	Mean	n	Mean	n	Mean	n	Mean
Potassium									
	Pork	7	4010 ± 116	7	3188 ± 508		-		-
	5000 ppm K	6	5008 ± 35	4	5088 ± 139		-		-
	Beef	4	1805 ± 38	4	1420 ± 119	2	1808	2	1840
	Pork	3	4213 ± 18	4	3337 ± 155	2	4351	3	4175 ± 18
Sodium									
	Pork	7	483 ± 63	7	835 ± 208		-		-
	1000 ppm Na	6	997 ± 150	4	792 ± 87		-		-
	Beef	4	475 ± 19	4	589 ± 66	2	479	2	458
	Pork	3	591 ± 7	4	1080 ± 62	2	617	3	634 ± 26

Values presented are means ± standard deviations (in parts per million = ppm) resulting from repeated analyses of each sample.
n = number of observations.

Some of these suggested difficulties could be overcome by the use of chemical ashing as a method of comparison. In this method, high temperatures were unnecessary and the organic material was destroyed. Table 2 shows the results following chemical ashing. Chemical ashing and a fourth procedure involving boiling of the samples gave estimations of the sodium and potassium content of the tissues that agreed with those obtained from the TCA homogenization method, but disagreed with the results obtained after oven ashing. It is not known why the oven ashing procedure, which has apparently been successfully employed in many laboratories, gave erroneous results in the present experiments. Possibly, better results would have been obtained if platinum crucibles had been used. The results suggested that the TCA homogenization method was reliable. The trend for decreasing variability of results with the increased use of the method suggested that the estimations were increasing in accuracy as experience was gained in using the procedure.

SUMMARY AND CONCLUSIONS

A comparison was made of four methods of extracting potassium and sodium from muscle samples. The methods included homogenization in 2% TCA, oven ashing, acid ashing or boiling in water followed by acidification of the solution. Results suggested that oven ashing was inaccurate as an extraction procedure. Extraction by homogenization in a 2% TCA solution was found to be reliable and readily adaptable to the equipment available.

EXPERIMENT I. LOS ALAMOS LAMBS

EXPERIMENTAL

Counter

The Los Alamos four pi liquid scintillation counter (Fig. I, II) was used to measure gamma activity. The Los Alamos counter is a well-type counter which can accommodate samples weighing up to 300 lb. Because the scintillation solution surrounds the sample, the geometrical efficiency approaches 100% and makes a very short counting interval possible.

Anderson (1958) has described the counter as follows: "The counter itself is a cylindrical steel tank 6 ft long and 30 in. in diameter." (see Fig. III). "An axial well 18 in. in diameter accommodates the subject or sample which is surrounded by a layer of liquid scintillation solution (terphenyl and POPOP in toluene) 6 in. thick. The counter is shielded by 5 in. of lead. Scintillations are detected by 108 photomultipliers (2 in. diameter cathodes), which observe the solution through ports in the outer wall. The photomultipliers are connected in two banks of fifty-four tubes each, which are operated in coincidence in the usual manner. The energy resolution of the system is not good, but is adequate to separate gamma-rays whose energies differ by a factor of 2 or more. Thus, the machine is usually operated for simultaneous counting in two energy channels: 1 - 2 MeV, giving ordinarily only the natural K^{40} (1.45 MeV gamma) activity, and the 0.5 - 0.8 MeV, giving some K^{40} activity (easily calculable from the upper channel count) and also any Cs^{137} activity (0.66 MeV gamma) present in the sample."

In the present experiment the potassium determinations from the upper channel were based on the gamma rays which deposit more than 0.8

Figure I. General view of Los Alamos human counter.

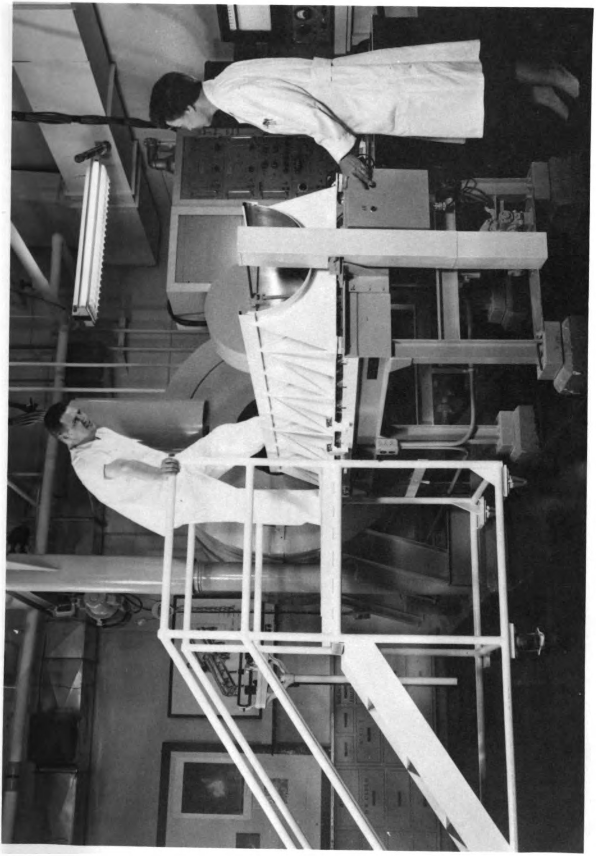


Figure II. Method of restraining animals for counting.

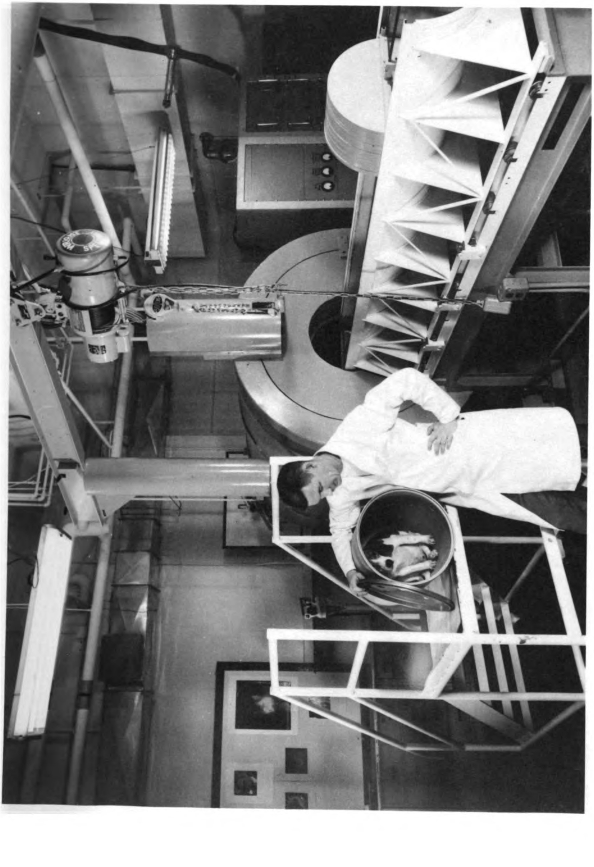
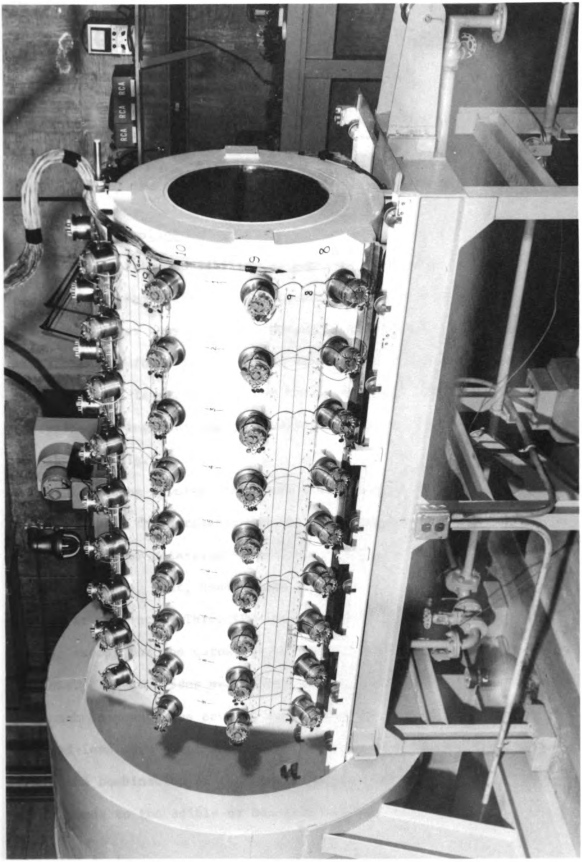


Figure III. The Los Alamos human counter outside its lead shield showing the banks of photomultiplier tubes.



Mev. in the scintillator.

Animals

Ten recently shorn, blackfaced lambs with a mean liveweight of 88 lb. were purchased from a feedlot at LaJara in southern Colorado in March of 1960, and transported to Los Alamos, New Mexico.

Counting and Sampling

Because of the possibility of potassium and other radioactive contamination on the wool and skin, counts were made on the live lambs both before and after they had been thoroughly washed with detergent and warm water. It was found that a second washing did not reduce the gamma activity below the level accomplished by one washing. The animals were restrained during counting in a cardboard drum in the same manner as the dog shown in Fig. II.

After being counted, the lambs were slaughtered and dressed in the usual manner. The carcasses and the non-carcass components of the animals were counted for potassium-40 activity. The non-carcass components included the hide, feet, head, blood and also all of the internal organs and their contents. Three 100-second counting periods were used in all cases, except for the carcasses which were counted for five 100-second periods. The carcasses were physically separated into fat, lean and bone and each component was counted separately for potassium-40 activity. The fat and lean were then ground separately and sampled for chemical analyses. The combination of the separable fat plus lean tissue of each lamb corresponds to the edible or boneless carcass.

Chemical Analyses

Three to 7 gm. samples of ground tissue were taken for analysis of water and ether-extract. Water was determined by difference after drying for 24 hours at 100°C (Benne et al., 1956). Ether-extract was determined on the water-free sample, which was dried in a disposable aluminum dish and then extracted with diethyl ether in a Goldfisch apparatus (Hall, 1953). Protein was determined on 1-3 gm. samples of tissue by the Kjeldahl method as described by Benne et al. (1956).

Potassium and Sodium Estimations

The total potassium content of the live lambs and their various components was estimated from potassium-40 counts. The raw counting data and efficiency calibrations were entered on punched cards, and calculations of total grams of potassium per kilogram of material were carried out on an I.B.M. electronic computer, which tabulated and printed the results (Anderson et al., 1959). It should be noted that 1 gm. of potassium per kilogram of tissue is equal to 0.1% potassium or 1000 ppm potassium.

The potassium and sodium content of the separable lean and fat from the carcasses was determined by flame photometry on the samples that had been taken for gross chemical analyses. The method of extraction of the potassium and sodium from the tissues by homogenization in 2% TCA and the conditions used for flame photometry have been described earlier in this thesis. Duplicate analyses were carried out on each tissue sample.

Cesium-137 Estimations

The raw counting data obtained from the lower channel setting were also entered onto I.B.M. cards, and the computer tabulated and printed the results as micromicrocuries ($\mu\mu c$) of cesium per gram of potassium.

RESULTS AND DISCUSSION

Animal Composition

The weights and composition of the lambs are presented in table 3. It may be noted that the variation in liveweight was not extreme. The range of separable components in the carcass amounted to about 18% in fat content and 10% in lean content. The data on the chemical composition of the boneless carcasses show that the range in percentage for both ether-extract and moisture was 20.1 and for protein 4.3.

