PART B - Vol. II

AN EVALUATION OF NUTRITIONAL MARKERS

NAS9-10955

T. D. Luckey
University of Missouri - Columbia

CR-115125

VOLUME II

AN EVALUATION OF NUTRITIONAL MARKERS

BY

A. R. KOTB AND T. D. LUCKEY

PREPARED FOR

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER
Houston, Texas 77058

NASA CONTRACT NAS9-10955

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF MISSOURI SCHOOL OF MEDICINE
Columbia, Missouri 65201

TABLE OF CONTENTS

TABLES

MARKERS IN NUTRITION

INTRODUCTION

Marker, indicator, tracer, reference substance, or index substance are terms applied by workers in nutrition and physiology to a number of materials used in the qualitative or quantitative determination (usually indirect) of physiologic or nutritional phenomena. The diversified use of markers has accumulated much information which merits a comprehensive review of the subject. A variety of materials and procedure have been used repeatedly but the authors have found no complete review of the topic. The scope of this review is limited to dietary markers, markers which would be put into the diet, which would occur in the food eaten by the animals or which would be taken orally at any time. Excluded from consideration are injected markers and most metabolites which originate within the animals. The review also excludes dyes and radioactive materials used to trace animal movements in the wildlife and a great number of substances, radioactive and non-radioactive, used only as qualitative or quantitative indicators in different medical diagnostic techniques, for example, the study of the function of some organs and the localization of certain tumors in the body. Also excluded are materials which have been used as indicators to indirectly determine body composition *in vivo*, e.g., tritium and deuterium have been used in the "dilution technique" to determine total body water and the determination of the body ^{40}K content has been used to estimate "lean body mass".

This review presents an historical perspective of the use of markers in nutrition and some characteristics of individual markers with an evaluation of their usefulness in studies of food intake, food passage and food absorption in the gastro-intestinal tract of man and animals. The techniques of feces sampling when the marker method is used, are also discussed. The extensive application of a number of indicators to study the rate of passage of food residues in the digestive tract makes this a major consideration herein. Consumption and digestibility of forage by grazing animals has been an area of interest and challenge to nutritionists; the indicator technique has contributed greatly to this area. Food utilization studies in space suggest interesting problems which could utilize new application of the indicator method.

An early use of markers was the study of the rate of passage of food residues through the digestive tract of animals and man. Glass beads and small seeds were typical early markers. The use of these primitive markers has practically ceased; today, the use of radioactive isotopes and radioactivation analysis provide added convenience and precision to marker techniques. A variety of food utilization studies have taken advantage of the indicator technique. Studies of digestion, absorption, and retention of different food nutrients were found to be more convenient, less costly, and sometimes more precise with the use of suitable indicators than the conventional method.

CLASSIFICATION OF MARKERS

The markers used in food utilization or rate of food passage may be grouped various ways; completeness and clarity suggest the classification given in Table I. A second grouping is useful from a physiologic viewpoint. Theoretically, any substance which can be classified in any of the following groups could be used as a marker in nutrition.

A. Absorbable

1. Completely absorbed from the alimentary tract and recovered in the urine. Substances that fit in this group may be referred to as "urinary indicators". Sweat and gaseous exchange are possible excretory paths for these compounds. A variable portion may be stored and some may be lost from skin functions.

2. Partly absorbed from the alimentary tract with a fixed percentage recovered unchanged in the urine.

B. Non-absorbable fecal markers

Substances in this category are absorbed minimally from the alimentary tract and may be virtually completely recovered unchanged in the feces. These substances are referred to as "fecal markers". The residue of compounds partially absorbed (A-2 above) could be useful as fecal markers.

Most of the work with markers has involved the use of sub-

stances belonging to category B due to the availability of many substances that are known to fit in this group. Very limited examples are known of materials in category A that for practical reasons and under a variety of conditions may be accepted for quantitative use. Materials within category B are discussed herein according to the following categorization:

1. External Markers
    a. Particulates
    b. Dyes
    c. Metal oxides
    d. Mineral salts
    e. Soluble markers
    f. Radioactive markers
    g. Inert metals
    h. Microorganisms
    i. Others
2. Internal Markers
    a. Silica
    b. Lignin
    c. Chromogen
    d. Fecal nitrogen
    e. The acid soluble fecal fraction
    f. Methoxyl and fiber

DEFINITION OF TERMS AND CRITERIA

A variety of terms have been used in this field. A group of these are presented with definition and/or example to illustrate

their meaning in this review.

Extent of passage: The point which undigested residue of the food comes to or has passed through the alimentary tract may be determined by the use of markers without otherwise disturbing the subject. Under certain conditions of digestive system malfunction or disorder, the food residue may be blocked at certain points along the digestive tract. The term extent of passage is used to describe how far a given ingested material traveled or was allowed to pass.

Passage of ingesta: Many terms have been used to describe the passage of ingested material through the alimentary tract. Most important of these are "transit time", "retention time", "rate of passage", "rate of flow", and "rate of transport". These terms have been frequently used interchangeably; this has caused much confusion. To eliminate this confusion, we propose the following definitions to be compatible with the derivation of terms used to indicate passage.

1. Transit time - This is the time it takes the digesta of a given meal to pass through the alimentary tract or certain segments of it. This time also represents the retention time of this digesta in the tract or the particular segment. A simple way of calculating this time is by recording the time of first or last appearance of the marked residue of a meal. Other more useful methods to calculate retention time include "mean retention time" (Castle, 1956a), "mean time" (Blaxter, Graham

and Wainman, 1956), and "turnover time" (Hungate, 1966). See pages 78-84.

2. Rate of passage - This refers to the quantity of digesta (as grams or % of quantity) that passes a certain point along the alimentary tract in a given amount of time.
3. Rate of flow - This term denotes the quantity of digesta (as grams or % of quantity) that travels a certain distance in a given amount of time (e.g., g/m/hr).
4. Rate of transport - This term denotes the distance (as centimeters or % of length) traveled by the digesta of a given meal through the alimentary tract (or certain segments of it) in a given amount of time.

Digesta: Food and ingested material which is subjected to digestion within the digestive tract. Technically, it would include secretions, and excretions (mucosal cells) from different digestive organs.

External indicator: An indicator or a marker which is added to the diet or taken orally, e.g., chromic oxide.

Internal indicator: An indicator or a marker which occurs naturally in the diet, e.g., lignin.

Grab sampling: This is a technique for the sampling of feces where a sample is taken manually from the rectum or from a recent fecal pat. This method is mostly used with large animals.

Sward sampling: This is another technique for feces sampling specifically used with grazing animals. In this method fecal samples are

collected from the sward of grazed areas.

Ratio technique: This method is used for determination of digestibility when markers are used. The marker/nutrient ratio is determined in the food and feces.

Fecal index method: This is another method for determination of digestibility especially used with grazing animals. A regression equation relating digestibility to the concentration of a fecal component (e.g., internal marker) is established from a conventional digestion trial. Subsequently, this equation is used to calculate digestibility under similar conditions where only concentration of this particular fecal component is determined.

A nutritional marker may be administered in one of the following ways depending on the nature of both the marker and the study:

a. it may be taken with the food after mixing with part or all of the diet, or drink,
b. it may be taken in a pill or capsule when its color or taste is undesirable or when it is unchewable,
c. it may be prepared as a powder or solution to be taken orally or via suppository or fistula,
d. internal markers occur naturally in the diet and are therefore ingested as a part of the diet.

The criteria of nutritional markers for effective study were presented by Alvarez (1948). These requirements have been modified and augmented herein. For a given material to qualify as a marker in nutritional studies, it should:

a. be inert with no toxic, physiologic or psychologic effects,

b. be neither absorbable nor metabolized (within the alimentary tract),

c. have no appreciable bulk,

d. mix intimately with the usual food and remain uniformly distributed in the digesta,

e. have no influence on alimentary secretion, digestion, absorption, normal motility of the digestive tract, or excretion,

f. have no discernible influence upon the microflora of the alimentary tract,

g. have qualities that allow ready, precise quantitative measurements and,

h. have physical-chemical properties which make it discernible throughout the digestive process.

These criteria are applicable for the quantity used with due understanding that there can be negligible actions of small amounts of a compound which would be unacceptable in larger quantities.

Non-digestibility, complete recovery and ease of determination have been the characteristics of major concern in the search for ideal nutritional markers. Less systematic knowledge has accumulated on other criteria. However, it will be noted that large quantities of some markers (e.g., $BaSO_4$ or charcoal) do affect intestinal motility (Alvarez, 1948), some microbial cells may affect the quantity of nutrients (e.g., live yeast cells can absorb thiamine while killed yeast provide much nutrients before the "empty cell" is excreted,

Garber, Marquett, and Parsons, 1949; Ness, Price, and Parsons, 1950), some markers may bind or absorb nutrients (e.g., cholesterol level of serum and liver is lowered when lignin is added to the diet, de Somer and Eyssen, 1971) and some markers may affect the alimentary flora (e.g., in vitro studies show that 1-10 µg/ml of rare earth minerals give LD_{50} for E. coli, Maier, 1968). Thus, all criteria should be examined before complete acceptance can be given for any nutritional marker.

PROPERTIES AND EVALUATION OF MARKERS

EXTERNAL MARKERS

Particulates

Earlier markers included glass beads, small seeds and charcoal. In 1904 Elliott and Barclay-Smith used colored glass beads in their study of the distribution of food along the digestive tract of rabbit. Hoelzel reported (1930) that in 1915 he ingested about 300 g of glass beads in place of a meal and as a consequence a serious condition of constipation developed suggesting that glass beads pass through the digestive tract much slower than normal food residues. This impression was put to a further experimental test immediately after the appearance of a publication by Alvarez and Freedlander in 1924 on the use of colored beads as a test material to determine the rate of progress of food residues in the digestive tract of man. Glass beads (about 2mm in diameter) and a group of other materials were tested by Hoelzel on himself. He concluded (1924) that glass beads pass through the digestive tract slower than food and material which is more like ordinary

food residues (e.g., cotton thread knots and small seeds) and that the rate of passage of the various substances was roughly inversely proportional to their specific gravity, i.e., heavier materials pass more slowly than do lighter materials. However, in view of the stand taken by Alvarez (1928) in which he reiterated the findings obtained with glass beads as evidence of the normal intestinal rate, Hoelzel decided to examine the problem more extensively. In 1930 he summarized his experience with glass beads and a group of other inert materials including rubber, cellulose (cotton knots), small seeds (tomato, millet, grape, sunflower, and psyllium), small pieces of metal (aluminum, steel, silver, and gold), gravel, barium sulfate, ferric oxide, and carmine as indicators of the rate of passage through the digestive tract of rabbits, guinea pigs, dogs, cats, rats, mice, monkey, pigeons, hens as well as himself. After sacrificing the animals and segmenting their digestive tract, using fluoroscopic observations and x-ray, he was able to determine the location of the test material. Stereoroentogenograms were made to determine the location in man. He reaffirmed his earlier conclusions on glass beads and the relationship between the specific gravity and the rate of passage and was able to locate the main sites of stasis of the heavier materials in different species.

Alvarez (1948) found that when glass beads of different colors were given to each of a group of patients on three successive days, many of the later ones caught up with or passed the first ones. In spite of these critical disadvantages, some workers continue to use

glass beads as a marker. Recently, Baily (1968) used colored glass beads in his study of the rate of food passage in rabbits and assumed that glass beads pass through the digestive tract of the rabbit at a rate approximating the passage of natural foods.

In 1921 Burnett gave his subjects a different substance after each of the three daily meals; rape seed was ingested at 8 a.m., charcoal at 2 p.m., and millet seed at 8 p.m. He concluded that with slow intestinal rate the three substances were so commingled in the feces that it was difficult to recognize any one of them before the others and that charcoal may sometimes cause an unnatural retardation of the rate of passage. He preferred millet seed as the test material and used it in subsequent studies (1921, 1923). He pointed out that this seed is not unlike that of tomatoes and berries, foods that are frequently ingested and they are readily recognized as white dots on the dark brown field of the feces.

Hoelzel (1930) reported that when millet seeds and thread knots were administered simultaneously, the initial rates (taken from the first appearance) for the seeds and knots were alike but the final rate for the seeds was about 24 hours longer than the final rate for the knots. However, tests made with charcoal and knots by Hoelzel (1930) showed neither initial nor gross final retardation of the charcoal. So, when 50 cc of French millet seed, 100 knots, and 1.5 gm of charcoal were ingested together by man, the knots and charcoal passed in equal time

while the final passage of the seeds was delayed as in the other tests. Van Liere, Stickney, and Northup (1945) gave 50 cc of a mixture of 10% charcoal suspension in 10% gum acacia in water by stomach tube to dogs. They assumed that these two materials were physiologically inert but they conceded that their test meal did not satisfy the criteria suggested by Alvarez (1948) for a marker. Charcoal was also used by Guernsey and Evvard (1914) in digestion studies with swine.

Other materials which may belong to this group of markers include rubber and plastics. Ewing and Smith (1917) stated that bone black, carmine, finely ground charcoal, or bismuth compounds were unsuitable for ruminants and used rubber discs in their studies of food passage and digestion with cattle. King and Moore (1957) used plastic particles with cattle and humans. Campling and Freer (1962) used rubber and plastic particles with cattle and showed the importance of particle size and specific gravity in determining retention time of these particles in the alimentary tract. A comparison between polystyrene particles and stained food suggested that the former was unlikely to provide an alternative method for estimating retention time of food in the digestive tract of the cow (Campling and Freer, 1962). On the other hand, work by Chandler, Kesler and McCarthy (1964) indicated that polyethylene was an acceptable marker for digestibility studies. When this material (particle size, 50 mesh; density, 0.916) was fed to calves at the level of 5% of the diet (dry matter basis) recovery was 100.3 $\pm$ 9.3% and dry matter digestibility determined by

the marker method agreed with that determined by the conventional method of total collection. From the study of the distribution of digesta, dry matter, and polyethylene along the alimentary tract, these authors suggested that this material may also be used to study the changes in dry matter occurring within the tract or the changes in nutrients associated with the dry matter in movement and distribution.

Newborn animals are known to absorb large molecules and particulates for several hours following birth. Payne's (1964) review of particulate absorption from the alimentary canal indicates a minute quantity of bacteria and most radioactive particulates are taken up in the tonsils of adult animals and man. Large quantities of radioactivity were found in alimentary tract lymph nodes when the particle size was less than 5 μ in diameter. When the particle size was 20-30 μ in diameter, absorption was negligible with resins impregnated with 198 Au.

Dyes

Carmine, a red or purplish-red pigment obtained mainly from cochineal, is one of the earliest materials used to determine rate of food passage. Alvarez and Freedlander (1924) pointed out that five grains (0.3 g) of carmine given to puppies came through so completely in 24 hours that none can be seen macroscopically after that. When 10 grains were given, the puppies developed diarrhea. Based on these observations these workers questioned the suitability of carmine for measuring the normal rate of progress of the food residues. Mulinos (1935) gave each of 140 men and 10 women a capsule

containing 10 grains of carmine and found that a third of his patients passed most of it with the first stool from 6 to 48 hours later. Half of the patients passed most of the dye with the second stool, from 9 to 98 hours after ingestion. Alvarez (1948) commented that these results made him sure that the carmine method was very crude. Under certain conditions, carmine may have some undesirable side effects. Hoelzel (1930) reported that he discontinued using carmine after finding that it caused nausea when taken during a fast.

Lutwak and Burton (1964) attempted to devise a marker which would permit sharp differentiation between short metabolic periods and would move in the intestine at a rate similar to that of the fecal bolus. They found that the administration of brilliant blue (disodium salt of 4-{[4-N-ethyl-p-sulfobenzylamino) phenyl] (2-sulfoniumphenyl)-methylene}[1-(N-ethyl-N-p-sulfobenzyl)-$\Delta^{2,5}$-cyclohexadienimine], consistently resulted in the passage of brightly colored stools ranging in hue from grass green to bright blue, depending on the amount of bile pigments present. However, the propensity of this dye to spread out in the stool for several days passage was similar to that of carmine. They found that when brilliant blue or carmine was suspended in an aqueous gel of methylcellulose, a stable homogeneous preparation resulted which did not stain mucous membranes even when held in the mouth for periods up to 5 minutes. After drying, the mixture was found to consist of dye adsorbed onto the methylcellulose. The dry mixture was the form in which the preparations were used experimentally. When the two dye preparations were compared, some differences were observed in the time required for the complete passage and were attributed to the fact that brilliant blue is more readily visible in the stool than carmine and thus, could be observed long after traces of carmine become obscured by the inherent color of the stool.

Kindel (1960) tested a total of 23 dyes and dye-materials for marking the feces of sheep. Four dyes were found to impart a good stain to the feces; methylene blue produced a blue-green stain,

crystal violet produced a violet stain, basic fuchsin produced a red stain, and aniline blue produced a deep blue stain. The dyes in powder form were mixed with dry ground feed and salt, and were fed to sheep. Methylene blue was fed in 1-, 2-, and 3-gram doses which produced similar staining results. All other dyes were fed in single doses of approximately 10 grams. The effective dyes appeared in the feces of sheep approximately 24 hours after ingestion and continued to appear for 2 to 4 days afterwards.

The foregoing discussion included a group of dyes and staining materials which can only be determined qualitatively by observing the first and last appearance of the marker in the feces or at any point along the digestive tract. This, however, allows some quantitative measurements to be made such as the time it takes the marker to travel a certain distance or to reach a certain point.

Sometimes, the quantitative determination of the dye itself is desirable. Corbin and Forbes (1951) have used anthraquinone violet as a reference material in their digestibility studies with sheep. They found that this dye was readily soluble in benzene and easily extracted and its characteristic light absorption spectrum allowed it to be readily measured spectrophotometrically at 578 mμ. When 0.5 g of anthraquinone violet was given in gelatine capsules to lambs twice daily before feeding, the average recovery of the dye in the feces of 9 lambs was 100.5% (96.4-106.0%). On the other hand, Flatt, Horvath, DeCosta, Stewart, and Warner (1957) reported that anthraquinone violet was absorbed from the rumen and the rate of absorption was too variable for the dye to be of value as an

indicator. These authors reported a recovery of less than 70% for this dye in preliminary studies with mink and rabbit. In studies with pigs, they showed that feeding anthraquinone violet at the level of 0.025% of the ration resulted in coloring the fat of the carcass; the color persisted for 6 - 7 months after the dye had been withheld. Digestibility was consistently lower when determined with anthraquinone violet than with chromic oxide; a greater difference was noted when the diet contained added fat. Further work by Ellis (1971, personal communication) indicated that anthraquinone violet was unsuitable for determination of rumen volume in tied off rumen preparations. The stratum corneum was found stained and the dye could not be removed by extraction with benzene suggesting adsorption to the mucosa. Absorption was also indicated from the stained vascular system within the submucosa.

Phenol red has been used for measurement of absorption and transit in the digestive tract of the rat (Reynell and Spray, 1956) and for determination of rumen fluid volume (Hecker, Budtz-Olsen, and Ostwald, 1964). Reynell and Spray (1956) suggested binding in the stomach as the reason for incomplete recovery of this dye while Hecker et al. (1964) suggested that phenol red was not broken down in rumen fluid on the basis of complete recovery with in vitro techniques.

Monastral blue (copper phthalocyanin) is another dye suggested for digestibility studies by Coup and Lancaster (1952). Its quantitative determination requires analysis for copper since it con-

tains about 11% of this element. This is a distinct disadvantage because copper is a natural food nutrient and a constituent of the body.

The use of heavy inert indicators has been criticized by some workers because of errors that may be introduced due to differential rates of passage of food residue and indicators (Balch, 1950; Johnson, Dinusson, and Bolin, 1964; Drennan, Holmes, and Garrett, 1970). In the stained particle technique, feed particles serve as the marker after being stained with the appropriate dye.

Lenkeit and Habeck (1930) described a method to study food passage through the digestive tract of ruminants in which the marker was either hay or straw colored with magenta or acid fuchsin; the stained particles in the feces were collected and counted. Other early reports on this area include that of Habeck (1930), Lenkeit (1932), Usuelli (1933), and Columbus (1936). Balch (1950) has adopted the stained-particle technique, which he made semi-quantitative, to measure the rate of passage through the gastrointestinal tract of ruminants. A small proportion of the meal (about 5%) was stained with a suitable dye and mixed thoroughly with the rest of the diet. Samples of digesta or feces are sieved to make possible visual identification of the stained particles which can be counted and weighed. The recovery of stained residues can be expressed in several ways as presented by Castle (1956) and reviewed by Balch and Campling (1965).

Ellis and Huston (1967; 1968), however, described the stained particle technique as laborious and time consuming and raised serious questions concerning the significance which can be attached to measurements obtained by this technique. Three different size particles of ground alfalfa hay were stained different colors and fed together in a single meal to sheep. It was concluded that mean retention times based on counting stained fecal particles collected on a 60-mesh sieve as compared to a 40-mesh sieve were significantly less for the smaller size feed particles and not significantly different for the two larger size feed particles (Table 2). Observations regarding the faster passage of the fine particles in comparison with the coarse ones were also shown by Balch and Campling (1965) and were confirmed by means of uniformly ^{14}C-labeled neutral detergent fiber (cell walls) with different particle size (Smith, Waldo, Moore, Leffel, and Van Soest, 1967) and by studies of radioactive cerium (Ellis and Huston, 1968). These results are important since many investigators used for collecting stained fecal particles a cotton-gauze filter or similar materials of relatively coarse and undefined porosity (Balch, 1950; Castle, 1956) and others used a 40-mesh sieve (Eng, Riewe, Craig, and Smith, 1964). Ellis and Huston (1967) stated that on the basis of their results they expect that appreciably different retention time would be obtained from the use of a finer-mesh sieve as contrasted to coarser-mesh gauze. Balch and Campling (1965) demonstrated that the retention time of stained ruminant feeds is influenced by many

factors including size and specific gravity of the particles, but found polystyrene unsatisfactory and finely ground chromic oxide unsuitable as markers for determination of retention time in the digestive system of ruminants.

A modification of the stained particle technique has been introduced by Asplund and Harris (1970). In this procedure the laborious counting of the stained particles is replaced by quantitative recovery of the dye. The major difficulty encountered in this work was the masking of dye color by chromogens in feed and feces. The dye used in this study was Sudan III which is extractable with acetone. A better procedure for determination of this dye or a better dye will make this a desirable technique.

Metal Oxides

The main compounds in this group that have been considered as fecal markers are chromic oxide, ferric oxide, and titanium oxide. Their use as fecal markers has been mostly in studies of food utilization.

Chromic oxide

A group of chromium-containing compounds has been shown to possess characteristics of inert indicators. Among these are chromium sesquioxide (chromic oxide, Cr_2O_3), chromium chloride ($^{51}CrCl_3$), sodium chromate ($Na_2{}^{51}CrO_4$), and ^{51}Cr-labeled erythrocytes or hemoglobin. Of all these, only chromic oxide has been widely used in both radioactive and non-radioactive forms in studies of food utilization.

Adult humans have chromium always in the lung but not always

in other tissues. Wild and domestic animals have more chromium in tissues than man. Foods contain little chromium, generally less than 0.1 µg/g fresh weight except for thyme 10 µg, and black pepper 3.7 µg (Schroeder, Balassa, and Tipton, 1962).

Chromic oxide (chromium sesquioxide, Cr_2O_3) with a molecular weight of 152.02 is light to dark green in color and practically insoluble in water, alcohol, acetone, but slightly soluble in acids and alkalis (Merck Index, 1968). The chemical determination of Cr_2O_3 in the feces (Whitby and Lang, 1960; Daly and Anstall, 1964; Cheong and Salt, 1968; Mink, Schefmman-Van Neer, and Habets, 1969) is somewhat tedious and there are some doubts about the adequacy of the chemical analytical methods. The use of radioactive ^{51}Cr, however, simplified the determination procedure considerably (Sassoon, 1966).

Since commercial chromic oxide contains small amounts of dichromate, purification has been recommended before use (Whitby and Lang, 1960; Sassoon, 1966).

Whitby and Lang (1960) reported that some rare examples of acute appendicitis have been directly attributed to the use of barium contrast materials in x-ray examinations and since Cr_2O_3 is more dense than barium sulfate, side effects due to the use of Cr_2O_3 have been of interest to some investigators. They pointed out that there were no side effects directly attributable to the administration of Cr_2O_3. Schroeder, Vinton, and Balassa (1963a,b) found that the addition of Cr to the diets of mice and rats at the level of 5 ppm resulted in increased weight gain.

Survival was not affected by the added metal in all groups and mortality of young male mice was reduced. In a later experiment, however, Schroeder, Balassa, and Vinton (1964) found that giving Cr to mice in drinking water at the level of 5 ppm from weaning till death resulted in increased mortality and decreased longevity in males but not in females.

The use of chromic oxide as a fecal marker was first proposed by Edin (1918). Workers have found it unabsorbable with essentially complete recovery in the feces which was shown by conventional methods of chemical analysis. Kane, Jacobson, and Moore (1950), using dairy cows, showed that it was possible to recover 99.9% (96.7-102.1%) of the chromic oxide in the feces of ruminants. Studies on humans, however, showed a recovery of only 93% (80-103%); Rose (1964) attributed the lower recoveries to either the short duration of the study in some cases or to fecal losses which were not recognized in other cases. More recently, however, Sharpe and Robinson (1970) have shown that mean recovery of ingested chromic oxide by young women was 101% (100-103%) which is in line with results on ruminants by Kane et al. (1950). Complete recovery was also confirmed by means of radioactive isotopes (Visek, Whitney, Kuhn, and Comar, 1953; Kane, Jacobson, and Damewood, 1959; MacKenzie, Anwar, Byerrum, and Hoppert, 1959; Donaldson and Barreras, 1966; Pearson, 1966; Sassoon, 1966; and Utley, Boling, Bradley, and Tucker, 1970; see discussion on ^{51}Cr).

Digestibility data obtained by the chromic oxide ratio technique were compared to those obtained by the conventional method. Hamilton,

Mitchell, Kick, and Carman (1927-1928) found in experiments with sheep good agreement between the conventional method of total collection and the chromic oxide method when the duration of the collection period was 3 days or longer. Satisfactory results were also obtained with sheep, calves and pigs by Barnicoat (1945) using the chromic oxide method. There were no significant differences between the conventional and the chromic oxide method for determination of apparent digestibility in dairy cattle as reported by Kane, Jacobson, and Moore (1949, 1950, 1952); Kane, Ely, Jacobson, and Moore (1953); Kane, Jacobson, Ely, and Moore (1953); Kane, Jacobson, and Damewood (1957, 1959); and Davis, Byers, and Luber (1958), in sheep as reported by Axelsson and Kivimäe (1951) and Elam, Reynolds, Davis, and Everson (1962), and in beef heifers as reported by Utley et al. (1970). Successful use of this indicator has also been reported in horses (Olsson, Kihlen, and Gagell, 1949), rats (Schürch, Lloyd, and Crampton, 1950), and poultry (Mueller, 1956).

