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Title: Digesta Passage Rates in the Rat

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Michael H. Penner

The movements of digestion markers in the gut of the rat were investigated and the results of the investigation are reported in two manuscripts. The objective of the first study was to determine the influence of particle size of dietary wheat bran, chosen as a representative insoluble fiber, on the mean retention time (MRT) of digesta in the rat. The study design included a control group to detect the influence of particle size of digestion marker on the observed retention times. Additionally, a comparison was made between bran and the soluble fiber pectin. Both the soluble cobalt EDTA (Co EDTA) and insoluble chromium mordanted bran (CrMB), of two size ranges were administered to 4 groups of rats fed a semi-purified diet supplemented with wheat bran or pectin (10% dilution). Bran size and particulate marker size were large/large, large/small, small/small and pectin/small for the 4 groups. Mean retention times for CrMB were significantly longer in the pectin than the bran supplemented groups. Differences were not significantly different within the bran supplemented group. No significant differences were found between the rate of passage of CrMB and Co EDTA in any group.

The second study was designed to identify pooling of markers in the rat gut. A mathematical model was developed as a system of equations which predicted the marker distribution along the gut following dosing. The model assumed that two pools, the stomach and cecum, were kinetically recognizable in the rat. The predicted distribution of CrMB and Co EDTA was compared to *in vivo* distribution at 4 h intervals by sequential termination and dissection of the animals. The smaller particle bran had a longer MRT in the cecum and a shorter MRT in the proximal colon. Total MRT to the mid-colon was similar in all bran supplemented groups. Pectin fed animals had significantly enlarged gut organs which explained the slower movement of marker in these animals. The cecum was found to delay the transit of marker from the small intestine to the proximal colon as much as 2.1 h. This delay resulted in the gut appearing as a single pool overall and therefore led the model to overpredict the initial rate of appearance of marker distal to the cecum.

A streamlined system was developed to digest the fecal samples in preparation for atomic absorption spectroscopy, the method used to quantify digestion marker recovery. The development of the method is discussed and supporting data are presented on the reproducibility and limits of the method.

Digesta Passage Rates in the Rat

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Bret R. Luick

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INTRODUCTION

Dietary fiber has been investigated in relation to prevention of gastrointestinal diseases, circulatory diseases and mineral balance in humans [1]. Dietary fiber, both from kind and quantity, is known to influence the rate of passage of digesta in many species of animals [2].

The mechanism by which fiber influences rate of passage is complex and may involve fluid retention in the gut, fecal bulking, changes in viscosity of the digesta, microbial growth and production of short chain fatty acids [3]. Fiber particle size is regarded as a key determinant of rate of passage of digesta in many species of animals. In specific species particles are separated within the gastrointestinal tract by anatomical structures or gut motility patterns that discriminate between digesta particles based on size, solubility and density.

In ruminants, where fiber passage has been most well studied, the majority of separation of digesta by size occurs in the reticulo-rumen [4, 5, 6]. Motility patterns and colonic haustra in the hindgut of the rabbit [7] and sieving action at the entrance to the cecum of the chicken [8] result in differential passage rates based on particle size in these species.

In humans, rate of passage is known to be dependent on the particle size of dietary fiber [9,10,11,12]. Surprisingly, very little information is available on particle size and rate of passage parameters for the rat [13,14,15]. Unlike humans, the rat has a distinct forestomach [16] and the cecum is well

developed. The presence of such anatomical differentiation indicates the possibility for measurable discrimination of passage rates of digesta based on particle size within the rat. Since the particle size of dietary fiber can differ considerably based on source and preparation it is important to address this issue.

The first experiment conducted as part of this study was an investigation of the behavior of digestion markers with varying particle size of dietary bran. In order to determine if the markers were tracking the behavior of the bulk of the diet, combinations of dietary bran particle sizes and marker particle sizes were studied. In addition, the behavior of digestion markers when administered to rats fed pectin, a gel forming polysaccharide, were determined.

The paradigm by which digestion marker kinetics are interpreted involves a system of pools which are connected by pipes. Pools are considered mixing vats subjected to a constant inflow and outflow. Observations of the system begin when a marker is pulse dosed into a point in the system. When digestion markers are administered to an animal, the concentration of marker that appears in the feces has a time profile very similar to that produced from combinations of pools and pipes. Much success has been had by modelling the digestive tracts of various species of animals and is commonly referred to as compartmental analysis.

With this in mind, the second experiment performed with rats was to investigate the time distribution of marker within the rat in attempt to identify regions behaving as pools. It is possible that important kinetic events occur in the digestive tract of these animals that will not be visible by analysis of the

excreted marker. A set of equations was developed, based on known anatomical compartmentalization of the rat, to model the expexted movement of digesta within individual sections of the intestinal tract. The actual events were then compared to this model.

Finally, analysis of the markers themselves presented a problem. Digestion markers were selected which were considered safe to handle, readily available and which met the stringent requirement of stability in the digestive tract. Chromium mordants and cobalt EDTA were selected for this purpose and, being metals, were analyzed by atomic absorption spectroscopy (AAS). The techniques for preparing organic samples for AAS are numerous, but not uniformly applicable. A streamlined system of sample preparation and analysis was developed from published methods which appears to be applicable to fecal and tissue analysis.

REVIEW OF LITERATURE

Dietary Fiber

The extensive occurrence of dietary fiber reports in the current nutrition literature can be readily traced to publications by Burkitt [17,18] and Burkitt and Trowell [19]. These reports called widespread attention to the possible positive role of fiber in human nutrition. Summing the past 20 years a great many publications have explored and expanded on this theme. The following brief review is limited in scope to those reports, especially concerning humans and rats, which call attention to the importance of particle size of fiber in its physiological interactions with mammalian digestive systems. Some consideration is given to soluble fiber as it pertains to my research. Some discussion is made of papers where both soluble and insoluble digestion markers were employed.

Dietary fiber is generally viewed as complex polysaccharide substances that are resistant to mammalian enzymes. This includes non-starch polysaccharides such as cellulose and hemicellulose and soluble polysaccharides such as gums and pectins which are not at all fibrous. Other substances classified as dietary fiber are lignins, tannins, cutins, maillard reaction products, some fatty substances and mineral complexes. Digestible substances such as starch and protein which escape upper gut assimilation or originate in the upper gut may be classified as fiber. The definition and composition and physiological effects of fiber have been reviewed by many authors [1, 3, 20, 21, 22].

Dietary fiber has been investigated in relation to prevention of gastrointestinal diseases, circulatory diseases and mineral and vitamin balance in humans [1].

Dietary fiber, both from kind and quantity is known to influence the rate of passage of digesta in many species of animals [2]. The mechanism by which fiber influences rate of passage is complex and may involve fluid retention in the gut, fecal bulking, changes in viscosity of the digesta, microbial growth and production of short chain fatty acids [3]. Fiber particle size is regarded as a key determinant of rate of passage of digesta in many species of animals.

Particles are separated within the gastrointestinal tract by anatomical structures or gut motility patterns that discriminate between digesta particles based on size, solubility and density. For example, in ruminants the majority of separation of digesta by size occurs in the reticulo-rumen [4]. Smaller and less dense particles are preferentially lost from the reticulo-rumen and therefore experience a faster rate of passage [4, 5, 6]. The llama, a member of the camelidae, has a forestomach similar to ruminants and again smaller particles have a faster rate of passage [23]. Because of specialized motility patterns and colonic haustra in the hindgut of the rabbit [7] and because of sieving action of the entrance to the ceca of the chicken [8], smaller particles experience a slower rate of passage in these species. In hatchling chicks this effect is not apparent since the ceca begin functioning only after one or more weeks of age [24].

Particle Size and Water Binding

Reduction in particle size of fiber generally causes a reduction in fecal bulking and water binding properties [11, 25, 26]. In man, increased particle size on two types of wheat bran, Canadian hard red and French soft white, significantly increased daily stool weight in both cases [12] . These results have been corroborated [9, 11, 25, 26]. However, the effect was not found in the rat [27]. This latter result can be readily criticized in that fecal collections were done on a

daily basis and it is unlikely that the samples were representative of freshly voided feces.

That water binding based on particle size is not similar between man and rat is surprising. The particle size of wheat bran is comparable in the human and rat studies. Even though one study has shown the fermentation of unclassified wheat bran to be comparable between humans and rats [28], it is possible that the well developed cecum of the rat results in enough fermentation of wheat bran to alter the composition of the fiber differentially to particle size. Particle size influences in vitro and in vivo fermentation. For instance, pangola grass stems ground to 1 mm and 0.1 mm particle size showed differential rates of degradation when digested with a fungal cellulase [29] and smaller particles of total cell walls are the more rapidly solublized class.

For subjects fed a coarse versus fine bran supplemented diet, Ehle et al. [30] found that fecal short chain fatty acids were higher in the former group but not statistically different. Fecal inoculations derived from the two groups for in vitro cultures did not consistently differ in fermentation performance on various substrates, including bran. Acid detergent analysis indicates that the digestibility of wheat bran in rats is greatest for particles of 0.85 mm [31], meaning that an optimum fiber size for fermentability can exist. Nyman and Asp found no correlation between digestibility of wheat bran and particle size in rats [32]. The composition of wheat bran has been found to be constant within limits over a range of particle sizes. As delivered coarse American Association of Cereal Chemists (AACC) soft white wheat bran and that ground to pass a #40 sieve have similar neutral detergent (38.8%, 40.5%, respectively) and acid detergent (10.8%, 11.3%, respectively) fiber values [11]. Similar consistancy has been

seen for hard red wheat bran [33]. Gut contents of oat bran supplemented rats show increased water content with decreased particle size [34].

Robertson and Eastwood [35] examined factors of water binding in fibers by centrifugation. Water may be either trapped in the fiber or bound to it. They found water holding capacity to be a function of fiber structure and less so of chemical composition. Bound water is a function of chemical composition and since they found that the preparation of the fiber influenced the water holding capacity their results imply that the structure of the dietary fiber is key to fecal bulking. Rate of passage in the hindgut of the rat slows with age and could well result in changes in the pattern of fecal matter moisture content [36]. In this regard, studies of cecectomized rats have shown that rate of passage and fecal water content are significantly increased above sham operated controls [37, 38].

In general, different fibers contribute differently to stool weight. In rats 8% pectin or guar result in daily fecal dry matter output similar to fiber free fed rats [39]. Oat bran and cellulose produce increasingly heavier fecal weights. Among the highest daily weights was wheat bran supplement, however it was fed at the 21% level to compensate for its limited dietary fiber content (38%). When the unabsorbable marker chromic oxide was incorporated into the diet (0.4%) dilution of the marker in the hind gut and feces followed the same pattern. In effect the more fermentable the fiber the less residual diluting capacity it had. For oat bran both wet weight of hindgut contents and the ratio wet to dry matter was higher than in fiber free controls [34]. Comparisons between man and rat have shown similar responses in fiber breakdown and bulking capacity [28]. Fecal bulking was found to be inversely related to the water solubility of the fiber. In descending order of ability to induce fecal bulking were wheat bran,

apple, cabbage, carrot and guar gum. The increasing extent of in vivo fermentation was found to follow the same order. Fecal weight and water were observed to increase linearly with additions of the purified fiber "Solkafloc" to the diet of dogs [40]. Because bran is a poor source of calories, its addition to a diet results in caloric dilution. For a series of brans fed to rats, fecal bulking increased linearly with decreasing digestibility of the bran [41]. This brought forth the interesting point that whole wheat flour, because of its 95% digestibility, is a poor agent for fecal bulking.

In man, increased fiber intake correlates with increased rate of passage [42, 43, 44, 45]. There are sex differences in that women on like diets exhibit lower stool weights and longer transit times [44]. Stool weight is known to correlate inversely with rate of passage [46]. Cooking of wheat bran can diminish these effects compared to raw bran [47]. Stephen et al. addressed the question of whether pharmacologically reducing or extending transit time in humans while consuming a common high fiber diet would result in a change in microbial mass [48]. Indeed it was found that a linear response occurred of decreased microbial mass with decreased transit time. These results indicate that conversion of fiber to microbial mass will depend at least in part on residence time in vivo.

Particle Size and Rate of Passage

The literature concerning rate of passage studies has a stimulating array of terminology. Briefly, the term "transit time" (TT) refers to the elapsed time from mouth to anus of a marker. The stop watch is started when the dose is swallowed and stopped at the first appearance of marker in the feces. This measurement can be variable and tells little of the dynamics within the gut [2], but it is widely reported. Unfortunately, the term transit time is also used for

many other measurements of rate of passage such as the time for 80% recovery of a marker dose. Mean retention time (MRT), also called mean transit time (MTT), is the single most useful measurement of rate of passage. This number represents the average time a particle has spent in the digestive environment. By comparison, transit time is the minimum time any particle could spend in the digestive environment.

In humans, rate of passage is faster (shorter mean transit time) in groups fed diets supplemented with bran of larger particle size versus smaller particle size [9, 10, 11, 12]. When comparing large and small particle wheat bran ground from Canadian red spring wheat or French soft wheat and fed to two different groups it was found that rate of passage was faster in people fed the large particle bran supplemented diet [12]. Similar results have been found with AACC soft white wheat [11]. Kirwan et al. [10] found that only coarse bran increased rate of passage compared to a diet without the bran supplement. They concluded that the water binding capacity of the larger bran particle caused increased fecal bulking and accounted for the increased rate of passage. Van Dokkum et al. [25] report a distinctly shorter rate of passage (44 versus 62 hours for time of 80% marker recovery) in subjects fed coarse versus fine bran supplemented diets but the differences were not significant. Wrick et al. report the same combination of particle size and mean retention time but the differences were not significant [9]. Separation of particles of dissimilar sizes within humans has not been demonstrated. Comparison of simultaneously administered soluble and insoluble markers in humans when fed either large or small particle bran supplemented diets revealed that the mean retention time of the soluble marker was longer but not statistically different [9]. A few studies have reported on the interaction of particle size and passage rates of digesta in

the rat. Wheat bran, when ground to three levels of fineness, did not result in a significantly different rate of cumulative loss of a water soluble marker [13]. In a lifespan study of the rat, soluble markers moved more rapidly than insoluble markers in both sexes at 7 and 18 months of age [14]. Females were found to have shorter mean retention times. In a recent well executed study rats were fed a high roughage diet (50% alfalfa, 15% wheat bran) [15]. In this case soluble and insoluble markers were found to have similar mean retention times. Unlike humans, the rat has a distinct forestomach [16] and the cecum is well developed [2]. The presence of such anatomical differentiation indicates the possibility for measurable discrimination passage rates of digesta based on particle size within the rat. Rate of passage in individual gut segment in rats have been found to be age dependent [38]. While the stomach and small intestine passage rates remain constant, the cecal mean retention time increases five fold and the large intestinal transit time increases 70% between weanling age and 20 months. Fiber studies using rats are generally done with quite young animals and it is possible that differential particle movement will not be detectable until the adult gut motility pattern is established. Determination of mean retention time by compartmental analysis depends on a relatively well developed marker excretion curve. If fecal excretion is irregular, less confidence can be put into the calculated kinetic constants. For rats on a high roughage diet exhibiting normal levels of feed intake, the separation of liquid and particle fractions may be less because of a rapid flow of the bulk of the digesta. Conversely, when a low roughage diet is fed, the lack of residue in the hindgut may result in considerable delays of marker excretion. Accumulation of dietary and intestinal residues in the distal colon may obscure any separation of markers that occured in proximal regions of the gut. Delay of dietary residue in the distal colon of rats on a variety of diets has been observed [49]. Consequently, there are conditions

that can prevail in the gut which cannot be interpreted by compartmental analysis.