Table 3. Weight and composition of the lambs

Item	Mean	S.D.	Range
Liveweight (lb.)	87.9	9.5	76.6 - 105.5
Hot carcass weight (lb.)	45.7	5.9	38.2 - 56.3
Non-carcass components (lb.)	42.3	3.9	35.6 - 48.5
Carcass composition			
Separable fat (lb.)	9.7	3.5	4.1 - 14.1
Separable fat %	20.8	5.9	9.7 - 27.8
Separable lean (lb.)	27.1	3.1	23.4 - 33.0
Separable lean %	59.4	3.2	55.3 - 65.5
Bone (lb.)	7.5	0.9	6.1 - 8.4
Bone %	16.5	2.1	14.4 - 20.4
Edible carcass composition			
Ether-extract %	22.7	6.9	9.1 - 32.5
Protein %	15.7	1.4	14.0 - 18.3
Water %	60.9	5.6	52.7 - 72.8

Potassium Content and Composition

The total estimated potassium content of the live lambs and their components is presented in table 4. The mean potassium content was estimated to be 108 gm. in the unwashed lambs and 71 gm. in the washed lambs. Washing the lambs reduced the potassium content from 0.271 to 0.177% or

Table 4. Potassium content of 10 live lambs and their components as estimated from potassium-40 counts and from flame photometry

Item	Mean % K	S. D.	Range	Counting errors[a] S.D. (%)
Potassium-40				
Live unwashed lambs	0.271	0.030	0.229 - 0.316	2.29
Live washed lambs	0.177	0.020	0.147 - 0.209	3.56
Dressed carcass	0.225	0.033	0.171 - 0.262	3.68
Non-carcass components	0.178	0.039	0.094 - 0.233	6.56
Separable fat	0.070	0.044	0.013 - 0.154	66.41
Separable lean	0.298	0.020	0.267 - 0.319	5.35
Separable bone	0.141	0.048	0.071 - 0.206	34.85
Flame photometry				
Edible carcass	0.255	0.028	0.219 - 0.297	--
Separable lean, run 1	0.313	0.014	0.298 - 0.340	--
Separable lean, run 2	0.316	0.016	0.286 - 0.340	--
Separable lean, run 2, fat-free basis	0.340	0.017	0.306 - 0.363	--
Separable fat	0.082	0.023	0.056 - 0.126	--
Separable fat, fat-free basis	0.239	0.031	0.209 - 0.310	--

[a]Standard deviation computed by the method described by Comar (1955) expressed as a percent of total counts. Based on mean sample count (10 observations) and mean background count.

on average 37 gm. of potassium was removed from each animal by washing. Analysis of variance showed that the difference in potassium content between the washed and unwashed lambs was highly significant. While the potassium activity dropped to 0.65 of its original value with a standard deviation of 0.07, the apparent total amount of cesium-137 present remained at 0.99 of the unwashed level, with a standard deviation of 0.12 (Anderson, personal communication). This strongly suggests that the radioactivity removed was almost entirely potassium. The natural activities of radium and thorium contribute many more counts to the lower energy channel than does potassium, and the main fission products in fallout such as ruthenium-103 and rhodium-106 will be counted almost exclusively in the lower channel. If any of these activities were being removed, the apparent cesium-137 activity would drop significantly. The presence of potassium in the wool is not difficult to account for, since the external secretion of the sheep are rich in potassium, much of which is trapped in the wool. In fact, Kulwich _et_ _al_. (1960b) suggested that the radioactivity of wool measured in the upper channel (potassium setting) of a low level gamma ray detector might be used as a non-destructive method for measuring the impurities present in unscoured wool.

No satisfactory explanation can be given for the fact that the total potassium content on adding the carcass and non-carcass components together was greater than the potassium content for the live washed lambs. Any self absorption of the gamma rays that occurred in the larger samples, such as for the live animals, should have been allowed for in the calibration of the counter. A highly significant correlation of 0.84 was found between the potassium content of the carcass and the non-carcass components. Since much of the intracellular fluid, which contains potassium, is present in the non-carcass components, a positive relationship would necessarily exist between the potassium content of the carcass and non-carcass components in order to accurately predict carcass composition from the gamma counts of the live animal.

When the potassium content of the carcass was computed from the weight of the component tissues and their potassium content as estimated from potassium-40, it was found that a level of 0.222% of potassium was present. This value agreed well with the figure of 0.225% potassium when the radioactivity of the intact carcass was counted. This suggests that the average values for the potassium content of the separable fat and bone are approximately correct. The presence of potassium (or radioactive material) in fatty tissue and bone (table 4) has also been shown by Kulwich _et al_. (1961a, b) and Pfau _et al_. (1961). The counting errors given in table 4 show that the estimated potassium content of the fatty tissue or bone from any particular animal is likely to be inaccurate. This is because the small sample size in relation to the size of the counter resulted in a small sample count in relation to the large background count, which has some sort of a normal level for a given counter.

The variation of the means for separable lean and fat based on the 10 duplicate analyses (making up any of the flame photometry potassium means, table 4) was separated by analysis of variance into components due to differences between individual lambs and an error term. The standard error of the duplicate means was computed from the error mean square and found to range from 15 to 25 ppm of potassium, which suggested reasonable agreement between duplicate samples.

As shown in table 4, there was agreement in the average values obtained for the potassium content of the separable lean or of the fat as measured by flame photometry or by the natural gamma activity. For the lean, analysis of variance showed that although the difference between the flame photometry mean of run 2 and the potassium-40 mean was small, it was significant at the 5% level. Similar results were reported by Pfau _et al_. (1961), although they did not report on the variation of the two methods or whether the means had been tested for significant differences. The present results and those of Pfau _et al_. (1961) confirm the presence of potassium in fatty tissue at levels that cannot be ignored. Thus, the results from direct chemical analyses show that the statement by Anderson (1959) that, "There is no potassium in fat", is incorrect unless qualified. In the present experiment, the results for both lean and fatty tissues were found to have lower standard deviations for potassium content when measured by the flame photometric method than from the potassium-40 method.

Since the investigators suspected that there were important day-to-day variations in the flame photometry results during the course of the first run, a second set of potassium determinations was carried out on the lean tissues. Thus, all determinations in run 2 were carried out on the same day and were calculated from the same standard curve.

When the duplicate means of run 1 were correlated with the duplicate means of run 2, a highly significant correlation of 0.88 was found. However, the standard error of the regression equation for predicting the potassium content of the lean in run 1 from run 2 was found to be 70 ppm, which indicates that run-to-run errors in measurement are an important source of variation. Factors contributing to the differences will include sampling errors and slight differences which occur in the standard curves. The variation could also result from small differences in the rate of atomization of the solutions, and other factors apparently inherent in the photometer and which cannot be precisely controlled.

When the potassium content of the separable fat as measured by flame photometry was correlated with the potassium content of the separable fat as measured by potassium-40, a non-significant correlation of 0.40 was found. The potassium content of the separable lean as measured from potassium-40 was correlated with the flame photometrically determined potassium content of the lean in the two runs. Non-significant correlations of 0.57 and 0.52 were found in run 1 and run 2, respectively. However, a great deal of weight should not be placed on the lack of agreement between the individual values for the potassium content of the tissues as estimated by the two methods. The counting errors in table 4 show that the potassium-40 method would not be expected to give an accurate estimate of the potassium content of the separable fat or lean.

A range of 0.27 to 0.34% of potassium was observed in the separable lean of the lambs on combining the data from both methods. These results compare favorably with a range of 0.20 to 0.30% for lamb muscle as reported by Toscani and Buniak (1947), 0.27 and 0.31% reported by Harris et al. (1952) for sheep muscle, a figure of 0.30% for sheep muscle reported by Blaxter and Rook (1956), and mean figures in the order of 0.43% for the fat-free, blood-free biceps femoris muscle of sheep reported by Mounib and Evans (1960). As the separable lean in the present experiment contained from 4 to 10% chemical fat, the correction of the potassium content to a fat-free basis increased the potassium figures on average by 0.025% to give a value of 0.34%. Further work is needed to establish the potassium levels of different muscles of different breeds and species of animals before accurate comparisons can be made. In a similar manner, the conversion of the potassium content of the separable fat (which contained from 41.2 to 77.4% chemical fat) to a fat-free basis increased the calculated potassium content from 0.082% to 0.239%. It is interesting to note that on this basis the muscular tissue has a higher potassium content than the fatty tissue.

The K/N ratio has been suggested as a measure of the constancy of the potassium content of various tissues and organs. The mean K/N ratio (wt./wt.) in the separable lean was 0.1045 (S.D. = 0.0047; range = 0.0936 to 0.1115) and in the separable fat was 0.0753 (S.D. = 0.0049; range = 0.0677 to 0.0818). These results present the same picture as when the potassium content was expressed on a fat-free basis with the lower potassium content being found in the fatty tissue.

Table 5 shows the relationships between the potassium content and other variables for the lambs. The potassium content of the edible carcasses (by flame photometry) was computed from the percentages of potassium in the separable fat and lean (run 2) and the weights of these tissues.

Table 5. Correlations between the potassium content of live lambs and carcasses and carcass composition.

	From potassium-40			Flame photometry
Item	Live unwashed % K	Live washed % K	Dressed carcass % K	edible carcass % K
Live washed lambs, % K (K-40)	0.66*	-	-	-
Dressed carcass, % K (K-40)	0.30	0.40	-	-
Separable fat, % of dressed carcass	-0.79**	-0.73*	-0.38	-0.92**
Separable lean, % of dressed carcass	0.57	0.58	0.52	0.81**
Separable lean, % of dressed carcass, on a fat-free basis	0.66*	0.63	0.57	-
Separable bone, % of dressed carcass	0.86**	0.78**	0.14	0.81**
Ether-extract, % of edible carcass	-0.79**	-0.71*	-0.41	-0.87**
Water, % of edible carcass	0.77**	0.65*	0.40	0.81**
Protein, % of edible carcass	0.80**	0.83**	0.41	0.94**

* Correlation significant at 5% level.
**Correlation significant at 1% level.

While most of the potassium in the dressed carcass was present in the muscular tissue (82%), the correlation between the estimated potassium content of the live animal and the lean content of the carcass was not significant. If, however, the percentage lean in the carcass was corrected to a fat-free basis, the correlation between the potassium content of the unwashed live animals and percentage lean was just significant. These correlations, which were very much smaller than would have been anticipated from results that have been published in the literature, can perhaps be explained in part by the small variability of the percentage lean in this group of animals. The percent of separable lean of the 10 carcasses showed a standard deviation of 3.2% (table 3). However, the counting statistical error as given in table 4 is 5.4% for the lean and 3.7% for the carcass. Since the variability of the carcasses as determined by dissection is less than the precision of the potassium measurements, a significant correlation would not be expected.

Percent separable fat in the live animal or carcass would be expected to show a negative correlation with potassium concentration, since fat contains comparatively little potassium. As the dissection data show a larger variability for fat than lean (S.D. of 5.9% versus 3.2%, table 3), it was not surprising that significant correlations were found between potassium in the live animal and separable fat in the carcass (table 5) in contrast to the results for lean. The fact that the standard deviation for separable fat (5.9%) was larger than the error in estimation of potassium content of the live animals and carcasses (table 4) is in contrast to the data for separable lean. Although the correlations between separable fat in the carcass and the potassium content of the live animal were significant, they were not high enough for practical application.

The reason for the positive correlation between percent potassium (from potassium-40) and percent bone is not clear. It is known that percent bone tends to follow muscle percent in growing animals, but the variability of bone in these animals was even less than the variability in separable lean (S.D. of 2.1% versus 3.2%, respectively). Thus the positive correlation between potassium in the live animal and percent bone needs further verification.

Although it might be anticipated that skin and wool contamination of the unwashed lambs would reduce the relationship between the amount of potassium in the live animal and carcass composition, for some unknown reason the opposite situation was found to be the case. In general, washing lowered the correlations between the potassium content of the live animal and the various components.

The counts on both the live animals and carcasses have been related to carcass composition. In the case of live animals, it seemed likely that the potassium in the non-carcass components would lower the relationship with carcass composition. However, this was not found to be the case, as none of the correlations between carcass potassium content (as estimated from potassium-40) and the percent of the separable components of the carcasses were significant. This is in marked contrast with the comparable correlations with percent potassium in the edible (boneless) carcass as estimated from flame photometry and the percent of separable components in the dressed carcass. For all separable components, these correlations were highly significant. It is not considered that the exclusion of bone potassium, which is a small proportion (11%) of carcass potassium, is the explanation for the significant correlations observed. Evidence already presented suggests that bone is positively correlated with the potassium content of these lambs, and so the presence of bone potassium when the potassium-40 method was used to measure dressed carcass potassium content should not greatly alter these relationships. The explanation for the significant correlations when flame photometry was used to measure potassium content would appear to be due to the greater accuracy for estimation of potassium than for the potassium-40 method.