Reports on the use of chromic oxide as an indicator in absorption and metabolic balance studies in human are numerous. Edin, Kihlen, and Nordfeldt (1944) stated that they had successfully applied the chromic oxide method to humans. In a study on the absorption of carotene in man, Kruela (1947, 1950) reported that the quantitative collection of feces and the chromic oxide indicator method gave the same results of carotene excretions. Irwin and Crampton (1951) found chromic oxide satisfactory for the determination of apparent digestibility of food dry matter in human. Stanley and

Cheng (1956, 1957) reported the successful use of chromic oxide in the study of cholesterol absorption. Metabolic balances for calcium, phosphorus, and magnesium using the chromic oxide method have been reported by Whitby and Lang (1960), Rose (1964), and by Sharpe and Robinson (1970). It is of interest to note that when Whitby and Lang (1960), and Rose (1964) recovered only 93% of chromic oxide in the feces, they assumed that 7% of the feces was lost and correction of data for this loss was made. Davignon, Simmonds, and Ahrens (1968) used chromic oxide to calculate pool size and turnover rates of human intestinal content.

In studies with humans, chromic oxide is usually administered orally in capsule form; a capsule contains 500 mg. For metabolic balance studies, capsules of chromic oxide are given 3 times daily, one with each of the main meals beginning when the subjects start their balance regimen (Whitby and Lang, 1960; Rose, 1964; Sharpe and Robinson, 1970). A daily dose of 300 mg was, however, found adequate by Davignon et al. (1968). In studies with ruminants, chromic oxide can be administered in capsules, impregnated in special paper, or as pellets. The dose for sheep is about 1 g per day and for cattle about 10-15 g per day when chromic oxide is used in any of these forms administered one or more times daily (Kane et al., 1953; Corbett, Greenhalgh, McDonald, and Florence, 1960; Langlands, Corbett, McDonald, and Reid, 1963; Harris, Lofgreen, Kercher, Raleigh, and Bohman, 1967; MacRae and Armstrong, 1969). When mixed with the diet of sheep and cattle, chromic oxide is added at the rate of 0.5% of the entire ration (Butcher and Harris, 1956;

Bradley, Forbes, Albert, Mitchell, and Neumann, 1958; Elam, Putnam, and Davis, 1959; Elam et al., 1962). For pigs, it has been added at the level of 1% of the diet (Schürch, Crampton, Haskill, and Lloyd, 1952; Moore, 1957) and the same level has been used for rats (Schürch, et al., 1950). A chromic oxide level of 1-2% of the diet has been used for chickens (Mueller, 1956).

It is concluded from the foregoing discussion that chromic oxide at levels used as a marker is nontoxic and almost quantitatively recoverable in the feces of man and animals and can, therefore, be used as an inert indicator in digestibility studies.

Ferric oxide

Ferric oxide (Fe_2O_3, N.W. 159.70) has reddish-brown to yellowish-orange color depending upon the size and shape of the particles and the amount of combined water (Merck Index, 1968). It was suggested by Bergeim (1924) for the study of intestinal reductions and later (1926) as a marker for nutrient utilization studies. He pointed out that ferric oxide is practically insoluble, unabsorbable and of suitable physical consistency to follow the food in the course of its digestion through the intestine and be excreted with practical completeness in the feces. Bergeim (1924) also reported that no detrimental side effects were noted in his experiment; he suggested it would be desirable to give with the ferric oxide an equal weight of powdered agar-agar to counteract any constipating tendency of the iron. He recommended iron administration not be continuous over longer periods of time than necessary.

Ferric oxide was also tried by Hoelzel (1930) on himself. He concluded that the ferric oxide slowed down the passage of intestinal contents and had an effect out of all proportion to the small amount taken. Ferric oxide has mostly been used as an indicator for the determination of apparent digestibility.

Although some workers reported that fairly accurate results were obtained when ferric oxide was used as a marker (Heller, Breedlove, and Likely, 1928; Gallup, 1928, 1929), many others reported the opposite effect (Gallup and Kuhlman, 1931; Moore and Winter, 1934; Knott, Hodgson, and Ellington, 1936; Hale, Duncan, and Huffman, 1939). Hale et al. (1939) stated that variations in the amount of ferric oxide passing through the digestive tract make it an unreliable indicator. One group (Heller et al., 1928) also reported that use of the normal iron content of diets was more satisfactory than ferric oxide additions. These contradictions cautioned against further use of this compound as an inert indicator of digestibility.

Titanium oxide

Titanium oxide (TiO_2, M.S. 79.90) possesses perhaps the greatest hiding power of all inorganic white pigments. It is insoluble in water, HCl, HNO_3 or diluted H_2SO_4 (Merck Index, 1968). Schroeder, Balassa and Tipton (1963) found titanium in human organs especially in lung where values tended to increase with age. Some foods were also found to contain titanium (e.g., butter, 2.5 ppm; pork, 1.8 ppm). Milk, cheese, eggs, beef, lamb and some cereals had little or none of this element. Titanium

oxide was proposed by Askew (1931) as a suitable reference substance for digestibility studies. This was based on studies with sheep ingesting 2-3 g daily of TiO_2. After three months, no ill effects were noticed and analysis showed complete elimination in the excreta and no storage in liver. TiO_2 was reintroduced by Fournier (1950) as a more suitable marker than ferric oxide for the study of quantitative intestinal absorption. The chemical procedure for analysis for this compound (Charlot and Bezier, 1945) was described by Fournier (1950) as accurate and sensitive. He indicated that TiO_2 was insoluble both in strong acids and bases, and could be recovered completely in the feces of rats. Feeding this oxide to rats in amounts equivalent to 1% of the body weight did not affect the appetite and it was not detected in the blood, liver, kidney or urine. The rate of passage through the digestive tract was compared to that of $CaCO_3$ and found to proceed at similar rates. After six hours of fasting, only a trace was found in the stomach of the rat and after 24 hours neither the stomach nor the intestines contained a trace of TiO_2 and only minute amounts of Ca. Later, Fournier and Dupuis (1953) found the rate of transport of TiO_2 was comparable to that of phosphorus. This marker was, therefore, frequently employed in studies of calcium and phosphorus absorption by Fournier and coworkers (Fournier, 1950a, 1950b, 1951a, 1951b, 1951c; Randoin, Susbielle, and Fournier, 1951; Fournier and Dupuis, 1953). Njaa (1961) found TiO_2 a useful marker in studies of protein digestion in rats since it can be readily determined in

Kjeldahl digests of food and feces. This author reported a mean recovery of about 98% for 78 studies. Further experimental evaluation of this compound through wider use as a marker in food utilization studies is needed before passing final judgement on its usefulness.

Mineral Salts

Two major compounds in this group have been tested and used as inert markers. They are barium sulfate and cuprous thiocyanate.

Barium sulfate ($BaSO_4$)

Barium sulfate is a white, fine, heavy (M.W. 233.43), odorless powder which is insoluble in water, dilute acids, and alcohol. Barium sulfate has long been used as a radiopaque medium for G.I. tract studies (Merck Index, 1968). Barium sulfate can be chemically determined by gravimetric methods (Dick, 1967; Figueroa, Gordan, and Bassett, 1968) or by emission flame photometry (Dick, 1969a). The availability of radioactive ^{131}Ba allows the use of radioactive techniques when desired (Seife, 1962; Najean and Ardaillou, 1963; Boender, Mulder, Ploem, deWael, and Verloop, 1967; Boender and Verloop, 1969).

As to the toxic effects of barium sulfate, Figueroa et al. (1968) reported that they have not noted any undesirable effects in their patients receiving a daily dose of 0.5 g of this marker and cited support where 5 g of barium sulfate was administered daily to each of 40+ patients for a period up to

one year without adverse effects.

Alvarez and Freedlander (1924) pointed out that giving a large amount of indigestible material like barium salts with a small meal of gruel or milk must bring about unusual and abnormal conditions. When 60 g of barium sulfate in milk was given to each of a number of healthy students, quantitative analysis of the stools for barium revealed that, although most of it is extruded in the first 24 or 48 hours, a considerable amount remains and is excreted slowly during the next few days. They regarded 60 g of barium sulfate as a large amount and attributed the faster passage of glass beads taken with barium sulfate to the latter's bulk. Hoelzel (1930) found that 25 g or more of barium sulfate helped sweep out other test materials but slowed down the passage of light test materials. Alvarez (1948) concluded that the giving of large amounts (e.g., 60 g) of barium sulfate acts like a large dose of agar or mineral oil; it hurries food residues through the bowl and temporarily cures constipation. Also, enough barium appears to be absorbed to stimulate contraction of the intestinal muscle.

Whitson, Carrick, Roberts, and Hauge (1943) were first to report the use of barium sulfate as an inert indicator in the study with chicks. They reported that barium sulfate mixed with the diet at the level of 0.5% was an appropriate inert indicator because it did not influence the palatability of the feed, had no apparent effect upon the chickens, and was readily deter-

mined chemically. These authors, however, did not report any recovery data to show the non-absorbability of the marker. Barium sulfate was also used by Bokori (1968) to study transit time of different feeds in the digestive tract of chickens.

Barium sulfate was tested as an inert indicator in studies with humans by Bassett, Tuttle, Figueroa, and Jordan (1962); Dick (1967); and more recently by Figueroa et al. (1968). Dick (1967) administered 0.5 g of barium sulfate in gelatine capsules three times daily, a dose which was well tolerated by the patients, and recovered an average of 97% of it in the stools. Figueroa et al. (1968) described Dick's method for the determination of barium as more time consuming compared with their method which required only the exact weighing of the $BaSO_4$. These workers also used only 0.5 g of barium sulfate divided into three equal capsules, one of which was taken with each meal. The recovery of barium sulfate in the stools ranged from 97.7 to 103.0% with a means of 100.4%.

Radioactive studies using $^{131}BaSO_4$ showed no significant exchange of the material across the bowel of mice (Seife, 1962) and less than 1% of orally administered $^{131}BaSO_4$ was found in the bones of mice (Najean and Ardaillou, 1963). Studies with man using $^{131}BaSO_4$ showed an average of 100.5% of the administered dose was recovered in the stools (Boender et al., 1967). Further details on the use of ^{131}Ba is given later.

Barium sulfate has been successfully used as an inert indicator in nutrient utilization studies after it was established that this compound was nontoxic, nonabsorbable from the digestive tract, and

completely recoverable in the feces of man and animals. Whitson et al. (1943) found it possible to study fat utilization in chickens by adding barium sulfate to the diet at the level of 0.5%. These workers did not, however, compare this procedure to the conventional method.

Barium sulfate has been used as an indicator in studies of calcium utilization in man (Bassett et al., 1962; Figueroa, et al., 1968). These workers suggested that in order to get an accurate assessment of calcium balance one should correct for variability of fecal flow to give a 100% recovery of the marker. The impact of this correction is shown in Table 3.

Cuprous thiocyanate

Cuprous thiocyanate (CuSCN, M.W. 121.62) is a white or yellowish-white powder, insoluble in water or alcohol, soluble in ammonia, and is decomposed by concentrated mineral acids (Merck Index, 1968).

Dick (1969 b) introduced cuprous thiocyanate as an inert fecal marker. He pointed out that the determination in the feces of chromic oxide or barium sulfate requires time consuming procedures. Cuprous thiocyanate is a compound with solubility characteristics in water similar to those of barium sulfate. Cuprous thiocyanate, however, is readily decomposed by 50% nitric acid at the boiling point. Thus, to estimate cuprous thiocyanate in feces, it is only necessary to add nitric acid, boil and filter in order to obtain a solution for analysis of Cu. This may be estimated by conventional chemical methods or more simply by

atomic absorption spectroscopy. The miminum recovery in any individual was 97.0% and the maximum 102.8% of the administered dose. This investigator suggested that cuprous thiocyanate starts its transit as a compound insoluble in stomach acid, but is partially changed in the lower gut to a compound (Cu_2O) insoluble under neutral or alkaline conditions. The absorption of thiocyanate from the compound is a disadvantage; however, it is a normal metabolite and Dick reported the use of 1 g of CuSCN per day for periods up to 38 days without serious toxic reactions but suggested the use of 500 mg/day for a limited time. Lee, Temperley, and Dick (1969) used 250 mg/day of cuprous thiocyanate in divided doses for estimating fecal fat excretion in humans. These workers indicated that, using the cuprous thiocyanate method, laboratory estimations are much simpler and the results are reproducible. More work with this compound is needed, especially in light of the reported thiocyanate absorption before it can be completely evaluated.

The Water-Soluble Markers (Polyethylene glycol and Cr-EDTA)

In addition to their use as digestibility indicators, the water soluble markers have been most useful in study of water balance and volume in the rumen. Mathematical treatments of the use of these markers for these purposes have been given by Hydén (1961), Ulyatt (1964), Warner (1966), Weston and Hogan (1967), and Warner and Stacy (1968).

A series of products known as the polyethylene glycol (PEG) compounds are manufactured through the reaction of ethylene oxide

with water, ethylene glycol, or diethylene glycol, the latter materials furnishing functional groups (in this case hydroxyl) for the propagation of the reaction. The process results in a mixture of diols of different chain lengths, in which the numbers of molecules of various sizes are presumed to be represented by Poisson's distribution (Shaffer, Critchfield, and Nair, 1950b). Polyethylene glycols 200, 300, 400, and 600 are fluids; compounds 1000, 1500, 1540, 4000, 6000 and 10,000 are solids of increasing firmness. The number gives the approximate average molecular weight.

A gravimetric procedure for the quantitative determination of PEG was devised by Shaffer and Critchfield (1947) and was later modified by Sperber, Hydén, and Eckman (1953). A faster and more accurate method is the turbidimetric method introduced by Hydén (1956a) which has been modified by several workers (Corbett, Greenhalgh, Gwynn, and Walker, 1958; Smith, 1959; Ulyatt, 1964).

The absorption and excretion of different polyethylene glycols have been reported in the animal and human body by Shaffer and Critchfield (1947) and by Shaffer *et al.* (1950a). It was shown that PEG compounds of molecular weights above 1000 were not absorbed from the gastrointestinal tract of the rat and man. The toxicology of PEG has been studied by Smyth, Carpenter, and Weil (1950) who suggested a safe dose for rats of 1.5 and 0.06 g/kg of body weight of PEG 1500 and 4000, respectively.

Polyethylene glycol (PEG) has been frequently used as a water-soluble marker in studies of absorption in man and animals (Sperber

et al., 1953; Hyden, 1955, 1956, 1961; Corbett, Miller, Clarke, and Florence, 1956; Corbett, Greenhalgh, Gwynn, and Walker, 1958; Corbett, Greenhalgh, and Florence, 1959; Corbett, Greenhalgh, McDonald, and Florence, 1960; Borgstrom, Dahlqvist, Lundh, and Sjövall, 1957; Lundh, 1958; Smith, 1959; Gray, Jones, and Pilgrim, 1960; Oyaert and Bouckaert, 1961; Weller, Pilgrim, and Gray, 1962; Ulyatt, 1964; Nicholson and Sutton, 1969; MacRae and Armstrong, 1969; Sinha, Martz, Johnson, and Hahn, 1970).

In 1953 Sperber et al. reported the use of PEG as a reference substance in studies of ruminant digestion and found that it was neither absorbed nor destroyed to any considerable extent in the digestive tract and reported more than 90% recovery in the feces. It was later reported by Corbett et al. (1956) to be suitable as a reference for the estimation of fecal output in the cow.

Borgstrom et al. (1957) used it in their study of intestinal digestion and absorption in humans and found that after giving a test meal containing PEG, replicate samples taken from the same freshly drawn intestinal content gave a constant ratio of fat to PEG. They concluded that it was justifiable to use the water soluble PEG with a molecular weight of 3000 - 3700 as a reference material in studies of human digestion and absorption of water soluble substances as well as fat when the latter (fat) is present in the test meal in a finely emulsified form. Lundh (1958) used 1.2 - 1.5 g of PEG with a molecular weight of around 3800 as an indicator of the test meal and as a non-absorbable reference substance.

Although several studies have shown that this material, when

used in high enough molecular weight (about 4000) is neither absorbed nor destroyed to any considerable extent, many reports indicated difficulty to achieve complete recovery in the feces.

Corbett et al. (1958) compared the patterns of excretion of PEG and Cr_2O_3 in cows. It was concluded that the concentrations of Cr_2O_3 in the dry matter of separate defecations varied by a maximum of approximately ± 10% from the 24 hour weighted mean concentrations. Maximum variations in PEG concentrations, on a similar basis, were ± 40-60%. It was suggested from this study and later confirmed (Corbett et al., 1959) that, because of its association with the water of the digesta, PEG was cleared from the reticulo-rumen much more rapidly than Cr_2O_3, giving rise to wider variations in the concentration of PEG in the DM of feces. The passage of PEG and lignin from the rumen of sheep was compared by Weller et al. (1962) after injecting 10 g of PEG (4000 mw) into the rumen 4 hours after start of feeding. Within about 1 1/2 hours after injection the PEG would be distributed evenly through the water of digesta but the lignin present in the coarse plant fiber would only gradually be added to the digesta and would, therefore, pass more slowly to the omasum.

In a recent publication by Nicholson and Sutton (1969) PEG and Cr_2O_3 were used as indicators to study the effect of diet composition and the level of feeding on digestion in the stomach and intestines of sheep fitted with rumen and re-entrant duodenal cannulas. It was pointed out that although both the water soluble polyethylene glycol (PEG, average molecular weight 4000) and water insoluble Cr_2O_3

gave similar estimates of variations in duodenal flow, disadvantages were found with PEG. First, its recovery in the feces was not complete since only 94.8% was recovered compared to 103.5% for Cr_2O_3. Second, at the concentration and the rate of infusion used, PEG formed an appreciable fraction of the dry matter and organic matter of the digesta and feces. To overcome these disadvantages, the authors proposed the use of a radioactive form of PEG that could be administered in much lower concentrations. Such radioactive material in the form of tritium-labeled PEG has been introduced (Ghanem and Westermark, 1962; Till and Downes, 1965; Neudoerffer, McLaughlin, and Horney, 1970). Tritium was not lost by exchange reactions and only small amount of the radioactivity (2-3% of the dose) was recovered in the urine of sheep (Till and Downes, 1965). Recent studies with dairy cows by Sinha et al. (1970) showed that approximate fill and weight of rumen contents can be determined using PEG as a reference substance, but the use of this material to determine digestibility is doubtful. These observations may be explained on the grounds that PEG associates itself with the liquid phase and not the solid phase of digesta (Hydén, 1956; Corbett et al., 1958, 1959; and Sinha et al., 1970). The extent of PEG recovery seems to be related to the diet. Clark and Hembry (1967) obtained much lower recoveries when sheep and steers were fed cottonseed hulls compared to alfalfa hay as dietary sources of fiber.

The lack of a specific, sensitive and accurate method for the analysis of PEG has been seen as a serious limitation in the use of this material as a reference substance. This may partly explain

the occasional failure by some workers to achieve complete recovery or reproduce results. The use of radioactive PEG has been proposed to overcome this difficulty. The complex of ^{51}Cr with ethylenediamine tetraacetic acid (^{51}Cr EDTA) has been studied and suggested by Downes and McDonald (1964) as a satisfactory substitute for PEG. After dosing sheep with a mixture of ^{51}Cr EDTA and PEG through a rumen fistula about 95% of the dose of ^{51}Cr (corrected for the amount taken in rumen liquor samples) appeared in the feces and 2.5% (also corrected) in the urine during the five days after dosing; the recovery of PEG in the feces was slighly lower than that of the ^{51}Cr but no significant quantity of PEG could be detected in the urine. Values as high as 4.7% of the ^{51}Cr EDTA dose were observed in the urine of some animals. The disappearance of the two markers from the rumen and their use to estimate the volume of rumen liquor were studied; results agreed within experimental error. These authors concluded that ^{51}Cr EDTA is a satisfactory soluble marker in spite of the slight absorption and subsequent excretion in the urine and that the estimation of ^{51}Cr is simple, accurate, and specific. The use of EDTA as a chelating agent has been reviewed by Broad (1971).

Hogan (1964) used ^{51}Cr EDTA to determine rate of flow of digesta from the reticulum and from the abomasum in sheep. Less than 5% of the dose administered was excreted in the urine. The marker was distributed through almost all the water of the digesta leaving the reticulum and was not adsorbed onto the particulate

material of digesta. Water balance in the rumen has also been studied by means of the ^{51}Cr EDTA complex (Weston and Hogan, 1967; Warner and Stacy, 1968a,b). Later, Warner (1969) found, under conditions as yet undefined, that some of the ^{51}Cr EDTA complex became bound to particulate matter in the rumen of sheep. Rumen volume calculated from the extrapolated zero time concentration of marker (Warner and Stacy, 1968) would be relatively unaffected by the binding. On the other hand, net inflow and outflow rates, calculated from the slope of the marker concentration vs time curve, would be overestimated, sometimes grossly, when binding occurred. Difficulty in demonstrating this phenomenon with in vitro techniques suggested that the binding of ^{51}Cr EDTA requires the presence of some special microbial activity and that in vitro techniques fail to maintain a truly normal rumen microbial population.

Work by Stacy and Thornburn (1966) with sheep suggested that ^{51}Cr EDTA may be useful in studies of renal function. Unlike Ca EDTA, continuous injection of large quantities of stable Cr EDTA to rats for 25 days did not affect the kidney function.

Under conditions where the use of the radioactive indicator is undesirable, the stable Cr EDTA complex can be used. Binnerts, Klooster, and Frens (1968) studied the stable indicator for digestion experiments with ruminants and described a method for determination by atomic absorption. It was indicated that a small percentage of the dose (always lower than 5% and mostly around 3% for sheep or lower for cattle) is absorbed and excreted in the urine.

This is in agreement with results obtained by Downes and McDonald (1964) for the radioactive indicator. The ability to account for this small urinary excretion makes it possible to apply simple corrections in digestion experiments.

^{125}I-diatrizoate sodium is another radioactive soluble marker which has been tested by Tan, Weston, Warner, and Hogan (1968-69).

Radioactive Markers

The use of glass beads, small seeds, dyes and inert indicators such as chromic oxide, polyethylene glycol, or barium sulfate was shown to have several disadvantages as previously discussed. The main disadvantages include variations in the rate of passage and recovery and the difficulty in quantitative analysis.

The use of radioactive indicators could offer several important benefits. Among these are:

1. The radioisotope measuring methods are relatively easy to carry out with precision.
2. The amounts of the radioactive indicator to be used is very small and has no effect on the bulk of the food and no undesirable side effects.
3. It is possible to obtain a detailed picture of the passage of material through the gastrointestinal tract.

^{51}Cr and ^{131}Ba are discussed in this section. Other radioactive markers are discussed elsewhere in this review, e.g., ^{51}Cr EDTA is discussed with polyethylene glycol and ^{106}Ru, ^{91}Y, ^{140}La, ^{95}Zr, ^{46}Sc, ^{47}Sc, ^{144}Ce and ^{198}Au are discussed with the inert metals group.

Chromium-51 (^{51}Cr)

The use of this radioisotope (half life 27.8 days) as a biological tracer has been reported by Gray and Sterling (1950) who showed that the isotope could be used as a tag for plasma proteins or erythrocytes depending upon the valence state. The use of ^{51}Cr to study the distribution of chromium in animal tissues was stimulated by reports on chromium contamination of drinking water as a result of industrial use of chromium and disposal of its wastes. The distribution of ^{51}Cr in the tissues of rats for intervals up to 24 hours following the intravenous injection of the tracer in isotonic acetate buffer has been reported by Krantz, and Talmage (1952). The absorption of ^{51}Cr from the gastrointestinal tract has been studied (Visek, Whitney, Kuhn, and Comar, 1953; MacKenzie, Anwar, Byerrum, and Hoppert, 1959; Donaldson and Barreras, 1966; Nutrition Rev., 1967). Visek et al. (1953) fed trivalent chromium 51, $^{51}CrCl_3$ to rats and found that most was excreted in the feces within 4 days and only about 0.5% was absorbed. MacKenzie et al. (1959) found that orally administered ^{51}Cr in the hexavalent form, $Na_2{}^{51}CrO_4$, was absorbed to the extent of about 6% in fasted rats and about 3% in nonfasted rats as judged by the excretion of chromium in the urine. In both fasted and nonfasted animals hexavalent chromium was absorbed to a much greater extent than trivalent chromium ($^{51}CrCl_3$). After feeding radioactive chromate, the liver showed a maximal uptake after one day of about 1% of the administered dose, whereas kidney and blood contained 0.1 - 0.2%. These quantities decreased over a

two week period. Chromium absorbed by the spleen appeared to be bound more tightly than in other tissues studied. These results were confirmed later by Donaldson and Barreras (1966) in studies with humans and rats.

Cr^{51} has been used as a fecal marker either as ^{51}Cr-labeled hemoglobin (Pearson, 1966) or as $^{51}Cr_2O_3$ (Kane, Jacobson, and Damewood, 1957, 1959; Brandt and Thacker, 1958; Sassoon, 1966; Davignon, Simmonds, and Ahrens, 1968; Utley et al., 1970).

Cr_2O_3 has been widely used as an inert marker but the difficulty in chemical analysis has been to dissolve the Cr_2O_3 completely. Brandt and Thacker (1958) used radioactive $^{51}Cr_2O_3$ in their studies of food passage through the digestive tract of rabbits. Kane et al. (1959) used the radioactive marker and found it as precise as the stable compound for determination of digestibility in cattle. Sassoon (1966) succeeded in partly standardizing Cr_2O_3 powder for purity and crystal size by cyclone elutriation. The tendency of aggregation of Cr_2O_3 was reduced by trituration with lactose, this provided evenly labeled ^{51}Cr. Sassoon found that twice-daily doses in the milligram range appeared to become as evenly dispersed in the feces of pigs and sheep as the physiological diurnal fluctuations would permit. The mean recovery of 8 single doses of 100 mg of purified $^{51}Cr_2O_3$-lactose from four 25 kg pigs in two 10-day periods was 100.1%. This is in agreement with an earlier statement by Harrison, Fraser, and Mullan (1961) that all the ingested ^{51}Cr by rats was fully recovered in the excreta after administration as $^{51}Cr_2O_3$ in a gelatine capsule into

the stomach through a stomach tube. The recovery reported by Sassoon (1966) is, however, better than that reported by Pearson (1966) for ^{51}Cr-labeled hemoglobin where only 94.9% was recovered in the feces of humans. Davignon et al. (1968) used radioactive chromic oxide in studies with humans and reported a mean recovery of 97.8%. From studies of first order kinetics of $^{51}Cr_2O_3$ excretion, these workers suggested that the marker was evenly distributed in a homogeneous pool, and that pool size did not change during this period of observation. Studies with ruminants showed no detectable radioactivity in the blood or urine of heifers after oral administration of 146 μc of $^{51}Cr_2O_3$ (Utley et al., 1970).