Ehle et al. [30] found that the half time of excretion of chromium mordanted bran was longer from pigs fed large versus small particle bran supplemented diets. However, the large particle bran was fed at a lower level to compensate for a higher cell wall content. Since it has been reported that rate of passage decreases in pigs with increasing dietary residue neutral detergent fiber (NDF) [50], I recalculated the results based on total bran content of the diet. This narrows but does not reconcile the difference in excretion rate. Interestingly, mean retention time and time of first appearance (transit time) were similar between the two groups resulting from opposing combinations of transit time and turnover time (the rate of pool emptying). Since the digestibilities of the two diets were similar the faster transit time might be attributed to the enhanced fecal bulking of large particle bran. Ehle et al. [30] comment that the pig can be used only cautiously as a model of human digestion. This point has been confirmed with comparative in vitro digestibility studies [51].

Other Effects Of Fiber Particle Size

Particle size of dietary wheat bran has been shown to influence the plasma levels of vitamins A and E [52]. Plasma retinyl palmitate was significantly higher in large particle bran fed rats but the opposite was observed for alpha-tocopherol. In neither case did plasma levels consistently reflect hepatic levels of the vitamins. The authors felt the lowered plasma vitamin E levels resulted from impairment of vitamin E absorption as a result of interaction between the particle and the absorptive site. In another study unclassified wheat bran showed no effect on plasma or liver vitamin E levels [53].

Purified cellulose and hemicellulose had no effects on gastric emptying of lipids, small intestine lipolysis, lipid and cholesterol content of the small intestine contents nor in plasma levels of lipid [54]. However, differences were evident if whole bran or wheat germ were fed [54] which lends weight to the importance of the physical form of fiber in nutrient interactions. Others have not been able to demonstrate changes in serum cholesterol by rice or wheat bran supplementation [55]. Lowering of serum lipids has been noted in rats fed increasingly finer ground bran, however total diet intake as well as lipid and cholesterol intake were similarly reduced [56]. In fact, others have found no change or increased serum cholesterol in wheat bran fed rats [57,12]. Pectin has been reported to reduce serum cholesterol [58] in humans, perhaps through binding of bile salts in the small intestine or by delaying or impeding absorption from viscosity effects.

Fiber Type and Rate of Passage

Studies with rats have demonstrated that the movement of markers in individual segments of the gut correlate with the fiber composition of the diet [49, 59]. Dietary supplementation of guar gum decreases rate of passage of a radioisotope marker through the stomach and small intestine of the rat compared to a fiber-free diet. Radiopaque markers, when followed throughout the gastrointestinal tract of rats fed cellulose, fiber-free, guar gum, oat bran, pectin and wheat bran diets, show increased rate of passage in the order given [49]. Pectin, cellulose and wheat bran led to decreasing halftime of excretion of a digestion marker relative to a fiber free diet in rats fed a diet supplemented with these fibers [60]. Others have found pectin to retard marker transit time relative to guar gum [61] although different levels were fed and the sample size

was small. In humans pectin has been reported to not affect gut rate of passage since it can be effectively fermented by gut bacteria. Mean retention time decreased using a diet supplemented with the effectively unfermentable fibers alpha cellulose and lignin [62].

Rats fed 10% wheat bran had significantly shorter gastrointestinal transit times than fiber free control animals [63, 64]. Interestingly, germ-free rats gave results similar to the conventional animal [35] which indicates that the physical nature of the bran itself exerts a substantial physiological effect independent of fermentation. If the level of a fermentable fiber is within the ability of the gut microflora to process it, the fiber may not result in substantial fecal bulking or decreased transit time [65, 66]. Conversely, reduction of transit time with the addition of a fiber may not imply that microbial fermentation was not on going, especially in the case of a mixed fiber diet.

II DIGESTION KINETICS

Historical Perspective

Digestion kinetics refers to the movement of digesta through the alimentary canal. Exploration of digestion kinetics blossomed in the 1950's when several models were forwarded. Originally Balch [67] suggested the use of the times at which 5% and 80% of total marker was excreted as indicative of the time feed residues spend in regions of the cow. This approach was purely descriptive and provided no insight to the actual movements of digesta within the animal. The first complete mathematical derivation for the distribution of a pulse dosed digestion marker in an animals was given by Blaxter et al. [68] working with sheep. This model was derived on a purely theoretical basis and provided no proof that the rate constants had true biological relevance. The form of the model was biexponential, meaning the mathematical equation was fundamentally two exponential terms. This paper was followed almost immediately by the work of Brandt and Thacker [69] who, using rabbits as their model animal, arrived at a mathematically identical description. Kotb and Luckey [70] reviewed the markers that have come into use in the study of the digestive tract. Shipley and Clarke [71] summarized the use of tracers for in vivo studies, generalized for any marker in any system of pools. Grovum and Williams avoided direct sampling of the pools (by rumen fistula, for example) by showing that digestion pools could be adequately described by analysis of the marker appearing in the feces [72]. They further gave evidence of the biological relevance of the derived rate constants. Grovum and Phillips [73] compared results from sheep to a bench top hydraulic model and confirmed the validity of marker studies in the ruminant digestive tract. This included the interpretation of rate constant in terms of mean retention time (MRT) of the markers in the

animals. These authors could find no theoretical relevance to the use of the arbitrary 5% or 80% excretion point suggested by Balch. Warner [2] summarized the approaches in the literature for calculating MRT and gave tables of MRT for gut segments and whole gut transit from all available studies on birds and mammals. Particles within the digestive tract do not behave as inert markers, for instance many change in particle size and solubility during their sojourn through the gut. Quiroz et al. [74] forwarded a model that describes an "age-dependency" term to a particle. However, they found that the statistical difference between the two models was small although the age dependant terms do improve the fit. There have been many other models forwarded in the same vein, especially in the last 10 years, which attempt to describe the behavior of particles in the gut in greater detail. The current trend is to adapt the approach of chemical reactor theory to animal digestion [75]. Here, the intent is to determine the rate at which nutrients are assimilated and to understand the architecture of animals digestive systems. Chemical reactor theory is very adaptable to unusual configurations and non-steady state conditions.

Hydraulic Model

The following discussion briefly introduces the terminology of digestion marker kinetics. The exponential model for digestion kinetics is drawn from a simple hydraulic model, also an exponential model, where a dose of dye is imagined to be added to a tank of water of constant volume. The dose of dye mixes instantly with the water and elutes from the tank via a steady flow of water through the system. The model can be expanded as much as desired, several pools (tanks) are possible of any size, flow can be in any direction and blind pools may be added. In the study of animal digestion, the concentration of marker in the

outflow of the system, that is the fecal matter, can be used to adequately determine the kinetics of the digesta flow within the animal.

As it happens, a system more complex than two pools is not often necessary, nor easy to distinguish from a two pool system. This is because other pools will not greatly affect the overall behavior of the marker as seen in the outflow of the system. That is, to solve a complex system it is almost necessary to monitor concentrations of the marker at various points in the system. This could be done with fistulas or cannulas. Figure II.1 shows a simple one pool model of digesta flow.

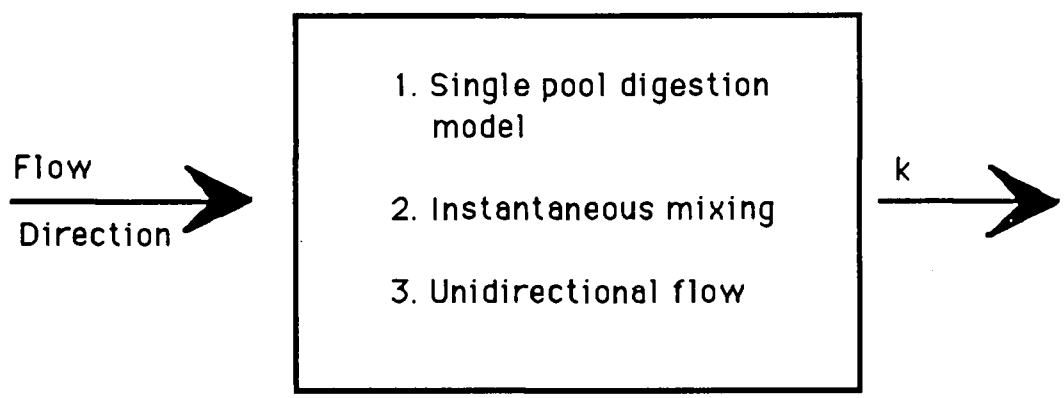

Figure II.1 Diagrammatic model of a single pool system.

The value k is a rate constant having units 1/hours. Mathematically the system is described by the differential equation

$$dq(t)/dt = q(t)*k, \qquad \{1\}$$

where "q" is the quantity of dose in the pool and "t" is time. The idea is that any particle within the pool has an equal statistical chance of being part of the outflow. Therefore the quantity of particles that are lost at a given time is a product of the quantity particles in the system and a probability coefficient. This

is equally true of the bulk matter in the system and the small amount of marker added into the system. The probability coefficient for the marker and the bulk matter will be the same if the two quantities have the same behavior in the system. For instance, if a very dense marker is used that rests undisturbed in the bottom of a gut compartment, it cannot be expected to represent the flow of other less dense or fluid components of the system. In the integrated form equation {1} becomes

$$q_p(t) = q_p(0) * e^{-kt} \qquad \{2\}$$

where q_p is the quantity of marker in the pool at some time and e is the base of the natural logarithms. This is the familiar equation for first order losses, such as radiation decay. To save confusion I will drop the term $q_p(0)$, since it is the initial quantity of dose, and can be normalized to 1. The natural log of this equation has the form of a linear equation

$$\ln(q_p(t)) = -kt, \qquad \{3\}$$

where the negative of the slope is k, the rate constant (1/h). The reciprocal of the slope is turnover time (h) which is described as the time required for a volume equal to the pool size to pass through the pool. This depends on the flow rate and pool size. Halftime ($t_{1/2}$) is the time required for the concentration of marker within the pool to fall by half and is equal to ln(2)/k. The mean time, or mean retention time that a marker spends in a pool turns out to be equal to the turnover time. The above equations can be recast in terms of concentrations rather than quantities of markers, however volume appears on both sides of the equation and can therefore be divided out.

Figure II.2 is a hypothetical plot on linear coordinates of the amount of marker remaining in the pool versus time. Figure II.3 shows similar hypothetical data

expressed as the natural logarithm of maker in the pool versus time. The plot is linear with slope of -1/k.

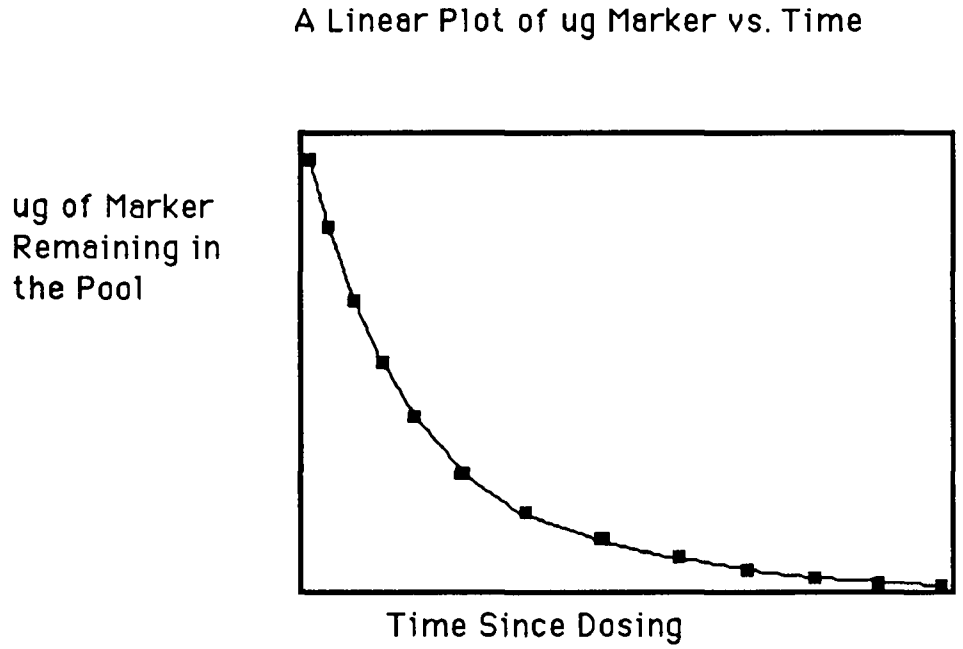

Figure II.2 The natural logarithm of marker versus time in a single pool with constant volume and flow.