The correlations between the potassium content of the 10 lambs and the chemical composition of the edible portion of their carcasses are also presented in table 5. As was the case for the separable tissues, significant relationships were found with the radioactivity counts for the live animals, but not for their carcasses. The highest relationships were found between the potassium content of the live animal and the percent protein in the edible tissue. This would be expected if most of the potassium were present in the muscular tissue or lean. Since most of the body potassium is present in the intracellular fluid (Wolstenholme and O'Connor, 1958; Conway, 1957; Robinson, 1960) and most of the carcass intracellular fluid is present in muscular tissue, it might be expected that the highest relationships to potassium-40 would be with percent lean in the carcass and/or with percent protein in the edible carcass. The highly significant correlations between the percent potassium (flame photometry) in the edible carcass and its composition were in contrast with the non-significant correlations between the potassium content (from potassium-40) for the dressed carcass and the composition of the edible carcass. This again suggests that the use of flame photometry resulted in more accurate estimations of the potassium content than the use of the potassium-40 method under the conditions of application.

Table 6 gives the regression equations which could be used for predicting carcass composition from the potassium content of the live animals

Table 6. Regression equations for predicting carcass composition

Dependent variable	Independent variable	Regression equation	$S_{y.x}$
Dressed carcass			
% Separable lean (fat-free basis)	% K (K-40, live unwashed)	Y = 85.4X + 31.99	3.0%
% Separable lean	% K (Flame, edible carcass)	Y = 92.8X + 35.74	2.5%
% Separable fat	% K (K-40, live unwashed)	Y = 62.36 - 153.6X	4.0%
% Separable fat	% K (Flame, edible carcass)	Y = 70.25 - 194.2X	2.4%
% Bone	% K (K-40, live unwashed)	Y = 60.3X + 0.20	1.1%
% Bone	% K (Flame, edible carcass)	Y = 59.9X + 1.26	1.3%
Edible carcass			
% Protein	% K (K-40, live unwashed)	Y = 38.0X + 5.40	1.0%
% Protein	% K (K-40, live washed)	Y = 59.3X + 5.17	0.8%
% Protein	% K (Flame, edible carcass)	Y = 47.2X + 3.65	0.5%
% Fat	% K (K-40, live unwashed)	Y = 72.53 - 183.9X	4.5%
% Fat	% K (K-40, live washed)	Y = 66.39 - 246.9X	5.1%
% Fat	% K (Flame, edible carcass)	Y = 76.74 - 212.3X	3.6%
% Water	% K (K-40, live unwashed)	Y = 145.1X + 21.55	3.8%
% Water	% K (Flame, edible carcass)	Y = 162.2X + 19.58	3.4%

and their carcasses. The standard errors of these equations give an indication of the accuracy with which they could be applied to estimate the composition of lamb carcasses. When these standard errors are compared with the range of carcass components shown in table 3, it can be seen that the non-destructive potassium-40 method for estimation of carcass composition would not discriminate very well between animals. Although flame photometry apparently gave a more accurate estimation of potassium content, even this method did not result in an accurate prediction of carcass composition. For example, the total range of protein in the carcasses of these lambs was only 4.3% although the most accurate equation for predicting carcass protein has a standard error of 0.5%. However, the range of composition of these lambs is one over which an experimenter may wish to determine treatment effects in a critical experiment. It must also be remembered that flame photometry is a destructive technique, which was used solely to investigate the relationships present and has no practical significance.

Results show that significant relationships exist between the potassium content of the lambs and their carcass composition. However, the natural variation in the potassium content of the different tissues shown in table 4 is probably sufficient to prevent any marked reduction in the errors of prediction. This suggests that if the results found in this experiment are typical for the sheep, then the potassium-40 method is not likely to be very useful for predicting the composition of this species.

Sodium Content and Composition

The data on the sodium content of the lamb carcasses are presented in table 7. The standard error of the duplicate means was found from

Table 7. Sodium content of the carcasses and some separable components of ten lambs as measured by flame photometry.

Item	Mean % Na	S. D.	Range
Edible carcass	0.073	0.006	0.063 - 0.083
Separable lean	0.075	0.005	0.069 - 0.081
Separable lean (fat-free basis)	0.080	0.005	0.074 - 0.087
Separable fat	0.070	0.017	0.051 - 0.111
Separable fat (fat-free basis)	0.206	0.014	0.183 - 0.225

analysis of variance to be 9 and 10 ppm of sodium for the separable lean and separable fat, respectively. It was interesting to note that when the chemical fat was removed (mathematically) from the separable fat, the remaining material (protein, water and ash) had a greatly increased sodium content. On correcting to a fat-free basis, there was more sodium in the separable fat than in the separable lean, whereas, for potassium the reverse was true.

A range of 0.069 - 0.081% of sodium was observed in the separable lean of the lambs. These values are lower than the range of 0.079 - 0.140% for lamb muscle reported by Toscani and Buniak (1947), but agree with the figures of 0.073 and 0.074% for sheep muscle observed by Blaxter and Rook (1956). However the values obtained for sodium in the present experiment are higher than the figures of 0.062 and 0.064% reported for sheep muscle by Harris et al. (1952) and the mean figures of 0.050 and 0.045% sodium in the fat-free, blood-free biceps femoris muscles of sheep reported by Mounib and Evans (1960). These results suggest that differences exist between muscles or between breeds of sheep and perhaps between methods of analysis. Further work is needed to clarify the factors involved.

The relationships between the sodium content of the edible carcass and other carcass components are presented in table 8. It may be observed that with the exception of bone, all the correlations between the sodium content of the edible carcass and carcass composition were significant.

Table 8. Correlations between the percent sodium in the edible carcasses of ten lambs and other carcass variables.

Item	r
Edible carcass	
% water	0.82**
% ether-extract	- .80**
% protein	0.70*
Dressed carcass	
% separable lean	0.79**
% separable fat	- .78**
% separable bone	0.48

* Correlation significant at 5% level
**Correlation significant at 1% level

However, these correlations tended to be the same or lower than the equivalent potassium correlation coefficient (cf. table 5). The sodium relationships are likely to be of less interest in meat animals as radioactive sodium isotopes are not naturally present in the tissues. Therefore, the addition of isotopes would probably render the meat unsuitable for human consumption. Yet these relationships may be of interest in work with experimental material.

Cesium Content and Composition

The cesium-137 content of the lambs and their components is presented in table 9. As is customary in the literature, the cesium content has been expressed as the cesium/potassium ratio and as the cesium content of

the tissue. It can be seen that washing the lambs increased the cesium/potassium ratio. As was explained earlier, this occurred because washing removed potassium from the skin and wool without markedly affecting the cesium content of the lambs (see cesium/Kg. tissue, table 9).

Table 9. Cesium-137 content of ten live lambs and their components

Item	Mean	S.D.	Range
Live unwashed (μμc cesium-137/gm. K)	58.0	10.6	41.8 - 74.5
Live washed (μμc cesium-137/gm. K)	84.8	17.9	60.2 - 121.0
Dressed carcass (μμc cesium-137/gm. K)	81.5	23.0	43.0 - 122.4
Non-carcass components (μμc cesium-137/ gm. K)	96.8(9)[a]	59.4	25.9 - 189.2
Separable lean (μμc cesium-137/gm. K)	79.5	18.1	59.1 - 107.7
Live unwashed (μμc cesium-137/Kg. tissue)	155.5	24.1	126.3 - 182.8
Live washed (μμc cesium-137/Kg. tissue)	148.7	21.4	118.8 - 183.3
Separable lean (μμc cesium-137/Kg. tissue)	236.6	54.3	167.8 - 323.1

Counts for separable fat and bone on the lower channel were so close to background that several of the samples were estimated to have a negative cesium content and all results on these tissues were obviously inaccurate. For this reason they have not been reported.
[a]Mean is based on 9 observations as one negative value was discarded.

In the section on <u>Potassium Content and Composition</u> it was stated that "the apparent total amount of cesium-137 present remained at 0.99 of the unwashed level, with a standard deviation of 0.12 (Anderson, personal communication)", whereas the figures in table 9 suggest that 0.96 would be a better estimate. The difference between these estimates is due to the fact that two sets of potassium and cesium figures were available on two of the washed lambs. These were washed twice in order to determine whether all the external potassium was removed by one washing. The figures in table 9 include those obtained after one washing, which was

comparable to the situation on the remaining lambs. However, Anderson used the figures obtained after two washings, which for some unknown reason gave a somewhat higher estimate of cesium. It is probable that the increase in cesium count was a chance occurrence.

It can be seen from table 9 that the cesium/potassium ratio in the washed lambs and their components was fairly constant which would be expected as cesium is metabolically similar to potassium (Langham, 1961). This resulted in a higher concentration of cesium per unit of muscular tissue than in the live lambs, because the lean tissue also had a higher concentration of potassium (table 4).

The levels of cesium reported are similar to those found in cattle tissue in 1957 - 1959 in the U.S.A. (Van Dilla et al., 1961), in pigs and calves prior to 1962 in Canada (Green et al., 1961; McNeill and Robinson, 1962) and in human beings, largely from the U.S.A. (Langham, 1961). However, these values are very much lower than those observed in Norway and Sweden in beef, horse, mutton, pork, reindeer and to a lesser extent in man (Hvinden and Lillegraven, 1961a, b; Baarli et al., 1961; Liden, 1961). As all these observations were made prior to the recent nuclear weapons test series of the U.S.S.R. and the U.S.A. beginning in August of 1961, it would appear that these levels resulted from earlier weapons tests. Differences may be due to different world fallout patterns and different eating habits. For example, the cesium/potassium ratio in Norwegians (Baarli et al., 1961) was very much lower on average than in Laplanders Liden, 1961). Differences in sheep, cattle and reindeer (Hvinden and Lillegraven, 1961b; Liden, 1961) were attributed to variation in grazing habits.

Some correlations between the cesium content of the live lambs and carcass composition are presented in table 10. Components were chosen which were highly correlated with potassium content in the present experiment, and also lean content, which has been shown by other workers (Kulwich et al., 1961a, b; Pfau et al., 1961) to be significantly related to

Table 10. Correlations between the cesium content of live lambs ($\mu\mu c$ cesium-137/Kg. tissue) and some carcass components

Item	Live unwashed cesium content	Live washed cesium content
% Protein (edible carcass)	-.240	-.063
% Separable fat (dressed carcass)	0.042	-.183
% Separable lean (dressed carcass)	-.094	0.257

cesium content. None of the correlations in table 10 were significant. However, Pfau et al. (1961) and Kulwich et al. (1961a, b) have shown that cesium-137 is less closely related to lean content than potassium, and the latter workers concluded that cesium could not provide a useful estimate of leanness. As none of the potassium relationships (from potassium -40) with carcass composition in the present experiment were very high, the lack of relationship with cesium-137 content is not too surprising. It has been shown that the individual estimates of potassium from potassium-40 were not very accurate in the present experiment and as the same conditions were used for cesium determinations, a significant relationship would not be expected. The literature shows that the cesium content of animals may vary from laboratory to laboratory and will change with continuing nuclear weapons tests. For this reason cesium is potentially less useful than potassium-40, which is a constant proportion of all potassium. The differing levels of cesium would make a comparison of the results from different laboratories more difficult than is the case for potassium.

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SUMMARY AND CONCLUSIONS

Ten lambs with a mean live weight of 88 lb. were used to determine the accuracy with which their carcass composition could be measured from their potassium-40 content. It was found that on average the live lambs contained 108 gm. of potassium which was reduced to 71 gm. by washing, which indicated the presence of appreciable amounts of potassium on the skin and wool. The potassium content of the live lambs when estimated from potassium-40 was significantly related to carcass composition. However, carcass potassium as estimated by the potassium-40 method was not significantly related to physical or chemical composition. In contrast, the flame photometrically determined potassium content of the carcass was significantly related to carcass composition. This suggested that the carcass potassium content was more accurately determined from flame photometry than from potassium-40. However, it should be noted in this experiment, that potassium did not give an accurate estimate of carcass composition, regardless of the method of measurement.

It was shown that the sodium content of the carcasses was less closely related to carcass composition than was potassium. The sodium and potassium contents of fatty and muscular tissue were found to be in general agreement with the results of other workers. The cesium-137 content of lamb and its possible use for predicting composition were discussed. It was concluded that cesium was likely to be less useful than potassium for measuring the composition of live animals and the carcass parts.

EXPERIMENT II. GROUND PORK AND LAMB

The relationship between the potassium content and both physical and chemical composition in Experiment I was lower than was anticipated from the literature. It was decided to test the potassium-40 method again on homogeneous tissue samples of exactly the same weight and with a wider range in composition than was present in the lambs. Under these more ideal conditions, it was anticipated that the relationships would be closer than would normally be expected if the potassium-40 method was adopted on a routine basis for measuring composition. Flame photometry was used as an alternative method to check the accuracy of the potassium determinations, and in addition, to measure the sodium content of the samples.