Barium-131 (^{131}Ba)

As previously pointed out, barium in the form of barium sulfate has been used as an inert indicator. Confirmation of its non-absorbability came from studies with radioactive ^{131}Ba. This isotope decays to ^{131}Cs by means of electron capture with associated γ-emission and has a half-life of approximately 12 days. Daughter element ^{131}Cs decays to the nonradioactive ^{131}Xe by means of electron capture and has a half-life of approximately 10 days (Seife, 1962). Tests in mice each fed 15 to 25 μc of $^{131}BaSO_4$ and sacrificed at times ranging from 24 to 100 hours after feeding revealed that there was no significant exchange of ^{131}Ba across the bowel (Seife, 1962). Najean and Ardaillou (1963) stated that less than 1% of the orally administered $^{131}BaSO_4$ was found in the bones of mice. Boender, Mulder, Ploem, deWael, and Verloop (1967) reported a recovery of 100.5% (96.5 - 106.5%) after the administration of

$^{131}BaSO_4$ to humans. These workers confirmed the nonabsorbability of this material by means of the whole body counter. Similar results were also reported by Boender and Verloop (1969) from their studies on absorption, loss and retention of iron in man.

The absorption of ^{131}Ba-labeled nutrients can be studied by means of $^{131}BaSO_4$ as a marker using a 2 channel gamma ray spectrometer system which can differentiate the emanation of ^{131}Ba from the emanations of the ^{131}I-labeled nutrient. Seife (1962) conducted fat balance studies in dogs by the conventional and the $^{131}BaSO_4$ methods with ^{131}I-labeled triolein as the dietary test fat; there was agreement between the two methods (Table 4). Iron utilization in man has also been studied by means of the $^{131}BaSO_4$ method (Boender et al., 1967; Boender and Verloop, 1969). Boender et al. (1967) indicated that with the aid of an oral test dose of $^{59}FeSO_4$ and $^{131}BaSO_4$, the percentage of iron absorption can be rapidly and easily calculated without the need for quantitative collection of feces. In order to determine the ultimate retention of Fe^{59} in the organism after an oral test dose of ^{59}Fe, however, it is necessary either to make quantitative determinations of fecal ^{59}Fe or to establish with the aid of a whole body counter how much ^{59}Fe remains in the organisms two weeks or more after administration of a test dose of radioactive iron (Table 5).

Inert Metals

Ellis (1968) has discussed a number of properties of the rare earth elements which suggested their advantageous use as indigestible markers. Certain of these, in addition to being essentially indiges-

tible by mammals (Hamilton, 1947; Garner, Jones, and Ekman, 1960; Bell, 1963; Ellis,1968) become tightly bound to plant material (Morgan, 1959; Ellis, 1968; Ellis and Huston, 1968) and, therefore, might be expected to flow through the gastrointestinal tract in close association with indigestible feed residues. Such an association would be desirable in reducing variation in fecal marker concentration attributable to differential flow of feed residue and marker from the reticulorumen (Corbett, Greenhalgh, and Florence, 1959).

On the other hand, the rare earths, as most metals, are harmful to bacteria. This oligodynamic action expresses itself in a LD_{50} for Escherichia coli B and Pseudomonas chlororaphis of 9, 4; 5, 4; and 0.4, 4 µg/ml for cerium, europium, and lutetium, respectively (Maier, 1968). Further work on this specific problem has been reported by Johnson and Kyker (1966), Talburt and Johnson (1967), and Maier (1968).

The magnitude of absorption of different elements from the alimentary tract of man has been compiled by the International Commission on Radiological Protection (1959) and routes of excretion of these elements in laboratory mammals by Altman and Dittmer (1968).

Scandium ($^{46,47}Sc$), yttrium (^{91}Y), lanthanum (^{140}La), cerium (^{144}Ce), europium (Eu), and dysprosium (Dy), are members of the rare-earth group for which data are available to establish their criteria as fecal markers. Other elements discussed here include zirconium (^{95}Zr), ruthenium (^{106}Ru), and gold.

Ruthenium-106 (^{106}Ru)

Ruthenium-106 has a half-life of 1 year and decays by beta emission to ^{106}Ru which has a half-life for beta decay of 30 sec. Although the ^{106}Ru beta particle is very weak (maximum energy 0.04 mev) it is easily counted by virtue of the short half-life of its daughter ^{106}Rh whose predominant beta particle emission has a maximum energy of 3.5 mev.

Thompson and Hollis (1958) killed rats at intervals from 35 min to 30 hours following intragastric administration of 8 μc of a solution of ^{106}Ru chloride. Distribution of ^{106}Ru within the various segments of the GI tract and distribution of tract contents were determined by counting and autoradiography. It was concluded that the radioactivity was associated with the food residues in the tract and that there was no significant holdup of the radioisotope due to absorption on the intestinal wall. ^{106}Ru was also used by Sikov, Thomas, and Mahlum (1969) in their study of the passage of tracers through the gastrointestinal tract of neonatal and adult rats. Their techniques were similar to those reported by Thompson and Hollis (1958). A dose of 9.5 μc was administered by stomach tube and results were expressed both as distance traveled and percent of length traveled.

^{103}Ru-phenanthroline complex has been tested by Tan, Weston, Warner, and Hogan (1968-69) and was found to adsorb to solids of digesta.

Yttrium-91 (^{91}Y)

Marcus and Lengemann (1962) introduced ^{91}Y as a radioisotope marker for the study of food movement in the small intestine after they established that it remained with the food and it was non-absorbable. In an *in vitro* experiment, they labeled ground rat food with ^{91}Y and made a suspension after covering it with an excess of water and shaking. A range of pH 3.0 - 8.0 was tested. After one hour the samples were centrifuged and the solid and supernatant counted separately. At pH 3 about 85% of ^{91}Y remained combined with the ground rat food and as the pH increased, about 96% of ^{91}Y was found combined with the food at pH 8.0. In vivo experiments with rats showed that after feeding 1 μc of ^{91}Y with the diet, the total recovery of ^{91}Y in the digestive tracts averaged 97.9±0.8%.

Lanthanum-140 (^{140}La)

Lanthanum-140, which was introduced by Hayes, Carlton, and Nelson (1964) as an unabsorbable tracer, was used to verify the the completeness of stool collection and to study absorption of iron-59. Because of its abundant high-energy radiation, ^{140}La can be assayed directly in the presence of most other radionuclides when appropriate energy discrimination and geometric correction factors are used. When 20 μc of ^{140}La and 2 μc of ^{59}Fe were given to patients with an oral dose of other substances, the recovery of La averaged 96 - 104% and a distinct retardation of ^{59}Fe excretion was observed. These workers recommended the use of ^{140}La in studying this phenomenon.

^{140}La was also used by Francois, Compère, and Rondia (1968) in their study of the rate of passage through the digestive tract of rats and found to be essentially indigestible.

Zirconium-95 (^{95}Zr)

^{95}Zr oxide was chosen by MacDougall (1964) to estimate the fat absorption from random stool specimens for the following reasons:

1. It is not absorbed from the intestinal tract and does not have the potentially irritant effect of chromium. Its degradation product is niobium which is also non-absorbable and has a spectrum similar to ^{95}Zr.
2. The product can be obtained directly as ^{95}Zr oxide and requires no further chemical preparation.
3. It has a sufficiently long half-life (63 days) to be of practical value in clinical studies.
4. No toxic side effects have been reported in animals or humans, apart from rare skin sensitivity after prolonged local application.
5. ^{95}Zr has a spectrum which would permit analysis in conjunction with ^{131}I, ^{59}Fe, and ^{60}Co using differential counting techniques.

Using ^{95}Zr, ^{131}I-labeled leic acid and carmine, MacDougall determined fat absorption by three different methods.

1. Chemical analysis of a 48-hour stool collection.
2. $^{95}Zr/^{131}I$ ratio in food and individual stool specimens over a 2-day period.

3. By expressing the total ^{131}I recovery in stools as percentage of the total dose given.

Doses of 0.25 μc of ^{95}Zr and 1.75 μc of ^{131}I were given 3 times daily to dogs and to children 4 to 16 years old. Stool collections were obtained as from the appearance of the first colored stool and continued until no further radioactivity was demonstrable. It was concluded that intestinal fat absorption can be accurately estimated from a single stool specimen. When the first marked stool was excluded from the calculations, results obtained by the random stool method showed no significant difference from those obtained by chemical analysis of a 48-hour stool collection (Table 6). About 75 - 100% of the total ^{95}Zr dose was recovered in stools within 48-72 hours of the last dose given to most dogs and patients; no data on complete recovery were reported.

Scandium-46,47 ($^{46,47}Sc$)

Because of its favorable radiation characteristics (half-life 3.4 days and only emits a single γ-ray of 0.15 mev.) and unabsorbability, it was suggested that ^{47}Sc is a suitable isotope to use as a fecal marker in metabolic work. ^{51}Cr and ^{47}Sc were introduced as inert fecal markers by Pearson (1966) who showed that average fecal recoveries in human of ^{51}Cr (administered as labeled hemoglobin) and ^{47}Sc were 94.9±3.97 and 98.6±4.46%, respectively, after simultaneous oral administration of 10 μc of each. There was no significant difference in their gastrointestinal transit time. Pearson also pointed out that isotopes of rare-earth elements for

use as nonabsorbable fecal markers are more convenient than ^{51}CrHb since these tracers require no special chemical preparation.

^{47}Sc is the daughter of ^{47}Ca and its presence is usually regarded as a complicating factor in counting procedures for ^{47}Ca. The presence of ^{47}Sc, however, was found by Ogg, Pearson, and Veall (1967) to be an advantage because ^{47}Ca, in effect, carries its own inert marker and this can be exploited to estimate net ^{47}Ca absorption from an orally administered dose by measuring a single active sample of feces. The oral dose of ^{47}Ca contains a certain ratio ^{47}Ca/^{47}Sc which will depend on the growth and decay curves for the two isotopes. If a proportion of the ^{47}Ca is absorbed, it leaves its associate ^{47}Sc remaining in the gut, so that there is an excess of ^{47}Sc in the feces. This excess is related to the proportion of the ^{47}Ca which has been absorbed. After oral administration of approximately 10 μc of ^{47}Ca in fruit juice to patients, Ogg *et al.* (1967) concluded that there was no systematic difference between values obtained by this method and values obtained by the usual cumulative excretion technique, but results obtained using small fecal samples less than were variable. This was attributed by these workers to inhomogeneous distribution of the two isotopes in the gut, a difficulty which might be overcome by sampling from previously homogenized pooled specimens.

The long-lived ^{46}Sc (half-life 84 days) has also been studied (Spencer and Rosoff, 1963; Miller and Byrne, 1970). In studies with cattle, Miller and Byrne (1970) compared ^{46}Sc with ^{144}Ce as

nonabsorbable markers. It was shown that neither radioisotope was detected in blood or urine after oral dosage and recovery in feces was almost complete; it was concluded that ^{46}Sc is as suitable as ^{144}Ce for use as a nonabsorbable reference material for cattle and it could substitute in mixtures with certain other radioisotopes which ^{144}Ce would be unsuitable because of its counting characteristics.

Cerium-144 (^{144}Ce)

Cerium-144 has been studied more extensively than other members of this group as a fecal marker (Garner, et al., 1960; Chandler and Cragle, 1962; Miller and Cragle, 1965; Padgitt, Martz, and Graham, 1966; Miller, 1967; Miller, Perry, Chandler, and Cragle, 1967; Huston and Ellis, 1968; Ellis and Huston, 1968; Francois et al., 1968; Miller, Moss, Hall, and Gorman, 1969; Miller and Byrne, 1970).

^{144}Ce is a fission product and a component of world-wide fall-out and is practically nonabsorbable from the gastrointestinal tract of animals (DuBois, 1956; Garner et al., 1960). Its half-life of 284 days is long enough for physical decay to be neglected during short-term experiments (Garner et al, 1960). Garner et al. (1960) suggested the use of ^{144}Ce as a marker in digestibility and similar trials. They reported that equilibrium conditions were reached after 5-6 days on twice daily doses with cows. Similar results were obtained by Chandler and Cragle (1962) in their study of the gastrointestinal sites of absorption and endogenous secretion of calcium and phosphorus in dairy calves.

Padgitt et al. (1966) found that ^{144}Ce concentration in the feces reached equilibrium within 3 days in rabbits. On the seventh day after ingestion, the rabbits were killed and no radioactive cerium was detected in the urine, blood, muscle, bone, liver, kidney, spleen, heart, or lungs. When one rabbit was switched to untreated diet, the radioactivity in the whole body dropped to background count within 3 days. ^{144}Ce was also tried by Francois et al. (1968) in their study of food passage through the digestive tract of rats and sheep and found to be essentially nonabsorbable.

Further evaluation of radioactive cerium as a non-absorbed marker became available from comparison with other well known markers. Miller and Cragle (1965) obtained comparable results when using $^{144}CeCl_3$ and Cr_2O_3 in their study of gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. Later, Miller et al. (1967) showed that the excretion curves of ^{141}Ce, ^{144}Ce, and Cr_2O_3 were essentially similar in cattle (fig. 1). Huston and Ellis (1968) compared ^{144}Ce with chromic oxide and polyethylene glycol (PEG) as fecal markers. The variation in each marker's fecal concentration attributable to analytical determination (duplicates), collection periods, and sheep is expressed as coefficients of variation in Table 7. These results indicated that there was considerably less variation between collection periods and between sheep in the fecal concentration of ^{144}Ce than for either chromic oxide or PEG. Huston and Ellis (1968) and Ellis and Huston (1968) evaluated further properties of radiocerium as a digesta marker for ruminants by in vivo and in vitro

studies. Results suggested that radioactive cerium remained in close physical association with indigestible residues during their transit of the ruminant gastrointestinal tract by continued adsorption onto indigestible particles or by readsorption onto other particles. Miller and Byrne (1970) stated that because of this adsorption and the possibility of secondary labeling, results obtained with a rare earth marker do not correspond to rate of passage as defined by Balch (1950) since residues from several meals could be labeled; valid comparisons, however, could be made using a rare earth as a flow rate marker for the total residues derived from the total diet. Padgitt et al. (1966) have shown from their experiments with rabbits that the average dry matter digestibility determined by the ^{144}Ce ratio technique was 31.9% compared with 34.8% for total collection. This difference of about 3 percentage points is large enough to cause some concern about the accuracy of the ^{144}Ce technique. However, the total number of animals in this experiment was only four; this is not enough to justify judging the accuracy of this marker to estimate digestibility on the basis of these results.

Gold

Bris, Dyer, and Teare (1967) used radioactive (^{198}Au) to study the passage of ingesta through the digestive tract of ruminants. Radiogold (^{198}Au) was orally administered to cattle and sheep and the time course of its excretion was followed. The excretion pattern was found reproducible. No radioactivity in the urine and no visible damage from irradiation in the digestive tract was observed. When a

muscle relaxant was administered, the passage of ingesta as described by the excretion pattern of ^{198}Au, was slowed down. These authors indicated that the half-life of ^{198}Au of 64.8 hours was long enough for rate of passage studies but short enough to make decontamination of equipment a relatively minor problem. They proposed the use of stable colloidal gold and assay by neutron activation analysis when it is undesirable to use radioactive isotopes. This proposal was put to experimental test by Martz, Asay, Wormington, Leddicotte, and Daniels (1969). These workers used a stable gold-resin complex as a marker to study the passage of ingesta in cattle employing neutron activation analysis for determination of gold. The gold particles were excreted at a faster rate than stained straw and this was attributed to the small particle size of the gold marker (200-400 mesh). The variations associated with the gold technique were approximately of the same magnitude as those when stained straw is used as a marker. This study showed that gold can be detected at a level of 10^{-6} g/g of dried feces with 5% accuracy between replicates.

Others

It has already been pointed out that the rare earth elements become tightly bound to plant material as a result of possessing adsorption properties. These adsorptive effects, as summarized by Ellis (1968), occur at concentrations approximating, or less than, the molar solubility of the corresponding hydroxides (in the order of 10^{-6} to 10^{-7} M) and at "radiocolloidal behavior" concentrations (below 10^{-6} to 10^{-7} and in the order of 10^{-11} M).

These concentrations are below the detection range of the usual methods of chemical analysis and, hence, radioisotopes of the elements have usually been employed in studying such behavior. The use of radioactive tracers, however, introduces the problems of cautious handling and waste disposal, especially in studies with large animals. The use of dysprosium as a marker and activation analysis as the technique for its determination overcomes these problems and offers the advantage of using a member of the rare earth group. It was suggested by Ellis (1968) that these problems of waste disposal may be circumvented by radioactivation analysis of those rare earth elements of sufficiently high nuclear cross section to provide the required analytical sensitivity in the feces of animals fed the nonradioactive isotope of the element. Dysprosium, a nonradioactive isotope and a member of the rare earth group, was used by Ellis (1968) as a nonabsorbable marker. He described a method for the instrumental radioactivation analysis of this element in forages, rumen contents and feces. It was shown when sheep were fed hay containing 36.2 ppm of Dy that its recovery in the feces averaged 99.8% (97.5 - 102.8%) and that the variation in fecal dysprosium concentration between and within daily samples of feces was between two and three times its variation in concentration on the ingested forage upon which it had been adsorbed. Ellis (1968) pointed out that the low within and between day variation in fecal Dy concentration suggests potential advantages for rare earth elements over other presently accepted indigestible markers.

Because of its short half-life, Dy might pose problems for some reactor facilities which are not well equipped for handling its activation analysis. Ellis (1968) proposed that europium may be more desirable where less automated facilities are available because it has a high nuclear cross section and reacts with thermal neutrons to produce high-energy gamma emitting radioisotopes (^{152}Eu and ^{154}Eu), having relatively long half-lives (5.3 years). Other candidates for activation analysis include terbium ytterbium, lutetium, and chromium.

Microorganisms

Bacterial spores have been used as a marker in studies of transit time through the digestive tract. Holman and Fernish (1923) fed spores of Bacillus anthracis to guinea pigs and found that the animals rarely become infected but pass anthrax spores for many days. Meyer and Easton (quoted by Alvarez and Freedlander, 1924) studied the passage of washed detoxified spores of B. botulinus through the digestive tracts of rabbits and guinea pigs. It was reported that these spores had little or no influence on the animal. Large numbers came through in 24 and 48 hours; a good many for the next week, and then a few at intervals for months afterward. More recently, Ducluzeau, Bellier, and Raibaud (1970) reported on their studies of the transit of the inocula of several bacterial strains through the digestive tract of mice. These workers used the spores of the strictly thermophilic Bacillus subtilis which are incapable of germinating at the temperature of the animal's body as a marker of transit through the digestive tract. It was found that

the speed of propulsion of the contents and the emptying of the intestine were more rapid in classic than in germfree animals. When the kinetics of the appearance and disappearance of the spores of this Bacillus in the feces of classic mice were compared with those of cells of 10 bacterial strains introduced "per os", it was found that Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri, Lactobacillus salvarious, and a streptomycin-resistant strain of Escherichia coli originating from the mouse digestive tract were eliminated at the same speed as the Bacillus spores; E. coli, $K_{12}S$, Welchia perfringens, and Pseudomonas sp. were eliminated more rapidly; Staphylococcus pyogenes and Sphaerophorus varius were eliminated more slowly than the Bacillus spores.

Contrepois and Gouet (1969) have devised a microbiological technique to measure the transit of microparticles in the digestive tract of ruminants using the spores of Bacillus subtilis. The method takes advantage of the fact that this strain is a strict aerobic thermophile; spores introduced into the anaerobic medium of the ruminant digestive tract at the temperature of 39°C cannot germinate. They, however, multiply rapidly at 60°C; this makes it easy to separate this strain from other bacteria of the rumen. Beside these characteristics, the authors claim that these spores have a particularly great resistance against chemical, physical, and enzymatic agents. The procedure has been described by them as simple, rapid, and accurate but suggested that a definitive judgement of the technique should await comparison with other methods of study.

It is recommended that application of such microbiological techniques in humans should not be allowed until completely proven safe in other monogastric animals.

Others

Stanley and Cheng (1956, 1957) suggested the use of inert indicators to study the absorption of such readily exchangeable substances as cholesterol. They used chromic oxide and ^{14}C-labeled cholesterol to determine cholesterol excretion. Later, Grundy, Ahrens, and Salen (1968) found that cholesterol and some other commonly ingested plant sterols, during passage through the intestine, are degraded to products which are no longer recognizable as neutral sterioids by their method of gas-liquid chromatography. Unlike cholesterol, plant sterols are negligibly absorbed in man, but the percentage of radioactivity from dietary cholesterol found in the feces is almost exactly the same as that of plant sterols. These workers were able, therefore, to use plant sterols as internal standard to correct for the unaccountable loss of cholesterol. To determine cholesterol excretion in the feces, radioactive cholesterol and β-sitosterol labeled with different isotopes (^{3}H or ^{14}C) were fed to the patients. Beta-sitosterol was chosen as the internal standard in man because it is only absorbed to a very slight degree (5% or less) and what is absorbed is rapidly returned to the intestinal lumen. This compound also passes through the intestine in the same physicochemical state as cholesterol; it accompanies cholesterol at every step of its isolation and chromatographic measurement, and it is lost to the same extent as cholesterol

due to bacterial degradation in the intestine.

This procedure was also used by Borgstrom (1969) for the quantification of cholesterol absorption in man after the feeding of a single isotope-labeled meal.

INTERNAL MARKERS

Silica

Wildt in 1874 suggested that silica could serve as a digestibility indicator by virtue of its nonabsorbability. He studied digestion in different parts of the digestive tract of sheep using naturally occurring silica as a reference substance. In this technique the animals were slaughtered after a period of standardized feed intake and the absorption and excretion of a number of feed constituents in relation to the silica were then determined. More than 50 years later, Gallup (1929) reintroduced silica as a suitable digestibility indicator and a possible substitute for iron oxide. In a study with rats, silica and iron oxide were compared to the conventional method; data for protein digestibility agreed closely. Later, Gallup and Kuhlman (1931) repeated this work with large animals (cattle) and confirmed the earlier conclusion of the validity of silica as an indicator of protein digestibility. They further concluded that the silica naturally contained in the feed served as a better index of the digestibility of the other substances (fat, fiber, and nitrogen-free extract) than did the added iron marker. Whitson, Carrick, Roberts, and Hauge (1943), however, pointed out that the silica method did not seem applicable to

poultry due to the high mineral, low silica content of the diet, possible retention of silica particles in the gizzard, and the occurrence of silica in the feathers. Studies conducted later by Gallup, Hobbs, and Briggs (1945) with cattle and sheep indicated that the recovery of silica in the feces is not quantitative under all conditions as shown in Table 8. Estimates of forage intake of grazing animals using silica as marker were more variable than those obtained with lignin (Van Dyne and Meyer, 1964). Two major factors may account for variability in recovery of silica. First, some of the ingested silica is absorbed and excreted in the urine (Sauer, Laughland and Davidson, 1959; Forman and Sauer, 1962). Second, contamination of the food consumed by animals with dust is noted. The degree of this contamination depends, of course, on the surrounding environment.

Lignin

Dietrich and Konig (1871) quoted by Csonka, Phillips, and Jones (1929) claimed that lignin was not digested in the animal body. Paloheimo (1925) stated that lignin reappears quantitatively and unchanged in the feces. Rogozinski and Starzeuska (1926, 1928) found that the lignin in oat straw is not digested by sheep. Rubner (1928), however, reported a loss of lignin in humans and dogs. Csonka et al. (1929) indicated that lignin suffers a loss of the methoxyl group in passing through the animal body, and hence, is broken down by the animal organism. This degradation takes place in the stomach and is not brought about by

bacteria but rather by some other agent. Hale, Duncan, and Huffman (1939) showed that, in cows, lignin was digested in variable amounts up to 24% and that digestion did not take place in the rumen but after passing from the rumen. Forbes and Swift (1943) found lignin to vary in digestibility from negative values to plus 29% in cattle, and Ely, Kane, Jacobson, and Moore (1953) found the apparent digestion coefficients of lignin in cattle fed rations containing orchard grass cut at four stages of maturity ranged from 4 to 16%. A range of -7 to 42% was reported by Smith, Turner, and Harris (1956) for apparent digestibility of lignin in nine forage species by deer. Lignin of alfalfa hay was digested to the extent of 2 to 11% by deer and 9 to 15% by sheep.

Ellis, Matrone, and Maynard (1946) suggested an improved method for lignin determination - the 72% sulfuric acid method - and showed that lignin was not digested by the cow, sheep or rabbit. Recovery range was 94 - 106% for a group of feeds tested. Data for digestibility as calculated by the lignin ratio and conventional methods compared favorably. Swift, Thacker, Black, Bratzler, and James (1947) reached the same conclusion for lignin as a digestibility index in sheep. Forbes and Garrigus (1948) applied the lignin ratio technique to the study of digestibility of pasture forages by steers and wethers. The average recovery of lignin was 102% in digestion trials with steers. Consumption of dry matter, digestible protein and total digestible nutrients (TDN) were calculated for sheep and steers

in grazing trials using the lignin ratio method. Kane, Jacobson, and Moore (1950) reported a mean recovery of 98.8% (97.1 - 101.5%) for lignin as compared to 99.9% (99.0 - 101.2%) for chromic oxide in cows fed a ration with alfalfa as forage. There were no significant differences between digestibility coefficients obtained by the use of lignin and chromic oxide ratios and those calculated by the conventional procedure of total quantitative collection. The lignin of orchard grass, however, appeared to be digested to variable degrees. Recoveries of the lignin in orchard grass averaged 95.7% for 3 cows (Kane, Ely, Jacobson, and Moore, 1953). Losses of this size were seen by Kane, Jacobson, Ely, and Moore (1953) to limit the usefulness of the orchard grass lignin as a digestibility indicator. Elam and Davis (1961) reported a recovery of 87% for lignin of a mixed ration fed to cattle.

Variability in reports on digestibility and recovery of lignin may be attributable to the analytical methods used for determination of lignin (Balch, Balch, and Rowland, 1954; Van Soest, 1964). The incomplete knowledge of lignin structure limits the specificity of all lignin methods which are based to some extent on empiricism. This situation is further complicated by evidence indicating that fecal and dietary lignin are somewhat different in their chemical characteristics (Ely, Kane, Jacobson, and Moore, 1953; Balch et al., 1954; Elam and Davis, 1961). The impact of the method of determination of lignin on its use as a marker is clear from the following examples. Crampton and Jackson (1944) found that the "formaldehyde lignin" was negatively correlated while "lignin by difference" was

only slightly but positively correlated with digestibility of the dry matter. Ellis et al. (1946) improved lignin determination by introducing their "72% H_2SO_4" method. Results in Table 9 show the effect of slight variation in sulfuric acid concentration (Mueller, 1956). From digestibility trials with cows, Balch, Balch, and Rowland (1954) obtained digestibility coefficients of 9.3, 4.7, and 1.3% for crude lignin and 8.3, 9.1, and 4.4% for corrected lignin (for contaminating proteins) when determinations were carried out by the three methods of Norman and Jenkins (1934) as modified by Gray (1947); Ellis, Matrone, and Maynard (1946); and Armitage, Ashworth, and Ferguson (1948), respectively. Price, Lindahl, Frederiksen, Reynolds, and Cain (1964) compared the "72% H_2SO_4" method to the "acid-detergent lignin" (ADL) method (Van Soest, 1963) when lignin was used to estimate the dry matter intake of sheep from forage at different stages of maturity. Dry matter intake was consistently higher when the ADL method was used. Analysis for lignin is further complicated by the effect of drying and heating during the preparation of laboratory samples. Van Soest (1964) found that the drying techniques that are sometimes employed on the feed and feces produce serious heat damage (Table 10).