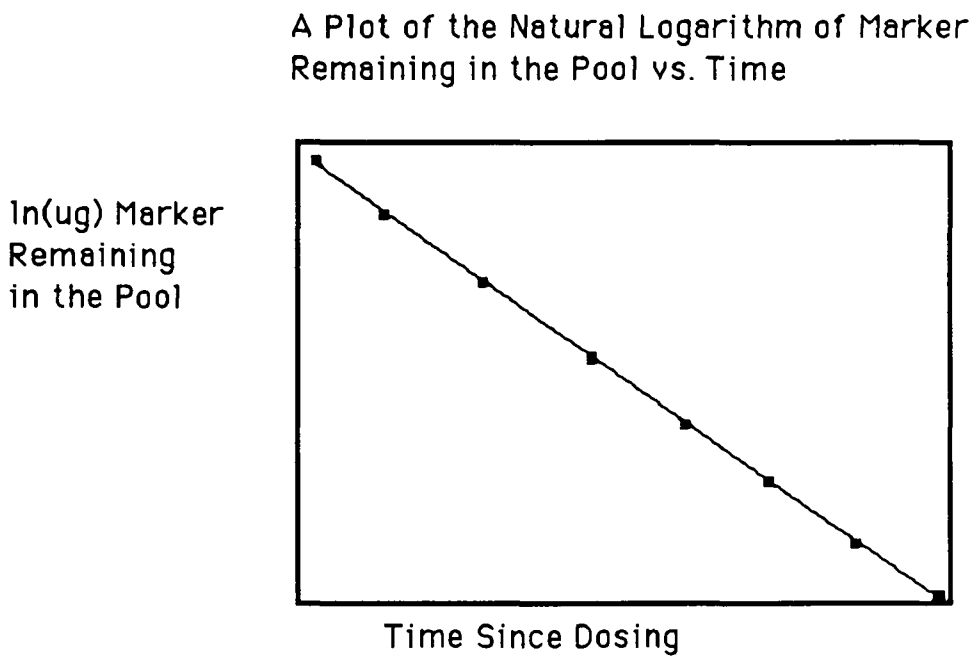

Figure II.3 The natural logarithm of marker versus time in a single pool with constant volume and flow.

A two pool model of the digestive system requires the addition of a second term. A diagram representing a two pool system is shown in Figure II.4.

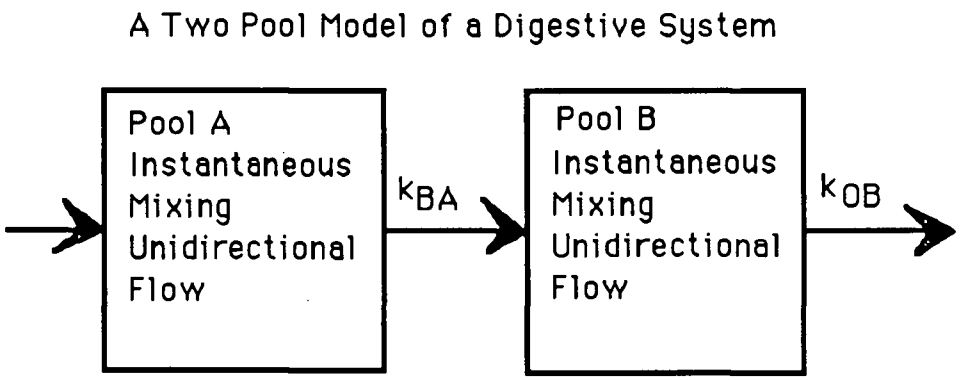

Figure II.4 A diagrammatic representation of a two pool system.

The pools are named A and B, the two rate constants are distinguished by subscripts which are read "to B from A" and " to Out from B." Since the flow is unidirectional, the rate constants can be referred to as k_a and k_b respectively. The kinetic description for the single pool system still holds for pool A. Pool B has two controlling kinetic constants, the quantity of marker in pool B is described as the sum of two terms:

$$dq_b(t)/dt = k_a q_a(t) - k_b q_b(t) \qquad \{4\}$$

The change in quantity of marker in pool B with time equals the inflow of marker from pool A less the outflow. The solution of this for total marker in pool B is

$$q_b(t) = (k_a/(k_a-k_b))^*(e^{-k_b t} - e^{-k_a t}). \qquad \{5\}$$

A plot of equation {5} is shown in Figure II.4. Often the rate constants are referred to as k_1 and k_2. As it happens, the plot looks the same no matter which rate constant is the larger. By convention the larger pool is assigned the rate constant k_1 and for this reason $k_2 > k_1$. If the constants are the same size equation {5} is undefined and must be written in a different form [5].

If there is a pipe (small intestine) which delays the transport of the marker the term t is adjusted by a constant equal to the delay. Hence t would appear as $(t-\Delta)$, where Δ represents a delay of some hours (zero to infinity are possibilities). This delay is called transit time (TT) and is equal to the shortest possible time that a marker can take to get through the system. In animal digestion this is the mouth to anus time, or time of marker first appearance in the feces after oral administration of the dose. Total mean retention time (TMRT) is the sum of the individual retention times and the delay or, $1/k_1 + 1/k_2 + TT$. This derivation is discussed in detail by Shipley and Clarke [71].

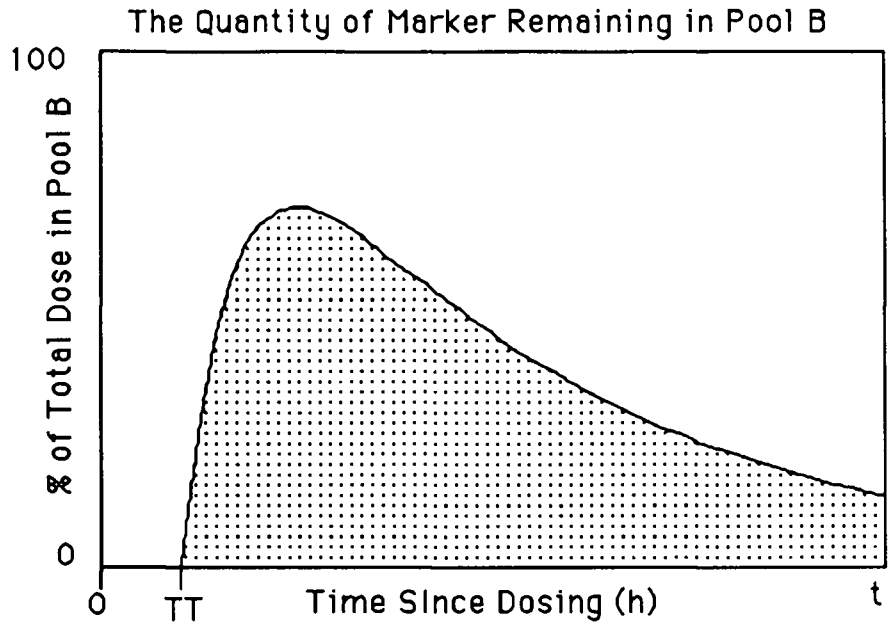

Figure II.5 The quantity of marker in pool B, the second pool of a two pool system.

Figure II.5 shows the percent of the dose administered to pool A that can be expected to occur in pool B over time as predicted by equation {5}. The transit time is shown as TT. If this figure were re-expressed on semi-logarithmic co-ordinates, the tailing section of the curve would approach a straight line. The slope of that asymptote is $-1/k_1$. If the asymptote were extended to the y axis and the difference between the y co-ordinate of each of the ascending points and this extended line taken and replotted as a point above the x axis, a new straight line would appear that has slope $-1/k_2$. The intersection of these lines will be at TT [4]. Computer routines are widely available that will fit a least squares line to a data set approximating Figure II.5.

A Mathematical Model

Chapter V is a manuscript which describes the quantity of marker that can be expected at any point in the digestive tract following oral administration of the

marker dose. The quantity is described by a mathematical model that is comprised of 15 equations. The derivations for these equations are not in the manuscript but are given here. The equations are given in Table II.1, which is adapted from the manuscript. The numbering of the equations starts with {M1}, which corresponds to equation {2} of the previous discussion. Equation {M5} of the table is equivalent to equation {5} of the previous discussion.

In Figure II.4, pool A can be taken as the stomach and pool B taken as the cecum in the rat. The arrow that connects A to B would be the small intestine. The arrow out of pool B is the colon and would in turn empty into a fecal collection tray. The amount of marker that is administered to a rat would eventually be recovered in that tray. The tray can be called pool C. I solved the system by use of the mathematical device called the Laplace transform. This transform is an integration which changes a differential equation into another equation that can be manipulated algebraically. The algebraically manipulated equation is then subjected to an inverse transform, itself actually another integration. The way the system of pools is solved is to set up simultaneous equations, the solution of the first become part of the next, and so on.

The stomach is the first pool, pool A. The Laplace transform of equation {1} is

$$sF_a(s) - f_a(0) = -k_a F_a(s),$$

where lower case a still refers to pool A or the rate constant for loss from pool A. After a little manipulation the equality becomes

$$F_a(s) = f_a(0)/(s+k_a).$$

and $f_a(0)$ is the value of the function at time zero. For pool A this is $q_a(0)$, or 1, the normalized dose. The inverse transform of this is equation {M1} of Table II.1.

Equation {4}, which describes the change in marker concentration in pool B with time, transforms to

$$sF_b(s) - f_b(0) = k_aF_a(s) - k_bF_b(s)$$

and after substituting in $F_a(s)$, setting $f_b(0)$ to zero (initially there is no marker in pool B) and rearranging, becomes

$$F_b(s) = k_a/(s+k_a)(s+k_b).$$

The inverse transform is equation {5} or {M5}.

The quantity of marker that accumulates in the feces is described as

$$dq_c(t)/dt = k_bq_b.$$

The transform of this is

$$sF_c(s) - f_c(0) = k_bF_b(s),$$

and making substitutions for $F_b(s)$ and setting $f_c(0)$ to zero, becomes

$$F_c(s) = k_bk_a/s(s+k_a)(s+k_b).$$

The inverse Laplace to this equation is any of equations {M7, M10 or M13}.

In Table II.1, equation {M2} states that the quantity gained by the small intestine equals the quantity lost by the stomach. Equation {M3} recognizes that after a delay, marker in the small intestine is lost to the cecum. Equation {M3} is simple to derive. It is assumed that the small intestine exhibits no pooling behavior, it is a simple conduit connecting the stomach to the cecum. therefore the marker leaves the small intestine in an identical fashion to the manner it enters, but only after the delay Δ. That is, after time Δ, the quantity of marker in the small intestine will be

$$q_{si}(t) = \left(1 - e^{-k_st}\right) - \left(1 - e^{-k_s(t-\Delta)}\right),$$

which after a cancellation and re-ordering of terms becomes equation {M3}. Equation {M4} states that no marker exits in the cecum until it gets there.

Equation {M5} has already been derived and also recognizes the delay term Δ. Equation {M7} corresponds to equation {M2} except that the colon is preceded by two pools. Notice that this equation has two time dependent terms. After a delay called μ, the proximal colon looses marker to the distal colon. The delay μ is measured from the time marker is first administered. Equation {M8} is developed in the same manner as equation {M3}. Equations {M10} and {M11} are the same as {M7} and {M8} where Ω, the Greek symbol meaning "the end" is transit time of the whole gut. This is the time of marker first appearance in the feces. Equation {M13} is the same as equation {M7}.

Equation {M14} is the formal definition of total mean retention time (TMRT). It is applicable to the whole system or any sub-region. The term Œ is time at which marker leaves the sub-region. If the sub-region is actually the entire system it become the mouth to anus transit time (TT), just referred to as Ω. The second right hand side term is the ratio of the time weighted "area under the curve" (the integral of the function) to the area under the curve. This equation is described in references [5,6,7].

Equation {M15} is developed by making the assumption that the total amount of marker in the system equals the combined quantities in pools A and B. The amounts in the small and large intestines can be ignored since they are automatically included in the delay terms. That is, equation {M15} is the sum of equations {M1} and {M5} evaluated at any time beyond Œ.

This model is not limited to given number of regions. If the gut were subdivided into several subsections, for instance the small intestine could be divided into

the duodenum, jejunum, and ileum, than an equal number of delay terms must be added. The real value of this model is not that it will predict what actually happens in the gut of the rat, but that is predicts what can be expected to happen if the gut were a perfect hydraulic system. The ways in which the model deviates from observed behavior of the gut points to unique characteristics of the gut. Refinements of the model will result in discovering more subtle characteristics of the gut.

Table II.1 The mathematical model of Chapter V.

Region	Time period	Function
{M1} Stom.	t(0) to ∞	$f(t) = e^{-k_s t}$
{M2} S.I.	t(0) to Δ	$f(t) = 1 - e^{-k_s t}$
{M3} S.I.	Δ to ∞	$f(t) = e^{-k_s t}(e^{-k_s \Delta} - 1)$
{M4} Cecum	t(0) to Δ	$f(t) = 0$
{M5} Cecum	Δ to ∞	$f(t) = A_1\left(e^{-k_c(t-\Delta)} - e^{-k_s(t-\Delta)}\right)$
{M6} P.C.	t(0) to Δ	$f(t) = 0$
{M7} P.C.	Δ to μ	$f(t) = 1 - A_1 e^{-k_c(t-\Delta)} - A_2 e^{-k_s(t-\Delta)}$
{M8} P.C.	μ to ∞	$f(t) = A_1 e^{-k_c t}\left(e^{-k_c \mu} - e^{-k_c \Delta}\right) + A_2 e^{-k_s t}\left(e^{-k_s \mu} - e^{-k_s \Delta}\right)$
{M9} D.C.	t(0) to μ	$f(t) = 0$
{M10} D.C.	μ to Ω	$f(t) = 1 - A_1 e^{-k_c(t-\mu)} - A_2(e^{-k_s(t-\mu)}$
{M11} D.C.	Ω to ∞	$f(t) = A_1 e^{-k_c t}\left(e^{-k_c \Omega} - e^{-k_c \mu}\right) + A_2 e^{-k_s t}\left(e^{-k_s \Omega} - e^{-k_s \mu}\right)$
{M12} Feces	t(0) to Ω	$f(t) = 0$
{M13} Feces	Ω to ∞	$f(t) = 1 - A_1 e^{-k_c(t-\Omega)} - A_2 e^{-k_s(t-\Omega)}$
{M14}		$TMRT = Œ + \int_{Œ}^{\infty} t\, f(t)\, dt \Big/ \int_{Œ}^{\infty} f(t)\, dt$
{M15}		$f(t) = A_3\, e^{-k_b(t-Œ)} - A_4 * e^{-k_a(t-Œ)}$

Symbols: k_s, k_c, rate constants (1/h) for marker loss from stomach and cecum; A_1, $k_s/(k_s - k_c)$; A_2, $k_c/(k_c - k_s)$; t, time since administering dose; ∞, infinite time; Δ, time of marker first appearance in cecum; μ, time of marker first appearance in distal colon; Ω, time of marker first appearance in feces. Colon divided midway on the middle colic artery

III DRY MATTER, CHROMIUM AND COBALT ANALYSIS

This chapter addresses the laboratory techniques which were used for the analysis of samples generated in the marker studies. Some analytical results are presented to illustrate the development of the methodology. The studies themselves are given as manuscripts in chapters IV and V.