EXPERIMENTAL

Pork and Lamb Samples

Both pork and lamb samples were obtained from several carcasses, which were boned out and prepared at the Michigan State University Meat Laboratory. No attempt was made to keep the meat separate from different carcasses within each species. The meat was ground into a homogeneous mixture in a "silent cutter", which is used for making sausage emulsions. The range in chemical composition of the samples approximated that which may be found in sausage emulsions. Exactly 19.0 lb. of the meat emulsion were packed separately into waterproof, cardboard cartons, which were about 10 inches high and 9.5 inches in diameter. At the same time, a sample was taken for chemical analysis. Each carton of meat emulsion was capped to prevent evaporation and frozen for ease of handling and to pre-

vent deterioration. Twenty cartons of pork and seventeen cartons of lamb were prepared.

Chemical Analyses

The samples taken for chemical analysis were frozen and stored in glass jars until analyzed for percent water, fat and protein by the methods described previously. The samples were then used for duplicate potassium and sodium analyses by flame photometry after being extracted by the TCA homogenization method described earlier.

Scintillation Counter

Radioactivity was measured with the Radiation Counter Laboratory Model 55400 Ratio Computer (Regas _et al._, 1959). It is a large scintillation detector with a centrally positioned well 12 inches in diameter and 24 inches deep. The counting well was optimistically described as being able to contain 90 lb. of meat. With the exception of the end areas, the sample is completely surrounded by the scintillation material, thus giving a counting geometry approximating a four _pi_ configuration. The detector tank was surrounded by a 3 inch lead shield to reduce the radiation background in the counter well. Eight photomultiplier tubes 5 inches in diameter view the scintillation material from the end areas. At the time that the experiment was carried out, only five of the eight photomultiplier tubes were functional. Pulse heights were counted in the range of 1.2 - 1.6 Mev., which included the potassium-40 peak (1.45 Mev.). Limiting the range reduced the possibility of counting the radioactivity from other elements.

Counting Methods

Two cartons were always counted at the same time; thus, the gamma activity of the meat was determined on 38-lb. batches. Each of the 19-lb. cartons was counted in two different combinations, giving a total of 20 observations for pork and 15 for lamb. Cartons were measured in pairs to increase the precision of counting. All samples and backgrounds were counted for two consecutive 5-minute periods. With the first 10 pork combinations, background counts were taken after each pair of cartons had been counted. Since background remained fairly constant, two pairs of cartons were counted between each background observation for the remaining pork samples. With lamb, background counts were made only after counting every third pair of cartons, or at about 40-minute intervals.

Potassium Standards

Four 19-lb. lots of commercial sucrose, which were shown to have no gamma activity, were put in cardboard cartons identical to those used for the meat samples. Three lots of chemically pure KCl containing 33, 66 and 132 gm. of potassium were added to three of the sugar cartons to act as potassium standards as outlined by Anderson (1959). Then each standard was counted with a carton of pure sugar giving 38-lb. standards. The potassium standards were counted for two 5-minute periods twice during the experiment.

RESULTS AND DISCUSSION

Potassium Standards

The counts per second (cps) on the potassium standards were related to the grams of potassium in the standards by the following linear equation:

$$K\ (\text{gm.}) = 2.6554\ \text{cps} + 0.52$$

Since the potassium standards fulfilled the requirements discussed by Anderson (1959), the above equation was used to predict the potassium content of the meat samples. The predicted potassium content was then expressed as a percentage of the fresh meat sample. The scintillation detector recorded an average of 0.3716 cps per gram of potassium. Since the natural mixture of potassium isotopes emits 2.96 gamma rays per second per gram (Anderson, 1959), the efficiency of the detector was 12%. It is probable that a higher counting efficiency would have been achieved if all eight photomultiplier tubes had been functioning. Conceivably, this lowered efficiency could have reduced the accuracy of the determinations.

Gross Meat Composition

The gross chemical composition of the meat samples is presented in table 11. The means, standard deviations and ranges are based on the 38-lb. samples that were counted for gamma activity. Thus, each individual observation comprises the mean composition of the contents of each pair of 19-lb. cartons that were counted together. It can be seen that there was quite a wide range in composition, with the variation for pork and lamb being about the same. The summation of the individual components does not total 100%, because the ash content of the samples was not determined.

Table 11. Gross chemical composition of the counted meat samples

Item	Mean	S.D.	Range
Pork (20 observations)			
Water %	51.1	7.4	37.7 - 63.1
Fat %	33.5	9.5	17.5 - 50.7
Protein %	14.5	2.2	10.8 - 18.3
Lamb (15 observations)			
Water %	53.7	7.7	36.6 - 63.1
Fat %	30.0	10.0	18.4 - 52.5
Protein %	15.8	1.8	11.5 - 18.0

Potassium and Sodium Content of the Ground Meat Samples and Relationships with Composition

The mean counts per second observed on the pork samples were 15.7 (S.D. = 3.2; range = 9.7 - 20.8) and on the lamb samples were 16.5 (S.D. = 3.0; range = 10.9 - 20.8). With both pork and lamb, the counting error (S.D.) was approximately 3.6, when expressed as a percentage of the total number of counts. Since the background was determined more frequently with pork, the over-all accuracy may have been greater than with lamb. The counts were converted to potassium content of the meat samples as described earlier.

The standard error of the mean of the duplicate potassium analyses by flame photometry was found from the error mean square of analysis of variance to be 23 ppm for the pork samples and 16 ppm for the lamb samples, respectively. The standard error of the mean for the duplicate sodium analyses was found from the error mean square of analysis of variance to be 5 ppm for the pork samples and 7 ppm for the lamb samples, respectively. It must be re-emphasized that these figures take no account of day-to-day variation in the operation of the flame photometer, as the duplicate analyses were always carried out on the same day. However, it should be noted that for potassium, the standard error would have to be 88 ppm to give a standard error of 3.6%, which could be considered equivalent to a counting error of 3.6%. This suggests that under the conditions of use, the flame photometry estimations of potassium were probably more accurate.

The data on the potassium content of the ground pork and lamb as measured by flame photometry and as measured from the potassium-40 content are presented in table 12. It may be observed that both methods

Table 12. Potassium and sodium content of ground pork and lamb samples as measured by flame photometry or from potassium-40

Species	Method	Mean %	n	S. D.	Range
Potassium					
Pork	Flame photometry	0.245	20	0.038	0.175 - 0.307
Pork	Potassium-40	0.244	20	0.050	0.153 - 0.323
Pork	Flame photometry, fat-free basis	0.367	20	0.008	0.352 - 0.382
Lamb	Flame photometry	0.244	15	0.038	0.163 - 0.287
Lamb	Potassium-40	0.256	15	0.046	0.171 - 0.323
Lamb	Flame photometry, fat-free basis	0.348	15	0.008	0.337 - 0.361
Sodium					
Pork	Flame photometry	0.042	20	0.003	0.035 - 0.047
Pork	Flame photometry, fat-free basis	0.064	20	0.005	0.056 - 0.072
Lamb	Flame photometry	0.062	15	0.006	0.049 - 0.070
Lamb	Flame photometry, fat-free basis	0.089	15	0.006	0.081 - 0.104

estimated the same potassium content of pork. However, analysis of variance showed that the difference between the means of the potassium content of lamb, although small (120 ppm), was significant ($P < .05$). As was noted in the experiment with the live lambs and their components, the use of flame photometry resulted in a smaller standard deviation for the potassium content of both species, than when potassium-40 was used.

Correction of the potassium content of the ground pork and lamb to a fat-free basis permitted a comparison to be made of potassium levels. It is interesting to note that on this basis the potassium content of the ground lamb (0.348%) was almost identical with the potassium content of separable lamb lean (0.340%, table 4) observed earlier. However, in contrast, it was shown by a "t" test that the difference in potassium content between the pork and lamb on a fat-free basis was highly significant ($P < .001$). Further work is needed to investigate whether pork normally has a higher potassium content than lamb, and to determine if factors such as age and sex may influence potassium levels as has been found true for humans (Anderson and Langham, 1959). All such factors would have to be considered before any potassium-based technique could achieve accuracy.

The data on the sodium content of the ground meat samples are also presented in table 12. Application of a "t" test showed that the sodium content of ground lamb was greater than for pork on a fat-free basis ($P < .001$). This was the opposite of the results for potassium. The sodium content of the ground lamb on a fat-free basis was higher than similar values for lamb muscular tissue observed in table 7. This can probably be explained by the high sodium content of the non-fatty material found in separable fat (table 7), as a considerable amount of separable fat was present in many of the ground lamb samples.

Table 13 shows the correlation and regression equations used in predicting the composition of the meat samples from their potassium content

Table 13. Relationships between the percent potassium in ground meat samples (X) as estimated from potassium-40 and other chemical components

Dependent variate	Correlation[1]	Regression equation	$S_{y.x}$
Pork (20 observations)[2]			
% Water	0.977	Y = 144.89X + 15.70	1.61%
% Fat	-0.975	Y = 79.36 - 187.60X	2.20%
% Protein	0.962	Y = 42.14X + 4.20	0.61%
Lamb (15 observations)[2]			
% Water	0.917	Y = 153.13X + 14.39	3.20%
% Fat	-0.908	Y = 80.21 - 196.15X	4.35%
% Protein	0.883	Y = 34.93X + 6.62	0.89%

[1]All correlations significant at the 1% level
[2]Each observation was made on a pair of 19 lb. samples. The composition of these 38 lb. samples have been related to their potassium content.

as estimated from their natural radioactivity. In pork, the accuracy with which the composition of the samples could be predicted looked promising. The standard errors of the equations based on the gamma activity of the pork are almost exactly the same as those given by Kulwich et al. (1960a) from the beta activity of pork samples. In contrast, the error in estimating the composition of lambs was quite high. The magnitude was similar to that observed in regression equations for predicting the composition of lamb carcasses (table 6).

The relationships between the potassium content of the ground meat as measured by flame photometry and some of the other chemical constituents are presented in table 14. All correlations are larger than the corresponding relationships reported in table 13, when the potassium content of the samples was estimated from natural radioactivity. It can be seen from table 14 that the standard errors of the regression equations for predicting meat composition were very much smaller when flame photometry was used than when the natural gamma activity was used. These results suggest that part of the explanation for the relatively high standard errors resulting from the potassium-40 approach are due to errors involved in counting the gamma emissions. It is likely that better instrumentation and longer counting times than those used in the present experiments would reduce the errors involved. The precision indicated in table 14, where the standard error of estimate of the fat and water content of the pork was less than 1%, would be sufficiently accurate in a routine, non-destructive method of analysis. The results in tables 13 and 14 suggest that potassium is more closely related to the composition of pork than of lamb.

Table 14. Relationships between the percent potassium in ground meat samples (X) as measured by flame photometry and other chemical components

Dependent variate (Y)	Correlation	Regression equation	Sy.x (%)
Pork (20 observations)			
Water %	0.997	Y = 190.88X + 4.43	0.54
Fat %	- .996	Y = 94.00 - 247.35X	0.90
Protein %	0.986	Y = 55.71X + 0.88	0.38
Potassium % (from K-40)	0.977	Y = 1.261X - 0.064	0.0109
Lamb (15 observations)			
Water %	0.993	Y = 204.17X + 3.83	0.97
Fat %	- .990	Y = 94.22 - 263.36X	1.46
Protein %	0.975	Y = 47.52X + 3.98	0.42
Potassium % (from K-40)	0.936	Y = 1.153X - 0.0251	0.0168

The high correlation between the two methods of measuring potassium content shown in table 14 is in contrast to the lack of a significant relationship for the same comparison on the separable lean or fat for the Los Alamos lambs. It can be explained by two factors. First, the wider range in chemical composition in the second experiment probably would increase the magnitude of any correlation. A second and more important explanation, is the fact that the use of larger samples in relation to the size of the counter may have increased the statistical precision of counting in the second experiment. As was mentioned earlier, the Los Alamos counter was not designed to count samples as small as the separable fat from the lamb carcasses, and so the poor results for separable fat cannot be regarded as a criticism of the technique.

It was noted that the potassium content on a fat-free basis of the ground pork when measured by flame photometry was significantly related ($r = -.675$) to the chemically determined fat of the ground pork. In a similar manner, when the potassium content of the fat-free ground pork was estimated from the potassium-40 content, there was a negative correlation with percent fat of -.784. The potassium content of the ground lamb was also calculated to a fat-free basis from flame photometry and potassium-40 estimations. The potassium content on a fat-free basis was in both cases negatively and non-significantly correlated with the percent fat in the ground lamb.