In spite of these limitations, several workers continue to use lignin as a marker especially in studies of nutrient digestion in the rumen due to the fact that its rate of flow out of this organ is less variable than that of other widely used markers such as chromic oxide (Johnson, Dinusson, and Bolin, 1964; Drennan,

Holmes, and Garrett, 1970). An interesting method for lignin determination in acid-detergent fiber (ADF) has been introduced by Van Soest (1967). In this method, potassium permanganate is used to oxidize lignin at room temperature and removes it from the fiber residue. This method permits simultaneous determinations of lignin, cellulose, and silica.

Chromogen

The use of plant chromogen as a digestibility indicator for grazing animals was introduced by Reid (Reid, Woolfolk, Richards, Loosli, Turk, Miller, and Blaser, 1949; Reid, Woolfolk, Richards, Kaufman, Loosli, Turk, Miller, and Blaser, 1950). Spectral examination of acetone extracts of the forages studied and their corresponding fecal products revealed that some chromogen(s) absorbing light at 406 mμ was completely recoverable in the feces. The pigments have been identified as mostly chlorophylls and their degradation products, chiefly pheophytins (Smart, Sherwood, Matrone, and Wise, 1953). Recovery averaged 100.5% (94.4 - 106.2%) in studies with sheep and cattle (Reid et al., 1950). Data on digestibility and dry matter consumption as derived by the chromogen ratio method were in close agreement with those derived by the conventional method.

Cook and Harris (1951) compared the lignin and the chromogen ratio methods for determining consumption and digestibility of forage by sheep. The coefficient of variation for digestibility among animals was much smaller by the lignin than by the chromogen determinations. It was concluded that the chromogen method, although

satisfactory for alfalfa was not suited for determining digestibility of winter range forage, since, in some cases, there was considerable less chromogen recovered in the feces than actually consumed. Later, Kane et al. (1953) obtained comparable digestibility coefficients with a standard 10-day consumption-excretion method and the chromic oxide and chromogen ratio techniques when calculated with both the total collection samples and the averages of 3 days' partial collection (grab) samples.

In 1952 Reid, Woolfolk, Hardison, Martin, Brundage, and Kaufman stated that the use of the chromogen method as suggested by Reid et al. (1950) is feasible only under conditions in which the samples of feed obtained for analysis truly represent the feed consumed by the animals, a situation that exists only in trials in which hand-feeding is practiced.

Conventional digestion trials were conducted in which clipped, whole forages of different botanical composition and of various stages of growth were hand-fed. The relationship between the chromogen/dry matter ratio of the feces and that of the forage consumed was established mathematically by the equation: $Y = (9.9025 X + 137.3 \text{ Log } X) - 242.12$; where Y = units of chromogen/g of forage (dry basis), and X = units of chromogen/g of feces (dry basis). The study included 18 pasture forage mixtures ranging in dry matter digestibility from 51.6 to 74%. This relationship allowed the circumvention of the manual sampling of forage, as it was found that the chromogen concentration of the forage could be predicted from a knowledge of the fecal chromogen level. Chromogen concen-

tration so estimated can be used in the usual ratio technique for determination of digestibility of consumed forage under conditions of grazing. These investigators also suggested an alternate procedure for the direct computation of digestibility from the chromogen concentration of the feces by the equation: $Y = 32.74 + 0.0168 X + 8.47 \text{ Log } X$; where Y = % of dry matter digested, and X = units of chromogen/g of feces (dry basis). The coefficient of correlation between digestion coefficients computed from this relationship and those derived from the conventional digestion trial was 0.96.

This chromogen formula and the chromic oxide ratio were found by Kane et al. (1953) to compare favorably with the standard total collection procedure in predicting nutrient digestibility in cows. Later, Lancaster, and Bartrum (1954) found that the method of analysis for chromogen as suggested by Reid et al. (1952) could be a source of error in using this material as an internal marker. They suggested that in order to obtain reproducible estimates of the mixed chromogens in acetone extracts of feces, light should be excluded both during and after extraction and observation of optical density should be made within 6 hours from the preparation of the extract.

Fecal Nitrogen

Blaxter and Mitchell (1948) showed that fecal nitrogen excretion was related to dry matter consumption. Gallup and Briggs (1948) suggested that the feed consumption of grazing animals could be determined by taking advantage of the relationship between fecal nitrogen excretion and dry matter intake. Raymond (1948) reported that

nitrogen concentration in the feces of sheep was related to that of the grass consumed and later Lancaster (1949) pointed out that fecal nitrogen concentration was related to the digestibility of forage. Forbes (1949) pointed out that total fecal nitrogen varies too widely to be of practical use in the manner suggested by Gallup and Briggs. Lancaster (1949) divided the forages into two classes based on their protein contents in using fecal nitrogen as an indicator of digestibility in grazing animals. He found that the nitrogen excreted in the feces per unit intake of pasture organic matter was constant. This relationship provided a means for measuring organic matter digestibility in herbage according to the formula: organic matter digestibility coefficient (%) = 100 $(1 - \frac{C}{N})$; where C is a constant and N is % of nitrogen in the ash-free feces. Based on data from 52 digestibility trials with sheep reported from widely dispersed centers in New Zealand, C was found to be 0.83 $\pm$ 0.102 g nitrogen per 100 g of pasture organic matter consumed provided that the protein content of the pasture consumed was not less than 15%. Further calculations based on the data from another 101 digestibility trials reported from different parts of the world showed that C was equal to 0.80 $\pm$ 0.081 and 0.67 $\pm$ 0.120 when the protein contents of the pasture consumed were 15% or more and below 15%, respectively. Forbes (1949) considered this as an improvement over the method of Gallup and Briggs (1948) since it recognizes that the forage protein level may have some effect on the amount of fecal nitrogen per unit of dry matter intake. Forbes (1949),

however, still criticizes Lancaster's method because a sharp break in the relationship between fecal nitrogen and organic matter intake is not expected. He (1950) proposed the use of protein as an indicator of pasture forage digestibility but concluded that his method may be used with a satisfactory degree of accuracy for the determination of digestibility of dry matter by grazing steers and not lambs. Forbes' formula to calculate protein digestibility, $\% = 100 - \frac{\% \text{ protein in feed}}{\% \text{ protein in feces}} \times [100 - 42.64 (\% \text{ protein in feed} - 5\%)^{0.2148}]$ was later used by Kane et al. (1953) in a comparison involving the total collection method, the chromic oxide ratio, and Reid's chromogen formula (Reid et al., 1952) to determine nutrient digestibility and dry matter consumption of forage by grazing cattle. These workers concluded that the determination of digestibility by chromic oxide ratios, by chromogen formula, and by the protein formula is equally valid with standard total collection procedure. They also indicated that the simultaneous use of two digestibility indicators (one internal and one external) gives excellent results in measuring both dry matter consumption and digestibility of forage by grazing animals.

Later, Lancaster (1954) developed a linear equation for cattle: $Y = X + 0.9$, where Y = feed/feces organic matter ratio and X = fecal nitrogen (dry basis). In view of the criticism of his method by Forbes (1949) and Woolfolk, Richards, Kaufman, Martin, and Reid (1950), Lancaster showed that the method leads to estimations of intake of sufficient accuracy for a wide variety of purposes and

the dependence of the method on a reliable and simple chemical estimation, like that of nitrogen, makes it much more satisfactory for large-scale field work than other methods such as those based on chromogen or lignin which have yielded more precise estimates in closely controlled digestibility trials.

Later, Kennedy, Carter, and Lancaster (1959) found that the evidence strongly favors use of a quadratic or logarithmic regression formula rather than a linear one as proposed by Lancaster (1954). The "best" formula for the prediction of the feed/feces organic matter ratio (Y) were:

$$Y = 10.57 \log X_2 - 1.74 \pm 0.32 \ (7.8\%)$$

$$Y = 4.32 X_3 - 0.42 X_3^2 - 5.51 \pm 0.32 \ (7.7\%)$$

$$Y = 10.34 \log X_3 - 1.33 \pm 0.35 \ (8.4\%)$$

$$Y = 3.81 \log X_1 + 2.24 \pm 0.44 \ (10.6\%)$$

$$Y = 7.96 \log X_2 + 1.06 \log X_1 - 0.82 \pm 0.31 \ (7.4\%)$$

where X_2 and X_3 measure fecal nitrogen in undried and dried feces, respectively, and X_1 measures fecal pigments at 415 mμ. Each equation is followed by the standard deviation and the coefficient of variation. It was found that the formula proposed by Reid *et al.* (1952) was in general not satisfactory for predicting dry matter digestibility from fecal pigment concentrations in 37 consecutive weekly trials with cows fed cut pasture herbage. For estimating the feed/feces organic matter ratio, fecal nitrogen (with a prediction error of 8%) provided a more reliable index than fecal pigments (error, 11%). However, the use of fecal pigments in addition to fecal nitrogen led to a small but significant reduction in the error

of prediction (to 7%). The effects of species and seasonal variations on the regression of digestibility on fecal nitrogen concentration have been studied (Lambourne et al., 1962, 1963; Langlands et al., 1963). The different equations obtained by different investigators under different conditions demonstrate the necessity for using a regression obtained under the specific condition of the study.

The acid soluble fecal fraction (ASFF)

The fecal index method as described in the foregoing discussion has been criticized by Owen (1961) on the grounds that regression equations developed at one level of intake indoors are applied to studies of grazing animals at different levels of intake. An alternate procedure was therefore suggested for estimating the dry matter intake of grazing animals. He reported from studies with sheep that a particular fraction of the feces, readily dissolved by treatment with dilute acid, was closely related to the intake of dry matter. To determine this fecal fraction, feces were quantitatively collected and 1 g of the ground dried feces was allowed to stand in 150 ml of 0.2 N hydrochloric acid for 18 hours at room temperature. After filtering through a paper disc in a Gooch crucible, the amount of this dissolved fecal fraction was determined and the total daily production calculated. From further studies involving a range of feeds, including fresh and dried herbage, fed at different levels of intake to ewes and wethers, it was found that for eleven of these feeds, including grass-clover mixtures, the relationship between daily dry matter

intake and the daily dissolved fecal fraction output in grams can be expressed by the following equation:

$Y = 250 + 17.8\ X$, where Y = dry matter intake, (g), and X = dissolved fecal fraction output, (g). For two other legume feeds, lucerne and a clover mixture, the author reported that this fecal fraction output was higher than that for grass and grass-legume mixtures at comparable levels of dry-matter intake, but no data was given.

After analyzing the data statistically, it was found that only 2 - 3% error was associated with this procedure when using five animals or more per group and Owen (1961), therefore, recommended that it should be suitable for estimating the free grazing intake of sheep without the need to develop separate "local" regressions for each type of sward grazed.

This method has been further evaluated by Langlands and Corbett (1964) in studies with sheep and cattle. The relationship between daily dry matter intake (Y) and daily excretion of the acid soluble fecal fraction (X_1) both measured in g in sheep was:

$$Y_1 = 512 + 16.3\ X_1 \qquad (1)$$

The 95% confidence limits for the regression coefficient included the value of 17.8 used by Owen (1961). When this value was adopted, the relationship became:

$$Y_1 = 465 + 17.8\ X \qquad (2)$$

The intercept in this equation (465g) is much higher than the value of 250 g reported by Owen (1961).

These linear relationships were criticized by Langlands and Corbett (1964) on the grounds that they imply no ASFF excretion at intakes of less than 512 g (equation 1) or 465 g (equation 2) dry matter per day. A logarithmic form was, therefore, proposed for this relationship by these workers combining observations of both sheep and cattle:

$Y_2 = 1.6734 + 0.907\ X_2$, where $Y_2 = \log_{10}$ daily dry matter intake, g, and $X_2 = \log_{10}$ daily ASFF excretion, g. When the relationships for sheep, cows, and steers were examined separately, the regression coefficients could be pooled, but the intercepts could not ($P < 0.001$). When the relationship between the intake factor (100/100 - organic matter digestibility, this factor when multiplied by fecal O.M. output gives O.M. intake) and the percent ASFF in the dry matter was examined, significant differences were found when one pasture was cut at different seasons of the year, when different species of herbage were given at the same seasons of the year, and when sheep, steers, and cows were given the same herbage. The precision of the two indicators, fecal nitrogen and ASFF was studied by comparing their correlations with the intake factor. The precision of the fecal nitrogen relationships was similar to or greater than that of the ASFF relationships (Table 11). Langlands and Corbett (1964) concluded that their studies do not endorse Owen's conclusions (1961) that the ASFF would provide a fecal index applicable to a wide range of herbage and animals and would overcome the need to derive specific equations for estimating intake restricted to individual growths of herbage. They

found that even with relationships restricted to single growths, ASFF was less precise and less reliable than the fecal nitrogen technique.

Methoxyl and Fiber

Methoxyl is contained in lignin in a firm attachment (Rogozinski and Starzewska, 1927; Phillips, Weihe, Jones, and Csonka, 1929; Phillips, 1934) and the methoxyl and lignin contents of plants increase as the plants mature (Phillips and Goss, 1935; Phillips, Goss, Davis, and Stevens, 1939; Forbes and Garrigus, 1950; Ely, Kane, Jacobson, and Moore, 1953). Attempts have, therefore, been made to use methoxyl groups as a digestibility index in forage evaluation especially since methoxyl is a more clearly defined chemical entity and is more easily measured than lignin (Richards and Reid, 1952; Ely et al., 1953; Richards and Weaver, 1957; Anthony and Reid, 1958). Richards and Reid (1952) found in a study with three different growth stages of pasture herbage that the digestibility of forage dry matter was negatively correlated with the amount of lignin (-0.99) and of methoxyl (-0.99) in the forage consumed by steers. When fecal lignin and fecal methoxyl contents were correlated with dry matter digestibility, the respective correlation coefficients were -.097 and 0.43. In another study, Richards and Weaver (1957) reported a lower negative correlation coefficient for the relationship between dry matter digestibility and methoxyl content of the feces (-0.73). This is similar to the correlation coefficient of -0.74 reported for cattle by Anthony and Reid (1958) from 107 observations obtained from digestibility trials with three forages of different botanical compo-

sition which were harvested at different stages of maturity.

These authors concluded that the availability of more precise techniques such as the chromogen method precludes wide use of fecal methoxyl as an indicator of forage digestibility. They, however, suggested that fecal methoxyl may be employed to screen forages differing markedly in digestibility (Reid, 1962).

A study of the correlation between herbage digestibility and the fiber content of the feces was suggested by Raymond (1949). Later, Richards and Reid (1953) pointed out that the fecal content of crude fiber is correlated with herbage digestibility. The use of "macerate crude fiber" as a fecal indicator was proposed by Raymond, Kemp, Kemp, and Harris (1954) who established linear regression based on data from 40 herbage feeds relating digestibility and fecal content of "macerate crude fiber". These authors suggested that "macerate crude fiber" could be used alone as a fecal indicator or in combination with chromogen in a multiple regression to improve the precision of the chromogen method. Later, "normal-acid fiber" was proposed as a fecal index for the estimation of herbage digestibility (Walker and Hepburn, 1955; Raymond, Jones, and Harris, 1955; Griffith and Thomas, 1955). Raymond et al. (1955) found that the correlation coefficient between fecal "normal-acid fiber" and organic matter digestibility of herbage was -0.93, but the relationship did not apply to all type of herbage.

The following discussion is an attempt to give a summarized

evaluation of internal markers. Conner, Bohman, Lesperance, and Kinsinger (1963) and Ridley, Lesperance, Jensen, and Bohman (1963) concluded that the chromogen technique was more reliable than the lignin method for the determination of apparent digestibility and forage intake of range cattle. McCullough (1959) noted that the validity of the chromogen method for the measurement of indigestibility has been supported by numerous conventional digestion trials with both cattle and sheep and that the simplicity and accuracy of this technique make it the technique of choice in digestibility trials with most forages. Raymond, Kemp, Kemp, and Harris (1954) and Kennedy et al. (1959), however, showed that fecal nitrogen was a more satisfactory indicator in predicting dry matter digestibility than chromogen. Since the marker ratio technique is preferred to the fecal index method, chromogen was earlier considered a better indicator than fecal nitrogen because it (chromogen) can be used in the ratio technique (because it is essentially indigestible) while fecal nitrogen had to be used only in the fecal index method which requires the establishment of regression equations prior to the study under grazing conditions. Later, Reid et al. (1952) found that the use of the chromogen ratio technique is feasible only in trials in which hand-feeding is practiced. Based on data available by Smith and Reid (1955) and by Kennedy et al. (1959) and until a better indicator is found for digestibility studies with grazing animals, the use of either indicator, chromogen or fecal nitrogen, in the fecal index method is equally valid. Kennedy et al. (1959) has suggested that

the use of the two indicators combined increased the accuracy.

The acid soluble fecal fraction (Owen, 1961) is another indicator for use under grazing conditions in the fecal index method. Since the two reports available on the use of this indicator show clear contradiction regarding its precision, further work including direct comparisons with the chromogen and fecal nitrogen method is required to verify its usefulness.

The use of silica, lignin, methoxyl, and fiber as internal markers should be minimized until better methods to justify their use become available. When lignin is used in studies of digestion in the rumen, its recovery should be confirmed.

APPLICATIONS

The variety of information provided by markers in nutritional studies includes: (a) the amount of food or specific nutrient eaten; (b) the extent of passage; (c) the rate of passage of ingesta through all or any part of the alimentary tract; (d) the digestibility of all or part of the food; and (e) the utilization of nutrients or food in a balance study. These are discussed here. The special techniques used to determine consumption and digestibility of forage by grazing animals are presented separately. The use of markers for determination of the volume of aqueous media (e.g., rumen contents) is discussed at the end of this section.

FOOD INTAKE

Excepting the use of markers to determine the amount of

forage eaten by grazing animals, dietary markers have been little used to determine the amount of food intake of animals or humans. The inexact status of the usual direct and indirect methods for evaluating food intake in humans suggests that dietary markers might play a role in future studies for evaluating food intake. It is interesting that even when a small group of persons is completely isolated as in simulated astronaut conditions, the records of food intake may be far from 100% accurate and open to question. Since there are other times when it is virtually impossible for food intake to be monitored exactly, i.e., the diet of astronauts during space flights, a new method for evaluation of diet intake is proposed based upon the use of nutrient indicators for each nutrient to be studied. We are in the process of evaluating this method.

The nutrient utilization system would make use of inert markers for the determination of total food or a given nutrient intake in a manner comparable to that which has been used to study the food digestibility of grazing animals. The marker to be used would be added to the diet in the ratio predetermined for either the total quantity of food or any given nutrient which it is desirable to follow, i.e., protein, fat or carbohydrate. The total fecal output would be determined directly if possible by quantitative collection or indirectly by the use of markers. Analysis for the marker quantity in the feces will allow calculation of the quantity of that nutrient ingested and information could be obtained to allow calculation of digestibility of either

the complete diet or any nutrient of the diet. This proposition faces one major challenge regarding the proper mixing of minute amounts (e.g., ppm or ppb) of the selected marker with various foods to give a uniform nutrient/marker (or food/marker) ratio. Today's advancing technology should be able to provide a satisfactory solution to this problem.

Markers can also be used in a qualitative way to check on the ingestion of certain drugs. Riboflavin has been used as a marker for checking on the ingestion of isoniazid pills by tubercular patients. The consumption of isoniazid pills containing 0.8 mg of riboflavin per kg of body weight produced a urinary excretion of riboflavin exceeding the normal level even at the end of the twenty-fourth hour. When another pill was taken on the second day, the level of riboflavin in the urine continued to rise. Analysis of urine samples can, therefore, be used in this method to evaluate patients' ability to follow a regular program of drug therapy on an out-patient basis (Hobby and Deuschle, 1959; Deuschle, Jordahl, and Hobby, 1960; Berry, Ross, and Deuschle, 1963; Nutrition Rev., 1965).

The easy way for the determination of exact food intake in experimental animals has not been very satisfactory in these reviewers opinion. If a metabolism cage with the diet compartment removed from the main cage is not used, then one must contend with spillage of food into the area of the feces and/or the urine. Separation of food from feces is inexact unless hard pellets are voided. Unless the animal is restricted, most of the

laboratory animals will exhibit coprophagy which should not be allowed in strict observation of food intake unless this material were also quantitative before it was ingested. All methods of restriction are open to some criticism concerning the physiological and psychological state of the animal. Restricting the movement of rats or mice has not resulted in acceptable growth. Liquid diets, pelleted diets and diet in capsule or diet which flows may be introduced directly via stomach tube into the stomach of animals. This allows exact measurement of the quantity of food and has been used extensively by some investigators. Some animals may regurgitate part of the food and not allow sufficient food to be given by stomach tube to allow growth and normal survival. Another criticism of this method is the amount of manpower time involved in feeding individual animals on a daily basis. Feeding of animals or people through oral or nasal tubes on a continuous basis has not been satisfactory and is not used extensively despite the fact that this could provide an exactly monitored intake.

The method proposed for labeling food to give an exact quantity of food or any one nutrient has not been experimentally worked out in animals. It has hereby been proposed for animals and for human work.

Domestic or other grazing animals can be maintained in pens with measured food intake, urine and feces, as discussed above for laboratory animals. However, practical considerations present

a greater challenge. How much forage does a grazing animal eat in the field and what is the digestibility of that material? Food consumption and digestibility studies in grazing animals have been so closely interlinked that separate discussions would involve needless repetition. Therefore, discussion of the food intake of grazing animals is deferred to the section entitled "Determination of the Consumption and Digestibility of Forage by Grazing Animals".

EXTENT OF PASSAGE

Having determined qualitatively (i.e., that the pills prescribed were taken) or quantitatively that material was ingested, the next procedure is to determine qualitatively that the material did pass through most or all of the alimentary tract. Classically, this problem has been approached with markers to determine if, as well as when, material is passed completely through the alimentary tract. The appearance of the stool itself is adequate evidence that complete blockage is not occurring. Radioactive markers given to experimental animals and radio-opaque material in general use are tools used to determine not only the rate of passage of ingested markers or the placement of the bulk of the marker at any given time, but also whether the material has stopped for any reason. The bolus of digesta may be stopped by a functional disturbance, i.e., spasms, atonicity, atrophagia or dysphagia. Or the blockage may have an anatomical base, i.e., diverticula, constrictions, volvulus, intususeption or other obstruction.

The common procedures are to ingest iodized oil or barium sulfate prior to fluoroscopy or an x-ray photograph. Radioelemeter capsules may also be useful. These procedures are equally valuable and more frequently used for rate of passage studies.

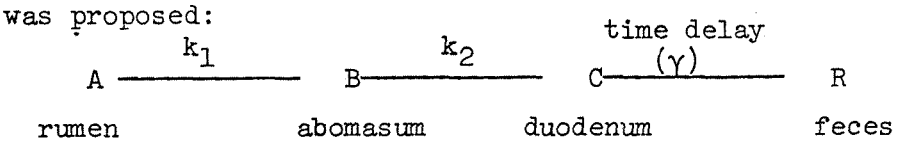
PASSAGE OF INGESTA

Terms used to describe the passage of ingested material through the digestive tract include transit time, retention time, rate of passage, rate of flow, rate of transport. Revised definitions of these terms have been presented earlier in this review. From the nutritional viewpoint, the transit time of digesta is one of the important factors that determines the efficiency of utilization of a given amount of food; other important factors include the rate of digestion, the nature of the absorbed end-products of digestion, and the requirements of the animal (Balch and Campling, 1965). From the physiological point of view, studies of food passage have contributed to the understanding of the nature of movements along the digestive tract and the influence of various drugs on these movements.

Mathematical models and analyses of food passage have been presented by Blaxter, Graham, and Waiman (1956), Brandt and Thacker (1958), Marcus and Lengemann (1962), Hungate (1966), and Sikov, Thomas, and Mahlum (1969).

Based on earlier conclusions (Balch, 1950; Phillipson, 1952; Piana, 1952) Blaxter, Graham, and Wainman (1956) suggested that the passage of ingesta through the digestive tract of ruminants be

regarded as a kinetic process. The following model system was proposed:



where A, B, C, and R represent amounts of a unit of food in the compartments concerned. Results obtained using the stained particle technique fitted a simple kinetic equation with three constants (k_1, k_2, γ). The subsequent manipulation of this equation permitted estimation of diurnal variation in feces production. However, direct experimentation was not carried out to show that these constants actually represent events taking place in the specific parts of the tract.

A model based on a hydraulic flow through two volumes in which complete mixing takes place was proposed by Brandt and Thacker (1958). Using radioactive chromic oxide ($^{51}Cr_2O_3$), they showed that the passage of digesta is an exponential function and that a single volume model with a single "half time" was sufficient to describe the flow of feed residues in the rabbit. For ruminants, however, a two-volume model with two half times was proposed. The equation describing this ruminant model is essentially similar to that developed by Blaxter et al. (1956).

Hungate (1966) suggested a two pool model for ruminants where the coarse particles in the pool to be ruminated have not yet entered the pool of material leaving the rumen, but constitute a separate pool which feeds it. The average rate at which small

particles leave this rumination pool is proportional to the concentration of coarse particles in the pool, and in consequence, the passage from the coarse to the small particle pool follows the kinetics of a first-order reaction. This precedes the passage of the comminuted particle from the rumen liquid-small particle pool into the omasum. Total passage of initially large particles from the rumen is thus the resultant of two sequential first-order reactions. This is in line with the mathematical model suggested earlier by Blaxter and associates (1956).

Several methods have been used to calculate retention time of food residues in the digestive tract or certain segments of it. Balch (1950) used the stained particle technique with cows and suggested the "5% excretion time" as a measure of the time required for food residues to traverse the omasum, abomasum and intestines and the interval of time between excretion of 5 and 80% of the particles as indicative of the time taken for passage through the ruminoreticulum. These excretion times are calculated from the cumulative excretion curve (Fig. 2).