Analysis of Fecal Dry Matter

The marker study protocol was, in effect, to offer rats a small amount of food mixed with the digestion markers, followed by serial collection of feces until the entire marker dose was presumed recovered (6 days). Individual fecal samples were placed in labelled sealable plastic bags. Moisture determinations of the fecal samples were taken as time permitted during the course of the trial. To do this the samples were weighed directly from the bag on aluminum or plastic weighing boats and then dried at 67° C for 12 h. This time and temperature combination was found to be adequate to achieve constant weight. The samples were then put into a fresh plastic bag and stored at room temperature awaiting metal analysis. Higher drying temperatures were not employed as I had previously found that drying cabbage and apple samples at 100° C for 12 h resulted in significant browning of the sample and probable loss of organic matter. This latter point was confirmed by drying the cabbage at room temperature in a vacuum desiccator over sulfuric acid. Fecal samples may not be as sensitive to drying as fresh vegetation.

It was difficult to accurately assess the moisture content of the fresh fecal samples. Firstly, it was impossible to keep up with all the weighings in the face of the fecal collection schedule. Towards the end of the marker study there was

a considerable delay (about 2 days) between fecal collection and wet weight estimation. Undoubtedly there was some loss of moisture from the samples. Secondly, the fecal samples appeared to dry somewhat in the metabolism cage even before collection.

Table III.1 shows the wet and dry weights of fecal samples collected on a 2 and 24 hour basis. The 2 hour samples were taken during the first two days of the first marker study. In all cases the % moisture is seen to be substantially higher in these samples and probably more representative of true fresh fecal moisture content.

Table III.1 The influence of collection interval on the observed moisture content of feces collected from 12 rats on a 10% wheat bran supplemented diet (mean ± SE).

Collection Interval	Wet wt. g	Dry wt. g	Moisture %
24 h	1.24 ± 0.05	0.82 ± 0.03	30.5 ± 1.4
n	82	84	82
2 h	0.22 ± 0.01	0.12 ± 0.01	41.2 ± 1.08
n	152	152	152

Analysis of Digestion Markers

The design of the feeding trial necessarily generated many samples for individual chromium and cobalt analysis. Over the six days of the trial each of the 48 animals produced more than 20 fecal samples resulting in about 1000 individual analyses for AAS. During the second feeding trial an additional fifty fecal samples and 240 gut segments were generated. Combined with the daily

standards it was apparent that quite a few runs on the AAS were required and a streamlined and reliable protocol was needed.

Prior to admitting a sample into a flame spectrometer the sample to be analyzed must be in a soluble form. This is because the sample is aspirated into the burner via a capillary tube. All particulate matter must be either destroyed or of small enough diameter to freely pass through the aspirator. In general, two methods of preparation for samples containing organic matter are employed in flame AAS. In the first, samples are dry ashed in a muffle furnace and the ash solublized in mineral acid. In the second method extremely harsh acidic conditions are employed to achieve total destruction of organic matter. This methodology generally employs one or all of perchloric, nitric, hydrochloric or sulfuric acids and sometimes addition of 30-70% solutions of hydrogen peroxide. Because of the ready availability of dry ashing equipment and a recent publication involving digestion markers analyzed using this approach [15], I first experimented with metal analysis using dry ash methodology.

The protocol was developed for chromic oxide [76] (Cr_2O_3) but has been used for chromium mordanted bran (CrMB) and cobalt EDTA (Co EDTA) [15] . Briefly, one gram of a dried sample is dry ashed at 600° C for 1.5 hours in a porcelain crucible. The ash is taken up in an oxidizing acidic solution of potassium bromate, manganese sulfate and phosphoric acid and digested with heat for 5-7 min. The solution is then filtered into a volumetric flask and, following dilution, is assayed for metals by AAS.

Table III.1 shows the results of several tests of chromium and cobalt recovery using the dry ash method of Williams et al. [76]. Each crucible contained 1.00 g

of plain wheat bran as a source of organic matter to which variable amounts of potassium dichromate ($K_2Cr_2O_7$) or Co EDTA were added. Potassium dichromate is the commonly used standard in chromium analysis and the use of a salt was prefered over CRMB since the precise amount of chromium added could be known.

Table III.2 Combined results from various assays of chromium and cobalt recovery using atomic absorption spectroscopy following dry ashing at 600° C for 1.5 hours.

	Quantity of Metal (ug)			
Sample	Chromium added	Chromium recovered	Cobalt added	Cobalt recovered
11	0	4	0	5
12	0	4	0	8
13	10	9.3	10	2
14	10	9.3	10	1
15	16	21	16	15
16	16	19	16	16
17	100	88	100	67
18	100	86	100	73
19	1000	690	1000	600
20	1000	690	1000	600

Surprisingly, the duplicate samples agreed fairly well at all levels of metal. The low values of cobalt recovery for samples 13 and 14 are unexplained. However, as the amount of metal increased the degree of underestimate of metal became substantial. Using the same ashing time but lowering furnace temperature to 550° C improved chromium recovery to 710 ± 50 ug (SD, n=8) at the 1000 ug addition level. This indicates that chromium recovery is sensitive to furnace temperature possibly as a result of volatilization. A trial was run to determine if chromium recovery varied with the quantity of dry matter. Wheat bran was added from 0 to 1.00 gram at 0.25 gram intervals along with 1000 ug of

chromium. Chromium recovery was 584 ± 65 ug (SD, n= 8) and showed very little correlation to the quantity of bran added ($r^2 = 0.20$). The mean recovery of 18 samples containing 1000 ug of chromium was 652 ± 81(SD). In my hands at least, the dry ash methodology, besides proving tedious, time consuming and tenuous (the ash is readily made airborne), was inaccurate.

Chromium compounds are known to be volatile at dry ash temperatures with losses enhanced at elevated temperatures [77, 78] . Aggett and O'Brien found that the degree of chromium loss depends on the other components of the ash [77] . Potassium dichromate alone slowly decomposed at 800° C but by 20 minutes about 10% chromium loss had been detected. In the presence of potassium sulfate more than a 40% loss was found in the same period. Conversely, potassium chloride effectively halted volatilization of $K_2Cr_2O_7$. Nonetheless, others have found dry ash methodology satisfactory for recovery of chromium from biological samples [79, 80]. In general, dry ashing of samples is considered simple but inferior to wet ashing because of volatilization of metals [81].

The alternative to dry ash sample preparation, as previously noted, is wet ashing. The standard way to do this is with perchloric acid which, because of its nearly unequalled oxidative prowess, is explosive. Perchloric acid digestion can only be safely carried out with specifically designated equipment. As remarked by Fisher et al. [80] "In our laboratory, the method of Whitby and Lang [perchloric digestion] was used effectively for many years, until a severe explosion occurred, probably originating in the flue of the fume cupboard. Thus alternative markers, or an alternative analytical technique for Cr_2O_3 were considered." By using more rigorous treatment of the sample with nitric and

sulfuric acid it is possible to obtain thorough destruction of organic matter. This approach has been used since the 1800's [82].

A wet ash protocol for foodstuffs using a kjeldahl flask is given by Jackson et al. [83] From 5-50 grams of beverage is heated with 10 ml nitric and then 5 ml sulfuric acid, both concentrated, until the solution is rendered clear. Following essentially the same acid protocol but using an erlenmeyer flask and a hot plate, Arafat and Glooschenko [84] extend the method to plant matter. Following acid digestion of 0.5 g dry matter, 2 ml of 70% hydrogen peroxide is added to the cold flask and once the initial reaction subsides, the flask is heated for 30 minutes. Farre and Lagarda [85] use only nitric acid but enhance oxidation with a $KMnO_4$ step. Ferrando et al. [86] have found nitric and sulfuric acids (7 : 3 ratio) adequately decompose chicken fecal matter. This same group [8] used the protocol successfully with chicken digesta.

I did not find enhanced destruction of organic matter with the addition of a few millileters of 30% hydrogen peroxide to the sulfuric acid residue although there was a vigorous reaction. Inadequate digestion of organic materials is evidenced by floating globules of fat or even hardened waxy material once the flask is cooled and diluted. However, sulfuric acid boils at about 290° C, and with patience and judicious additions of nitric acid, even the most resistant materials can be destroyed. Brown fumes resulting from the reaction of nitric acid are evolved from the digestion flask as long as organic matter is present [82]. Pretreatment of the sample in heated saturated KOH did not lessen the resistance of fatty substances.

I found that 100 ml volumetric flasks worked quite well as digestion flasks with samples of less than 1.5 grams of dry matter. Various protocols which are designed for the assay of naturally occurring chromium in foodstuffs generally call for large quantities of dry matter. Generally there is a concentrating step where the chromium is extracted from the acidic residue with an organic solvent such as acetylacetone. For the purposes of a marker study 1 gram of dry matter is more than adequate for a sample size as metal is added to the animal and therefore appears in the feces at relatively high levels. No concentrating steps are required. By using the volumetric flask as a digestion chamber the protocol for sample preparation is much reduced in duration. Once the digestion is complete, about 1.5 hours, the flask is diluted to volume and is ready for AAS. The dilution step actually takes about 0.5 hours since sulfuric acid liberates substantial heat on dilution as well as a contraction of volume. Dilution must be done with cooling and extensive mixing. Furthermore, the specific gravity of the acidic solution is nearly twice that of distilled water and the two phases are distinct even after 5 inversions of the volumetric flask. The two layers are visible to the eye but their presence can be analytically demonstrated by raising and lowering the AAS capillary uptake tube in the volumetric flask. The metals reside in the dense acidic layer.

Both nitric and sulfuric acid reduce the apparent recovery of chromium compared to standards made up in distilled water. This is brought about by matrix effects. The viscosity of sulfuric acid reduces the ability of the atomizer ("nebulizer" in the parlance of flame AAS) to disperse the sample and the larger droplets of sample are excluded from the flame by the action of the flow spoiler (a fan shaped device that separates particles by centrifugal dispersal). Furthermore, the increased specific gravity of the sample impedes the flow of

sample into the burner as it is drawn by vacuum through the nebulizer. To correct for nitric and sulfuric acid interference, I constructed blanks with a small amount of fecal matter and submitted the blanks to the same digestion protocol as the samples. Table III.3 shows the results of a trial using the nitric and sulfuric acid wet ash protocol.

Table III.3 The consistancy of atomic absorption spectroscopic signals of chromium standards following digestion with 0.1 grams dry weight of rat fecal matter.

Concentration (PPM)	n	Mean Reading[1]	Standard Deviation	Coefficient of Variation
0	15	0.395	0.102	25.9%
1	5	6.78	0.077	1.13%
2	5	13.16	0.102	0.77%
5	5	30.20	0.251	0.83%
10	5	55.11	0.426	0.77%

1 Atomic absorption is non-linear, units are given in the arbitrary scale of % absorption. This unit can be converted to absorbance, which is the unit of Beer's law, from tables provided by the instruments manufacturer.

The larger coefficient of variation seen in the vicinity of 0 ppm is a result of approaching the sensitivity limits of the protocol. The standard deviation may not be a reasonable estimate of variation in this region since the signal mean is close to zero and negative values cannot be taken. To reduce this problem I set the signal to 0 with distilled water thereby generating positive values for sample blanks. Therefore the magnitude of the signal is arbitrary but the relative error between dilute and concentrated samples remains constant. In effect, the coefficient of variation for repeated samples is quite low except in the region immediate to 0 ppm. In practice the lower limit of interpretable results was about 0.1 ppm. This observation was confirmed statistically by analysis of detection limits [81].

A determination was made of the independence of blank values to increasing quantities of dry matter. This point was important for interpretation of the experimental results since fecal samples ranged from less than 10 mg to 1.5 g.

It was found that the instrument zero remained constant for cobalt analysis for fecal samples up to the maximum weight tested of 1.5 g. The same could not be said for chromium. Chromium appeared in unmarked fecal samples at the level of 50 ug/g. Back calculation of the chromium level in the mineral mix, the level of mix in the diet and the average daily intake of the diet by the rats showed that the background level of chromium in the feces could be accounted for by dietary intake. Although chromium is an essential nutrient, the incorporation of the metal in a short term diet study is unlikely to be consequential. It would therefore seem advisable to avoid a mineral mix which includes chromium in its makeup when a study is undertaken involving chromium as a digestion marker.

An investigation was made of the repeatability and consistancy of sample analysis when increasingly larger subsamples were analyzed. To do this, a portion of chromium mordanted bran was ground to a dust in a coffee mill and combined at 0.9% with an equally fine grind of wheat bran. The combined materials were mixed thoroughly in a large bottle and subsampled. This dilution step was necessary since the mordanted bran was 11% chromium and 100 ml's of a 1 ppm solution would require the weighing of 0.00091 g. Dilution of more concentrated digestion was not done as it would defeat the purpose of co-digestion of the standards. Previous determinations of the chromium content of the mordant revealed that subsamples had different chromium content, ranging from 10-12%. Grinding and dilution of the mordant sample helped reduce that problem.

Analysis of 4 samples with a mean weight of 0.1005 g had a mean chromium content of 7791 ug/g, SD of 424 and CV of 5.4%. Analysis of 4 samples of mean weight 0.1839 g had a chromium content of 8359 ug/g, SD = 264 and CV =

3.2%. In this run the higher level of metal was beyond the upper limit of the calibration curve. The means cannot be compared per se because atomic absorption spectroscopy is inherently non-linear across concentration ranges. All samples must be bracketed by standards to derive accurate results. However, the coefficient of variance still has a degree of meaning and as might be expected, samples of greater weight are analyzed with less variance. There are two reasons for this. One, measurement errors are worse near the limits of an instrument, on this account both the balance and the spectrometer contributed. Two, there was a decreased likelihood of getting a non-representative subsample from the parent sample bottle as a larger sample was taken.