It can be seen from table 15 that the sodium content of the ground meat was closely related to composition. However, the sodium content was in all cases less closely related to composition than the potassium content (cf., table 14), when the same method was used for estimating both sodium and potassium. It was interesting to note that by expressing the sodium content on a fat-free basis, the signs of all correlation coefficients were reversed. This was apparently due to the high sodium content of the non-fatty material (water, protein and ash) found in the separable fat, which tends to increase in weight directly with the weight of chemical fat. As this non-fatty material in the case of lamb (table 7), has two and a half times the sodium content of the fat-free separable lean, the percentage of sodium in the fat-free ground tissues increased as the percentage of fat increases.

Table 15. Correlations between the percent sodium in ground meat samples and other chemical components

Item	% sodium	% sodium (fat-free basis)
Pork (20 observations)		
Water %	0.934	-.931
Fat %	-.928	0.934
Protein %	0.912	-.933
Lamb (15 observations)		
Water %	0.967	-.938
Fat %	-.968	0.941
Protein %	0.972	-.920

It is noted that the relationships observed are likely to be much closer in a homogeneous material, such as ground tissue, than in live animals or their carcasses. As potassium is more closely related to composition than sodium and is at the same time naturally radioactive, it is considered that the use of potassium is likely to prove more fruitful than sodium analyses in the investigation of body and carcass composition, unless the intracellular and extracellular fluid spaces are to be investigated separately.

A practical application of the potassium-40 counting method to the meat industry has been suggested by Anderson (1959). He pointed out that the counting method might have an outstanding advantage over chemical analysis because of its adaptability to continuous systems. He stated, "If the sample to be analysed (e.g., comminuted meat) can be passed through the counter in a continuous stream of constant bulk density, the counter will provide through a rate-meter a continuous measurement of the lean content of the meat. The use of such a system for control of the ratio of lean/fat input to the grinders is obvious. Whether or not such a system is feasible depends on the precision and response time requirements." Later in the same paper Anderson states "there must be sufficient mixing and grinding before the counter to ensure a homogenous material at the point of counting." Anderson indicated some of the problems that would have to be overcome before the potassium-40 method could be used.

In the light of the present experiment, it would appear that the counting method would not be sufficiently accurate for lamb and may not be accurate enough for pork. Anderson (1959) did not mention how background determinations would be fitted into a continuous system. It is known that the frequency of background determinations greatly influence the accuracy of any radioactivity determinations where background is not constant. It should also be remembered that the statistical precision of the counting method which was discussed by Anderson, applies only to the accuracy with which potassium can be estimated, and takes no account of the errors in estimating meat composition, if the potassium content is known with complete accuracy. These errors would be due to the biological variability of the potassium content of meat. Finally, it should be noted that in the present experiment it took 10 minutes to obtain a relatively inaccurate estimate of the composition of homogeneous meat samples.

SUMMARY AND CONCLUSIONS

The potassium content of 20 lots of ground pork and 15 lots of ground lamb, each weighing 38 lb. was estimated from the potassium-40 content and from flame photometry. A wide range of gross chemical composition was found in the 38-lb. lots of ground tissue from both species. The ground pork was found to contain significantly more potassium (0.37%) than the ground lamb (0.35%) on a fat-free basis.

Both methods of potassium estimation gave approximately the same potassium content of the individual samples. However, the flame photometrically determined potassium content was more closely related to the chemical composition of the samples in terms of percentage water, fat and protein, than was potassium content as determined by the natural gamma activity. The data suggest that such relationships are closer for pork than for lamb. The sodium content of the samples as measured by flame photometry was also very closely related to the composition of the samples, but the relationship was not as good as that observed for potassium.

Results of the potassium analyses using flame photometry demonstrated that a close relationship exists between potassium content and chemical composition. If a non-destructive method (such as potassium-40) is to be sufficiently accurate to be useful, then a degree of precision at least comparable to that obtained by flame photometry is needed.

EXPERIMENT III. TWENTY FOUR PIG BODIES

INTRODUCTION

The potassium content of animal tissues was more closely related to the gross composition of the samples when estimated from flame photometry than from potassium-40 (Experiments I and II). The tissue samples utilized in this study came from the bodies and carcasses of 24 pigs for which the major chemical components had been determined (Gnaedinger, 1962). Although the size of the pigs would tend to make sampling for chemical analysis more difficult and less accurate than in previous experiments in which flame photometry was used to measure the potassium content, these samples were considered suitable for investigating the relationships between the gross composition and potassium content by flame photometry. Unfortunately, facilities were not available for measuring the potassium content of the live pigs from their natural gamma radioactivity. It was, however, considered that the relationships established by flame photometry would give an indication as to whether the non-destructive potassium-40 technique was likely to be useful in estimating the composition of live pigs and their carcasses. Sodium analyses by flame photometry were also conducted.

EXPERIMENTAL

Animals and Their Gross Analyses

Twenty four pigs ranging in liveweight from 181 - 220 lb. were used in this experiment. A description of the slaughter procedure and methods of gross chemical analysis (fat, protein, water and ash) has been published by Gnaedinger (1962). At slaughter each animal was divided into 7 compartments. These included the carcass (including the skin), hair (including scurf and toenails), head (including the tongue), blood, viscera (liver, lungs, esophagus, trachea, heart, kidney and spleen), empty G.I. tract (stomach, intestines, caul and ruffle fat), and the contents of the G.I. tract. It should be noted that the eviscerated carcass containing the skin is equivalent to a commercially dressed carcass with the exception of leaf (kidney) fat which is normally removed under commercial conditions. The commercial practice of scalding the carcasses in 142°F water prior to scraping and dehairing was followed. This would undoubtedly remove some of the sodium and potassium from the exterior of the ani-m als and from the skin and hair. Each body fraction was frozen, sawed into thin strips, ground until homogeneous and samples were taken for subsequent chemical analyses (Gnaedinger, 1962). The samples were stored in a frozen condition in glass jars until thawed for analyses. Water, ether-extract and protein were determined by the methods described earlier. Ash content was determined on samples weighing approximately 5 gm. which were ashed for 24 hours at 525°C as described by Gnaedinger (1962).

An anomoly was noted in the data on pig 1 from the point of view of the present experiment. This pig was skinned, whereas, the skins were left on the remaining 23 hogs as is the commercial practice. The skin (plus some subcutaneous fat) of pig 1 weighed 23.4 lb. and the dressed carcass weighed 139.6 lb. For purposes of analysis, Gnaedinger included the skin of pig 1 under the hair classification. This compartment averaged 1.1 lb. in weight on the remaining animals. This had no effect when total body composition was computed, but made some differences to carcass composition in terms of both the gross chemical composition of carcass 1 and also its electrolyte content. As compared to the hair, scurf and toenails of the remaining pigs, the skin of pig 1 weighed a great deal more and had more water and fat, and less protein and ash. Thus, two sets of calculations will be presented in many of the statistical analyses related to the carcasses of these pigs. In one set, the frozen carcass composition figures presented by Gnaedinger (1962) which excluded the skin and hair of pig 1, will be used, and in the second set of calculations, the skin components of pig 1 will be added to the frozen carcass components. As Gnaedinger (1962) was mainly interested in the body composition of live pigs, the classification of the skin of pig 1 was not a matter of importance providing that it was included in the total body compartments.

It may be noted that the original experiment of Gnaedinger was not planned from the standpoint of determining the potassium and sodium content of the pigs. Blood was not available for all animals as some of the samples had been completely utilized in earlier analyses. As the blood samples were prevented from coagulating by the use of sodium citrate, they were unsuitable for sodium analyses. Three further blood samples were obtained from three mature hogs at the time of slaughter and these were analysed for sodium and potassium. Coagulation was prevented in these samples by the use of citric acid.

Potassium and Sodium Analyses

Potassium and sodium analyses were made after the samples had been extracted and prepared by homogenization in 2% TCA as described earlier. Unlike the two previous experiments, ground bone was also present in the carcass and head samples. Dean (1960) has shown that over 100 ppm calcium can depress potassium readings and enhance sodium readings in the range that these elements were being determined in this experiment. As it was possible that the method of extraction may dissolve a little of the finely ground bone, a preliminary analysis was carried out for calcium on ground material from carcass 1. Extraction and dilution was carried out in the usual manner. Analysis indicated that about 12 ppm of calcium was present in the solution analysed, and so it was assumed that the calcium level would not seriously influence the readings.

For samples other than those obtained from the carcasses, it was found necessary to prepare a new standard solution with a lower K:Na ratio than 5:1, thus, a ratio of 1:1 was chosen. This gave a range of 0 - 30 ppm potassium and sodium for the standard curves.

The potassium and sodium content of the empty bodies of these pigs were computed from the weights of the body components and their potassium and sodium contents. It was realized that this method of determining the potassium and sodium content of the empty body would underestimate these elements, since some of these elements may be trapped by particles of bone and teeth from the head and carcass.

RESULTS AND DISCUSSION

The weights of the major components of the bodies of 24 pigs are presented in table 16. It may be noted that the frozen carcasses comprised 76% of the liveweight of the animals. The value recorded for blood represents the difference in weight of the pig body before and after bleeding and is not a measure of the total blood volume of the animal. Dukes (1955) suggests the blood volume of swine is on average 7.4% of body weight.

Table 16. Body weight and components from 24 pigs (from Gnaedinger, 1962)

Item	Mean (lb.)	%	Range (lb.)
Live weight	198.4	100.0	181.0 - 220.0
Hot dressed carcass	156.2	78.7	140.2 - 173.6
Frozen dressed carcass	151.3	76.3	136.0 - 169.6
Head and tongue	10.2	5.1	8.3 - 13.0
Gastrointestinal tract + caul fat	10.5	5.3	8.7 - 12.9
Remaining viscera[a]	7.8	3.9	6.5 - 9.0
Hair, scurf and toenails	1.1 (23)	0.6	0.8 - 1.5
Blood	7.5	3.8	4.2 - 9.8
Gastrointestinal contents	3.4	1.7	1.7 - 5.4

[a]Liver, lungs, esophagus, trachea, heart, kidney and spleen.

However, the blood weight recorded represents the loss in body weight due to bleeding that might be expected in a commercial slaughter operation.

The chemical composition of the frozen carcasses (excluding the skin of pig 1) and the empty bodies (the whole animal excluding the contents of the G.I. tract) of the 24 pigs are presented in table 17. It may be noted that the range in body composition is less than the range in carcass composition. Similarly, the range in protein and ash is quite small.

Table 17. Composition of the empty bodies and frozen carcasses of 24 pigs (from Gnaedinger, 1962)

Item	Frozen carcass		Empty body	
	Mean	Range	Mean	Range
Water %	44.4	36.4 - 48.2	48.5	43. 6 - 52.5
Ether-extract %	38.6	32.8 - 48.3	33.5	28.4 - 41.5
Protein %	13.6	12.3 - 14.8	13.8	12.5 - 14.8
Ash %	2.8	2.1 - 3.3	2.7	2.2 - 3.2

The potassium content of the empty bodies and their components are given in table 18. The empty bodies of these pigs contained 0.20% potassium, or 0.30% on a fat-free basis. Green _et al_. (1961) have estimated 0.24% potassium in pigs of unspecified age and weight, while Spray and Widdowson (1950) have estimated that the fat-free bodies of adult pigs contained about 0.28% potassium. These values agree fairly well with the results of the present experiment, which may slightly underestimate potassium content because of the potassium trapped by bone fragments.

Table 18. Potassium content of the empty bodies and their components from 24 pigs

Item	n	Mean %	S.D.	Range
Frozen carcass[a]	24	0.2098	0.0186	0.1737 - 0.2455
Head and tongue	24	0.1470	0.0092	0.1253 - 0.1668
G.I. tract + caul fat	24	0.1873	0.0159	0.1617 - 0.2275
Remaining viscera	24	0.2571	0.0110	0.2343 - 0.2887
Hair etc.[b]	1	0.0978	--	-- --
Blood[c]	14	0.2199	0.0090	0.2061 - 0.2368
Empty body[d]	24	0.2013	0.0146	0.1734 - 0.2316

[a]If the skin etc. of pig 1 was included in the composition of this carcass, the mean potassium content of the 24 hog carcasses was reduced to 0.2089%.
[b]A composite sample from several animals.
[c]Eleven samples from the 24 hogs (range 0.2061 - 0.2386) plus 3 additional samples from 3 more hogs (range 0.2092 - 0.2155) and the mean value was used in computing body composition.
[d]In computing empty body composition the loss of weight between the hot carcass and frozen carcass was assumed to be water containing no potassium or sodium. The contents of the G.I. tract have been excluded from the computations.