The "mean retention time" was suggested by Castle (1956a) to facilitate comparison between different studies. Excretion curves for the marker are established as shown in Fig. 2. The "R" value which is directly proportional to the area to the left of the curve was calculated by adding together the time of excretion from 5 to 95% at intervals of 10%, taken from the graph, and dividing the sume by 10. This value is taken as an arbitrary measure of

the mean retention time of the marker in the alimentary tract. A somewhat similar term is the "mean time" used by Blaxter, Graham, and Wainmann (1956) which is calculated as the sum of times which individual stained particles spent in the tract divided by the number of stained particles excreted. This value may be slightly different from Castle's "mean retention time" because it gives weight to particles excreted beyond the 95% excretion time. A comparable concept to that of Blaxter and associates (1956) is the "food dry matter point" which was introduced by Mäkelä (1956).

The use of a single value (e.g., mean retention time or mean time) was seen by Brandt and Thacker (1958) as inadequate to describe the shape of the flow curve for the ruminant. They suggested that the expression of the parameters as half time is a concept which is easier to grasp. The use of two half times was suggested by these authors as an alternative to Balch's 5 and 80-5% excretion times on the grounds that a two volume model with two half times provides convenience in handling experimental data and that Balch's measures have no mathematical basis.

A comparison of Balch's 5% and 80-5% excretion times, Castle's mean retention time (R), Blaxter and associates' mean time, and Brandt and Thacker's t_1 and t_2 as measures of retention time has been reported by Shellenberger and Kesler (1961) who used the stained particle technique with cows. The results of this comparison as shown in Table 12 show little or no difference between

"mean retention time" and "mean time".

Another parameter that has been used as an indicator of retention time is the turnover time. Turnover time has been defined by Hungate (1966) as the time required for entrance of an amount of feed equal to that represented in the rumen. Warner (1966) used the term "mean residence time" to mean turnover time. This was defined as the average time spent by a particle in the rumen which may be calculated as the time needed for the number of particles leaving the rumen to equal the average number in the rumen during the period of observation, provided there is no destruction of particles within the rumen. The turnover time is, therefore, equal to the average time that particles of digesta remain in the rumen. Mathematical analysis of the turnover time has been presented by Hungate (1966). To calculate turnover time by Hungate's equation, the fraction of the original marker retained in the animal at any time is plotted against time after consumption of the marked diet. The turnover time is then calculated from the times at which certain percentages of the marker have appeared in the feces. Hungate calculated turnover time from Castle's data (1956) obtained with goats using the stained particle technique on the basis of 5 and 95% retention times. Turnover times so obtained agreed with Castle's "mean retention time". The half-life may be used as an indicator of the turnover time since it is approximately 0.693 of the latter. Hungate (1966) pointed out that of the various parameters, turnover time is most useful

because it is easily related to the daily food intake, rate of food passage, and amount of digesta in the rumen.

Passage of markers through the various segments of the digestive tract of the rat has been studied by Thompson and Hollis (1958), Marcus and Lengemann (1962), and Sikov et al. (1969). Marcus and Lengemann (1962) used segmental "transport rate" and segmental "half times" as measures of the passage of ^{91}Y through different segments of the tract. To accomplish this, rats were killed at predetermined time intervals after feeding and the percentage of the ^{91}Y dose present in a particular protion of the digestive tract was plotted against time for each of the consecutive alimentary segments; each curve was then resolved into input and outgo components. From the input curves, segmental transit half times were determined. This value is the time at which one-half of the dose has left the segment since input to one segment equals output from the preceding segment.

The foregoing discussion demonstrates the need for a standard system to express retention time of food residue in the alimentary tract. If passage in the tract is indeed an exponential function as suggested by Brandt and Thacker (1958) then the use of the "half time" concept is the appropriate way to express the rate of this passage. To give a complete description of retention time, we propose the following system. For ruminants the turnover time is calculated as presented by Hungate (1966) to give an account of the average retention time. In addition to the turnover time, the two half times as suggested by Brandt and Thacker

(1958) are calculated to describe the shape of the flow curve. For monogastric animals, only the half time is calculated as presented by Brandt and Thacker (1958) for the rabbit. This one half time should be sufficient to describe the flow curve for these animals and when divided by 0.693, it should give a value equivalent to that of the turnover time or average retention time.

DIGESTIBILITY STUDIES

In general there are two major advantages sought by those who use the indicator method in digestibility studies. First, the possibility of eliminating total quantitative collection of the feces and the use of, instead, random sampling (grab) methods. Especially in studies with large animals, this advantage has great appeal in terms of cost and labor. In fact, if the indicator method failed to offer this advantage, it will become useless in large animal studies. For the indicator method to offer this advantage, the sampling error should be very small so that random grab samples will be representative of the 24-hr average. This problem will be discussed in the section of feces sampling. The second advantage of the use of inert indicators in digestibility and retention studies is the ability of the investigator to correct the data for fecal losses. This is a very important procedure especially in human metabolic balance studies since collection of feces are usually incomplete because of loss on toilet paper and in general manipulation. A recovery of less than 100% of the indicator may be due to fecal losses which may

have gone unrecognized and a correction may, therefore, be necessary. So, when Whitby and Lang (1960) and Rose (1964) recovered only 93% of the nonabsorbable inert marker, chromic oxide, they assumed 7% fecal loss and made the necessary correction. In addition to these advantages, the inert indicator method was found more suitable than the conventional intake-excretion method in the study of absorption of such readily exchangeable substance as cholesterol (Stanley and Cheng, 1957).

The conventional "time-collection" method for determination of the apparent digestibility of the diet and various nutrients requires complete collection of the feces for the period of the digestion trial. In studies with humans, some laboratories (Nordin, 1962) arbitrarily discard stool collection of the first 24 to 48 hours after the start of the study and analyze those obtained thereafter, thus assuming that the residues from a meal progress through the gastrointestinal tract within a day or two. This problem is even more complicated in ruminant studies in view of the more complicated nature of the digestive tract.

The "time-marker" method was employed by many investigators to help identify the residues of a given meal and, therefore, allow a more accurate quantitative collection of the feces. In this method, as described by Bergeim (1926), a carmine capsule is given at breakfast of the first day of the study and a uniform diet is then fed for a period of 5-7 days, another carmine capsule being given at the last meal of the period. Feces are collected and that lying between the two carmine markers and including that

colored with the first carmine are kept for analysis. The success of this method depends upon the clear recognition of the marker in the feces and the accurate collection of all feces passed during the period of study. Periods of less than 5 days have not generally proven practicable because of the difficulty of obtaining a satisfactory separation of the feces for the experimental diet (Bergeim, 1926; Reifenstein, 1945). The disadvantages of using a nonabsorbable dye for this purpose have been discussed in the previous section. Note that carmine may become mixed with the diets of preceding and following days, and, in certain cases where the feces are of a very soft consistency, the error thus introduced may be considerable. Additional difficulties, such as food scattering and contamination of the food with the excreta and the feces with urine, have limited the use of this method in the small laboratory animal (Bergeim, 1926). Many of these difficulties were overcome by the use of a suitable inert indicator.

The "Inert-Indicator" Ratio Method

As early as 1847 Wildt proposed the use of an inert material in studies of food utilization. Edin in 1918 was first to suggest Cr_2O_3 as a digestibility index. A summarized description of the "Edin's Indicator Method" for the determination of the digestibility of feeds and feed mixtures was later published in English by Edin, Kihlen, and Nordfeldt in 1944. Bergeim (1924) suggested iron oxide as an indicator for

the study of intestinal reductions and later (1926) as a marker for nutrient utilization studies.

These two investigators were mainly responsible for the development of the indicator ratio method. The method has been used by many investigators, and many other markers have been added to the list as previously presented.

By combining the "time-markers" and the "inert-indicator" ratio methods, Rose (1964) introduced a new improvement in the technique. This new method combines the advantages of the two methods thus allowing more accuracy in the identification and measurement of the fecal output for a particular intake of food. Rose (1964) used carmine red and chromic oxide, and Sharpe and Robinson (1970) used brilliant blue and chromic oxide.

The theory of the inert-indicator ratio method for the determination of digestibility has been discussed by Bergeim (1926), Stanley and Cheng (1957), and by Kleiber (1961). The theory as outlined by Kleiber will be given here.

If:

I = daily intake of food

F = amount of feces excreted per day

A_i and A_f = concentrations of the indicator in food and feces, respectively,

then:

amount of indicator taken in per day = $I \cdot A_i$

amount of indicator excreted daily = $F \cdot A_f$

Since the indicator is indigestible, the amount excreted must equal the amount ingested. Thus,

$$I \cdot A_i = F \cdot A_f \quad \text{or} \quad \frac{F}{I} = \frac{A_i}{A_f} \qquad (1)$$

The equation for apparent digestibility (D) is $D = \frac{I - F}{I} = 1 - \frac{F}{I}$ (2)

or, replacing F/I by A_i/A_f (equation 1), $D = 1 - A_i/A_f$ (3)

Therefore, the apparent digestibility is equivalent to one minus the ratio of the indicator concentration in food and in feces. The above derivations assume the "dry basis" analysis of food and feces and the digestibility given in equation 3 is, therefore, for the dry matter of the food.

Now consider the digestibility of a given nutrient, nitrogen for example. If:

n_i and n_f = concentrations of nitrogen in food and feces, respectively,

then:

$I \cdot n_i$ = daily nitrogen intake, and

$F \cdot n_f$ = daily nitrogen excretion.

Then the apparent digestibility of nitrogen is:

$$\frac{I \cdot n_i - F \cdot n_f}{I \cdot n_i} = 1 \frac{F \cdot n_f}{I \cdot n_i} \qquad (4)$$

The ratio (F/I) may be replaced by the ratio of the indicator concentrations (A_i/A_f), therefore,

$$D_n = 1 - \frac{n_f}{n_i} \times \frac{A_i}{A_f}$$

where:

D_n = apparent digestibility of nitrogen

n_i and n_f = nitrogen concentrations in food and feces (on dry basis), respectively

A_i and A_f = concentrations of indicator in food and feces (on dry basis), respectively

Therefore, the apparent digestibility coefficient of a given nutrient is:

$$\text{Digestibility, \%} = 100 - 100\left(\frac{\text{\% indicator in food}}{\text{\% indicator in feces}} \times \frac{\text{\% nutrient in feces}}{\text{\% nutrient in food}}\right)$$

The foregoing discussion is based on the assumption that the indicator is completely inert and non-absorbable and, after reaching equilibrium the excretion of the marker equals the intake. When the indicator is ingested, its content in feces rises from zero to a level which becomes fairly constant for a given individual fed a particular diet. The time needed for fecal excretion of a suitable indicator to become steady depends partly upon the dietary regiment and partly on the individual (Whitby and Lang, 1960).

A period of "adjustment" is, therefore, required before starting the experimental period. This adjustment period in human was found to be two days or longer by Stanley and Cheng (1957). Whitby and Lang (1960) using Cr_2O_3 pointed out that the steady level of Cr_2O_3 excretion has failed to be attained within 72 hrs of starting Cr_2O_3 dosage in approximately one-quarter of the balances. Sharpe and Robinson (1970) showed that 6 days were required before the output equaled the intake of Cr_2O_3 and, therefore, recommend administering the marker for a 6-day preliminary period.

In view of the complexity of the digestive system in ruminants, more variations have been reported and preliminary

periods of at least 10 days have been suggested (Brisson, Pigden, and Sylvestre, 1957; Davis, Byers, and Luber, 1958; MacRae and Armstrong, 1969).

Clearly, on no single day is the excretion of indicator likely to be exactly equal to the intake but over a period of time the excretion of indicator will quite closely approximate the intake of this substance (Stanley and Cheng, 1957; MacRae and Armstrong, 1969). The length of the preliminary period would be a major factor in determining the length of time of the experimental or collection period in order to eliminate errors in sampling, and therefore, achieve a 100% recovery (Davis et al., 1958). Because of the known variability in the frequency and amount of human daily fecal excretion, 6 to 9 day collection periods have been recommended (Reifenstein, Albright, and Wells, 1945; Stanley and Cheng, 1957). Whitby and Lang (1960), however, showed that, after an adequate preliminary period on the balance regimen, it is practicable to base each investigation on two collection periods, each of three days, although a third 3-day period would act as a safeguard by revealing the presence of a trend due to some unforeseen and uncontrolled variable in the experiment. In ruminants, no significant differences were found between data of 6-day and 10-day total collection periods. With Cr_2O_3 as an inert indicator, a 100% recovery was reported with cattle for a 5-day collection (Corbett, Greenhalgh, Gwynn, and Walker, 1958) and a 10-day collection (Davis et al., 1958). A 6-day preliminary

and a 6-day collection period seem reasonable in human nutrition (Rose, 1964; Sharpe and Robinson, 1970). In ruminants, however, a 14-day preliminary period should precede a 7-day collection period (MacRae and Armstrong, 1969).

BALANCE STUDIES

If it is desired to determine the amount of a given nutrient retained in the body, one should account for all losses of this nutrient in the excreta. Feces, urine, integument losses, sweat and the expired gases are routes by which different nutrients or their metabolites are excreted. The latter two routes become of some significance only under certain conditions (e.g., under hot environmental conditions and when the retention study involves metabolites or certain elements as selenium of which a significant portion is carried out by the expired air). The discussion here applies only to those nutrients that are excreted in the feces and the urine under mild environmental conditions (negligible sweat).

In the conventional balance method, the urine and the feces are quantitatively collected and analyzed for the nutrient under study. The food is also analyzed and the nutrient intake is determined. The difference between the intake and the excretion gives the nutrient retention.

An inert marker can be used to determine the amount which is excreted in the feces as has been previously discussed, and the urine can be quantitatively collected and sampled for analysis.

Recently, Furuya, Takahashi, and Kameoka (1970) have devised a method using a urinary indicator for the estimation of the pro-

portion of absorbed nitrogen that was retained in rats and pigs. Using this method, they were able to eliminate the necessity of quantitative collection of feces and urine. Chromic oxide was used as a fecal marker and dietary potassium as a urinary indicator. They suggested the following formula to calculate nitrogen retention:

Retained N (% of absorbed) =

$$100 - \frac{(1 - D_{cr}/F_{cr} \times F_I/D_I}{(1 - D_{cr}/F_{cr} \times F_N/D_N} \times \frac{U_N}{U_I} \times R$$

where:

D_{cr}, D_N, D_I = concentration of Cr_2O_3, N, and indicator (K), respectively, in the diet

F_{cr}, F_N, F_I = concentrations of Cr_2O_3, N, and indicator (K), respectively, in the feces

U_N, U_I = concentrations of N and K, respectively, in the urine

R = the recovery of the indicator, which shows the percentage of indicator (K) excreted in urine relative to that of the indicator absorbed during the experimental period.

The recovery of potassium was calculated as follows:

$$\text{Recovery of K (\%)}, R = \frac{K_{ab} - K_{ret}}{K_{ab}} \times 100$$

Where:

K_{ab} = the amount of absorbed K as determined by Cr_2O_3

K_{ret} = the amount of retained K

The amount of absorbed K was calculated as follows:

$$K_{ab} = FI \times D_K \times (1 - D_{cr} / F_{cr} \times F_N / D_N)$$

where:

FI = the amount of food intake

D_K = K content in the diet

Linear relationships were established between potassium retention and body weight gain for the two species:

$$Y = (2.44 \times 10^{-3}) (X) - 0.002 \quad \text{rats}$$

$$Y = (1.83 \times 10^{-3}) (X) \quad \text{pigs}$$

where:

Y = amount of retained K in grams

X = body weight gain in grams

When the conventional method was compared to this method in determining N retention (% of absorbed), a close agreement was found as reflected by a correlation coefficient of 0.97 for both rats and pigs.

It was pointed out by these authors (Furuya et al., 1970) that the urinary indicator to be used in this method should satisfy the following requirements:

1. It should be easily absorbed into the body and should not be decomposed.
2. It should have a negligible retention in the body or retention is easily determined.
3. There should be little variation with time in the nutrient/indi cator ratio in the urine since it is desirable to obtain a representative sample at as few

collecting times as possible.

4. The indicator should be easily and uniformly mixed with other components of the diet and should be easily and accurately determined.

They pointed out that potassium was chosen as their urinary indicator because it is highly absorbable, its recovery is assumed to be about 100% in the adult and in the growing animal its retention can be estimated from body weight gain, and it is easily and accurately determined by flame photometry.

The disadvantages of using potassium as a urinary indicator should not, however, be overlooked. First, K is not 100% absorbed and the use of a fecal marker to determine its digestibility might introduce an error in the quantitative recovery of K. Second, potassium is partly retained in the body especially by growing animals. The use of an indirect parameter, e.g., weight gain, to determine retention introduces another error and requires the establishment of linear equations in a preliminary trial in which total urine collections have to be made. The linear equations should be derived under the specific conditions of the study. And last, conditions that result in body K loss in urine or dietary K in sweat will constitute an error in the quantitative recovery of K. It should be pointed out here that the authors (Furuya et al., 1970) did not report any data on uniformity of K excretion in urine and the sampling errors involved. This information is promised in a later publication. This is very

important because it has bearing on the main advantage of this procedure, namely to eliminate total urine collection.

A urinary indicator that satisfies the requirements previously suggested and eliminates the errors discussed, would certainly make this procedure of great use in metabolic balance studies.

CONSUMPTION AND DIGESTIBILITY OF FORAGE IN GRAZING ANIMALS

The search for a satisfactory indirect method for determining the consumption and apparent digestibility of forages in grazing animals has been in progress for many years. Available methods have been reviewed by Valentine (1956) and in "Inter-society Forage Evaluation Symposium" (1959) and "Pasture and Range Research Techniques" (1962). The latter two publications were prepared through joint efforts by the American Society of Agronomy, the American Dairy Science Association, the American Society of Animal Production, and the American Society of Range Management. Other related works include that by Harris, Lofgreen, Kercher, Raleigh, and Bohman (1967) and by McDonald (1968). Two main classes of markers have been used in this type of study: internal markers which occur naturally in feedstuffs such as lignin, chromogen, nitrogen or silica and external indicators which originate outside the diet such as chromic oxide.

The introduction of the internal indicator concept made the measurement of nutrient digestibility of forage grazed by animals simpler and less costly. In general, a good internal indicator should possess the following features (Reid, Woolfolk, Richards,

Kaufman, Loosli, Turk, Miller, and Blaser, 1950):

a) It occurs naturally and in a measureable quantity in the feedstuffs.

b) It should be indigestible, and therefore, completely recoverable in the feces.

c) Its chemical analysis is simple, accurate, and rapid.

d) Its recovery from the feces must not be influenced by treatment of the feed (curing methods, heat, etc.), by stage of maturity or by irregular passage of the indicator through the gut.

e) Its equilibrium in the feces with that in the feed must be established soon after feeding is started in order that short time trials may be used.

With the use of a suitable internal indicator, nutrient digestibility can be determined by either one of two methods:

1. The ratio technique: The concentrations of the indicator used and the nutrient in question are determined in the feed and feces and the apparent digestibility coefficient is then calculated as previously discussed.

2. The fecal index method: The use of the ratio technique was seen by Reid, Woolfolk, Hardison, Martin, Brundage, and Kaufman (1952) as feasible only under conditions in which the samples of feed obtained for analysis truly represent the feed consumed by the experimental animals, a situation that exists only in trials in which hand-feeding is practiced. This problem can be solved by the fecal index method where the forage is clipped and fed to the experi-

mental animals in a conventional digestion trial in which the forage consumed and the feces voided are quantitatively measured. Representative samples of the feed and feces are analyzed for the internal indicator and the nutrients in question. The relationship between the digestibility of a given nutrient and the concentration of the internal indicator in the feces is established by a regression equation. Representative samples of the feces voided by animals grazing this type of forage can then be analyzed only for the indicator. The concentration of the indicator so obtained can be inserted into the regression equation to calculate the apparent digestibility. It should be pointed out here that a regression equation established under a given condition does not necessarily apply under other conditions.

Unlike the ratio technique, internal indicators used in the fecal index method need not be completely indigestible. A wide range of indicators have, therefore, been used including fecal nitrogen (Lancaster, 1949; Raymond, Kemp, Kemp, and Harris, 1954), plant chromogens (Reid et al., 1952), methoxyl (Richards and Reid, 1952), and fiber (Raymond et al., 1954; Raymond, Jones, and Harris, 1955). Of these, only fecal nitrogen and plant chromogens have been extensively studied and are considered more reliable than others. Although the fecal index method is considered more reliable than the ratio method (Raymond et al., 1954; Reid, 1962), two main problems related to representative sampling pose a threat to the precision of this procedure. First, there is the problem of fecal

sampling due to diurnal fluctuations. A discussion of the different methods of feces sampling is presented later. The second problem is related to forage sampling. The sampling of forage under study for chemical analysis is carried out only during the pre-grazing conventional trial to establish the regression equation. Since selective grazing is a natural animal behavior (King, 1962), it is difficult to precisely correlate events on pasture with those of hand-feeding in pens. The error introduced by selective grazing may be of significant magnitude (Hardison, Reid, Martin, and Woolfolk, 1954; Raymond et al., 1954; Bath, Weir, and Torell, 1956; Lofgreen, Meyer, and Peterson, 1956; Meyer, Lofgreen, and Hull, 1956; Weir and Torell, 1959; Reid, 1962; McDonald, 1968). For example, Hardison et al. (1954) found that the average diet selected by grazing animals from 23 swards representing a variety of plants and growth stages contained 23% more crude protein, 37% more fat, 26% more ash and 17% less crude fibers than the whole herbage cut from the same source at a stubble height of 2 inches. Discussions on the problem of herbage sampling for chemical analysis have been given by Weir et al. (1959), Kennedy (1962), and McDonald (1968).

The amount of forage consumed by grazing animals can be determined by either of the following two methods:

1. Total fecal output

After calculating the digestibility by either of the two methods outlined above, one needs to know the total fecal

output to be able to calculate forage consumption. This can be done by one of two methods: a) direct, with the use of a harness and a fecal bag, feces can be quantitatively collected and b) indirect, an external indicator such as chromic oxide can be administered orally in known amounts to the grazing animals and total fecal excretions can, therefore, be calculated from the concentration of the external indicator in representative fecal samples.

2. The fecal index method

This can be carried out as described for calculation of digestibility except that the regression equation here will establish the relationship between forage intake and the concentration of the internal indicator in the feces. The acid soluble fecal fraction has been used in this method as the indicator (Owen, 1961; Langlands and Corbett, 1964).

VOLUME DETERMINATION

Under conditions where direct measurement of the volume of aqueous media becomes unfeasible (e.g., urine collections of astronauts during space flight or rumen contents) the use of markers allows the determination of the volume indirectly. The marker must be neither adsorbed nor absorbed and must be either water soluble or completely dispersed in water.

The technique is essentially a dilution method in which a given quantity of a marker is added to the system and the volume can, therefore, be calculated from the concentration of the marker after uniform distribution in the material has

occurred. This is a simple procedure in closed systems such as urine collection bags, but in open systems (e.g., the rumen) a few assumptions have to be made and mathematical treatment becomes necessary.

The volume of the rumen has been the focus interest of workers studying absorption and nutrient utilization in this organ. The use of polyethylene glycol (PEG) for the study of water movements in the rumen was introduced by Sperber, Hydén, and Ekman (1953). Later, Hydén (1961) presented an extensive description of the theoretical and practical aspects of the use of PEG for the study of water movement in the rumen. In his mathematical treatment, Hydén (1961a) assumed the steady-state condition where the volume of water in the rumen remains constant during the experiment and the rate of flow of water into and out of the rumen is continuous and constant. To accept results collected by this method, one should, therefore, examine the experimental conditions that have any bearing on these two conditions.

In this technique a known amount of the marker is administered directly into the rumen and samples of the rumen contents are removed at different intervals of time after administration. Assuming a steady-state condition and a rapid uniform mixing of the marker, an exponential relationship between marker concentration and time was suggested (Hydén, 1961a) where a straight line may be obtained by plotting the natural logarithm of marker concentration against time. When the straight line is extrapolated back to zero time, an estimate of marker concentration at the time of dosing may be obtained.

Ulyatt (1964) found that this relationship tended to be curvilinear rather than linear and that a quadratic fit increased the statistical precision of estimating initial marker concentration. Warner and Stacy (1968a), however, obtained linear relationships and suggested this linearity as a criteria for the steady-state condition.

Warner and Stacy (1968) used ^{51}CrEDTA complex as a soluble marker in the study of water balance in the rumen of sheep. Their mathematical treatment of the concept was extended over that of Hydén's to cover non-steady-state conditions. These workers stressed the importance of avoiding discontinuity in the curve over the period of extrapolation. This means that conditions in the rumen should be changing at constant rates (if not steady-state) throughout the entire period between administration of the dose and establishment of the curve. Discontinuities in the marker concentration curve may be caused by eating or drinking; this becomes especially important in animals which eat and/or drink fast. On this basis, Warner and Stacy (1968a) criticized those who give the marker dose immediately before feeding. Further details of the technique, the underlying assumptions, and practical limitations have been given by these authors (1968a). They applied the technique in a study of water balance in the rumen of sheep throughout the feeding cycle (1968b).

FECES SAMPLING

It was pointed out that for determination of apparent digestibility of a given nutrient by the indicator ratio technique, one needs only to know the concentrations of the indicator and nutrient in both the diet and the feces. If the indicator were excreted in the feces uniformly or in a predictable manner, the total collection of feces could be eliminated and only periodic sampling could be used. This was seen by many workers in this field especially those who work with large animals as the biggest advantage of the indicator-ratio technique, because if proven valid, it will greatly reduce labor and cost usually required for a conventional trial.

In studies with humans, Irwin and Crampton (1951) found that administration of chromic oxide thrice daily resulted in uniform mixing of the indicator in the feces whereas once daily dosage did not. Whitby and Lang (1960) also concluded that thrice daily dose with chromic oxide capsules gave satisfactory mixing of the indicator in the feces. Very limited examples have been presented by Whitby and Lang (1960) and by Rose (1964) to show the usefulness of random sampling of stools in human. Macdougall (1964) in a study with dogs and humans concluded that an estimate of intestinal fat absorption can be made with accuracy from a single stool specimen. These workers used zirconium-95, iodine-131, and carmine as markers and found that, if the first marked stool is excluded from the calculations,

results obtained by the random stool method show no significant difference from those obtained by chemical analysis of a 48-hour stool collection. Rose (1964), however, pointed out that while occasional analysis of a random sample of stool for chromic oxide, Ca, and P would provide a very rapid and labor-saving method of calculating fecal Ca and P, it is not suggested that this is the technique of choice for accurate balance work. For such work, it still seems desirable to collect all the stools and pool several days' stools.