This experiment was repeated using a fresh preparation of sample mix and using a range of 7 subsamples from 0.1016 g to 1.0305 g. Linear regression of chromium concentration (y) versus sample weight (x) revealed a downslope of apparent chromium content with increasing sample weight. However the squared correlation coefficient was only 0.14 and the slope was not statistically different from zero by the F test [87]. Once again the greatest variability was generated by the smallest samples.

Figure III.1 shows the regression of absorbance versus concentration of the samples tallied in Table III.3. The full range of samples is not given because atomic absorption is inherently non-linear, even after conversion of instrument units to absorbance units. Over short spans absorbance approximates a straight line quite closely. Since most of the samples I analyzed were less than 5 ppm in concentration it is appropriate to use this segment of data for analysis of variance in the standard curve.

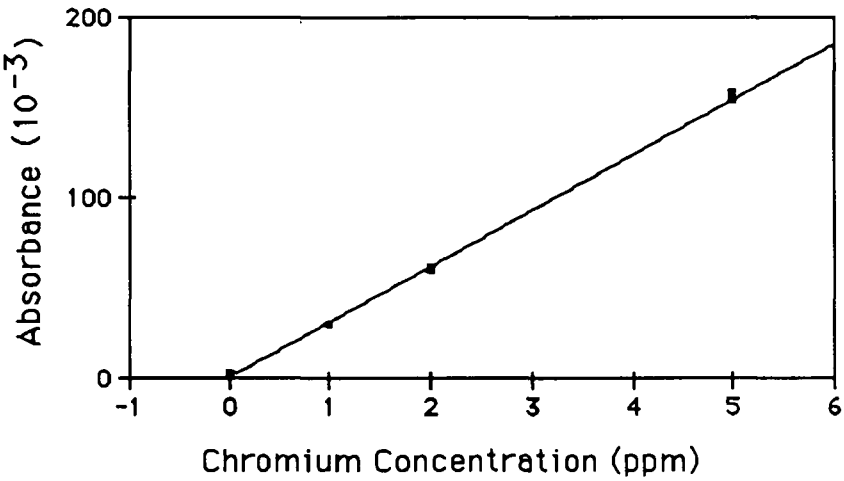

Figure III.1 The regression of absorbance on concentration of chromium in a Perkin-Elmer model 303 atomic absorption spectrometer.

Linear regression analysis of Table III.3, with the units converted to absorbance (see footnote 1 of Table III.3), gives a regression slope of 0.031 and a squared correlation coefficient of 0.999. Since the maximum useful absorbance on the spectrometer was about 0.52, the useful analytical range for the data set of Table III.2 was 0.52/0.031, or about 17 ppm chromium. The upper limit of 0.52 is imposed by a flat signal response at higher concentrations of sample. In effect, the maximum sensitivity of the machine was not employed as analysis was only extended to 10 ppm. In general I did not employ maximum sensitivity as the stability of the calibration curve was extended when sensitivity was reduced. As it was, the calibration curve rarely remained stabile beyond 10 minutes. The high correlation coefficient indicates that virtually all the variance in the data is explained by the regression model.

Analytical sensitivity can be estimated as the ratio of the slope of the analytical curve at some point where repeated calibrations have been made, to the standard deviation of those repeated measures [81]. The inverse of this ratio has units of ppm and, in the 1 ppm range for chromium, has a value of 0.01 ppm. This then, is the minimum difference that must exist between two unknowns before they can be reliably distinguished. For cobalt the analytical sensitivity was 0.026/0.00094, or 27.7. The inverse is 0.04 ppm. That is, about a 0.04 ppm difference between samples in the 1 ppm range can be detected.

When digestion markers are employed in an experiment, the level of chromium or cobalt in a sample can be made quite high by increasing the dose the animal is given. For this reason detection of metals in the range of 0.01 ppm, and therefore calibration curves with repeated measures, are unnecessary. For practical purposes, the wet ash protocol described above is rapid and sensitive and gives good reproducibility. By using replicates of standards it is possible to detect levels of chromium at the limits of the sensitivity characteristic of atomic absorption. This means that the method could be extended for routine analysis of chromium and cobalt, and likely other metals, of samples where chromium had not been added. In this case use of atomic absorption grade purity acids would be indicated.

IV Particle Size and Rate of Passage of Digesta in Rats

Abstract

The influence of bran particle size on the rate of passage of digesta through the gut of the rat was investigated. Three bran treatments were used which differed in the particle size of the bran and the particulate digesta marker. Additionally, a pectin treatment was included to compare the behavior of the digesta markers in a non-particulate environment. Chromium mordanted bran and cobalt-EDTA served as particulate and soluble digesta markers, respectively. Dietary bran particle size and marker particle size were either matched of contrasted. Rate of passage parameters were determined fro analysis of the time profiles of the excreted markers. Upon completion of the study gut organ wights ere determined. Dietary bran particle size had no significant effect on calculated transit times, halftimes and mean retention times. Furthermore, utilization of widely different particle sizes for the particulate marker did not significantly effect the calculated rate of passage parameters in groups fed a common bran particle size diet. Pectin supplemented animals had similar transit times to the bran fed animals but had significantly increased mean retention times of particulate and soluble marker. The increased mean retention times could be accounted for by increased gut pool volumes in the pectin supplemented animals. No significant differences were found between the rate of passage parameters for the soluble and particulate markers in any dietary treatment, suggesting that net separation of particles based on size and solubility was minimal.

Key words: rate of passage, particle size, wheat bran, pectin, rat

Introduction

Dietary fiber has been implicated as positively effecting the etiology of several pathological conditions prevalent in western societies, including diabetes, heart disease and colo-rectal cancer (1). Mechanisms through which fiber may exert its putative beneficial effects are not agreed upon and none have been clearly demonstrated experimentally. Experimental verification of fiber mechanisms is complicated by the inherent complexity of the disease processes themselves and the diversity and complex nature of dietary fiber. The experimental systems used to investigate these relationships are generally based on laboratory animals models, often the laboratory rat. To understand the role of fiber in these experimental systems it is essential to have a clear understanding of its influence on relevant physiological processes in the experimental animal being used. The effect of fiber particle size on the rate of passage of intestinal contents in the rat is one such nutritional interrelationship which is not clearly understood.

Dietary fiber, both in kind and quantity, is known to influence the rate of passage of digesta in many species of animals (2). The mechanism by which fiber influences passage rate is complex potentially involving fluid retention, fecal bulking, changes in digesta viscosity and microbial growth/metabolism (3). Fiber particle size is regarded as a key determinant of rate of passage of digesta in many species. Digesta particles are separated within the gastrointestinal tract by anatomical structures or gut motility patterns that discriminate between digesta particles based on size, solubility and density. In ruminants, where fiber passage has been most well studied, the majority of separation of digesta is reported to occur in the reticulo-rumen (4). Smaller and less dense particles are preferentially lost from the reticulo-rumen and therefore

experience a faster passage rate (4, 5, 6). Because of specialized motility patterns and colonic haustra in the hindgut of the rabbit (7) and because of sieving action of the entrance to the cecum of the chicken (8), smaller particles experience a slower passage rates in these species. In humans, separation of particles based on size has not been demonstrated. However, the particle size of fiber supplement appears to correlate with rate of passage where passage rates of both liquid and solid phases of digesta appear faster in humans supplemented with large versus small particle size bran (9, 10, 11, 12). .

Conflicting conclusions have been reached with respect to the relationship between the passage rates of liquid and solid digesta phases through the whole gut of the rat. Fisher et al (14) found soluble markers to move more rapidly than the corresponding insoluble markers while Sakaguchi et al. (15) have found the passage rates of particulate and soluble markers to be indistinguishable. Unlike humans, the rat has a distinct forestomach and the cecum is well developed (16). The presence of such anatomical differentiation indicates the possibility for measurable discrimination of digesta passage rates based on particle size within this species. Similar to humans, the quantity and source of dietary fiber are known to influence specific rate of passage parameters in the rat. Fisher et al. (14), utilizing soluble and insoluble digesta markers, have demonstrated an inverse relationship between fiber intake and digesta transit times. Lupton and Meacher (49), utilizing an insoluble radiopaque marker, have shown that diets containing equivalent percentages of chemically different dietary fibers may differentially effect gastrointestinal transit time. The influence of particle size on digesta rate of passage parameters is not well documented. Forsythe et al (13) have reported that variations in wheat bran particle size do not influence the time required for 90% excretion of a soluble

digesta marker. However, the rates of passage were not reported in their component rate constants and it is therefore not possible to determine if the results were a fortuitous combination of transit time and turnover time.

The purpose of the present study was to determine the relationship between fiber particle size and the rate of passage of particulate and soluble digesta in the rat. The experimental design allowed simultaneous assessment of the significance of the particle size of the particulate marker used to measure digesta transit. Wheat ran was chosen as the model fiber due to its widespread use in experimental fiber studies (88, 89, 90, 91, 92). A pectin supplemented treatment was included in the experimental design to examine the behavior of soluble and particulate markers in a non-particulate matrix. The presented results are of direct relevance to experimental studies in which the data are potentially affected by digesta transit times, such as those focussing on dietary fiber and chemically induced colon carcinogenesis in the rat (3).

Materials and Methods

Animals and Diets. Forty-eight male Wistar rats weighing approximately 125 g were randomly assigned to 4 experimental groups of 12 animals each. The rats were housed individually in wire-bottomed metabolism cages under a 12 hour light-dark cycle and allowed to adjust to diets for 9 days. The animal room was maintained at 23° C. One day after taking the final fecal collection the animals were anesthetized with 15 mg of sodium pentobarbital and terminated with bilateral lesions to the diaphragm. The gut was divided into the stomach, small intestine, cecum, and proximal and distal colon and their wet weights with contents taken. The colon was divided mid-way along the branching of the middle colic artery.

Three distinct diets were prepared (Table 1) which differed in fiber size and kind. The first and second diets were nine parts of AIN-76 (93) fiber-free purified diet for rats diluted with 1 part of AACC soft white wheat bran sorted between 1.18 mm (#16 U.S. sieve) and 0.83 mm sieve openings (#20 U.S. sieve) for the large bran diet and between a 0.36 mm (#45 U.S. sieve) and 0.10 mm sieve openings (#150 U.S. sieve) for the small bran diet. Citrus pectin (ICN Biochemicals, 6.7% methoxylated) was substituted for wheat bran in the third diet. There were 4 diet groups, the first 2 groups were fed the large bran diet and dosed with either large particle marker (LBLM) or small particle marker (LBSM). Animal groups 3 (SBSM) and 4 (PSM) were fed small bran and pectin diets respectively and both groups were dosed with small marker. The marker, chromium mordanted bran (CrMB) was sorted with the same sieves as the dietary bran. All groups were simultaneously dosed with Cobalt EDTA (Co EDTA) to estimate the kinetics of soluble gut contents.

Markers and dosing. Feed cups were removed from the cages two hours prior to the onset of the dark phase of the lighting cycle. At lights out, a prepared dose of 0.010 g Co EDTA and 0.020 g CrMB was mixed with two g of diet and placed in each respective cage. Two hours later the dose cup was replaced with the usual feed cup. Fecal collections began 2 h after initial presentation of the dose and continued every 2 h for 2 days. The collection interval was increased to 4h for a third day and daily collections continued for 3 additional days.

Chromium mordanted wheat bran was prepared as a solid phase digestion marker. The bran (ICN Biochemicals) was destarched before mordanting (94). Bran (400 g) was boiled in 4 l of 1N acetic acid for 60 minutes, vacuum filtered

through cheesecloth and rinsed with 4 l hot tapwater. The filtered mass was resuspended in 2 l of neutral detergent solution and simmered for 60 minutes in a steam kettle. Vapor losses were replaced with distilled water. The bran was filtered as above with extensive hot tapwater rinses followed by 95% ethanol and acetone rinses. Mordanting proceeded as in Uden et al. (95) with a yield 39% CrMB at 11.3 ± 0.2% chromium. Co EDTA was selected as a water soluble marker and was prepared (21) except that disodium EDTA was substituted for EDTA on an equimolar basis.

Analytical methods. Individual fecal collections from all animals were estimated for moisture and dry matter content (DM). Samples were dried at 67° C for 12 hours (constant weight), weighed to 0.01 g and stored in sealed plastic bags at room temperature. Chromium and cobalt content of the fecal collections were determined by atomic absorption spectroscopy (Perkin-Elmer 303) as follows. The samples were weighed to 0.1 mg prior to wet ash treatment. Wet ash was accomplished by heating an entire fecal sample in an 100 ml volumetric flask (Corning 5581) on a hotplate (Corning PC-351) with 10 ml of concentrated nitric acid per 0.5 g of dry matter. When the reaction subsided the flasks were cooled briefly and 5 ml of concentrated sulfuric acid was added. An additional period of heating rendered a clear solution which sometimes required additional 2 ml aliquots of nitric acid to make the solution nearly colorless. The flasks were diluted stepwise with extensive mixing and allowance for temperature equillibration. Standards for fecal chromium and cobalt analysis were made up daily and contained 0.100 g of fecal dry matter and were acid digested along side the samples. Because AIN-76 mineral mix contains chromium a dry matter correction was applied to all samples based on apparent

chromium content of feces taken prior to the marker study. Cobalt was not detected in fecal collections taken before the marker study.

Calculations. The parameters of rate of passage were estimated assuming a single pool model (69). The first order rate equation

$$C_f(t) = C_0 e^{-k(t-\Delta)}$$

was assumed for the model where $C_f(t)$ is the fecal concentration of marker (ug metal/g DM) as a function of time following peak marker concentration. The time of peak marker concentration is Δ, and was effectively equal to mouth to anus transit time (TT). The initial concentration of marker is C_0 and k is a rate constant (1/h) describing the fractional loss of marker from the pool. The negative of the regression slope of the natural logarithmic transformation of $C_f(t)$ plotted against the mid-points of fecal collection times was used as an estimate of k. Only the initial descending segment of this plot was used for kinetic analysis since deviation from linearity can eventually be expected as a result of recirculation of marker via coprophagy. The dry matter (DM) output during transit time was summed. The time of doubling of this sum was the cutoff point for observations included in the regression. Marker residence halftime ($t_{1/2}$) represents the time required for the concentration of marker within the pool to decrease by 50% and was calculated as ln(2)/k. Mean retention time (MRT) was calculated as the sum of TT and 1/k.