The frozen carcasses contained 0.21% potassium, or 0.34% on a fat-free basis. It was shown in table 12 that ground pork (lean and fat) contained 0.37% potassium on a fat-free basis. It is possible that the bone present in the carcasses in this study may be sufficient to explain the lower value for the entire carcasses.

Filer <u>et al</u>. (1960) presented some data from which it was possible to estimate the potassium content of 8 week old piglets. The animals were used in an experiment to study the influence of protein and fat content of the diet on body composition. For experimental purposes Filer et al. (1960) divided the animals into 8 groups, the lightest of which averaged 39 lb. and the heaviest 42 lb. liveweight. The average carcass potassium of 32 of the pigs which were fed on a high-fat diet was 0.18% and of 14 of the pigs fed on a low-fat diet was 0.21%. It appeared from the data that the difference in potassium content of the carcasses from the two feeding treatments was significant and that the lowered potassium content in the high-fat pigs was apparently due to the lower protein content in their carcasses. However, the potassium content of the pigs on the low-fat diet was approximately the same as that found in the carcasses from the 198 lb. pigs in the present experiment. When the potassium content of the carcasses of the piglets (Filer et al., 1960) was expressed on a fat-free basis, there was little difference between the animals on the high- and low-fat diets. The average potassium content was 0.26% on a fat-free basis. This is considerably lower than the 0.34% observed in the present experiment, and does not appear to be in agreement with the data of Spray and Widdowson (1950), which suggests that pigs reach chemical maturity in terms of sodium and potassium content at 3 weeks of age.

The low potassium content of the head (table 18) is probably the result of the high bone content (7.6% ash; Gnaedinger, 1962), even though pig brain is quite high in potassium (Widdowson and Dickerson, 1960). The low fat content (Gnaedinger, 1962) and high potassium content (Widdowson and Dickerson, 1960) of many organs explain the relatively high potassium content of the remaining viscera. The low potassium content of the hair, scurf and toenails may be at least partially due to the soaking that these components received during the scalding process.

The observed potassium content of the blood (0.22%) is very much higher than the value of 0.09% reported by Green et al. (1960). In this connection, it should be noted that McCance and Widdowson (1958) found 0.47 - 0.48% potassium in the erythrocytes from mature pigs. Widdowson and McCance (1956) found 0.02 - 0.03% potassium in mature pig serum. Assuming a haematocrit of 41.5 (Dukes, 1955) and using the above data to calculate the potassium content, gives an estimate of about 0.21% potassium in the blood of the mature pig. This suggests that either Green et al. (1960) had a group of pigs with widely different blood potassium from those in the present experiment or else the potassium-40 method of analysis was inaccurate on blood. It should be noted that in the sheep, strains have been found with blood levels of potassium which differ as much as for the groups of pigs discussed here (Evans, 1954; Kidwell et al., 1959), but the presence of high and low potassium strains of pigs have not yet been reported as far as the author is aware.

The sodium content of the 24 pigs and their various components is shown in table 19. It can be seen that the remainder of the body has a considerably higher sodium content than the carcass, with the head and the blood having the highest sodium content. When computed on a fat-free basis, the sodium content of the empty bodies was 0.124% and of the frozen carcasses was 0.116%. The former figure is less than 0.140% sodium indicated by Spray and Widdowson (1950) for the fat-free bodies of mature pigs. This latter figure agrees quite well with the results of Filer et al. (1960) on the carcasses of 46 piglets. Calculation from their data showed that the mean sodium content of the piglets on a fat-free basis was 0.127%. All results indicate that the sodium content of the entire carcasses is almost double that of the soft tissues of the carcass (fat and lean; see ground pork, table 12), because it was shown earlier

that ground pork contained 0.064% sodium on a fat-free basis. The increased amount of sodium in the entire carcass probably comes from the bone, which has been estimated to contain 36.5% of the total body sodium in man (Edelman, 1961). It should be emphasized that it is not known what proportion of bone sodium would be removed by the method of extraction used in the present experiment.

Table 19. Sodium content of 24 pigs and their components

Item	n	Mean %	S.D.	Range
Frozen carcass[a]	24	0.0715	0.0049	0.0611 - 0.0807
Head and tongue	24	0.1528	0.0161	0.1157 - 0.1852
G.I. tract and caul fat	24	0.1115	0.0122	0.0886 - 0.1439
Remaining viscera	24	0.1132	0.0044	0.1053 - 0.1205
Hair etc.[b]	1	0.0918	--	-- --
Blood[c]	3	0.1825	--	0.1760 - 0.1889
Empty body[d]	24	0.0825	0.0042	0.0730 - 0.0896

[a]If the skin of pig 1 was included in the composition of this carcass, the mean sodium content of the 24 hog carcasses was increased to 0.0717%.
[b]A composite sample from several animals.
[c]From 3 additional hogs as samples from the original 24 hogs were unsuitable for sodium analyses because coagulation was prevented by the use of sodium citrate.
[d]See footnote d to table 18.

If potassium-40 is to be used to non-destructively predict composition then several relationships are likely to be of interest. Workers in the field of animal production may wish to predict the composition of live animals and/or of their dressed carcasses, by making potassium-40 counts on the live animal. Similarly, workers interested in carcass composition may wish to estimate this from counts on the dressed carcass. The present data were used to investigate these relationships.

Table 20 indicates the relationships between the potassium content of the empty body which is approximately equivalent to the potassium content in the live animal and the potassium content of the frozen carcasses in relation to the gross chemical composition of the frozen carcasses.

Table 20. Correlations between the potassium content of the frozen carcasses and the empty bodies of 24 pigs and the components of the frozen carcasses

	Frozen carcass		Empty body	
Frozen carcass composition	% K (1)	% K (2)	% K (3)	% K (4)
% water	0.822**	0.809**	0.810**	0.813**
% ether-extract	-.831**	-.821**	-.818**	-.819**
% protein	0.712**	0.682**	0.674**	0.669**
% ash	0.469*	0.425*	0.417*	0.422*
% K (carcass)	-	-	0.985**	0.989**

(1) Skin of pig 1 omitted from both major components and potassium analyses of the carcass.
(2) Skin of pig 1 included in both major components and potassium analyses of the carcass.
(3) Skin of pig 1 omitted from major components of the carcass.
(4) Skin of pig 1 included in major components of carcass.

Two sets of data are presented, one of which includes the skin of pig 1 in the carcass compositional data and the other set omits the skin from the carcass. It can be seen in table 20 that this made very little difference to the relationships, even although the inclusion of the skin lowered the potassium content of carcass 1 by approximately 10%. It is interesting to note that the correlations between the potassium content of the empty body and carcass composition are almost as high as when carcass potassium content is related to carcass composition. This is not surprising, however, when it is noted that the empty body contained on average 176.8 gm. of potassium (S.D. = 14.4) and the frozen carcass contained 143.7 gm. (S.D. = 12.7), which is 81.3% of the total body potassium.

The regression equations that could be used for predicting carcass composition are given in table 21. The standard errors indicate that there is little difference in accuracy between body or carcass potassium content for predicting carcass composition. It should be noted that when carcass potassium is used to predict composition, plus or minus one standard error includes 29.8% of the total range in carcass water content, 29.6% of the range of fat content and 37.6% of the range in protein content. This suggests that the potassium content is not very accurate in discriminating between individual carcasses.

Table 21. Regression equations for predicting the composition of 24 frozen pig carcasses from the potassium content of the carcasses and of the empty bodies

Frozen carcass component (Y)[a]	Independent variable (X)	Regression equation	$S_{y.x}$
% water	% K frozen carcass[a]	Y = 133.9X + 16.1	1.76
% ether-extract	% K frozen carcass[a]	Y = 76.4 - 180.4X	2.30
% protein	% K frozen carcass[a]	Y = 25.3X + 8.3	0.47
% water	% K empty body	Y = 168.3X + 10.3	1.81
% ether-extract	% K empty body	Y = 84.2 - 226.6X	2.37
% protein	% K empty body	Y = 30.5X + 7.46	0.50
% potassium	% K empty body	Y = 1.257X-0.0432	0.0032

[a]Skin of pig 1 omitted.

Table 22 presents the relationships between the potassium content of the empty body and other body components. The correlations are similar to those observed on the carcass (table 21). Although the standard errors of the regression equations given in table 22 are lower than those in table 21, they do not discriminate between individual animals any better, because the range in body composition is lower than the range for the carcasses.

Table 22. Relationships between the percentage potassium (X) in 24 empty pig bodies and other body components

Dependent variable (Y)	r	Regression equation	$S_{y.x}$
% water	0.819**	Y = 145.8X + 19.2	1.52
% ether-extract	-.794**	Y = 71.3 - 187.9X	2.15
% protein	0.708**	Y = 27.6X + 8.2	0.41
% ash	0.439*		

The correlations between the sodium content of the carcasses of the pigs and their composition are presented in table 23. In this case, it

Table 23. Correlations between the sodium content of the frozen carcasses of 24 pigs and other carcass components

Frozen carcass component	% Na (1)	% Na (2)
% water	0.458*	0.526**
% ether-extract	-.508*	-.569**
% protein	0.472*	0.465*
% ash	0.349	0.468*

(1) Skin from pig 1 omitted from both major components and sodium analyses of the carcass.
(2) Skin from pig 1 included in both major components and sodium analyses of the carcass.

can be seen that the inclusion of the skin and some attached subcutaneous fat in the composition of the carcass of pig 1 increased the correlation, except in the case of protein where the correlation was essentially unchanged. It may also be noted that these sodium correlations were very much lower (except in the case of ash) than were their potassium counterparts. This agrees with the results from the earlier experiments.

The relationships between the sodium content of the empty bodies and the major chemical components of the empty bodies are given in table 24. The correlations for the empty body are higher than for the equivalent components on the carcass (cf. table 23). An explanation for this is not known unless the method of analysis removed some of the nonexchangable bone sodium, which would comprise a higher proportion of carcass sodium than of body sodium. It might be expected that exchangable sodium, which comprises a constant proportion of extracellular fluid, would be more closely related to body composition than total sodium. The sodium analyses indicated that the carcasses contained on average

Table 24. Relationships between percentage sodium (X) in 24 pigs (empty bodies) and other body components

Dependent variable (Y)	r	Regression equation	$S_{y.x}$
% water	0.700**	Y = 430.2X + 13.0	3.59
% ether-extract	-.655**	Y = 78.3 - 543.3X	2.64
% protein	0.597**	Y = 80.3X + 7.2	0.47
% ash	0.428*	-	-

47.4 ± 4.1 gm. of sodium and the bodies contained 72.5 ± 5.0 gm. This meant that the carcass contained 65.4% of total sodium, although the frozen carcass comprised 76.3% of body weight. If the standard errors of the regression equations in table 24 are compared with those in table 22, it can be seen that sodium is considerably less accurate than potassium for estimating body composition. It should be remembered, however, that a method that estimates exchangable sodium might be more closely related to body composition than the method used in this experiment, which may include some non-exchangable sodium.

SUMMARY AND CONCLUSIONS

The chemical composition of 24 pigs ranging in live weight from 181 - 220 lb. was determined. The animals were divided into 7 compartments at slaughter. These included the carcass (including the skin), hair (including scurf and toenails), head (including tongue), blood, viscera (liver, lungs, esophagus, trachea, heart, kidney and spleen), empty G.I. tract (stomach, intestines, caul and ruffle fat) and the contents of the G.I. tract. These compartments were frozen, sliced on a bandsaw, and ground until homogeneous. They were then sampled for analysis into water, protein, fat and ash. These same samples were also used in the present experiment for sodium and potassium determinations by flame photometry.

The empty bodies of the pigs contained on average 0.20% potassium (177 gm.) and their frozen carcasses contained 0.21% (144 gm.). When expressed on a fat-free basis these figures were increased to 0.30% and 0.34% potassium, respectively. The sodium content of the empty bodies was 0.083% and of the frozen carcasses was 0.072%.