On the other hand digestibility studies with ruminants have shown that chromic oxide exhibits a wide range of concentration in the feces at different times during a 24-hour period. Variation is decreased when twice daily doses are compared to once daily dosages (Hardison, Engel, Linkous, Sweeney, and Graf, 1956; Brisson, Pigden, and Sylvestre, 1957). The reduction was seen to be substantial by Hardison et al. (1956) in their studies with barn-fed dairy cows, and they suggested that random sampling of the feces could be used if the indicator were administered twice daily. On the other hand, Brisson et al. (1957), working with grazing cattle, and Davis, Byers, and Lubers (1958), working with barn-fed dairy cows, indicated that whether dosing once or twice daily, the variations were still large enough to preclude the safe recommendation of the grab-sampling technique. Davis et al. (1958), however, suggested that this can be overcome in digestion studies by using longer collection periods, e.g., 10 days. Earlier, Smith and Reid (1955) found that chromic oxide

of the feces taken rectally from grazing cows at 6 a.m. and 4 p.m. on seven consecutive days and bulked on an equal weight basis provided accurate estimate of total fecal output regardless of whether chromic oxide was administered in gelatin capsules or in a concentrate feed and whether it was administered once or twice daily. The work by Brisson et al. (1957) has also shown that when chromic oxide was administered 6 times daily, it was excreted at a constant rate which could be estimated from a fecal sample taken at any time during the day. Such a schedule of administration, however, is impractical as pointed out by the authors.

Since frequent dosing reduces these intra-day variations, it may be expected that mixing the indicator thoroughly with the entire ration would lead to a similar or better effect, especially when fed ad libitum. Elam, Putnam, and Davis (1959), however, fed a pelleted ration that contained 0.5% chromic oxide thoroughly mixed with its ingredients to heifers and found that a significant time-concentration variation occurred in the fecal pattern of chromic oxide excretion regardless of whether they were fed once a day, twice a day, or ad libitum (Fig. 3). It was concluded that the magnitude of the variation would preclude the indiscriminate sampling of feces for digestion trials as regards time.

There are some reports indicating different patterns of diurnal variations in the excretion of different indicators. Kane, Jacobson, and Moore (1952) reported that both lignin and chromic oxide exhibited diurnal variations in the dairy cow but that their

patterns of excretion were different. The chromic oxide content of the feces rose to the highest point at 9 a.m. where the lignin peak occurred in the vicinity of 8 p.m. Elam and Davis (1961) reported that the diurnal variations in lignin excretion were less than those for chromic oxide. Garner, Jones, and Ekman (1960) reported that the diurnal variation in concentration of cerium-144 administered twice daily with the feed was very similar to that of chromic oxide reported by Elam et al. (1959) except that the maximum concentration more frequently occurred in the mid-morning (11 a.m.) sample than in the afternoon sample.

Diurnal variation of chromic oxide has been studied in the goat by Kameoka, Takahashi, and Morimoto (1956) who also observed that inter-day variation is a natural occurrence in ruminants amounting to about 10%. Other species have also been shown to exhibit diurnal variations in the excretion of markers. Diurnal variations in chromic oxide excretion were observed in swine (Clawson, Reid, Sheffy, and Willman, 1955; Moore, 1957) and in chromic oxide and lignin excretions in chickens (Mueller, 1956).

In spite of the occurrence of the intra-day variations in the excretion of markers, many workers continue to use grab sampling because of the advantages offered by this technique as previously pointed out. To minimize errors caused by the diurnal variation when grab sampling is practiced, some workers devised special sampling schedules. Kane et al. (1952), using chromic oxide and lignin as indicators in studies with cows, suggested that sampling periods be from 1 to 3 p.m. or 4 to 6 a.m. or both

periods combined. These time intervals were chosen because both chromic oxide and lignin excretions of the cows approximated their averages for 24-hour cycle. These workers also indicated that by selecting periods of equal distance from the time when average values for the indicators are attained the morning and evening variation errors should cancel and a more accurate excretion rate for the indicator results. Based on this idea, they reported that digestibility data obtained in a 3-day trial using the two sampling periods of 10-12 a.m. and 2-4 p.m. agreed satisfactorily with data secured in conventional 10-day trials. Elam and Davis (1961), however, indicated that the time of feces sampling was of little importance when lignin was used to determine digestibility because the diurnal variations in its fecal concentration were slight.

Hardison and Reid (1953) and Smith and Reid (1955) reported that the bulking of equal weights of feces sampled at 6 a.m. and 4 p.m. for seven or more days would provide samples of which the chromic oxide concentration would allow estimates of the total fecal output. Similar systems have been used by many other investigators and satisfactory results were reported in comparison with the conventional method of total collection. However, the technique has been criticized by Raymond and Minson (1955) on the grounds that the diurnal pattern of chromic oxide excretion is not stable and varies with any change in the pattern of feeding behavior.

The above mentioned conclusions were based on the use of the marker in a quick-release form, usually as a fine powder in gelatin capsules or mixed with the diet. It has been shown (Corbett, Greenhalgh, Gwynn, and Walker, 1958; Corbett, Greenhalgh, and Florence, 1959) that the diurnal variation in chromic oxide excretion in the feces of ruminants, which have received regular concentrated doses of this marker is due primarily to uneven mixing of the marker with the contents of the reticulo-rumen and to its passage from this organ in advance of the food residues it is intended to mark (Corbett et al., 1958; Corbett, et al., 1959). This conclusion, however, is not entirely satisfactory in providing an explanation for the occurrence of diurnal variation in the excretion of markers in view of two reported examples. First, the use of chromic oxide which was thoroughly mixed with the ingredients of a pelleted ration did not eliminate or significantly reduce the diurnal variation in sheep even when fed ad libitum (Elam et al., 1959). Second, cattle exhibited a diurnal pattern when lignin, which is a natural constituent of the feed, is used as the indicator (Kane et al., 1952).

Any sampling procedure would be more reliable if the passage of chromic oxide through the alimentary tract and its excretion could be made more regular. Balch, Reid, and Stroud (1957) concluded that the administration of chromic oxide immediately before a single daily feed caused a more even excretion of chromic oxide in the feces than administration immediately after the feed. Since

Brisson et al. (1957) were able to induce substantial reduction in the diurnal variation by frequent dosing of the marker, and since 6 dosages a day is impractical, it was suggested that a capsule which would release chromic oxide at a uniform rate in the rumen and would simulate frequent dosing should be developed. Balch et al. (1957) suggested the use of the "macaroni" form used by Edin, Kihlen, and Nordfeldt (1944); Pigden and Brisson (1957) developed their "sustained-release pellet"; and Corbett et al. (1958), the chromic oxide paper. Langlands (1962) showed that the variability in the fecal concentration of chromic oxide tended to be greater when the macaroni rather than the paper was administered and that the macaroni was intermediate in variability between the paper form and the gelatin capsules.

The "sustained-release" pellet, developed by Pigden and Brisson (1957), consists of equal parts of chromic oxide and plaster of Paris moulded into a cylinder. This pellet dissolves in the rumen during a period of approximately 96 hours. An increase in the plaster/chromic oxide ratio produces a more durable pellet. The pellet was tested under laboratory trials as well as field trials with cattle when administered once daily. It was found that the maximum deviations from a 9-day average concentration were: laboratory animal no. 1, 94-104%; no. 2, 90-110%; field animal no. 1, 80-117%; and no. 2, 93-108%. These data show an improvement due to the use of this pellet when compared to those reported when using the gelatin capsule (e.g., 50-180% by Hardison and Reid, 1953; 65-141% by Smith and Reid, 1955).

The chromic oxide paper was developed by Corbett et al. (1958) who found that both the paper form and the "sustained-release pellet" gave a more uniform flow of chromic oxide through the duodenum of sheep than did the administration of chromic oxide powder in gelatin capsules. The chromic oxide paper was made of Kraft wood-pulp (prepared from wood by an alkali process), chromic oxide, and aluminum sulfate at the ratio of 100:75:2. Corbett, Greenhalgh, McDonald, and Florence (1960) tested two forms of this paper. One g of chromic oxide incorporated in a single sheet of paper of about 8 x 20 cm was compared to the same amount when the paper was shredded into strips of about 2 x 120 mm. Both forms were given in gelatin capsules. When sheep were dosed once a day, it was found that the intake and excretion of chromic oxide came into equilibrium about a week after dosing began with the chromic oxide paper and rather sooner when the marker was given as oil suspension in capsules. Subsequently, chromic oxide recoveries from the feces remained about 100% for all forms. During the last 12 days of the experiment, fecal samples were obtained directly from the rectum once daily at a different time, determined at random, each day. The twelve samples from each sheep provided estimates of the concentration of chromic oxide in the feces at 2-hour intervals for a period of 24 hours. It was found that variability in concentration was much less when paper strips were used than when the oil suspension was used; single sheets of paper were intermediate between the two.

Later, Langlands, Corbett, McDonald, and Reid (1963a) tested the chromic oxide paper to estimate fecal output with grazing steers and sheep. In this study doses of chromic oxide paper strips were each rolled up in a sheet of thin quarto paper for administration. It was concluded that the errors of estimates of fecal output were more stable when chromic oxide was administered in paper instead of the oil suspension capsules. It was indicated, however, that estimates of fecal output obtained from a regimen of dosing once daily and grab sampling at the same time of the day were unsatisfactory with both forms of the marker.

Another point of comparison between these chromic oxide forms is the problem of regurgitation. Pigden and Brisson (1957) reported that no evidence of regurgitation or of passage of the "sustained-release pellets" out of the rumen and through the digestive system before they have dissolved has been obtained in cattle. Regurgitation, however, was found to be a problem when these pellets were given to sheep (Pigden and Brisson, 1957; Corbett et al., 1960). On the other hand, no regurgitation problem has been observed with the paper form.

Harris, Lofgreen, Kercher, Raleigh, and Bohman (1967) reviewed work on the comparison of cottonseed meal and cellulose as Cr_2O_3 carriers to the free form of the marker administered twice daily to grazing steers. Estimates of fecal production from samples collected at 8 p.m. and 4 p.m. were 119%,

123%, and 138% of the actually measured outputs for the cottonseed meal, the cellulose capsules, and the free-form capsules, respectively. Further work was carried out on the marker when in mixture with cellulose. The technique was investigated with steers grazing range alone or supplemented with barley. Chromic oxide recovery was essentially 100% and fecal output was estimated accurately. In three other trials with grazing steers, big variations were experienced in two of these trials, but the mean of the 3 trials gave a 100% recovery and an accurate estimation of fecal output.

Sward sampling has been suggested as an alternative procedure to grab sampling since it is essentially a random procedure (Raymond and Minson, 1955). In using this procedure, however, there is the problem of identification of the defecations of each individual when a group of animals are grazing together. To overcome this difficulty, the dosing of each animal with differently colored particles of polystyrene has been used (Minson, Tayler, Alder, Raymond, and Rudman, 1960). The technique was used by Langlands et al. (1963b) to compare sward and grab sampling and also further evaluate the chromic oxide paper in estimating fecal output of grazing cattle. In sward sampling, the feces collected each day were generally from about ten to twelve defecations for each cow. Grab sampling was carried out twice daily at

milking times. Animals were dosed twice daily. It was concluded that the random error was appreciably less in the estimates of fecal output from the sward samples and that paper is to be preferred to usual capsules when grab sampling is practiced. The advantage of the paper when sward sampling is used may be relatively slight.

It is obvious that sward sampling as practiced by Langlands et al. (1963) is more laborious than grab sampling and may become impractical under some conditions. A modification of the procedure to make it easier without loss of accuracy is worth testing. Langlands et al. (1963) suggested that the sampling fraction, and therefore, the labor could be reduced without greatly increasing the total error of estimation of feces output by restriction of sampling to predetermined areas of the pasture as has been done by Raymond and Minson (1955). These latter workers devised the "ring-sampling" technique in which the feces dropped within certain marked areas are collected periodically and analyzed. This procedure was found more satisfactory than grab sampling for the estimation of fecal output in grazing sheep.

Other sampling procedures have also been examined. For example, the partial collection system which was tested by Hill, Repp, Watkins, and Knox (1961) requires the collection of feces for a limited time of each day of the trial by means of a harness and a fecal bag. Hill et al. (1961) studied the recovery of lignin in

confined heifers when feces were collected for 4 or 6 hours. Recoveries of about 105% and 106% were found for the 4 and 6 hour collection periods after multiplying by 6 and 4 to calculate the 24 hour lignin excretion, respectively. A 4 and 8 hour collection period were also compared by Hill (1965) with grazing cows using lignin as indicator. The system was found to be satisfactory in some cases, but failed to give reliable estimates in some other cases. It is obvious that this system does not offer any advantage over the total collection method from the point of view of the special equipment required. Besides, it might introduce large errors in view of the different patterns of diurnal variations.

In the foregoing discussion, the advantages and disadvantages of the different sampling systems were considered, especially from two viewpoints, e.g., ease and reliability. It is clear that grab sampling is the easiest and most practical of all systems, but its reliability is somewhat questionable. The other systems are not so easy and sometimes impractical; their reliability is also in question. As previously mentioned, grab sampling does not seem to be so important in human studies, but in animal studies, especially with large animals it offers the advantages of ease and low cost over the conventional method when indicators are used to study food utilization. It is, therefore, recommended that grab sampling be improved to eliminate the errors introduced by the diurnal variations.

CONCLUSIONS

Dietary markers are useful nutritional tools employed in studies of food utilization including food and nutrient intake, passage of ingesta through the digestive tract, digestibility, and absorption of nutrients in man and animals. The challenging problems in studies of food consumption and utilization under grazing conditions call for special application of the marker method. The following conclusions are drawn regarding the suitability of markers for different studies:

1. The stained particle technique is widely used to measure the rate of food passage in ruminants. The counting of the stained particles recovered in the feces is a laborious process and its validity is questioned. An alternative method is to use a nonabsorbed dye that can be completely recovered and quantitatively determined in the feces; this has yet to be used in a wide variety of trials.
2. Several dyes have been used to color the feces of ruminants. Best coloring was achieved by methylene blue, crystal violet, basic fuchsin, and aniline blue. Carmine has been used to color the feces of humans and monogastric animals to allow separation of experimental periods. A brilliant blue-methylcellulose mixture is recommended for this purpose in humans.
3. Chromic oxide has been the most widely used fecal marker in human and animal studies. In ruminants best results are

obtained when the marker is given in sustained-release form by mixing the marker with a suitable carrier. Availability of radioactive chromic oxide ($^{51}Cr_2O_3$) allows the use of minute amounts of the marker and eliminates the time consuming procedure of its chemical determination.

4. Titanium oxide has been used satisfactorily in studies of calcium and phosphorus absorption and protein digestibility. It can be determined directly or in Kjeldahl digests of food and feces.

5. Barium sulfate has been used in humans for extent and rate of passage studies and for chickens in food utilization studies. Radioactive $^{131}BaSO_4$ has been used to study the absorption of ^{59}Fe and ^{131}I-labled nutrients. Cuprous thiocyanate has also been successfully used in balance studies with humans.

6. A number of elements of the rare earths and other inert metals have been qualified as fecal markers and used successfully in studies of passage and absorption of food nutrients. Included in this group are the radioactive isotopes ^{144}Ce, $^{46,47}Sc$, ^{95}Zr, ^{140}La, ^{91}Y, ^{106}Ru, and ^{198}Au. When it becomes undesirable to use radioisotopes, dysprosium, europium and gold are non-radioactive members of this group that can be determined accurately with radioactivation analysis.

7. Soluble markers have been used in many physiological and nutritional studies in humans and animals. Variation in

results and the frequently reported failure to achieve complete recovery of polyethylene glycol (PEG) in the feces, especially in ruminants under certain nutritional conditions, suggest re-examination of this material as a nutritional marker. It is suggested that further studies be conducted using a radioactive form of this material. Chromium EDTA complex has been suggested as a satisfactory substitute for PEG. Radioactive ^{51}CrEDTA allows easy and precise measurement. Small quantities may be absorbed. Further work with this material especially on its toxicology is needed before it is recommended for wider use.

8. Microbiological techniques based on the use of strict thermophylic spores of bacteria such as B. subtilis to measure transit time in the digestive tract are interesting but have not been studied enough in regards to health and accuracy. The technique devised for ruminants should be evaluated in a comparison with other techniques.

9. Internal markers used for food utilization studies with grazing animals include silica, lignin, methoxyl, fiber, chromogens, fecal nitrogen, and the acid soluble fecal fraction. Only chromogen and fecal nitrogen seem to be reliable indices to predict digestibility of forage. The use of these two indicators may continue until a better marker becomes available. Because chromogen is not absorbed from the digestive tract, it can be used in both the ratio technique and the fecal index

method to predict digestibility. Fecal nitrogen, however, can be used only in the fecal index method. Available data suggest that the fecal index method is more reliable than the ratio technique in grazing studies.

10. It is recommended that the use of glass beads, small seeds, rubber, charcoal, and metal particles in studies of the rate of passage, and iron oxide, silica, anthraquinone violet, and monastral blue in digestibility studies should cease. This conclusion is drawn on the basis of contradiction in results and repeated failure to achieve complete recovery of some of these markers. Plastic particles have been used in studies of digestibility and food passage. Further work is needed to ascertain their usefulness. Colored plastic particles may, however, be used to mark individual defecations when sward sampling is used in grazing studies. Further studies are needed on particle size and specific gravity of metal particulates as nutritional markers.

REFERENCES

Altman, P. L. and Dittmer, D. S., Ed. (1968). Metabolism, pp. 418-422. Federation of American Societies for Experimental Biology, Bethesda, Maryland, U.S.A.

Alvarez, W. C. (1928). New England J. Med., 199, 858.

Alvarez, W. C. (1948). An Introduction to Gastroenterology, p. 617, P. Hoeber, New York.

Alvarez, W. C. and Freedlander, B. L. (1924). Amer. Med. Assoc. J., 83, 576.

Anderson, J. and Weinbren, I. (1961). Clin. Chim. Acta, 6, 648.

Anthony, W. B. and Reid, J. T. (1958). J. Dairy Sci., 41, 1715.

Armitage, E. R., Ashworth, R. B. and Ferguson, W. S. (1948). J. Soc. Chem. Indust., 67, 241.

Askew, H. O. (1931). N. Z. J. Sci. Tech., 13, 76.

Asplund, J. M. and Harris, L. E. (1970). J. Animal Sci., 31, 1199.

Axelsson, J. and Kivimäe, A. (1951). Acta Agr. Scandinavica 1, 282.

Baily, J. A. (1968). J. Mammal., 49, 340.

Balch, C. C. (1950). Brit. J. Nutr., 4, 361.

Balch, D. A., Balch, C. C., and Rowland, S. J. (1954). J. Sci. Food Agr., 5, 584.

Balch, C. C. and Campling, R. C. (1965). Rate of passage of digesta through the ruminant digestive tract. Second International Symposium on the Physiology of Digestion in the Ruminant, pp. 108-123. Dougherty, R. W., R. S. Allen, Wise Burroughs, N. L. Jacobson, and A. D. McGilliard, Ed., Butterworths, Washington.

Balch, C. C., Reid, J. T., and Stroud, J. W. (1957). Brit. J. Nutr. 11, 184.

Barnicoat, C. R. (1945). N.Z.J. Sci. Technol., 27, 202.

Bassett, S. H., Tuttle, S. G., Figuroa, W. G., and Jordan, T. (1962). Endocrine Soc., 43.

Bath, D. L., Weir, W. C., and Torell, D. T. (1956). J. Animal Sci., 15, 1166.

Bell, M. C. (1963). Sixth Intern. Congr. Nutr., Edinburgh, Scotland, p. 185.

Bergeim, O. (1924). J. Biol. Chem., 62, 49.

Bergeim, O. (1926). J. Biol. Chem., 70, 29.

Berry, D., Ross, A., and Deuschle, K. (1963). Amer. Rev. Resp. Dis., 88, 769.

Binnerts, W. T., Klooster, A. Th. van't, and Frenz, A. M. (1968). Vet. Rec., 82, 470.

Blaxter, K. L., McGraham, N., and Wainman, F. W. (1956). Brit. J. Nutr., 10, 69.

Blaxter, K. L. and Mitchell, H. H. (1948). J. Animal Sci., 7, 351.

Bloom, S., Jacobson, N. L., Allen, R. S., McGilliard, L. D., and Homeyer, P. G. (1957). J. Dairy Sci., 40, 240.

Boender, C. A., Mulder, E., Ploem, J. F., de Wael, J., and Verloop, M. C. (1967). Nature, 213, 1236.

Boender, C. A. and Verloop, M. C. (1969). Brit. J. Haemat., 17, 45.

Bokori, J. (1968). Acta. Vet. Acad. Sci., Hung., 18, 81.

Bolin, D. W., King, R. P., and Klosterman, E. W. (1952). Science, 116, 634.

Borgstrom, B. (1969). J. Lipid Res. 10, 331.

Borgstrom, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). J. Clin. Invest. 36, 1521.

Bradley, N. W., Forbes, R. M., Albert, W. W., Mitchell, G. E. Jr., and Neumann, A. L. (1958). J. Animal Sci., 17, 1199.

Brandt, C. S. and Thacker, E. J. (1958). J. Animal Sci., 17, 218.

Brannon, W. F., Reid, J. T., and Miller, J. I. (1954). J. Animal Sci., 13, 535.

Bris, E. J., Dyer, I. A., and Teare, I. D. (1967). Agronomy J. 59, 255.

Brisson, G. J., Pigden, W. J., and Sylvestre, P. E. (1957). Canadian J. Animal Sci., 37, 90.

Broad, W. C. (1971). Amer. Lab. March 1971.

Burnett, F. L. (1921). Boston Med. Surg. J., 184, 371;415.

Burnett, F. L. (1923). Amer. J. Roetgenol., 10, 599.

Butcher, J. E. and Harris, L. E. (1956). Proc. Amer. Soc. Animal Prod., 7, XXV.

Campling, R. C. and Freer, M. (1962). Brit. J. Nutr., 16, 507.

Carter, J. F., Bolin, D. W., and Derickson, D. (1960). N. Dakota Agric. Exp. Stat. Bull. No. 426 (Tech) p. 56.

Castle, E. J. (1956a). Brit. J. Nutr., 10, 15-23.

Castle, E. J. (1956b). Brit. J. Nutr., 10, 115-125.

Chanda, R., Clapham, H. M., McNaught, M. L., and Owen, E. C. (1951). J. Agric. Sci., 41, 179.

Chanda, R., Clapham, H. M., McNaught, M. L., and Owan, E. C. (1952). Biochem. J., 50, 95.

Chandler, P. T. and Cragle, R. G. (1962). Proc. Soc. Exp. Biol. Med., 111, 431.

Chandler, P. T., Kesler, E. M., and McCarthy, R. D. (1964). J. Dairy Sci., 47, 1426.

Charlot, G. and Bezier, D. (1945). Quoted by Fournier 1950. Compt. Rend. Acad. Sci., 231, 1343.

Cheong, F. H. and Salt, F. J. A. (1968). Lab. Pract. 17, 199.

Christian, K. R. and Coup, M. R. (1954). N.Z.J. Sci. Technol.(A), 36, 328.

Clanton, D. C. (1962). J. Animal Sci., 21, 214.

Clark, J. L. and Hembry, F. G. (1967). Personal communication.

Clawson, A. J., Reid, J. T., Sheffy, B. E., and Williams, J. P. (1955). J. Animal Sci., 14, 700.

Columbus, A. (1936). Forschungsdienst, 2, 208.

Connor, J. M., Bohman, V. R., Lesperance, A. L., and Kinsinger, F. E. (1963). J. Animal Sci., 22, 961.

Contrepois, M. and Gouet, P. (1969). C. R. Acad. Sci. (Paris), 268, 1757.

Cook, C. W. and Harris, L. E. (1951). J. Animal Sci., 10, 565.

Coombe, J. B. and Kay, R. N. B. (1965). Brit. J. Nutr., 19, 325.

Corbett, J. L., Greenhalgh, J. F. D., and Florence, E. (1959). Brit. J. Nutrition, 13, 337.

Corbett, J. L., Greenhalgh, J. F. D., Gwynn, P. E., and Walker, D. (1958). Brit. J. Nutr., 12, 266.

Corbett, J. L., Greenhalgh, J. F. D., and MacDonald, A. P. (1958). Nature, 182, 1014.

Corbett, J. L. Greenhalgh, J. F. D., McDonald, I., and Florence, E. (1960). Brit. J. Nutr. 14, 289.

Corbett, J. L., Miller, T. D., Clarke, E. W., and Florence, E. (1956). Proc. Nutr. Soc. 15, V.

Corbin, J. E. and Forbes, R. M. (1951). J. Animal Sci. 10, 574.

Coup, M. R. (1950). Proc. Ann. Conf. New Zealand Soc. Animal Prod., 10, 3.

Coup, M. R. and Lancaster, R. J. (1952). N.Z.J. Sci. Tech., 34, 347.

Cowlishaw, S. J. and Alder, F. E. (1963). J. Brit. Grassland Soc., 18, 328.

Crampton, E. W. and Jackson, I. R. C. (1944). J. Animal Sci., 3, 333.

Crampton, E. W. and Lloyd, L. E. (1950). J. Nutr. 45, 319.

Csonka, F. A., Phillips, M., and Jones, B. (1929). J. Biol. Chem. 85, 65.

Daly, J. R. and Anstall, H. B. (1964). Clin. Acta, 9, 576.

Davignon, J., Simmonds, W. J., and Ahrens, E. H. (1968). J. Clin. Invest., 47, 127.

Davis, C. L., Byers, J. H., and Luber, L. E. (1958). J. Dairy Sci., 41, 152.

DeGrazia, J. A. and Rich, C. (1964). Metabolism 13, 650.

de Somer, P. and Eyssen, H. (1971). Symposium, Nutritional and Biochemical Aspects of Host-Microflora Interaction. 55th Annual Meeting, Federation of American Societies for Experimental Biology, Chicago, Illinois, U.S.A.

Deuschle, K. W., Jordahl, C., and Hobby, G. L. (1960). Amer. Rev. Resp. Dis., 82, 1.

Dick, M. (1967). J. Clin. Path. 20, 216.

Dick, M. (1969a). J. Clin. Path. 22, 378.

Dick, M. (1969b). Gut. 10, 408.

Donaldson, R. M., Jr. and Barreras, R. F. (1966). J. Lab. Clin. Med., 68, 484.

Downes, A. M. and McDonald, I. W. (1964). Brit. J. Nutr., 18, 153.

Drennan, M. J., Holmes, J. H. G., and Garrett, W. N. (1970). Brit. J. Nutr., 24, 961.

BuBois, K. P. (1956). U. S. Atomic Energy Comm. Report, Oak Ridge Institute for Nuclear Studies-12.

Ducluzeau, R., Bellier, M., Raibaud, P. (1970). Zentralblatt fur Bakteriologie, Parasitenkunde, InfektionsKranKheiten und Hygiene, I Orig. 213, 533.

Edin, H. (1918). Medd. Nr. 105 fran Centralanst. for forsok-svasendet pa jordbruKsomradet.

Edin. H, Kihlen, G., and Nordfelt, S. (1944). Kgl. Lantbruks-HogsKol. Ann. 12, 166.

Elam, C. J. and Davis, R. E. (1961). J. Animal Sci., 20, 484.