Statistical methods. Paired t-testing, covariance analysis, analysis of variance for a completely randomized design and planned comparisons were performed according to Snedecor and Cochran (87). Differences among means were considered significant when the pooled variance estimated t-value of a planned comparison corresponded to $p < 0.05$.

Results

Feed intake and growth performance for all animal groups are shown in Table 2 over the period day 0 to day 7, and day 7 to day 15. Initial weights were equal but by day 7 PSM rats had gained significantly less weight. Daily weight gain in the PSM group recovered in the second period although these animals ate significantly less food. This is reflected in the higher feed efficiency ratio (FER) of the PSM animals.

Figure 1 shows the cumulative fecal DM output for all groups for days 9 and 10. Circadian rhythmicity is evident in Figure 1 and is easily seen by holding a straight edge along the points of the figure. Covariance analysis using a linear model showed that the slopes of cumulative DM output versus time were not homogeneous ($p < 0.01$). The PSM animals had a significantly higher rate of DM output than the bran fed animals. The slopes of individual group regressions were 0.030, 0.032, 0.031 and 0.038 g DM/h for the LBLM, LBSM, SBSM and PSM groups, respectively.

The kinetic parameters derived from the analysis of marker excretion are summarized in Table 3. Bran fed animals had distinctly shorter CrMB $t_{1/2}$ and MRT then with the pectin fed animals ($p < 0.05$). However CrMB TT was equal among all diet groups. Co EDTA $t_{1/2}$, MRT and TT were similar to CrMB values for all groups. The mean individual differences of insoluble less soluble $t_{1/2}$, MRT and TT (CrMB - Co EDTA) calculated by animal showed no significant differences.

Table 4 shows the wet weights of the gut organs taken 1 day after the final fecal collection. The gastrointestinal tract of the pectin fed animals appeared distended and had numerous gas pockets. The digesta was paste-like and fecal pellets were not apparent except in the distal colon. These pellets, like those collected in the metabolism cages were not well formed. This was in marked contrast to the bran fed animals where well formed fecal pellets were evident from the proximal colon onwards. The mean small intestinal wet weight of the pectin fed animals was nearly double that of the bran fed animals and cecal weights were more than double. The differences were significant ($p < 0.05$).

Discussion

The reduced daily gain seen in the first period for pectin supplemented rats was overcome by day 9 although the mean bodyweight remained less. Reduced weight gains in rats fed 10% pectin have been reported (96, 60) whereas others have found normal growth (61, 97). In the present study food intake by the pectin fed rats was initially irregular and diarrhea was prevalent. These effects abated but the stool remained soft and infrequent.

The FER of the PSM animals during period 2 was higher than among the dietary bran supplemented animals. The increase of the relative gut weight in rats being fed the fermentable fiber seen here has been noted by others (98, 99) and contributed to the weight recovery.

In preliminary feedings it was found that some rats did not eat wheat bran particles held up on a #16 U.S. sieve (1.18 mm opening). Particles passing a # U.S.150 sieve (0.10 opening) may have a different composition from the larger particles (9, 11, 33). Therefore the particle size ranges selected were maximally

separated. Nevertheless, we found little evidence that particle size plays a large role in the rate of movement of gut contents in the rat. The LBLM and LBSM groups had identical diets, only the particle size of the CrMB digestion marker differed. Since $t_{1/2}$, MRT and TT were essentially the same between these groups it can be argued that the large and small marker move in consequence to the bulk of the diet.

The LBSM and SBSM groups were included in the experimental design to test the influence of particle size of an insoluble dietary fiber on the behavior of digestion markers. In this comparison small particle CrMB displayed similar behavior in both groups and was not sensitive to the particle size of the bulk of the diet. The water soluble marker Co EDTA might have the greatest opportunity for mobility within the gut and therefore could provide insight into the separation of digesta fractions within the gut. However it displayed essentially the same behavior as the solid phase marker. Neither the insoluble nor the soluble digestion markers were sensitive to the particle size of the dietary fiber. Figure 1 shows that DM output decreased during the illuminated hours. This indicates that the observed rate constants are somewhat dependent on the timing of marker dosing. This effect could be minimized by the use of constant lighting as rats are nocturnal animals. The effect of the fluctuation could be minimized by extending the regression over a longer period. This is possible only if the degree of coprophagy is low and the marker concentration in the feces is not approaching detection limits or background levels.

The plots of fecal marker concentration against time indicated that a single pool model was adequate to describe marker behavior. In view of the substantial stomach weights shown in Table 4 this is somewhat surprising. In the present

experiment the dose was offered when the stomach fill was at a minimum, especially since feed had been withheld for the previous 2 hours. This probably resulted in a relatively rapid transfer of dose from the stomach to the cecum and therefore minimized the influence of the stomach on marker kinetics. Had the dose been administered by gavage to a relatively full stomach it is probable that the stomach volume would measurably influence the total mean retention time of the markers. Mean retention times from 0.8 h [59] to 3.1 h [14] have been observed in the rat stomach.

Both TT and MRT may be expected to be lower in the pectin fed animals than the bran fed animals. This is because of a higher fiber intake and because of the higher rate of DM output indicates a more rapid flow of residue through the gut of the pectin fed animals. Pectin is 100% dietary fiber whereas AACC soft white wheat bran is only 39% dietary fiber, although the relative contribution to fecal DM in part depends on the extent of fermentation.

The average TT of PSM animals was not significantly longer than other groups which indicates that the gel forming characteristics of pectin did not simply slow the movement of markers. Substantial gut organ enlargement was observed in the PSM rats relative to the bran fed animals. Enlargement of gut organs in rats fed soluble fibers relative to insoluble fibers or fiber free diets has been noted elsewhere [32, 100, 55]. Since the rate constant k depends on the flow rate and gut volume, and since the flow rates were apparently higher in the pectin fed animals, the enlargement of the gut organs explains the lengthened $t_{1/2}$ and MRT seen for these animals.

It is concluded that in the current feeding regime, changing the size of grind of the dietary wheat bran did not result in significant changes in the passage rate of the soluble or insoluble markers. Furthermore neither the particle size of the marker nor its solubility resulted in significant differences in the measurement of the rate of passage in these rats. In contrast, the 10% pectin diet was associated with a significantly increased mean retention time of CrMB and Co EDTA.

Table IV.1 Composition of diets[1]

Constituent	Large Bran	Small bran	Pectin
		%	
Large Bran[2]	10	0	0
Small Bran	0	10	0
Pectin[3,4]	0	0	10
Sucrose[3]	49.5	49.5	49.5
Casein[3]	18	18	18
DL-Methionine[3]	0.27	0.27	0.27
Cornstarch[3]	13.5	13.5	13.5
Cornoil[5]	4.5	4.5	4.5
AIN Mineral mix[3]	3.15	3.15	3.15
AIN Vitamin mix[3]	0.9	0.9	0.9
Choline bitartrate[3]	0.18	0.18	0.18

1 Based on the AIN-76 purified diet for rats and mice.
2 Large and small bran prepared from AACC Soft White wheat bran, St. Paul, Mn. Large and small bran sieve opening ranges were 1.180 - 0.833 mm and 0.355 - 0.105 mm respectively.
3 ICN Biochemicals, Cleveland, OH.
4 6.7% methoxylated
5 Mazola corn oil (Best Foods, CPC International Inc., Englewood Cliffs, NJ).

Table IV.2 Body weight, food intake and feed efficiency of rats from all groups[1].

	Diet Group[2]				
	LBLM	LBSM	SBSM	PSM	MSE[3]
Day 0 weight (g)	125.3[a] ± 0.3	125.2[a] ± 0.2	125.1[a] ± 0.2	125.3[a] ± 1.1	0.71
Day 7 weight (g)	171.8[a] ± 1.6	172.6[a] ± 1.5	173.3[a] ± 1.3	159.9[b] ±6.9	30.9
Day 15 weight (g)	221.8[a] ± 2.2	222.8[a] ± 2.7	221.3[a] ± 2.4	209.8[b] ± 2.4	70.1
Period 1 gain[4] (g/d)	6.64[a] ± 0.21	6.77[a] ± 0.20	6.89[a] ± 0.17	4.95[b] ± 0.28	0.58
Period 2 gain[5] (g/d)	6.25[a] ± 0.21	6.27[a] ± 0.21	6.00[a] ± 0.21	6.09[a] ± 0.16	0.47
Total gain[6] (g/d)	6.43[a] ± 0.15	6.51[a] ± 0.17	6.42[a] ± 0.16	5.56[b] ± 0.14	0.29
Food intake[5] (g/d)	16.40[a] ± 0.29	16.23[a] ± 0.46	16.27[a] ± 0.35	15.24[b] ± 0.24	1.29
Period 2 FER[5,7] (%)	38.1[ab] ± 1.2	38.7[ab] ± 1.0	36.8[a] ± 1.1	40.0[b] ± 1.06	13.8

1 n = 12, mean ± SEM. Row means with a common superscript are not significantly different ($p > 0.05$).

2 LBLM = large particle bran diet, large particle marker; LBSM = large particle bran diet, small particle marker; SBSM = small particle bran diet, small particle marker; PSM = pectin supplemented diet, small particle marker.

3 Mean square error.

4 Day 0 to day 7.

5 Day 7 to day 15.

6 Day 0 to day 15.

7 Food efficiency ratio = 100 times grams of weight gain per gram feed.

Table IV.3 Halftime (hours) and transit time (hours) of particle and liquid markers in rats fed four diet regimes (Mean ± SEM). [1]

Diet Group[2]		LBLM	LBSM	SBSM	PSM	MSE[3]
Fiber		Large Bran	Large Bran	Small Bran	Pectin	
Solid Marker		Large CrMB	Small CrMB	Small CrMB	Small CrMB	
Liquid Marker		Co EDTA	Co EDTA	Co EDTA	Co EDTA	
CrMB[4]	$t_{1/2}$	$6.9^{a} \pm 1.0$	$7.2^{a} \pm 1.3$	$6.9^{a} \pm 0.8$	$11.3^{b} \pm 1.53$	15.8
	MRT	$20.3^{a} \pm 1.6$	$20.5^{a} \pm 1.8$	$20.1^{a} \pm 1.6$	$28.1^{b} \pm 2.1$	38.2
	TT	$10.3^{a} \pm 1.1$	$10.1^{a} \pm 1.5$	$10.5^{a} \pm 2.0$	$11.8^{a} \pm 0.9$	27.6
	n	12	11[5]	12	12	
Co EDTA[6]	$t_{1/2}$	$6.3^{a} \pm 0.7$	$8.3^{a} \pm 1.1$	$6.2^{a} \pm 0.9$	$12.6^{b} \pm 2.1$	19.7
	MRT	$18.0^{a} \pm 1.1$	$21.2^{a} \pm 2.0$	$21.3^{a} \pm 1.7$	$29.7^{b} \pm 3.4$	58.9
	TT	$8.8^{a} \pm 0.8$	$9.4^{a} \pm 1.4$	$12.3^{a} \pm 1.8$	$11.5^{a} \pm 1.4$	23.2
	n	12	11	12	12	
Contrast[7]	$t_{1/2}$	$0.6^{a} \pm 0.5$	$-1.0^{a} \pm 0.8$	$0.5^{a} \pm 0.6$	$-1.3^{a} \pm 1.6$	11.8
	MRT	$2.4^{a} \pm 0.9$	$-0.7^{a} \pm 1.9$	$-1.2^{a} \pm 1.2$	$-1.6^{a} \pm 2.8$	40.2
	TT	$1.5^{a} \pm 1.0$	$0.7^{a} \pm 1.3$	$-1.8^{a} \pm 1.1$	$-0.3^{a} \pm 1.3$	16.7
	n	12	11	12	12	

1 Means across rows with different superscripts are significantly different at $p<0.05$. Significance calculated by contrast analysis.

2 LBLM = large particle bran diet, large particle marker; LBSM = large particle bran diet, small particle marker; SBSM = small particle bran diet, small particle marker; PSM = pectin supplemented diet, small particle marker.

3 Mean square error.

4 Chromium mordanted bran. $t_{1/2}$ is halftime, TT is transit time, MRT is mean transit time (the sum of 1/k and TT).

5 One animal of the LBSM group refused the dose.

6 Co EDTA.

7 Calculated as group averages of individual differences of CrMB and Co EDTA observation.

Table IV.4 Organ wet weights (g) with contents[1].

Diet Group[2]	n	Stomach	Small Intestine	Cecum	Proximal[3] Colon	Distal Colon
LBLM	12	$6.6^{a} \pm 1.6$	$7.6^{a} \pm 0.2$	$2.3^{a} \pm 0.2$	$1.0^{a} \pm 0.2$	$1.0^{a} \pm 0.2$
LBSM	12	$5.8^{a} \pm 1.3$	$7.6^{a} \pm 0.2$	$2.4^{a} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.9^{a} \pm 0.1$
SBSM	12	$5.2^{a} \pm 1.4$	$7.3^{a} \pm 0.2$	$2.6^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$0.9^{a} \pm 0.1$
PSM	12	$6.9^{a} \pm 1.4$	$13.4^{b} \pm 0.8$	$6.0^{b} \pm 1.1$	$1.2^{a} \pm 0.2$	$1.1^{a} \pm 0.1$
MSE[4]		7.85	0.766	1.23	0.081	0.071

1 Mean ± SEM. Column means sharing a common superscript are not significantly different.

2 LBLM = large particle bran diet, large particle marker; LBSM = large particle bran diet, small particle marker; SBSM = small particle bran diet, small particle marker; PSM = pectin supplemented diet, small particle marker.