All correlations between the gross chemical composition of the frozen carcasses and either the potassium content of the empty bodies or frozen carcasses were significant. Similarly, all correlations between the gross chemical composition of the empty bodies and their potassium content were significant. The magnitude of the standard errors from the resulting regression equations suggests that potassium is of questionable value for predicting the chemical composition of pig bodies and carcasses, unless methods are found for reducing some of the errors in these relationships. These errors include any inaccuracies in the measurement of the potassium content of the pig.

Percent sodium was less closely related to the composition of the pigs than was potassium. It was shown that sodium was more closely related to the composition of the bodies than the carcasses.

GENERAL DISCUSSION

The usefulness of potassium-40 as a non-destructive method of estimating composition must be considered, firstly, in terms of its absolute accuracy, and secondly, in terms of possible alternative methods.

Sources of Errors in the Potassium-40 Method

In regard to accuracy, the standard errors of the regression for predicting composition from potassium-40 in the experiments reported herein, have been rather high. Where comparisons are available, the present experiments appear to agree with the results in the literature (Kulwich *et al.*, 1960a). The lack of precision may be due to errors in measuring potassium by natural radioactivity, or due to errors in measuring the actual composition from the true potassium content because of biological variability in the animals or, finally, to errors in measuring animal composition.

Two experiments were performed in which it was possible to compare potassium-40 with flame photometry for estimating potassium content. The fact that the composition of animal carcasses or ground meat samples could be more accurately estimated from flame photometry than from natural gamma activity suggests that flame photometry measures potassium more accurately. The accuracy of the potassium-40 method of estimating potassium can be improved by using longer counting times than those used in the present experiments. It is also possible that the use of improved electronic equipment and the utilization of more efficient liquid scintillation fluids may increase the efficiency of counting.

Even when flame photometry was used to measure potassium, the accuracy of estimating composition was generally unsatisfactory. The only experiment in which the standard errors of the regression equations were small enough to give the desired accuracy (standard error of 0.56% for water and 0.90% for fat) was when flame photometry was used to predict the composition of ground pork samples.

The problem of biological variability will always be a final limiting factor in relating potassium content to composition. For example, it was shown that when the potassium content of the lamb muscle was expressed on a fat-free basis, the standard deviation was 5% of the mean value, so that only approximately 66% of observations would fall within a range of 10% of the mean value. Similar results were observed when the K/N ratio was investigated. This suggests that potassium comprises a slightly different proportion of the muscular tissue of the different animals in the sheep. Because of this, the muscular tissue and other components would not be closely related to potassium content.

Evans (1954), Evans and King (1955) and Kidwell _et al_. (1959) have shown that the red cells of individual sheep could be classified as either high potassium (approximately 83-m-equiv. per liter of erythrocytes) or low potassium (approximately 23-m-equiv. per liter of erythrocytes). These differences were reported to have a genetic basis. With blood comprising approximately 8% of body weight in sheep (Dukes, 1955), the inclusion of both types of sheep in an experiment would be sufficient to introduce some error. If the widely different potassium levels should occur in the muscles of sheep then even larger errors would occur unless the types were studied separately. However, Mounib and Evans (1960) have shown that the potassium content of the skeletal muscle of sheep of the two different blood types was not significantly different, although it tended to vary slightly in the same direction as blood potassium. Further work is needed to establish the potassium content of different muscles in a given species of animal, and also to ascertain whether differences occur between various species of animals.

Several authors have shown that the potassium content of different organs of sheep (Mounib and Evans, 1960; Blaxter and Rook, 1956), cattle (Blaxter and Rook, 1956) and pigs (Widdowson and Dickerson, 1960; Green et al., 1961; McNeill and Robinson, 1962) differ considerably between organs. As the organs vary in weight within a given species, this may also add to biological variability. For the potassium approach to be successful in predicting composition, the over-all effects of different organs and glands must be the same. Other sources of biological variability may include potassium contamination on the skin, or in the case of sheep, on the wool. Another source of variation may be the potassium in the contents of the digestive tract. For ruminants, this may be especially important because of the relatively large proportion of the contents to body weight. Blaxter and Rook (1956) have shown that the proportion of potassium in the G.I. tract of cattle may vary considerably.

It has been shown in all species of mammals that have been investigated, that the potassium content initially increases until the animal reaches "chemical maturity" and then levels off and declines with advancing age. Spray and Widdowson, 1950; Anderson and Langham, 1959; Allen et al., 1960). The decline in potassium with age occurs even when the ratio of potassium to body composition is calculated after removing the effects of fat, water and bone mineral (Allen et al., 1960). This indicates that the reduction of potassium is not due to increasing fatness with age. The effect of age and maturity need to be further investigated in farm animals, so that corrections for these factors may be applied.

The lower potassium content in the fat-free carcasses (0.26%) of the 8 week old piglets of Filer et al. (1960) as compared to the higher values (0.34%) for the fat-free carcasses of the 198 lb. pigs in the present experiment may be due to age. However, the data of Spray and Widdowson (1950) do not support this hypothesis as chemical maturity in terms of electrolyte content appeared to be reached at about 3 weeks of age. Further work in this field is obviously needed.

The variation in potassium content due to sex may not be important from the point of view of prediction, as Spray and Widdowson (1950) found that when the bodies of male and female rats and rabbits were adjusted to a fat-free basis, no differences could be detected. In other words, any differences due to sex in the potassium content of these species were explicable on the basis of fat content. However, Allen et al., (1960) found that for human beings after 15 years of age, the ratio of potassium to body composition with the effects of fat, water and bone minerals removed, was less for females than males. If potassium content is influenced by sex in other species then an adjustment would be needed in order to compare or combine data on males and females. Therefore, there may be a large number of factors, apart from the variation of potassium content in the tissues of animals of the same age and sex, which can effect the biological relationships between potassium content and composition.

Another problem involved in estimating the absolute accuracy of prediction by the potassium-40 method concerns the errors in measuring the composition of the carcasses or the intact animals. Apart from weighing or other human errors, the most important source of inaccuracy in measuring physical composition in terms of separable or dissectible components comes from evaporative water losses. This can, however, be minimized by the use of damp towels.

In experiments which measure the composition of animals or carcasses in terms of gross chemical composition, sampling will likely be a source of error. It was shown by Kirton et al., (1962) that the standard error of the composition of a lamb carcass (based on twelve 50 gm. samples per carcass) was 0.41% for water, 0.54% for ether-extract and 0.22% for protein and ash. Taking duplicate samples from the carcass would not have greatly reduced the accuracy of the determinations of the chemical composition. The lamb carcasses averaged 39 lb. in weight and were not ground as finely as the pig carcasses (averaging 151 lb.) studied in the present experiment. In the case of the lambs, however, very much larger samples were analyzed. Results suggest that some of the error involved in measuring the relationship between the chemical composition of the carcasses or bodies and their potassium content may be due to inaccuracy in measuring the gross chemical composition.

Potassium-40 in Relation to some Alternative Non-Destructive Methods

The other main factor involved in ascertaining the potential usefulness of the potassium-40 method is the accuracy of possible alternative methods. Currently used methods for estimating composition include "eye" and "hand" appraisal and body weight measurement, which are known to be inaccurate. A few methods more recently suggested for indirectly determining body composition will be discussed. In all experiments cited, the actual body composition was determined by chemical analysis and related to the non-destructive method being investigated.

The technique used by Gnaedinger (1962) for determining live animal specific gravity from air displacement and helium dilution was not sufficiently accurate to permit the estimation of the body composition of pigs. In discussing the errors from the air displacement technique, Gnaedinger stated that, "The greatest source of error appeared to result from inaccuracies in reading relative humidity." He also reported, "The major difficulties involved in the helium dilution technique were caused by the activity of the experimental animals inside the chamber."

Similarly, Kay and Jones (1962) reported on the use of specific gravity (underwater weighing with helium dilution to determine head and lung volume) to predict the body fat content of pigs. The results were unsatisfactory when compared with the tritium dilution method of estimating body fat from the estimated body water. A correlation of 0.96 was reported between body water as determined by tritium dilution, and body fat. The mean difference between the predicted and actual body water content was 1.17%, which appears promising.

Panaretto and Till (1962) have shown that tritiated water is more accurate than antipyrine and its amino derivative, N-acetyl-4-aminoantipyrine (NAAP), for measuring the chemically determined body water content of goats. The use of antipyrine and NAAP resulted in biassed estimates of body water. The standard error of the regression equation for estimating body water from tritium dilution was 2.1% and for estimating body fat was 2.8%. Unfortunately, experiments relating potassium-40 content to complete body composition determinations have not been reported.

Doornenbal _et al._ (1962) have made some just criticisms of the potassium-40 method and suggested the use of chromium-51 determined red cell volume for predicting lean body mass in pigs. These workers defined lean body mass as carcass protein, which differs somewhat from the usual definition. Doornenbal et al. (1962) gave no indication of the accuracy for predicting carcass protein from red cell volume. On calculating the regression equation and standard error from the data presented by these workers, it was found that the standard error of the regression equation for predicting the weight of carcass protein was 1.23 lb. over a total range of 5.72 lb. Thus, one standard error included 22% of the total range, and discriminated little between animals. Nutritional and health-related factors could also greatly limit the application of this method.

Definite recommendations cannot be made until more experiments and comparisons have been performed. However, on the basis of the experiments reported in this thesis, it does not appear that the potassium-40 method is sufficiently accurate for most experimental purposes.

SUMMARY

The potassium, sodium and cesium content of animals and their parts were determined and related to their composition. Flame photometry and potassium-40 measurements were in essential agreement as to the potassium content of fatty tissue and muscular tissue from lamb carcasses. Similar results were found for ground pork and lamb samples. The composition of the animals and their tissues could be more accurately predicted from the flame photometrically determined potassium content than from potassium-40. Relationships between potassium content and composition were closer for pork than for lamb. In general, the standard errors of the regression equations for predicting composition from potassium content were too large to suggest that the potassium-40 method is likely to have useful application. Possible reasons for the magnitude of the standard errors have been fully discussed, as well as some possible non-destructive alternative methods for determining composition.

The sodium content of the various tissues was also determined by flame photometry. In contrast to potassium, the levels of sodium were higher in the non-carcass compartments than in the carcass of the pig. Sodium was found to be less closely related to composition than potassium.

The cesium-137 levels in lambs were found to agree with other data from North American sources. These levels were lower than some published from Scandanavia following nuclear weapons tests. The cesium content of the lambs was found to be unrelated to their composition.

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APPENDIX

Table 1. Comparison of different methods of extracting potassium and sodium

	Extraction method							
	TCA homogenization		Oven ashed		Boiling + HNO_3		Acid ashed	
Sample	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium
Pork 1	4097	433	3614	725				
	3934	431	2600	1107				
	4101	429	2492	1119				
	4040	438	3792	606				
	4034	534	3173	773				
	4084	572	3059	866				
	3782	542	3583	646				
Beef	1822	480	1340	673	1722	449	1855	465
	1825	450	1597	514	1843	508	1825	450
	1749	474	1359	594				
	1825	497	1384	575				
5000 ppm K 1000 ppm Na	5013	1295	5294	662				
	4987	996	4994	842				
	4987	918	5019	827				
	5013	908	5044	837				
	5072	908						
	4977	954						
Pork 2	4232	590	3551	942	4329	617	4188	634
	4211	596	3296	1073	4373	617	4183	608
	4197	586	3318	1030			4155	661
			3182	1075				

Values in ppm
Means and standard deviations presented in thesis Table 2.

Table 2. Slaughter and separation data from Los Alamos lambs

Lamb No.	Live weight	Hot carcass weight	Non-carcass components	Separable carcass components Lean	Fat	Bone
1	105.5	56.3	48.5	33.0	14.1	8.19
2	88.0	49.0	40.0	27.1	13.6	7.41
3	93.5	46.9	46.1	26.8	10.3	8.35
4	80.2	42.1	38.7	23.5	11.7	6.05
5	76.6	41.9	35.6	25.3	8.9	6.10
6	100.8	54.4	45.2	31.3	13.1	8.25
7	88.1	43.8	44.4	26.0	8.7	7.87
8	84.3	42.1	43.4	26.5	7.1	6.95
9	83.4	42.7	41.3	28.0	4.1	8.70
10	78.3	38.2	39.4	23.4	5.4	7.12

Weights in lb.
Means and standard deviations presented in thesis table 3.