Elam, C. J., Putnam, P. A., and Davis, R. E. (1959). J. Animal Sci., 18, 718.

Elam, C. J., Reynolds, P. J., Davis, R. E., and Everson, D. O. (1962). J. Animal Sci., 21, 189.

Elliott, T. R. and Barclay-Smith, E. (1904). J. Physiol. 31, 272.

Ellis, G. H., Matrone, G., and Maynard, L. A. (1946). J. Animal Sci., 5, 285.

Ellis, W. C. (1971). Personal communication.

Ellis, W. C. (1968). J. Agr. Food Chem. 16, 220.

Ellis, W. C. and Huston, G. E. (1967). J. Dairy Sci., 50, 1996.

Ellis, W. C. and Huston, G. E. (1968). J. Nutrition 95, 67.

Ely, R. E., Kane, E. A., Jacobson, W. C., and Moore, L. A. (1953). J. Dairy Sci., 36, 346.

Eng, K. S., Jr., Riewe, M. E., Craig, J. H., Jr., and Smith, J. C. (1964). J. Animal Sci., 23, 1129.

Ewing, P. V. and Smith, F. H. (1917). J. Agr. Res., 10, 55.

Figueroa, W. G., Gordan, T., and Bassett, S. H. (1968). Amer. J. Clin. Nutr., 21, 1239.

Flatt, W. P., Horvath, D. J., DeCosta, L. C., Stewart, D. G., and Warner, R. G. (1957). J. Animal Sci., 16, 688.

Forbes, E. B., Elliott, R. F., and Swift, R. W. (1946). J. Animal Sci., 6, 298.

Forbes, E. B. and Swift, R. W. (1943). Penna. State College Bull. 452, pp. 1-34.

Forbes, R. M. (1949). J. Animal Sci., 8, 19.

Forbes, R. M. (1950). J. Animal Sci., 9, 231.

Forbes, R. M. and Garrigus, W. P. (1948). J. Animal Sci. 7, 373.

Forman, S. A. and Sauer, F. (1962). Can. J. Animal Sci., 42, 9.

Fournier, P. (1950a). Compt. Rend. Acad. Sci., 231, 1343.

Fournier, P. (1950b). Compt. Rend. Acad. Sci., 231, 1556.

Fournier, P. (1951a). Compt. Rend. Acad. Sci., 232, 1019.

Fournier, P. (1951b). Compt. Rend. Acad. Sci., 232, 1593.

Fournier, P. (1951c). Compt. Rend. Acad. Sci., 232, 1769.

Fournier, P. and Dupuis, Y. (1953a). J. Physiol., Paris, 45, 443.

Fournier, P. and Dupuis, Y. (1953b). J. Physiol., Paris, 45, 451.

François, E., Compere, R., and Rondia, G. (1968). Bull. Recherches Agronom. Gembloux, 3, 655.

Furuya, S., Takahashi, S., and Kameoka, K. (1970). J. Nutr. 100, 671.

Gallup, W. D. (1928). J. Biol. Chem., 76, 43.

Gallup, W. D. (1929). J. Biol. Chem., 81, 321.

Gallup, W. D. and Briggs, H. M. (1948). J. Animal Sci., 7, 110.

Gallup, W. D. and Kuhlman, A. H. (1931). J. Ag. Res., 42, 665.

Gallup, W. D. and Kuhlman, A. H. (1936). J. Ag. Res., 52, 889.

Gallup, W. D., Hobbs, C. S., and Briggs, H. M. (1945). J. Animal Sci., 4, 68.

Garber, M., Marquette, M. M., and Parsons, H. (1949). J. Nutr., 38, 225.

Garner, R. J., Jones, H. G., and Eckman, L. (1960). J. Ag. Sci., 5, 107.

Ghanem, N. A. and Westermark, T. (1962). in Radioisotopes in The Physical Sciences and Industry, Vol. 3, p. 43, Vienna, International Atomic Energy Agency.

Gray, F. V. (1947). J. Exp. Biol. 24, 15.

Gray, F. V., Jones, G. B., and Pilgrim, A. F. (1960). Anst. J. Agr. Res. 11, 383.

Gray, S. J. and Sterling, K. (1950). J. Clin Invest., 29, 1604.

Greenhalgh, J. F. D. (1959). Ph.D. Thesis, University of Aberdeen.

Greenhalgh, J. F. D. and Corbett, J. L. (1960). J. Agric. Sci., 55, 371.

Greenhalgh, J. F. D., Corbett, J. L., and McDonald, I. (1960). J. Agric. Sci. 55, 377.

Griffith, G. ap., and Thomas, D. C. (1955). Agric. Progress, 30, 124.

Grundy, S. M., Ahrens, E. H, Jr., and Salen, G. (1968). J. Lipid Res. 9, 374.

Guernsey, S. C. and Evvard, J. M. (1914). Eiochem. Bul. 3, 369.

Gupta, B. N. and Majumdar, B. N. (1962). Ann. Biochem. Exp. Med. 22, 13.

Gupta, B. N. and Majumdar, B. N. (1963). Ann. Biochem. Exp. Med. 23, 145.

Habeck, R. (1930). Arch. Tierernahr. Tierz., 2, 626.

Hale, E. B., Duncan, C. W., and Huffman, C. F. (1939). Proc. Amer. Soc. Animal Prod. 32, 389.

Hamilton, J. G. (1947). Radiology, 49, 325.

Hamilton, T. S., Mitchell, H. H., Kick, C. H., and Carman, G. G. (1927). Ann. Rep., Ill. Agr. Expt. Sta., 119.

Hansky, J. and Connell, A. M. (1962). Gut, 3, 187.

Hardison, W. A., Engel, R. W., Linkous, W. N., Sweeney, H. C., and Graf, G. C. (1956). N. Nutr. 58, 11.

Hardison, W. A. and Reid, J. T. (1953). J. Nutr. 51, 35.

Hardison, W. A., Reid, J. T., Martin, C. M., and Woolfolk, P. G. (1954). J. Dairy Sci., 37, 89.

Harris, L. E., Cook, C. W., and Butcher, J. E. (1959). Agronomy J. 51, 226.

Harris, L. E., Lofgreen, G. P., Kercher, C. J., Raleigh, R. J., and Bohman, V. R. (1967). Utah Agr. Exp. Sta. Bul. No. 471.

Harrison, M., Fraser, R., and Mullan, B. (1961). Lancet, 1, 1015.

Hayes, R. L., Carlton, J. E., and Butler, W. R., Jr. (1963). Health Physics, 9, 915.

Hayes, R. L., Carlton, J. E., and Nelson, B. (1964). J. Nucl. Med. 5, 200.

Hecker, J. F., Budtz-Olsen, O. E., and Ostwald, M. (1964). Austr. J. Agr. Res., 15, 961.

Heller, B. G., Breedlove, C. H., and Likely, W. (1928). J. Biol. Chem. 79, 258.

Hervey, G. (1948). Radioactive Indicators, Interscience Publishers.

Hill, K. (1965). M.S. Thesis, New Mexico State University, U.S.A.

Hill, K. R., Repp, W. W., Watkins, W. E., and Knox, J. H. (1961). Proc. West. Sec. Amer. Soc. Animal Prod., 12, XLIV.

Hobby, G. L. and Deuschle, K. W. (1959). Amer. Rev. Resp. Dis., 80, 415.

Hodgson, R. E. and Knott, J. C. (1932). J. Agr. Res. 45, 557.

Hoelzel, F. (1930). Amer. J. Physiol. 92, 466.

Hoelzel, F. (1924). Quoted by Hoelzel 1930. Amer. J. Physiol. 92, 466.

Hogan, J. P. (1964). Aust. J. Agric. Res. 15, 384.

Hogan, J. P. and Weston, R. H. (1967). Aust. J. Agric. Res. 18, 803.

Holman, W. L. and Fernish, C. A. (1923). Am. J. Hyg. 3, 640.

Hungate, R. E. (1966). The Rumen and its Microbes, pp. 206-244. Academic Press, New York.

Huston, J. E. and Ellis, W. C. (1968). J. Agric. Food Chem. 16, 225.

Hydén, S. (1955). Ann. Agr. Coll. Sweden, 22, 139.

Hydén, S. (1956a). Kgl. Lantbruks-HogsKol. Ann. 22, 139.

Hydén, S. (1956b). Kgl. Lantbruks-HogsKol. Ann. 22, 411.

Hydén, S. (1961a). Kgl. Lantbruks-HogsKol. Ann. 27, 51.

Hydén, S. (1961b). Kgl. Lantbruks-HogsKol. Ann. 27, 273.

International Commission on Radiological Protection (1959). Report of Committee II on permissible dose for Internal Radiation, Pergamon Press, London, New York.

Intersociety Forage Evaluation Symposium (1959). Agronomy J., 51, 212.

Irwin, M. I. and Crampton, E. W. (1951). J. Nutr. 32, 77.

Johnson, R. R. and Dehority, B. A. (1968). J. Animal Sci., 27, 1738.

Johnson, D. E., Dinusson, W. E., and Bolin, D. W. (1964). J. Animal Sci., 23, 499.

Johnson, G. T. and Kyker, G. C. (1966). Mycologia, 58, 91.

Kameoka, K., Takahashi, S., and Morimoto, H. (1956). D. Dairy Sci., 39, 462.

Kane, E. A., Ely, R. E., Jacobson, W. C., and Moore, L. A. (1953). J. Dairy Sci., 36, 325.

Kane, E. A., Jacobson, W. C., and Moore, L. A. (1949). J. Animal Sci., 8, 623.

Kane, E. A., Jacobson, W. C., and Moore, L. A. (1950a). J. Dairy Sci., 33, 385.

Kane, E. A., Jacobson, W. C., and Moore, L. A. (1950b). J. Nutr., 41, 583.

Kane, E. A., Jacobson, W. C., and Moore, L. A. (1952). J. Nutr., 47, 263.

Kane, E. A., Jacobson, W. C., Ely, R. E., and Moore, L. A. (1953). J. Dairy Sci., 36, 637.

Kane, E. A., Jacobson, W. C., and Damewood, P. M., Jr., (1957). J. Dairy Sci., 40, 612.

Kane, E. A., Jacobson, W. C., and Damewood, P. M., Jr., (1959). J. Dairy Sci., 42, 1359.

Kennedy, W. K. (1962). in Pasture and Range Research Techniques (by a Joint Committee of the American Society of Agronomy and others), pp. 59-62, Comstock Publishing Associates, Ithaca, New York, U.S.A.

Kennedy, W. K., Carter, A. H., and Lancaster, R. J. (1959). NZJ. Agr. Res., 2, 627.

Kim, S. K. (1968). Amer. J. Roentgenol., 104, 522.

Kimura, T. K. and Miller, V. L. (1957). J. Agr. and Food Chem. 5, 216.

Kindel, F. (1960). J. Wildl. Mgmt., 24, 429.

King, K. W. and Moore, W. E. C. (1957). J. Dairy Sci., 40, 528.

King, W. A. (1962). in Pasture and Range Research Techniques (by a Joint Committee of the American Society of Agronomy and others), pp. 31-34. Comstock Publishing Associates, Ithaca, New York, U.S.A.

Kleiber, M. (1961). The Fire of Life, pp. 254-255. John Wiley and Sons, Inc., New York.

Knott, J. C., Hodgson, R. E., and Ellington, E. V. (1934). Washington Agr. Exp. Sta. Bul. 295.

Knott, J. C., Murer, H. K., and Hodgson, R. E. (1936). J. Agr. Res., 53, 553.

Kraintz, L. and Talmage, R. V. (1952). Proc. Soc. Exptl. Biol. Med., 81, 490.

Kreula, M. S. (1947). Biochem. J. 41, 269.

Kreula, M. S. (1950). Ann. Acad. Sci. Fennicae Ser. A. II Chem. No. 38, 7.

Lambourne, L. J. (1957). J. Agr. Sci., 48, 273.

Lambourne, L. J. (1957). J. Agr. Sci., 48, 415.

Lambourne, L. J. and Reardon, T. F. (1962). Nature, 196, 961.

Lambourne, L. J. and Reardon, T. F. (1963). Austr. J. Agr. Res., 14, 257.

Lancaster, R. J. (1949). N.Z.J. Sci. and Tech., 31, 31.

Lancaster, R. J. (1954). N.Z.J. Sci. and Tech., 36, 15.

Lancaster, R. J. and Bartrum, M. P. (1954). N.Z.J. Sci. Tech., 35, 489.

Lancaster, R. J., Coup, M. R., and Percival, J. C. (1953). N.Z.J. Sci. Tech., 35, p. 117.

Langlands, J. P. (1962). Quoted by Langlands, Corbett, McDonald, and Reid 193. Brit. J. Nutr., 17, 211.

Langlands, J. P. and Corbett, J. L. (1964). J. Agric. Sci., 63, 305.

Langlands, J. P., Corbett, J. L., and McDonald, I. (1963). J. Agric. Sci., 61, 221.

Langlands, J. P., Corbett, J. C., McDonald, I., and Reid, G. W. (1963a). Brit. J. Nutr., 17, 211.

Langlands, J. P., Corbett, J. L., McDonald, I., and Reid, G. W. (1963b). Brit. J. Nutr., 17, 219.

Lee, M. F., Temperley, J. M., and Dick, M. (1969). Brit. J. Surg., 56, 380.

Lenkeit, W. (1932). Berl. tierarztl. Wschr., 48, 17.

Lenkeit, W. and Habeck, R. (1930). Arch. Tierernahr. Tierz., 2, 517.

Lloyd, L. E., Peckham, H. E., and Crampton, E. W. (1956). J. Animal Sci., 15, 846.

Lofgreen, G. P., Meyer, G. H., and Peterson, M. L. (1956). J. Animal Sci., 15, 1158.

Lundh, G. (1958). Acta Chir. Scand., Suppl. 231, 15.

Lutwak, L. and Burton, B. T. (1964). Amer. J. Clin. Nutr., 14 109.

MacDougall, L. G. (1964). Amer. J. Dis. Child. 108, 139.

MacKenzie, R. D., Anwar, R. A., Byerrum, R. U., and Hoppert, C. A. (1959). Arch. Biochem. Biophys. 79, 200.

MacRae, J. C. and Armstrong, D. G. (1969). Brit. J. Nutr., 23, 15.

Maier, B. R. (1968). M.S. Thesis, University of Arkansas, U.S.A.

Majumdar, B. N., Gupta, B. N., and Kehar, N. D. (1962). Ann. Biochem. Exp. Med. 22, 13.

MaKela, A. (1956). Acta. Agral. Fennica, 85, 1.

Marcus, C. S. and Lengemann, C. W. (1962). J. Nutr. 76, 179.

Martz, F. A., Asay, K. H., Wormington, R. T., Leddicotte, G. W., and Daniels, L. B. (1969). J. Animal Sci., 29, 165.

McCullough, M. E. (1953). J. Dairy Sci., 36, 445.

McCullough, M. E. (1959). Agronomy J., 51, 219.

McDonald, I. W. (1968). Nutr. Abst. Rev. 38, 381.

Merck Index (1968). Merck and Co., Inc., Rahway, New Jersey, U.S.A.

Meyer, J. H., Lofgreen, G. P., and Hull, J. L. (1957). J. Animal Sci., 16, 766.

Miller, J. K. (1967). J. Nutr., 93, 386.

Miller, J. K. and Byrne, W. F. (1970). J. Nutr., 100, 1287.

Miller, J. K. and Cragle, R. G. (1965). J. Dairy Sci., 48, 370.

Miller, J. K. and Miller, G. (1965). J. Dairy Sci., 48, 988.

Miller, J. K., Moss, B. R., Hall, R. F., and Gorman, G. M. (1969). J. Dairy Sci., 52, 1643.

Miller, J. K., Perry, S. C., Chandler, P. T., and Cragle, R. G. (1967). J. Dairy Sci., 50, 355.

Mink, C. J. K., Schefmman-Van Neer, R. H. G. C., and Habets, L. (1969). Clin. Chim. Acta. 24, 183.

Minson, D. J., Tayler, J. C., Alder, F. E., Raymond, W. F., and Rudman, J. E. (1960). J. Brit. Grassl. Soc., 15, 86.

Moore, J. H. (1957). Brit. J. Nutr., 11, 273.

Moore, L. A. and Winter, O. B. (1934). J. Dairy Sci., 17, 297.

Morgan, A. (1959). U. K. Atomic Energy Res. Estab.-R3181, Harwell, Berkshire, England.

Mueller, W. J. (1956). J. Nutr., 58, 29.

Mulinos, M. G. (1935). Rev. Gastroenterol. 2, 292.

Najean, Y. and Ardaillou, N. (1963). Nouv. Rev. Franc. Hematol., 3, 82.

Ness, H. T., Price, E. L., and Parsons, H. T. (1950). Trans. Wisc. Acad. Sci., 40, 267.

Neudoerffer, T. S., McLaughlin, D. R., and Horney, F. D. (1970). J. Animal Sci., 31, 1042.

Nicholson, J. W. G. and Sutton, J. D. (1969). Brit. J. Nutr., 23, 585.

Njaa, L. R. (1961). Acta Agric. Scand., 11, 227.

Nordin, B. E. C. (1962). Amer. J. Clin. Nutr., 10, 384.

Norman, A. G. and Kenkins, S. H. (1934). Biochem. J., 28, 2147.

Norman, A. G. and Jenkins, S. H. (1934). Biochem. J., 28, 2160.

Nutrition Rev. (1965). 23, 40.

Nutrition Rev. (1967). 25, 76

Nutrition Rev. (1970). 28, 256.

Ogg, G. S., Pearson, J. D., and Veall, N. (1967). Strahlentherapie [Sonderb] 65, 415.

Olsson, N., Kihlen, G., and Gagell, W. (1949). Lantbrukshogskol. HusdjursforsoKsansfalt. Medd., 36

Owen, J. B. (1961). Nature (Lond.) 192, 92.

Oyaert, W. and Bouckaert, J. H. (1961). Res. Vet. Sci. 2, 41.

Padgitt, D. D., Martz, F. A., and Graham, E. R. (1966). J. Animal Sci., 25, 1249.

Paloheimo, L. (1925). Biochem. Z, 165, 463.

Pasture and Range Research Techniques (1962). Prepared by a Joint Committee of the American Society of Agronomy and others. Comstock Publishing Associates, Ithaca, New York, U.S.A.

Payne, J. M. (1964). Factors Affecting the Absorption and Distribution of Drugs, T. B. Binns, Ed., E. S. Livingston, Edinburgh.

Pazur, J. H. and DeLong, W. A. (1948). Sci. Agr., 28, 39.

Pearson, J. D. (1966). Int. J. Appl. Radiat., 17, 13.

Phillips, M. (1934). Chem. Revs. 14, 103.

Phillips, M. (1939). J. Assoc. Official Agr. Chemists, 22, 422.

Phillips, M. and Goss, M. J. (1935). J. Agr. Res., 51, 301.

Phillips, M, Goss, M. J., Davis, B. L., and Stevens, H. (1939). J. Agr. Res., 59, 319.

Phillips, M., Weihe, H., Jones, D. B., and Csonka, F. A. (1929). Proc. Soc. Exptl. Biol. Med., 26, 320.

Phillipson, A. T. (1952). J. Physiol., 116, 84.

Piana, C. (1952). Zootec. and Vet., 11, 12.

Pigden, W. J. and Brisson, G. J. (1956). Canad. J. Agric. Sci., 36, 146.

Pigden, W. J. and Brisson, G. J. (1957). Canad. J. Animal Sci., 37, 185.

Price, D. A., Lindahl, I. L., Fredericksen, K. R., Reynolds, P. J., and Cain, C. M. (1964). Proc. West Sec. Amer. Soc. Animal Prod., 15, LX.

Purser, D. B. and Moir, R. J. (1966). J. Animal Sci., 25, 509.

Putnam, P. A., Loosli, J. K., and Warner, R. G. (1958). J. Dairy Sci., 41, 1723.

Randoin, L., Susbielle, H., and Fournier, P. (1951). C. R. Acad. Sci., 232, 553.

Raymond, W. F. (1948). Nature, 161, 937.

Raymond, W. F. (1949). Agric. Prog., 24, 58.

Raymond, W. F. (1954). J. Brit. Grassl. Soc., 9, 61.

Raymond, W. F., Kemp, C. D., Kemp, A. W., and Harris, C. E. (1954). J. Brit. Grassl. Soc., 9, 69.

Raymond, W. F., Jones, E. C., and Harris, C. E. (1955). Agric. Progress, 30, 120.

Raymond, W. F. and Minson, D. J. (1955). J. Brit. Grassl. Soc., 10, 282.

Reid, J. T. (1962). in Pasture and Range Research Techniques (by a Joint Committee of the American Society of Agronomy and others) pp. 43-56. Comstock Publishing Associates, Ithaca, New York, U.S.A.

Reid, J. T., Woolfolk, P. G., Richards, C. R., Kaufman, R. W., Loosli, J. K., Turk, K. L., Miller, J. I., and Blaser, R. E. (1950). J. Dairy Sci., 33, 60.

Reid, J. T., Woolfolk, P. G., Hardison, W. A., Martin, C. M., Brundage, A. L., and Kaufman, R. W. (1952). J. Nutr., 46, 255.

Reid, J. T., Woolfolk, P. J., Richards, C. R., Loosli, J. K., Turk, K. L., Miller, J. I., and Blaser, R. E. (1949). J. Animal Sci., 8, 636.

Reifenstein, E. C., Jr., Allbright, F., and Wells, S. L. (1945). J. Clin. Endocrinol. 5, 367.

Reynell, P. C. and Spray, G. H. (1956). J. Physiol., 131, 452.

Richards, C. R. and Reid, J. T. (1952). J. Dairy Sci., 35, 595.

Richards, C. R. and Reid, J. T. (1953). J. Dairy Sci., 36, 1006.

Richards, C. R. and Weaver, H. G. (1957). J. Dairy Sci., 40, 618.

Ridley, J. R., Lesperance, A. L., Jensen, E. H., and Bohman, V. R. (1963). J. Animal Sci., 14, XXXV.

Ridley, J. R., Lesperance, A. L., Jensen, E. H., and Bohman, V. R. (1963). J. Dairy Sci., 46, 128.

Rogozinski, F. and Starzewska, M. (1926). Bull. Internat. Acad. Polonaise, Series B, 1-2, 157.

Rogozinski, F. and Starzewska, M. (1928). Acta. Biol. Expt. (Warsaw) 1.

Rose, G. A. (1964). Gut. 5, 274.

Rubner, M. (1928). Sitzungsber, Preuss, Adkad. Wissensh., 12, 127.

Sassoon, H. F. (1966). Int. J. Appl. Radiat., 17, 329.

Sauer, F., Laughland, D. H., and Davidson, W. M. (1959). Can. J. Biochem. and Physiol., 37, 183.

Sauer, F. D., Laughland, D. H., and Davidson, W. M. (1959). Can. J. Biochem. and Physiol., 37, 1173.

Schroeder, H. A., Balassa, J. J., and Tipton, I. H. (1962). J. Chron. Dis., 15, 941.

Schroeder, H. A., Balassa, J. J., and Tipton, I. H. (1963). J. Chron. Dis., 16, 55.

Schroeder, H. A., Balassa, J. J., and Vinton, W. H., Jr., (1964). J. Nutr. 83, 239.

Schroeder, H. A., Vinton, W. H., Jr., and Balassa, J. J. (1963a). J. Nutr., 80, 48.

Schroeder, H. A., Vinton, W. H., Jr., and Balassa, J. J. (1963b). J. Nutr., 80, 39.

Schürch, A. F., Crampton, E. W., Haskill, S. R., and Lloyd, L. E. (1952). J. Animal Sci., 11, 261.

Schürch, A. F., Lloyd, L. E., and Crampton, E. W. (1950). J. Nutr. 41, 629.

Seife, B. (1962). J. Lab. Clin. Med., 59, 513.

Shaffer, C. B. and Critchfield, F. H. (1947). J. Amer. Pharm. A. (Scient. Ed.), 36, 152.

Shaffer, C. B., Critchfield, F. H., and Nair, J. H. (1950a). J. Amer. Pharm. A. (Scient. Ed.), 39, 340.

Shaffer, C. B., Critchfield, F. H., and Nair, J. H. (1950b). J. Amer. Pharm. A., 39, 344.

Sharpe, S. J. and Robinson, M. F. (1970). Br. J. Nutr., 24, 489.

Shellenberger, P. R. and Kesler, E. M. (1961). J. Animal Sci., 20, 416.

Sikov, M. R., Thomas, J. M., and Mahlum, D. D. (1969). Growth, 33, 57.

Sinha, K. N., Martz, F. A., Johnson, H. D., and Hahn, L. (1970). J. Animal Sci., 30, 467.

Smart, W. W. G., Sherwood, F. W., Matrone, G., and Wise, G. H. (1953). J. Agr. Food Chem., 1, 318.

Smart, W. W. G., Jr., (1953). J. Animal Sci., 12, 941.

Smith, A. D., Turner, R. B., and Harris, G. E. (1956). J. Range Mangt., 9, 142.

Smith, A. M. and Reid, J. T. (1955). J. Dairy Sci., 38, 515.

Smith, L. W., Waldo, D. R., Moore, L. A., Leffel, E. C., and VanSoest, P. J. (1967). J. Dairy Sci., 50, 990.

Smith, R. H. (1959). J. Agr. Sci., 52, 72.

Smyth, H. F., Jr., Carpenter, C. P., and Weil, C. S. (1950). J. Amer. Pharm. A., 39, 349.

Spencer, H. and Rosoff, B. (1963). Diagnosis and Treatment of Radioactive Poisoning, pp. 171-189. International Atomic Energy Agency, Vienna.

Sperber, I., Hyden, S., and Eckman, J. (1953). Kgl. Lantbruks-HogsKol. Ann., 20, 337.

Stacy, B. D. and Thorburn, G. D. (1966). Science, 152, 1076.

Stanley, M. M. and Cheng, S. H. (1956). Gastroenterology, 30, 62.

Stanley, M. M. and Cheng, S. H. (1957). Amer. J. Dig. Dis., 2, 629.

Sullivan, J. T. (1955). J. Animal Sci., 14, 710.

Swift, R. W., Thacker, E. J., Black, A., Bratzler, J. W., and James, W. H. (1947). J. Animal Sci., 6, 432.

Talburt, D. E. and Johnson, G. T. (1967). Mycologia, 59, 492.

Tan, T. N., Weston, R. H, Warner, A. C. I., and Hogan, J. P. (1968-69). Annual Report, Division of Animal Physiology, Commonwealth Scientific and Industrial Research Organization, Sydney, Australia.

Thompson, R. C. and Hollis, O. L. (1958). Amer. J. Physiol., 194, 308.

Till, A. R. and Downes, A. M. (1965). Brit. J. Nutr., 18, 435.

Ulyatt, M. J. (1964). N.Z.J. Agric. Res., 7, 713.

Ulyatt, M. J. (1964). N.Z.J. Agric. Res., 7, 774.