3 Colon bisected midway along the middle colic artery.

4 Mean square error.

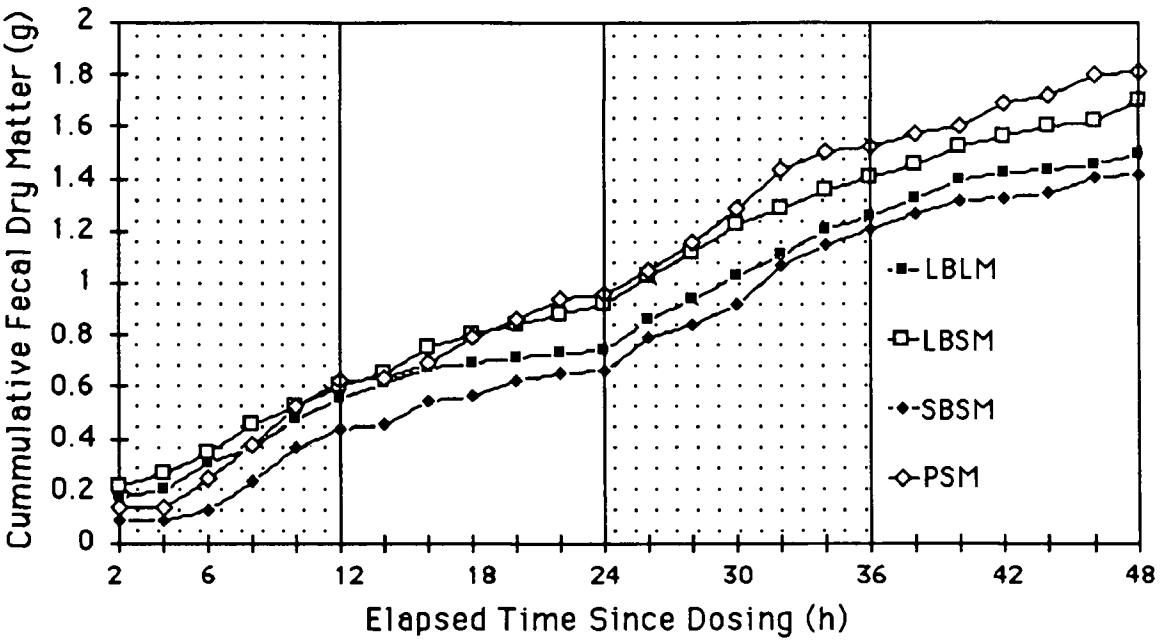

Figure IV.1 Cumulative fecal dry matter following administration of the marker dose. Shading indicates the dark phase of the 12 h/12 h lighting schedule.

V DIGESTION MARKER TRANSIT IN THE RAT GUT

Abstract

Rats were fed a semi-purified diet supplemented with 10% wheat bran of specified particle size, or 10% pectin. The distribution of chromium mordanted bran (CrMB) and the soluble marker cobalt EDTA was determined in the gut for 14 h by sequential slaughter. By 14 h the changes in marker concentration in bran supplemented groups were adequate to determine mean retention times (MRT) in regions of the gut proximal to the mid-colon but only proximal to the cecum in the pectin supplemented group. The smaller particle bran had a longer MRT in the cecum and a shorter MRT in the proximal colon. Total MRT to the mid-colon was similar in all bran supplemented groups. Pectin fed animals had significantly enlarged gut organs which explained the slower movement of marker in these animals. A 2 compartment model of the gut was used to predict the quantities of marker expected in each region of the gut at each termination time. The cecum was found to delay the transit of marker from the small intestine to the proximal colon as much as 2.1 h. This delay resulted in the gut appearing as a single pool overall and therefore lead the model to overpredict the initial rate of appearance of marker distal to the cecum.

Key words: Digestion markers, rate of passage, particle size, fiber

Introduction

Dietary fiber has considerable effects on the human large bowel especially by increasing fecal bulk and rate of passage [9, 66]. Many investigations into the effects of dietary fiber on rate of passage of digesta are conducted using the rat as a model system. Rate of passage is estimated with a variety of digestion markers selected for their presumed behavior in the gut [95, 70]. The rate of passage of a digestion marker depends on the gut compartmentalization and flow patterns that prevail within the animal [2]. Unless the flow and marker output in a dosed animal is very regular the precise interpretation of an excretion curve must be conservative. For the rat it is generally assumed that if multiple pools exist their turnover is fast enough such that all but the largest pool can be ignored. Various studies using the rat are available [16, 36, 59, 49] which study gastric emptying and gut transit time (TT) but the quantitative distribution of the marker throughout the gut following dosage is not well known. This information is of considerable importance since transit time amounts to the shortest possible route through the gut but it is mean retention time (MRT) that describes the overall passage of the gut contents.

Digesta is comprised of many fractions which may change in solubility and particle size. Therefore no single marker can adequately describe the passage history of a diet. The objective of this experiment was to study the progress of a soluble and an insoluble marker of two size ranges in the gut of the rat.

Materials and Methods

Animals and diets. Male Wistar rats weighing approximately 125 g were randomly assigned to 4 experimental groups of 12 animals each. The rats were housed in metabolism cages and allowed to adjust to their diets for 2 weeks. The animal room was lighted according to a 12 h/12 h light/dark lighting schedule. The rats were fed AIN-76 fiber-free purified diet for rats [93], diluted 10% with AACC soft white wheat bran or citrus pectin (ICN Biochemicals, 6.7% methoxylated). Groups 1 and 2 had large particle wheat bran sorted between 1.18 mm and 0.83 mm sieve. Group 3 had small particle wheat bran (0.36 mm - 0.10 mm sieve openings) and citrus pectin was substituted for wheat bran in the diet of the fourth group.

Markers. Chromium mordanted wheat bran (CrMB) was prepared as a solid phase digestion marker. The bran (ICN Biochemicals) was destarched before mordanting [94]. Bran (400 g) was boiled in 4 l of 1N acetic acid for 60 minutes, vacuum filtered through cheesecloth and rinsed with 4 l hot tapwater. The filtered mass was resuspended in 2 l of neutral detergent solution and simmered for 60 minutes in a steam kettle. Vapor losses were replaced with distilled water. The bran was filtered as above with extensive hot tapwater rinses followed by 95% ethanol and acetone rinses. Mordanting proceeded as in Uden et al. [95] with a yield of 39% CrMB at 11.3 $\pm$ 0.2% chromium.

Cobalt EDTA (Co EDTA) was selected as a water soluble marker and was prepared as in Uden et al. [95] except that disodium EDTA was substituted for EDTA on an equimolar basis. All groups received Co EDTA to estimate the kinetics of soluble gut contents.

In a preliminary run with four separate rats it was found that 0.58 g Co EDTA mixed with 2 g of AIN-76 fiber free diet produced a cathartic effect in all animals. Therefore the marker dose was lowered to 0.010 g of Co EDTA and 0.020 g CrMB. The level of Co EDTA might be safely doubled to extend detection of the marker. The CrMB was sized into the same large and small particle fractions as the diet. Group 1 received large particle CrMB and all other groups received small particle CrMB. Feed was withheld for the final 2 hours of the lighted period prior to offering the marker dose. The dose was placed in a clean feed cup fitted to the cage at the onset of the dark cycle. After two hours the cup was removed and replaced with the usual feed cup.

Sample collection and analysis. Fecal collections began 2 hours after initial presentation of the dose and continued every 2 hours. At the end of the 2 hour dose period a subgroup of 3 rats was selected from each group and anesthetized with 15 mg of sodium pentobarbital delivered intraperitoneally. The gut cavity was entered with a longitudinal incision on the ventromedial line following which the stomach, small intestines, cecum, upper and lower colon were rapidly isolated with ties. The segments were excised and put into individually labelled plastic bags and stored at -35° C. The rats were killed with bilatteral lesions to the diaphragm. Three more rats were selected from each group at 4 hour intervals for intestinal segmentation until all animals were terminated.

Individual fecal collections were dried at 67° C for 12 hours (constant weight), weighed to 0.01 g and stored in sealed plastic bags at room temperature. Chromium and cobalt content of the fecal collections were determined by

atomic absorption spectroscopy (AAS, Perkin-Elmer 303) as follows. The samples were weighed to 0.1 mg prior to wet ash treatment. Wet ash was accomplished by heating an entire fecal sample in an 100 ml volumetric flask (Corning 5581) on a hotplate (Corning PC-351) with 10 ml of concentrated nitric acid per gram dry matter. When the reaction subsided the flasks were cooled briefly and 5 ml of concentrated sulfuric acid was added. An additional period of heating rendered a clear solution of a pale yellow or green color. Additional 2 ml aliquots of nitric acid were used to render the solution nearly colorless. Tissue samples were weighed moist prior to oxidation. An entire tissue sample with contents was added to a 250 ml erlenmeyer flask followed by 20 ml of concentrated sulfuric acid. All larger samples were charred in the sulfuric acid to destroy fatty substances. Aliquots of nitric acid were added until brown fumes no longer evolved and the cooled acidic solution was finally decanted through a funnel into a 100 ml volumetric flask. In all cases the flasks were diluted stepwise with extensive mixing and allowance for temperature equillibration. Standards for fecal chromium and cobalt analysis were made up daily from stock solutions, each contained 0.10 g of fecal dry matter and were acid digested along side the samples. Standards for tissue chromium and cobalt analysis were made up in distilled water and calibration curves were drawn for acid interference. Because AIN-76 mineral mix contains chromium a dry matter correction was applied to all fecal samples based on apparent chromium content of feces taken prior to the marker study. Cobalt was not detected in fecal collections taken before the marker study.

Digestion model. A 2 compartment model was assumed for the gut of the animals with the stomach serving as the first compartment and the cecum as the second. The small and large intestines were assumed to cause a delay in

marker transport but not to have significant pooling effects. Table 1 shows the equations used to determine the expected quantity of marker in each segment of the gut. Since transit time (TT) generally refers to whole gut transit time, the terms Δ for time of passage from mouth to cecum, μ time of passage from mouth to an arbitrary point in the colon and $t(\Omega)$, the time of passage from mouth to anus were selected to distinguish the various transit times.

The use of Table 1 requires a knowledge of transit time along the gut and of the rate constants for the stomach and cecum. These terms can be calculated in 2 ways. First, the rate constants can be calculated directly from the relatively linear descending phase of a semi-logarithmic plot of the average % of total dose in a region versus time. A second approach involves summing the quantity of marker over adjacent regions. A hypothetical plot of summed data for several regions of the gut is shown in Figure 1. The quantity of the dose remaining in the stomach and small intestine for any time less than Δ is 100%. The time at which this sums departs from 100% is Δ, as shown in Figure 1, and can be estimated from the regression of the logarithm of the combined percent of marker in the small intestine and stomach over the period of time when this sum is decreasing. The point at which this regression equals ln(100) is taken as Δ and the slope is taken as $-k_{ssi}$, the rate constant for combined stomach and small intestinal emptying. If constant flow without axial mixing occurs in the small intestine, then k_{ssi} will equal k_s, the rate constant derived for the stomach alone. The value of k_{ssic} (the rate constant for stomach, small intestine and cecum combined) was estimated similarly but with the percent of marker in the cecum added to the sum. In principle k_c should equal k_{ssic}. However, the estimate of k_{ssic} will be more reliable in consequence to a wider range of values available for the regression. Only the "tail-end" of equation 5 produces accurate

estimates of k_c, whereas the summed points may start at 100% and range to a small value.

The value of μ can be estimated by simple regression only if $k_{ssi} >> k_{ssicp}$, the rate constant derived from data summed to the proximal colon inclusive. Otherwise μ can be estimated from the first appearance of marker in the distal colon. Ω is equal to the first appearance of marker in the feces.

Having determined these values, the MRT can be calculated from the serially combined totals for the segments. The total MRT [73] (TMRT) is calculated to each point, the regional MRT's are found by subtraction. For these purposes TMRT is calculated as

$$TMRT = \text{Œ} + \int_{\text{Œ}}^{\infty} f(t)\,dt \Big/ \int f(t)\,dt \qquad \{14\}$$

where Œ is the time when marker begins leaving the region in question. This approach is adapted from that suggested by Warner [2]. The use of fitted curves rather than direct use of the data avoids the requirement for total marker excretion. That is, marker behavior is predicted to infinite time from the measured behavior. The integrand $f(t)$ is an exponential term of the form of equation {1}, found by fitting a least squares line to the descending phase of a semi-logarithmic plot of summed results. If the descending phase shows significant non-linearity, multiple pools are probably present and additional terms will be required. For a 2 pool system, this curve will have the form

$$f(t) = A_3 * e^{-k_b(t-\text{Œ})} - A_4 * e^{-k_a(t-\text{Œ})} \qquad \{15\}$$

where k_a and k_b are the fractional rate constants of the 2 pools, A_3 is $k_a/(k_a-k_b)$, and A_4 is $k_b/(k_a-k_b)$. By convention $k_a > k_b$. Equation {15} is not a linearizable function and the constants will be most easily found by computer curve fitting routines. In the current model k_a and k_b ideally equal k_s and k_c.

Statistical analysis. Analysis of variance for a completely randomized design and planned comparisons were performed according to Snedecor and Cochran [87] Differences among means were considered significant when the pooled variance estimated t-value of a planned comparison corresponded to $p < 0.05$. Integrations were performed on an HP-28S hand-held calculator.

Results

Gut weights. Gut contents were relatively fluid in all animals up to the proximal colon. Distinct fecal pellets were visible in the distal end of this segment and further along in all bran supplemented animals. The gut contents of the pectin supplemented animals was fluid at all points and the fecal pellets were not well formed. The gut appeared turgid and gas pockets were visible.

The animals terminated at t(2.5) are the most appropriate choice for comparisons of organ weights as % of body weight (BW) since the degree of gut fill was likely to be at its lowest. The % BW of the gastrointestinal tract was 7.0 ± 0.3, 6.4 ± 0.2, 6.5 ± 0.2 and 10.3 ± 0.5 (%, n=3) for the groups 1 to 4, respectively. Group 4 was significantly higher ($p < 0.01$, MSE = 0.255).

Because of the number of animals it was not possible to determine if all rats began consuming the dose immediately after the dose was offered. However only a low level of marker remained in the stomachs of all groups by 2.5 h which

indicates that the dose was consumed very rapidly. A few rats did not consume the complete dose.

Marker distribution. The elapsed time from selecting an animal until the gut segments were isolated was 0.5 h. The elapsed times until the gut segments were actually isolated were 2.5, 6.5, 10.5 and 14.5 h after first offering the dose. The quantities of chromium and cobalt found in each region of the gut at each termination time are shown in Table 2 and Table 3 respectively. The markers moved rapidly from the stomach and small intestine to reside in the cecum. The markers did not increase in the proximal colon simultaneously with their appearance in the cecum. In all cases marker, once having appeared in the cecum, remained there in significant amounts for the duration of the trial. In many cases marker levels increased in the stomach simultaneously with their appearance in the feces. This phenomena was attributed to coprophagy and complicated the summing of the quantity of marker in the gut at later time points. For purposes of summing, stomach marker was considered as zero by 10.5 h.