Table 3. Chemical composition of separable fat and lean from Los Alamos lambs

Lamb No.	Separable lean Water	Ether-extract	Protein	Separable fat Water	Ether-extract	Protein
1	73.5	6.7	19.1	25.1	69.3	5.19
2	70.0	10.0	18.8	18.2	77.4	4.38
3	72.0	8.4	18.6	29.4	63.0	7.28
4	72.4	8.0	18.6	19.5	76.1	5.00
5	72.1	8.8	18.2	25.8	68.5	6.00
6	72.7	7.5	19.0	23.7	70.1	5.94
7	74.2	5.7	18.9	28.5	64.9	6.00
8	73.2	6.4	19.1	28.6	63.5	7.81
9	76.5	4.4	18.3	47.6	41.2	11.22
10	72.6	6.3	20.3	28.6	61.5	9.69

Composition values in %.
Means and standard deviations for edible carcass (separable fat + lean) presented in thesis table 3.

Table 4. Potassium content of Los Alamos lambs from potassium-40 counts

Lamb No.	Live washed	Carcass	Non-carcass	Separable carcass components: Lean	Fat	Bone	Live unwashed
1	0.177	0.262	0.233	0.297	0.056	0.135	0.250
2	0.174	0.194	0.151	0.287	0.014	0.123	0.229
3	0.179	0.171	0.094	0.267	0.042	0.071	0.270
4	0.151	0.230	0.175	0.308	0.074	0.199	0.267
5	0.147	0.220	0.171	0.271	0.013	0.094	0.237
6	0.162	0.181	0.166	0.314	0.096	0.147	0.268
7	0.183	0.249	0.214	0.284	0.089	0.194	0.293
8	0.194	0.256	0.171	0.319	0.112	0.155	0.261
9	0.196	0.235	0.188	0.316	0.154	0.206	0.316
10	0.209	0.250	0.216	0.318	0.052	0.087	0.314

Potassium content in %.
Means and standard deviations presented in thesis table 4.

Table 5. Sodium and potassium content of separable lean and fat from Los Alamos lambs as measured by flame photometry

Lamb. No.	Potassium: Separable lean, Run 1	Potassium: Separable lean, Run 2	Potassium: Separable fat	Sodium: Separable lean	Sodium: Separable fat
1	0.298	0.286	0.064	0.081	0.066
2	0.306	0.319	0.056	0.070	0.051
3	0.305	0.313	0.079	0.073	0.068
4	0.299	0.311	0.064	0.069	0.051
5	0.303	0.297	0.079	0.082	0.067
6	0.317	0.320	0.070	0.073	0.062
7	0.315	0.318	0.074	0.070	0.076
8	0.340	0.340	0.086	0.070	0.071
9	0.311	0.316	0.126	0.079	0.111
10	0.331	0.337	0.119	0.078	0.079

Sodium and potassium content in %. Values are the mean of duplicate analyses. Means and standard deviations for potassium have been presented in thesis table 4 and for sodium in thesis table 7.

Table 6. Cesium-137 content of Los Alamos lambs from gamma counts

Lamb No.	Live unwashed	Live washed	Carcass	Non-carcass	Separable carcass components: Lean	Fat	Bone
1	50.5	78.1	73.2	60.0	96.6	-51.5	124.9
2	62.4	86.8	84.4	49.2	107.7	727.9	400.6
3	67.7	83.1	122.4	145.6	80.7	909.4	401.5
4	68.2	89.8	81.4	175.2	104.9	176.3	234.5
5	74.5	121.0	75.2	-24.4	68.3	1.6	-69.9
6	61.2	99.6	92.6	189.2	72.1	39.3	75.2
7	47.7	64.9	50.9	55.3	59.1	62.5	153.3
8	48.8	70.6	96.9	101.7	64.0	-71.9	-74.6
9	57.2	93.5	95.1	69.0	80.9	74.4	76.4
10	41.8	60.2	43.0	25.9	60.3	510.6	14.1

Values presented are $\mu\mu c$ cesium/gm. potassium (potassium estimated from potassium-40).
Results on separable fat and bone are very unreliable because of the small number of counts.
Means and standard deviations are presented in thesis table 9.

Table 7. Composition of 38 lb. ground pork samples

Samples	Water	Ether-extract	Protein	Potassium: K-40	Potassium: Flame photometry	Sodium flame photometry
8 + 14	37.7	50.7	10.8	0.153	0.175	0.035
15 + 26	43.8	42.9	12.3	0.191	0.209	0.041
13 + 16	45.3	40.8	13.1	0.209	0.211	0.039
21 + 17	48.6	36.8	13.9	0.244	0.233	0.042
24 + 10	49.9	35.3	13.9	0.241	0.234	0.041
22 + 12	52.7	31.8	14.6	0.252	0.253	0.042
23 + 27	54.2	30.3	14.8	0.292	0.267	0.045
11 + 25	56.0	27.0	16.0	0.274	0.268	0.044
9 + 20	59.7	22.2	17.3	0.299	0.290	0.046
18 + 19	63.1	17.5	18.3	0.312	0.307	0.046
8 + 15	38.5	49.4	10.9	0.167	0.178	0.036
14 + 26	43.0	44.2	12.2	0.181	0.206	0.040
13 + 21	46.6	39.4	13.3	0.204	0.224	0.042
18 + 9	60.1	21.4	17.6	0.323	0.291	0.045
19 + 20	62.7	18.3	18.0	0.308	0.306	0.047
24 + 23	51.4	33.8	14.1	0.247	0.248	0.043
10 + 22	52.3	32.1	14.6	0.256	0.248	0.041
16 + 17	47.4	38.2	13.7	0.213	0.220	0.039
12 + 25	53.7	30.1	15.1	0.261	0.260	0.043
27 + 11	55.2	28.3	15.6	0.262	0.265	0.045

Composition figures in %.
Sample values the mean of two 19 lb. cartons of ground pork.
Means and standard deviations are presented in thesis tables 11 and 12.

Table 8. Composition of 38 lb. ground lamb samples

Samples	Water	Ether-extract	Protein	Potassium K-40	Potassium Flame photometry	Sodium flame photometry
1 + 2	36.6	52.5	11.5	0.171	0.163	0.049
3 + 31	49.0	35.5	14.6	0.217	0.218	0.059
29 + 34	54.1	30.1	15.5	0.277	0.249	0.064
32 + 35	63.1	18.4	17.6	0.323	0.287	0.066
4 + 28	58.8	23.2	16.7	0.310	0.277	0.064
6 + 36	57.3	25.2	16.8	0.271	0.262	0.064
7 + 33	55.5	26.8	16.1	0.259	0.247	0.064
5 + 30	55.3	27.5	16.1	0.270	0.257	0.063
28 + 37	58.9	23.1	16.8	0.318	0.270	0.067
1 + 3	40.4	46.7	12.4	0.180	0.182	0.051
2 + 31	45.2	41.3	13.7	0.209	0.198	0.057
32 + 37	62.2	18.6	18.0	0.270	0.283	0.070
29 + 30	54.2	29.5	15.3	0.263	0.254	0.061
33 + 34	54.7	28.1	16.0	0.236	0.242	0.066
35 + 36	59.6	22.9	16.6	0.272	0.272	0.066

Composition figures in %.
Sample values the mean from two 19 lb. cartons of ground lamb.
Means and standard deviations are presented in thesis tables 11 and 12.

Table 9. Potassium content of 24 pigs

Pig No.	Frozen carcass	Empty G.I. tract	Remaining viscera	Head	Blood[1]	Hair[2]	Empty body
1	0.2285	0.2024	0.2887	0.1668		0.0669	0.2063
2	0.2214	0.2275	0.2591	0.1538		0.0978	0.2121
3	0.2276	0.1923	0.2527	0.1577		0.0978	0.2166
4	0.2135	0.1918	0.2704	0.1529		0.0978	0.2074
5	0.1752	0.1904	0.2750	0.1433		0.0978	0.1746
6	0.2455	0.1988	0.2510	0.1560		0.0978	0.2316
7	0.2008	0.1796	0.2515	0.1440	0.2247	0.0978	0.1936
8	0.2375	0.1617	0.2513	0.1583	0.2168	0.0978	0.2211
9	0.2297	0.1597	0.2343	0.1436	0.2216	0.0978	0.2145
10	0.1872	0.2006	0.2512	0.1253	0.2061	0.0978	0.1830
11	0.2060	0.1872	0.2595	0.1503	0.2386	0.0978	0.1974
12	0.2164	0.1866	0.2577	0.1369	0.2238	0.0978	0.2063
13	0.2163	0.1698	0.2511	0.1531		0.0978	0.2067
14	0.2112	0.1856	0.2517	0.1569		0.0978	0.2043
15	0.2045	0.1821	0.2515	0.1434	0.2192	0.0978	0.1967
16	0.2205	0.1996	0.2556	0.1364	0.2117	0.0978	0.2110
17	0.1737	0.1984	0.2674	0.1442	0.2270	0.0978	0.1734
18	0.2193	0.1907	0.2526	0.1497	0.2308	0.0978	0.2106
19	0.1901	0.1684	0.2453	0.1351		0.0978	0.1845
20	0.2103	0.2062	0.2539	0.1380		0.0978	0.2027
21	0.2186	0.1966	0.2680	0.1481		0.0978	0.2097
22	0.1980	0.1674	0.2505	0.1458		0.0978	0.1908
23	0.1947	0.1689	0.2555	0.1426	0.2218	0.0978	0.1893
24	0.1891	0.1839	0.2644	0.1467		0.0978	0.1868

All figures presented are % potassium. Mean of duplicate analyses.

[1]The blood from the remaining samples had been completely used in previous analyses. Samples were collected from three additional mature pigs at slaughter and found to contain 0.2155, 0.2092 and 0.2111% potassium. The values for the 14 pigs were averaged and the mean value was used in computing the body potassium content.

[2]The value for pig 1 differs from the remaining value because the skin and some subcutaneous fat is included. For the remaining animals a composite sample was analysed.

The means and standard deviations are presented in thesis table 18.

The weights and chemical composition of the 24 pig bodies and their compartments have been presented in the appendix of Gnaedinger (1962).

Table 10. Sodium content of 24 pigs

Pig No.	Frozen carcass	Empty G.I. tract	Remaining viscera	Head	Blood[1]	Hair[2]	Empty body
1	0.0648	0.1180	0.1115	0.1852		0.0995	0.0830
2	0.0727	0.1346	0.1112	0.1673		0.0918	0.0844
3	0.0728	0.1439	0.1150	0.1573		0.0918	0.0865
4	0.0637	0.1081	0.1053	0.1521		0.0918	0.0756
5	0.0611	0.0973	0.1166	0.1391		0.0918	0.0730
6	0.0734	0.1245	0.1078	0.1647		0.0918	0.0868
7	0.0707	0.0997	0.1076	0.1435		0.0918	0.0801
8	0.0807	0.0886	0.1133	0.1569		0.0918	0.0896
9	0.0657	0.0993	0.1091	0.1513		0.0918	0.0770
10	0.0709	0.1097	0.1125	0.1537		0.0918	0.0832
11	0.0746	0.1076	0.1141	0.1611		0.0918	0.0849
12	0.0746	0.1039	0.1118	0.1157		0.0918	0.0830
13	0.0751	0.1139	0.1168	0.1609		0.0918	0.0851
14	0.0715	0.1252	0.1202	0.1603		0.0918	0.0818
15	0.0672	0.1021	0.1148	0.1481		0.0918	0.0793
16	0.0762	0.1201	0.1205	0.1194		0.0918	0.0863
17	0.0690	0.1107	0.1064	0.1540		0.0918	0.0789
18	0.0675	0.1103	0.1152	0.1564		0.0918	0.0789
19	0.0753	0.1127	0.1099	0.1297		0.0918	0.0835
20	0.0798	0.1162	0.1180	0.1346		0.0918	0.0889
21	0.0774	0.1140	0.1152	0.1716		0.0918	0.0876
22	0.0729	0.1047	0.1216	0.1666		0.0918	0.0830
23	0.0694	0.1033	0.1103	0.1612		0.0918	0.0793
24	0.0699	0.1076	0.1118	0.1566		0.0918	0.0804

All figures presented are % sodium. Mean of duplicate analyses.

[1]As the blood samples had been prevented from coagulating with sodium citrate, they were unsuitable for sodium analyses. Samples were collected from three additional mature pigs at slaughter and found to contain 0.1826, 0.1760 and 0.1889% sodium. The mean value was used in computing body sodium.

[2]See footnote 2 in appendix table 9.

The means and standard deviations are presented in thesis table 19.

The weights and chemical composition of the 24 pig bodies and their compartments have been presented in the appendix of Gnaedinger (1962).