Usuelli, F. (1933). Profilassi, 6, 7.

Utley, P. R., Boling, J. A., Bradley, N. W., and Tucker, R. E. (1970). J. Nutr., 100, 1227.

Vallentine, J. E. (1956). J. Range Mgt., 9, 235.

Van Dyne, G. M. and Meyer, J. H. (1964). J. Animal Sci., 23, 1108.

Van Liere, E. J., Stickney, J. C., and Northup, D. W. (1945). Castroenterology, 5, 37.

Van Soest, P. J. (1963). J. Assn. Official Agr. Chem., 46, 829.

Van Soest, P. J. (1964). J. Animal Sci., 23, 838.

Van Soest, P. J. and Wine, R. H. (1967). J. Animal Sci., 26, 940.

Visek, N. J., Whitney, I. B., Kuhn, U. S. G., and Comar, C. L. (1953). Proc. Soc. Exptl. Biol. Med., 84, 610.

Walker, D. N. and Hepburn, W. R. (1955). Agric. Progress, 30, 118.

Warner, A. C. I. (1966). J. Gen. Microbiol., 45, 213.

Warner, A. C. I. (1969). Vet. Rec., 84, 441.

Warner, A. C. I. and Stacy, B. D. (1968a). Brit. J. Nutr., 22, 369.

Warner, A. C. I. and Stacy, B. D. (1968b). Brit. J. Nutr., 22, 389.

Weir, W. C., Meyer, J. H., and Lofgreen, G. P. (1949). Agronomy J., 51, 235.

Weller, R. A., Pilgrim, A. F., and Gray, F. V. (1962). Brit. J. Nutr., 16, 83.

Weston, R. H. and Hogan, J. P. (1967). J. Agric. Res., 18, 789.

Whitby, L. G. and Lang, D. (1960). J. Clin. Invest., 39, 854.

Whitson, D., Carrick, C. W., Roberts, R. E., and Hauge, S. M. (1943). Poultry Sci., 22, 137.

Wildt, E. (1874). Jahrb. Landwirtschaftsges, 22, 1.

Woolfolk, P. G., Richards, C. R., Kaufman, R. W., Martin, C. M., and Reid, J. T. (1950). J. Dairy Sci., 33, 385.

Woolfolk, P. G. (1950). Ph.D. Thesis, Cornell University, Ithaca, New York, U.S.A.

TABLE 1

CLASSIFICATION OF MARKERS

A. Elements

1. Inert metals (heavy and rare earths)
2. Natural isotopes (^{40}K)
3. Artificial isotopes (^{144}Ce)

B. Compounds

1. Inorganic
   a. Metal oxides (Cr_2O_3, Fe_2O_3, TiO_2)
   b. Mineral salts ($BaSO_4$, CuSCN)
2. Organic
   a. Natural dyes (carmine, chromogen)
   b. Synthetic dyes (methylene blue, crystal violet, basic fuchsin, aniline blue, anthraquinone violet)
   c. Other (cellulose, lignin, plant sterols)

C. Particulates

1. Polymers (polyethylene glycol, plastics, glass, rubber)
2. Cells (yeast and bacteria)
3. Charcoal
4. Metal particles (i.e., sized aluminum particles)
5. Other particulates, seeds, cotton string

TABLE 2

MEAN RETENTION TIME OF DIFFERENT STAINED FECAL PARTICLES RETAINED ON 40 (0/40)- or 60 (0/60)-MESH SIEVE

(From Ellis and Huston, 1967. J. Dairy Sci., 50, 1996)

Dietary particle stained	Dietary marker fed	Fecal particles counted	Mean retention time, R			
			Sheep A	Sheep B	Sheep C	Mean*
		(mesh)	hr			
Stems, 2.5 cm	Green	0/40	50.1	46.1	53.6	49.9[a,b]
Stems, 2.5 cm	Green	0/60	45.5	43.9	50.7	46.7[a,b]
Composite, 2 mm-30 mm	Purple	0/40	61.7	50.1	50.7	54.2[b]
	Purple	0/60	54.1	51.2	51.4	52.2[b]
Fines, 2 mm	Blue	0/40	40.8	41.9	46.8	43.2[a]
Fines, 2mm	Blue	0/60	37.0	33.8	39.2	36.7[c]

* [a,b,c] Means with dissimilar superscripts are significantly different ($P = < 0.05$) as determined by "t" test, using data paried by animal.

TABLE 3

SUBJECT CB. CALCIUM BALANCE CORRECTED BY BARIUM SULFATE METHOD

(From Figueroa et al. 1968. Amer. J. Clin. Nutr., 21:1239)

Periods (5-day)	$BaSO_4$			Corrected Calcium Balance				Uncorrected Calcium Balance	
	Observed, g	Corrected, g	Factor	Intake, mg	Urine, mg	Stool, mg	Balance, mg	Stool, mg	Balance, mg
2	2.70	$\frac{2.50}{2.70}$	0.926	3,810 ± 14.5	60	3,631	+119	3,921	-171
3	2.51	$\frac{2.50}{2.51}$	0.996		67	4,053	-310	4,069	-326
4	1.84	$\frac{2.50}{1.84}$	1.358		55	3,830	-75	2,820	+935
5	2.89	$\frac{2.50}{2.89}$	0.865		31	4,081	-302	4,718	-939
6	2.56	$\frac{2.50}{2.56}$	0.976		20	3,987	-207	4,085	-305
7	2.39	$\frac{2.50}{2.39}$	1.046		13	4,016	-219	3,839	-42
8	2.58	$\frac{2.50}{2.58}$	0.968		2	4,130	-322	4,266	-458
Total	17.47					27,728		27,718	
Mean	2.4957 (for 5-day-periods), recovery, 99.9%								

TABLE 4

FAT BALANCE IN DOGS

(From Seife, 1962. J. Lab. Clin. Med., 59, 513)

	Metabolic balance study				
Dog No.	Food consumed 72 hrs. (Gm.)	Fat consumed 72 hrs. (Gm.)	Fecal fat (Gm.) 72 hrs.	Ingested fat absorbed (%)	Isotope method ingested fat absorbed (%)
1	3,000	162	14.8	91	92.9
2	3,000	162	14.5	91.9	91.4
3*	333	18	3.5	80.5	87.2
4	666	36	4.2	88.4	90.4
5	1,120	56	8.5	85	87
6	1,600	86	11.3	87	88.4
7	750	41	4.1	90	89
8	1,500	81	8.8	89	92.6
9	970	53	6.2	88	87.8
10	1,000	55	6.6	88	91

*When this small a quantity is ingested, excreted fecal fat becomes a considerable fraction of the total fecal fat.

TABLE 5

FAECAL ^{59} IRON AND FAECAL ^{131} BARIUM IN A NORMAL MAN (CASE 3)

(From Boender and Verloop, 1969. Brit. J. Haemat., 17, 45)

Days after administration of test dose	(oral dose in faeces		$^{59}Fe/^{131}Ba$* in faeces	^{59}Fe absorption** (% oral dose)
	^{59}Fe	^{131}Ba		
1	27.3	51.4	0.53	47
2	24.3	43.5	0.56	
3	5.8	6.0	0.97	
4	4.5	2.8	1.61	
5+6	4.3	0.4	10.75	
7+8	1.8	0		
9+10	2.3	0		
11+12+13	2.2	0		
Total in faeces (% oral dose)	72.5	104.1		
Ultimate retention of ^{59}Fe (% oral dose)	27.5			

*Ratio of ^{59}Fe to ^{131}Ba in the faeces. This value, multiplied by 100, indicates the per cent of the test dose iron excreted in the faeces. To calculate this percentage, the first highly radioactive portion of faeces was used.

**Iron absorption percentage calculated by subtracting the percentage of iron excreted from 100.

TABLE 6

FAT ABSORPTION RESULTS OBTAINED BY THREE METHODS

(From MacDongall, 1964. Amer. J. Dis. Child., 108, 139)

Dogs	Daily Fat Intake, Gm	% Fat Absorption by Chemical Analysis	% Fat Absorption by Random Stool Method	% Fat Absorption Assessed From Total I^{131} Recovery
1	33.5	80	(81.5) 80.3, 80.6*	Not done
2	43.0	80	(79.8) 78.7, 79.7	Not done
3	32.7	88.3	(84.4) 90.2, 88.7 (85.6)	88.6
4	45.5	87.4	(79.0) 86.8, 86.0, 88.7, 85.0 (77.3)	85.8
5	43.0	87.2	(80.0) 87.4, 87.2, 83.2, (84.7)	87.2
6	43.0	80	(87.6) 83.9, 83.8, (82.3)	88.0
7	43.0	86	(87.8) 88.4, 86.2	Not done
Patients				
C1	178	95	(90.8) 98 (x)	94.3
C2	77	98.3	(93.4) 98, (98.1)	98
C3	85	95.9	(93.5) 96.5, (95.0)	97.8
C4	76	96	(97.3) 98, 93.5, (96.9)	98
C5	75	97	(94.7) 96.2 (x)	96.4
C6	72	98.7	(93.7) 95.5, 97.4 (90.0)	97.1

* Figures in parenthesis Indicate first and last marked stools respectively

(x) Last makred stool discarded in error.

Table 7

COEFFICIENTS OF VARIATION FOR CONCENTRATION OF RADIOCERIUM, CHROMIC OXIDE, AND POLYETHYLENE GLYCOL IN 20 FECAL COLLECTIONS FROM EACH OF THREE SHEEP

(From Huston and Ellis, 1968. J. Agric. Food Chem., 16, 225)

Markers	Coefficients of Variation		
	Sheep	Collections	Duplicates
Radiocerium	12.4	6.4	2.7[a]
Chromic oxide	50.2	15.3	5.8
Polyethylene glycol	67.5	26.0	6.0

[a]There was no duplication of radiocerium activity counting due to limited feces. However, a pilot counting procedure gave an estimate of counting variation from which the coefficient of variation between duplicates was estimated.

TABLE 8

THE PERCENTAGE RECOVERY OF INGESTED SILICA FROM THE FECES OF STEERS AND SHEEP IN DIGESTION TRIALS CONDUCTED UNDER VARIOUS CONDITIONS

(From Gallup et al., 1945. J. Animal Sci., 4, 68)

Animals and experimental conditions	Number of trials	Roughages fed	Concentrates fed[1]	Average silica content of ration	Average recovery[2]	Standard deviation
				Percent	Percent	±
Steers in stanchions	8	prairie hay	none	6.18	103.0	7.06
Steers in stanchions	18	prairie hay	none	5.63	94.5	3.65
Steers in dry lot	9	native grass	none	6.38	107.0	6.17
Steers in dry lot	8	native grass	cottonseed meal	6.24	112.5	5.01
Steers in dry lot	15	silage	oil meals:grain	3.76	112.1	8.78
Steers on pasture[3]	8	native grass	none	6.37	136.3	16.90
Sheep in metabolism crates	8	prairie hay	none	4.76	111.6	6.23
Sheep in metabolism crates	32	prairie hay	oil meals	4.33	105.6	5.23

[1] The oil meals were cottonseed, soybean and peanut meals; the grain was barley and oats.

[2] Expressed as a percentage of the intake.

[3] Feed intake estimated from dry matter consumption: defecation ratios determined during adjacent periods when the steers were in dry lot.

TABLE 9

INFLUENCE OF SULFURIC ACID CONCENTRATION ON LIGNIN* CONTENT OF DIET AND EXCRETA OF CHICKENS

(From Mueller, 1956. J. Nutr., 58, 29).

	sulfuric acid concentration (weight per cent)			
	71.00%	71.83%	73.13%	74.15%
	%	%	%	%
Lignin in diet dry matter	3.99	2.96	2.81	2.78
Lignin in excreta dry matter	9.91	6.74	5.72	5.00
Lignin recovery (% of lignin intake)	104.0	94.4	84.0	74.7

*determined by the "72% sulfuric acid" method, Ellis et al. (1946).

TABLE 10

EFFECT OF OVEN DRYING ON APPARENT COMPOSITION OF LADINO CLOVER

(From Van Soest, 1964. J. Animal Sci., 23, 838).

Temperature, °C for 16 hr.	Lignin	Acid-detergent fiber	Initial moisture content
	%	%	%
20	5.5	31.8	80
40	5.4	31.9	80
50	5.6	31.6	80
60	6.6	32.1	80
80	7.0	32.2	80
100	9.4	35.8	80
100	7.4	32.4	10[a]
100[b]	14.4	41.4	80

[a]Air-dried at 20°C prior to oven treatment.

[b]Sample in covered container to reduce moisture escape.

TABLE 11

THE CORRELATIONS AND RESPECTIVE COEFFICIENTS OF DETERMINATION FOR THE RELATIONSHIP BETWEEN THE INTAKE FACTOR AND THE % ASFF IN THE DRY MATTER ($r_{y.x}$) AND % FAECAL NITROGEN IN THE ORGANIC MATTER ($r_{y.z}$)

(From Langlands and Corbett, 1964. J. Agric. Sci., 63, 305)

Trial No.	No. of pairs of observations	Correlations $r_{y.x.}$	Correlations $r_{y.z.}$	Coefficients of determination $r^2_{y.x.}$	Coefficients of determination $r^2_{y.z.}$
1	34	+0.950***	+0.946***	0.903	0.895
3	36	+0.938***	+0.927***	0.880	0.859
4	36	+0.299	+0.780***	0.089	0.608
5	22	+0.811***	+0.905***	0.658	0.819
7	6	+0.863*	+0.917*	0.745	0.841
8	12	+0.892***	+0.911***	0.796	0.830
9	9	+0.144	+0.812**	0.207	0.659
10	10	+0.649*	+0.920***	0.421	0.846
11	6	+0.286	+0.245	0.082	0.060

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

TABLE 12

RATE OF PASSAGE OF FEEDS THROUGH COWS OF HIGH AND LOW PRODUCTION[a]

(From Shellenberger and Kesler. 1961. J. Animal Sci., 20, 416)

	Measures of rate of passage (in hours)					
	Excretion times					
Production level	5%	80-5%	R	Mean time	t_1	t_2
High	20.9	54.6	51.9**	53.5*	9.5	21.4*
	±4.8[b]	±8.0	±7.9	±8.8	±4.9	±3.7
Low	26.2	59.5	63.2	62.8	9.9	26.2
	±7.0	±8.3	±6.6	±6.0	±4.1	±4.7
Difference	5.3	4.9	11.3	9.3	0.4	4.8

[a] Values are means of eight observations.

[b] Standard deviation from the mean

* Significantly faster than low production ($P<0.05$).

** Significantly faster than low production ($P<0.025$).

Fig. 1. Cumulative excretions of single doses of ^{141}Ce administered in water, ^{144}Ce adsorbed onto soybean meal, and chromic oxide as the powder. From Miller et al. (1967). J. Dairy Sci., 50, 355.

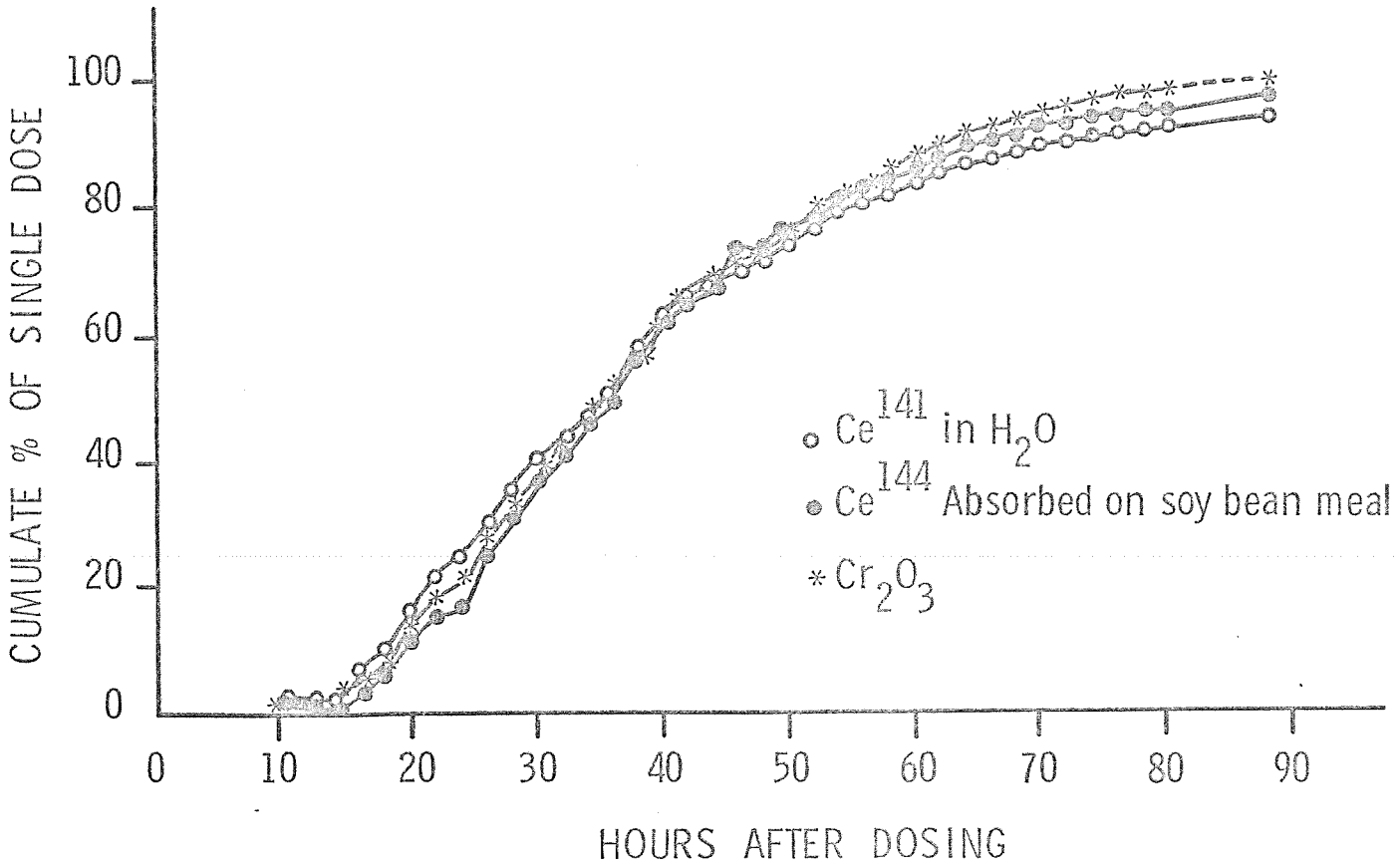

CUMULATE % OF SINGLE DOSE
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
HOURS AFTER DOSING
Ce^{141} in H_2O
Ce^{144} Absorbed on soy bean meal
Cr_2O_3

Fig. 2. Curve of excretion of undigested residue of stained hay by an adult goat. From Castle (1956a). Brit. J. Nutr., 10, 15.

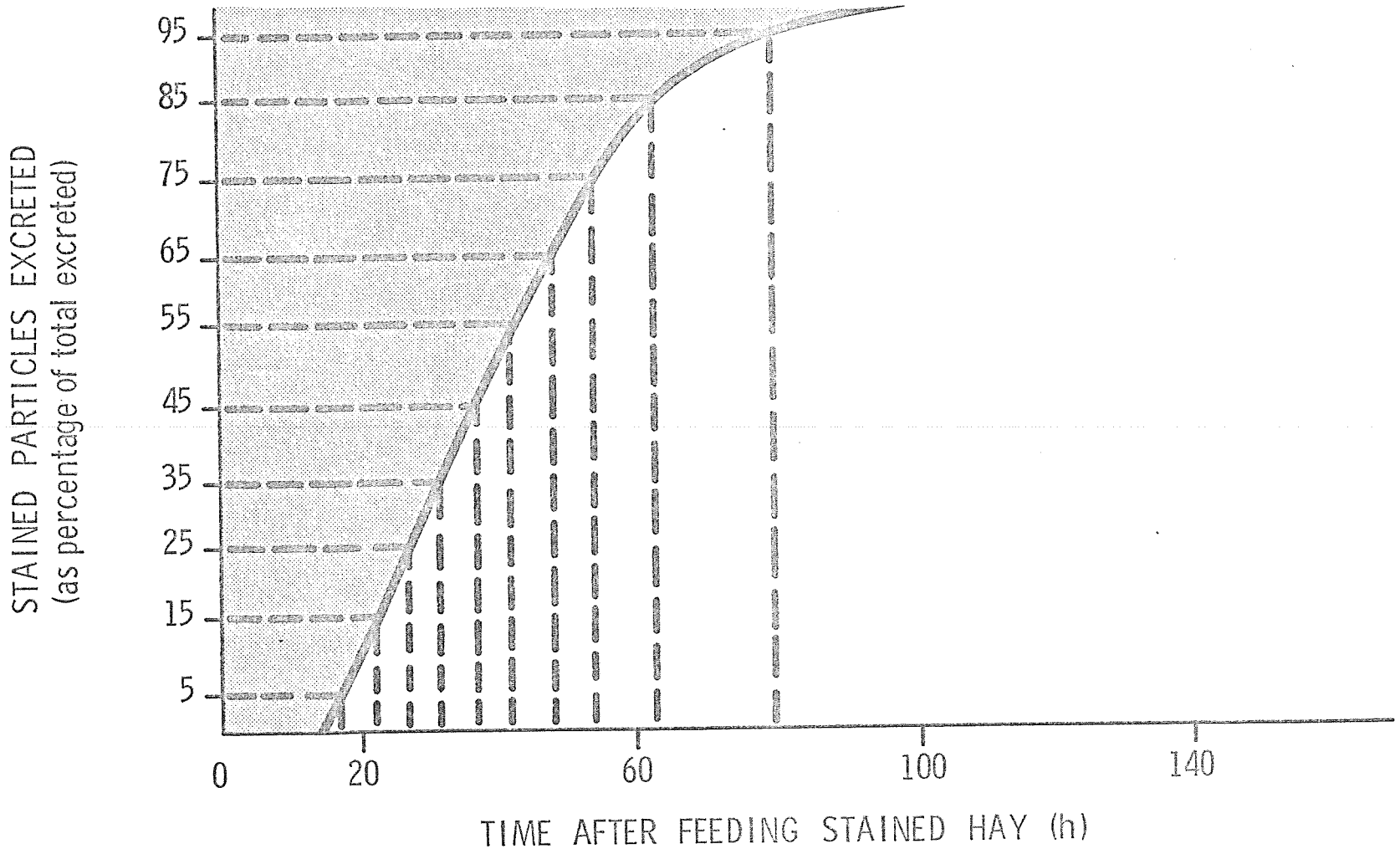
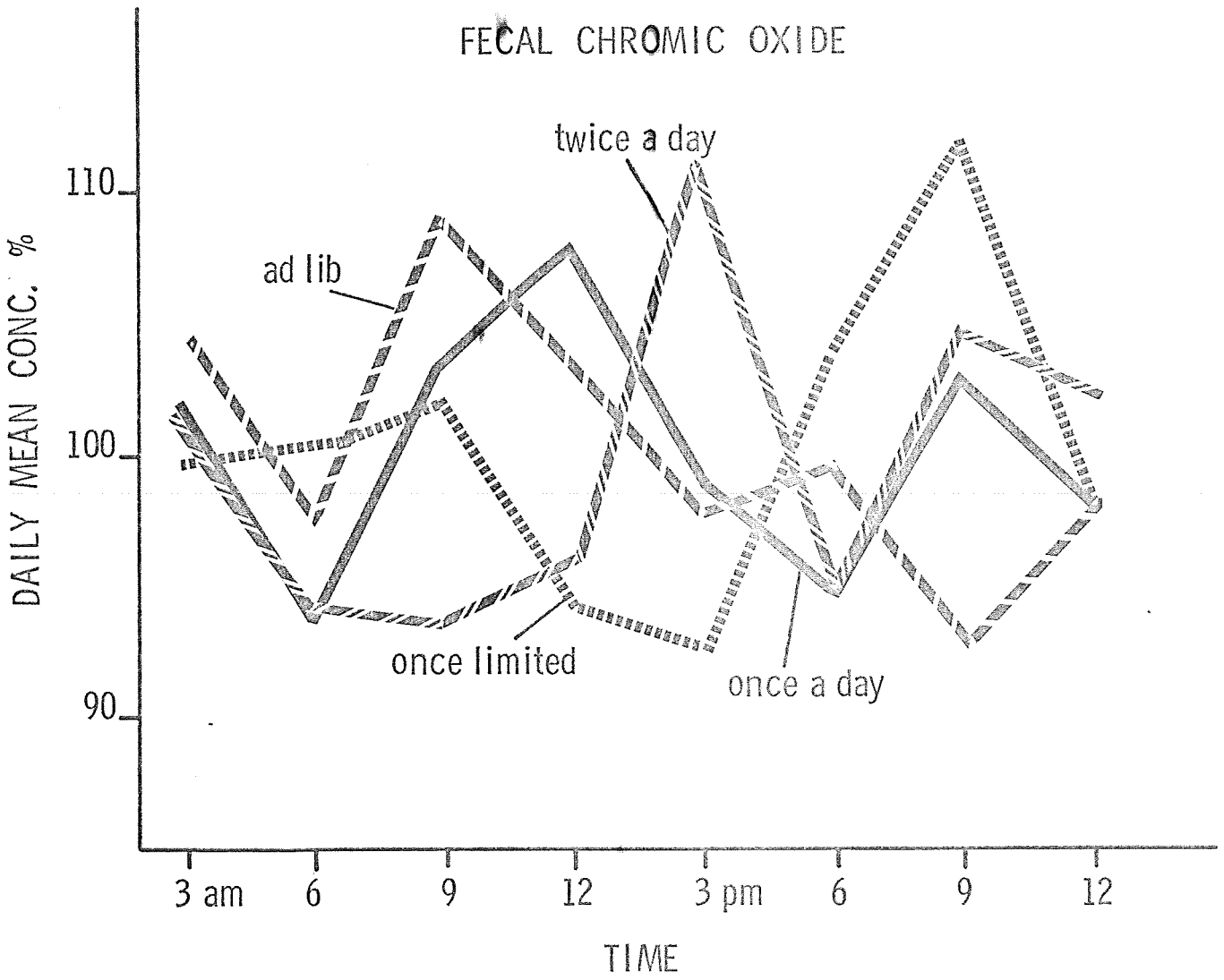

STAINED PARTICLES EXCRETED
(as percentage of total excreted)
95
85
75
65
55
45
35
25
15
5
0
20
60
100
140
TIME AFTER FEEDING STAINED HAY (h)

Fig. 3. The relative time-concentration variation of chromic oxide in the feces of heifers fed a pelleted ration. From Elam et al. (1959). J. Animal Science., 18, 718.


FECAL CHROMIC OXIDE
twice a day
ad lib
once limited
once a day
110
100
90
DAILY MEAN CONC. %
3 am
6
9
12
3 pm
6
9
12
TIME