Regressions were generated for all segments individually and sums of segments of the gut, where an apparently linear descending phase was evident on a semi-logarithmic plot of the data. All regressions had squared correlation coefficients above 0.80 and most were effectively 1.0. Figure 2 shows a representative plot of percent of marker by region plotted over time from offering the dose. The term π, the time of marker first appearance in the proximal colon was introduced since marker sometimes left the cecum at a time latter than first appearance. The transit times were estimated by extending the regression to the ordinate value of 100%.

Table 4 is a tabulation of MRT for combined and individual segments of the guts. The ability to calculate MRT depended on a distinctly descending and linear region of a semi-logarithmic plot of the data. This criteria was met for all segments up to the proximal colon inclusive in the bran supplemented animals, but only to the small intestine inclusive for the pectin supplemented animals. In several groups the percentage of the total dose was seen to increase in the stomach at the same time as marker appeared in the feces. This probably resulted from consumption of feces. In group 4 animals very little marker was detected in the feces at any time and no increase was seen in the percentage of marker in the stomach. Observations from the stomach and small intestine were omitted from the calculation of MRT where it was judged that the marker was present as a result of coprophagy. In general the stomach emptied too rapidly for an accurate estimate of marker residence time based on the observation alone. The estimate of stomach MRT therefore is based on the assumption that 100% of the marker existed in the stomach at t(0).

Figure 3 compares the observed results for marker content of stomach, small intestine and cecum combined, and the same including the proximal colon, the single term exponential fit to those data and the predicted fit based on Table 1. The use of a 2 termed equation, hence a 2 pool model, results in substantial overprediction of the quantity of marker residing in these regions of the gut.

Discussion

Preliminary work on marker recovery following dry ashing of the feces proved unreliable due to variable recovery of chromium. Various compounds of chromium are known to be volatile at dry ash temperatures [77, 78]. Although

sensitivity to chromium by atomic absorption spectroscopy was significantly higher in a reducing (optimized) flame the calibration curve was more stabile using an oxidizing flame. Because chromium was present at high levels (up to 20 ppm in the digestion flask) detection limits were generally not an issue and an oxidizing flame was therefore preferred. This finding is in agreement with Thompson [101].

In the present study and others [9, 102] the digestion markers moved rapidly from the stomach. Stomach MRT was calculated over the period t(0) to t(2.5) but this abbreviated regression span affected the estimated CrMB MRT very little. For Co EDTA the effect was dramatic an resulted in a large negative increase in the regression slope. This was likely the result of rapid loss of the soluble Co EDTA from the stomach. The regression over later time points, though highly correlated, was probably detecting a residual level of marker. Kunstyr et al. [16] found the liquid component to empty more rapidly in the rat. They attribute a biphasic loss of marker from the rat stomach to compartmentalization within the stomach. Calculating from their figure, MRT's of 1.7 and 5.0 h respectively were calculated from the 2 slopes. In the current data the Co EDTA MRT averaged 3.1 h in those cases where a residual slope was seen. Varga [36], using 15 μm diameter radiolabelled spheres, found the MRT in the rat stomach to be 0.7 h in comparable animals.

Pectin clearly increased the MRT of both markers in the stomach but may be a reflection of the time required to hydrate the diet. Brown et al. [59] gave rats baked beans mixed with varying levels of guar gum in an hydrated homogenate containing a colloidal radio-isotope by gavage. In this circumstance MRT

changed from 0.8 h for beans alone to 1.6 h with 1% w/v guar which indicates that viscous substances do correlate with slowed gastric emptying.

Diet groups 1 and 2 differed only in the particle size of the CrMB marker. The behavior of the marker was similar at all points in the foregut but the smaller marker had a longer MRT in the cecum. The behavior of the soluble marker was similar between these 2 groups up through the cecum. Diet groups 2 and 3 differed in the particle size of the bulk bran in the diet. The MRT in the fermentative environment of the cecum of CrMB in group 3 rats was 1.4 h longer. For the soluble marker the disparity was even greater. Because of the small sample size and because no repetitions were performed it is not possible to say with any certainty that these trends are significant. However it is interesting that smaller particles appear to be retained in the cecum somewhat longer. This trend might indicate that differential fermentation of fine particles can occur in the rat. There appears to be an inverse correlation between residence in the cecum and the remainder of the hindgut. Smaller particle bran is known to have a longer residence time in rabbits although this is attributable to a specific mechanism of the colon [103].

The behavior of markers in the ceca of pectin supplemented rats was in marked contrast to the other groups. The significantly enlarged ceca retained a large fraction of the dose and did not show an exponential decrease in marker content. For this reason cecal MRT could not be calculated in the group 4 animals. Slow cecal transit and accumulation of fecal matter in the proximal colon of rats on an 8% pectin supplemented diet has been reported [7]. Increase cecal wet weight and contents [104, 55] and gut transit time [61] in rats fed gel forming fibers has been reported. Viscous polysaccharides therefore

influence the rate of marker movement both by organ enlargement and diminished axial transport.

The equations of Table 1 were formulated under the assumption that both the small and large intestines act as simple conduits and that the stomach and cecum act as pools. All equations are for a dose normalized to 1. The quantity of marker in the stomach disappears according to the simple exponential function equation {1}. This loss is a gain to the small intestine until Δ when the small intestine begins loosing marker to the cecum. In principle, equation {5} is equivalent to equation {1} of Grovum and Williams [72] as are equations {7}, {10} and {13} equivalent to equation {4} of Grovum and Phillips [73]. Equation {5} describes the fraction of marker in the cecum, outflow from the cecum represents gain by the proximal colon until μ, when marker begins moving into the distal colon. Beginning at Ω all marker accumulates in the feces. If the small and large intestines are divided into numerous segments an equal number of transit time terms must be added.

The disagreement between predicted and actual quantities of marker in the various gut segments probably resulted from irregularities in the flow of digesta. In Table 2 is shown π, the time at which marker was predicted to first exit the cecum. In many cases this occured markedly later than cecal first appearance. This effect was clearly demonstrated by Lupton and Meacher [49] where a 21% wheat bran diet resulted in a 0.6 h cecal delay and a 8% pectin diet resulted in a delay of 1.5 h.

The prediction curve of Figure 3 decays to the observed curve as increasing values of k_S are employed. As k_S becomes large, equation {5} predicts the

cecum will empty as a single exponential function. If the small intestine effectively delivers the dose to the cecum before the cecum begins emptying, the dose can be considered to have been delivered as a pulse to the cecum. The excretion of both CrMB and Co EDTA from the rat has been reported to fit well to a single exponential term [15]. The period of withholding feed prior to administering a marker dose may enhance this effect by minimizing stomach volume. In contrast, a highly indigestible diet fed ad libitum would likely increase the ability of the stomach to act as a digestive pool both through increased volume and diminished rate of emptying.

It should be noted that the current data do not support the hypothesis that the stomach serves as the largest pool proximal to the cecum. From Table 2, the term SSI should equal the sum of S and Δ if the small intestine serves as a simple conduit. That is, Δ should equal SI. Generally this was not seen. The MRT of CrMB, attributable to the small intestine beyond transit time, is 1.4 times greater than the MRT of the stomach and 3.2 times greater for Co EDTA. This indicates that for particulate matter the small intestine acts somewhat as a conduit but that the water soluble fraction undergoes considerable mixing. The ratio of wet weights (3.4) of these organs across all groups at t(2.5) is similar to the ratio of MRT's, possibly indicating that Co EDTA has access to a gut volume close to total gut capacity. This point warrants further investigation as all assimilated fractions of the diet pass through a water soluble or solublized fraction at some point during absorption. Hence, on certain diets nutrients solublized in the ileum may still have access to absorption in proximal regions.

In spite of divergent transit time within each segment of the small intestine, the TMRT to the point of the distal colon shows remarkable consistancy among the

bran supplemented groups. This indicates that complex movements may occur within the digestive tract of the rat that will not be fully detectable by conventional digestion marker analysis.

Table V.1 Expected normalized quantities of marker in each gut segment[1].

Region	Time period	Function
{1} Stom.	t(0) to ∞	$f(t) = e^{-k_s t}$
{2} Sm. Int.	t(0) to Δ	$f(t) = 1 - e^{-k_s t}$
{3} Sm. Int.	Δ to ∞	$f(t) = e^{-k_s (t-\Delta)} - e^{-k_s t}$
{4} Cecum	t(0) to Δ	$f(t) = 0$
{5} Cecum	Δ to ∞	$f(t) = A_1 * \left(e^{-k_c (t-\Delta)} - e^{-k_s (t-\Delta)}\right)$
{6} Prx. Col.	t(0) to Δ	$f(t) = 0$
{7} Prx. Col.	Δ to μ	$f(t) = 1 - A_1 * e^{-k_c (t-\Delta)} - A_2 * e^{-k_s (t-\Delta)}$
{8} Prx. Col.	μ to ∞	$f(t) = A_1 * \left(e^{-k_c (t-\mu)} - e^{-k_c (t-\Delta)}\right) + A_2 * \left(e^{-k_s (t-\mu)} - e^{-k_s (t-\Delta)}\right)$
{9} Dt. Col.	t(0) to μ	$f(t) = 0$
{10} Dt. Col.	μ to Ω	$f(t) = 1 - A_1 * e^{-k_c (t-\mu)} - A_2 * (e^{-k_s (t-\mu)}$
{11} Dt. Col.	Ω to ∞	$f(t) = A_1 * \left(e^{-k_c (t-\Omega)} - e^{-k_c (t-\mu)}\right) + A_2 * (e^{-k_s (t-\Omega)} - e^{-k_s (t-\mu)})$
{12} Feces	t(0) to Ω	$f(t) = 0$
{13} Feces	Ω to ∞	$f(t) = 1 - A_1 * e^{-k_c (t-\Omega)} - A_2 * e^{-k_s (t-\Omega)}$

1 Symbols: k_s, k_c, rate constants (1/h) for marker loss from stomach and cecum; A_1, $k_s/(k_s - k_c)$; A_2, $k_c/(k_c - k_s)$; t, time since administering dose; ∞, infinite time; Δ, time of marker first appearance in cecum; μ, time of marker first appearance in distal colon; Ω, time of marker first appearance in feces. Colon divided midway on the middle colic artery.

Table V.2 The distribution of chromium mordanted bran in the rat gut at 2.5, 6.5, 10.5 and 14.5 hours after dosing1.

Time[2] (h)	Stomach Feces	Small Intestine	Cecum	Proximal Colon[3]	Distal Colon	
LBLM[4]						
2.5	19.4 ± 7.7	57.5 ± 4.3	19.4 ±10.2	2.0 ± 0.7	1.8 ± 0.6	*[5]
6.5	2.2 ± 1.6	12.9 ±10.9	42.2 ± 5.9	41.0 ± 7.9	1.7 ± 0.6	*
10.5	2.0 ± 0.7	1.94 ±1.0	29.8 ± 5.1	18.9 ± 6.0	46.5 ± 3.7	1.0 ± 0.2
14.5	15.7 ± 9.0	2.2 ± 0.9	18.2 ± 3.1	7.8 ± 4.5	29.4 ±14.4	26.8±14
LBSM						
2.5	28.8 ±12.5	60.5 ± 9.7	7.2 ± 3.4	2.0 ± 1.5	1.4 ± 0.3	*
6.5	2.5 ± 0.5	5.4 ± 2.9	35.6 ±16.3	42.6 ± 5.5	13.9 ±10.9	*
10.5	2.5 ± 0.7	1.9 ± 0.7	34.9 ± 3.3	17.8 ± 6.8	42.8 ± 5.8	0.2±0.02
14.5	13.0 ± 4.9	4.0 ± 2.5	25.6 ± 5.0	6.1 ± 2.7	34.7 ± 8.3	16.6±7.4
SBSM						
2.5	25.6 ± 9.0	41.0 ± 8.8	21.4 ± 4.4	5.1 ± 3.3	7.0 ± 3.4	*
6.5	3.5 ± 2.4	17.6 ± 4.7	53.3 ± 5.3	18.7 ± 4.2	6.5 ± 3.8	0.3± 0.2
10.5	9.2 ± 7.0	1.7 ± 1.0	30.3 ± 0.9	14.0 ± 4.7	31.9 ±16	12.9±12
14.5	11.0 ± 6.2	2.4 ± 1.0	20.6 ± 4.0	3.7 ± 1.9	27 ± 10	35 ±14
PSM						
2.5	20.3 ± 9.9	71.1 ±14	7.6 ± 6.0	0.5 ± 0.2	0.6 ± 0.3	*
6.5	6.2 ± 4.5	7.4 ± 3.1	49.9 ± 3.9	25.6 ± 4.7	10.7 ± 4.7	0.3 ± 0
10.5	2.1 ± 0.7	1.9 ± 0.9	57.7 ± 5.5	9.2 ± 4.3	28.9 ± 7.4	0.1 ± 0.1
14.5	4.0 ± 3.1	2.6 ± 1.9	65.1 ± 3.6	9.9 ± 0.8	15.7 ± 3.5	2.7 ±1.6

1 Mean ± SEM. n = 3 for all groups at all times.

2 Time since offering dose.

3 Colon bisected midway along the middle colic artery.

4 LBLM = large particle bran diet, large particle marker; LBSM = large particle bran diet, small particle marker; SBSM = small particle bran diet, small particle marker; PSM = pectin supplemented diet, small particle marker.

5 No net quantity of CrMB detected.

Table V.3 The distribution of cobalt EDTA in the rat gut at 2.5, 6.5, 10.5 and 14.5 hours after dosing1.

Time[2] (h)	Stomach	Small Intestine	Cecum	Proximal Colon[3]	Distal Colon	Feces
LBLM[3]						
2.5	5.7 ± 3.6	62.7 ± 7.5	29.3 ± 9.7	2.3 ± 1.6	*[4]	*
6.5	2.0 ± 0.5	3.3 ± 0.1	51.9 ± 5.5	36.7 ± 3.5	6.2 ± 2.3	*
10.5	0.3 ± 0.3	0.2 ± 0.1	37.2 ± 6.7	9.6 ± 8.1	42.0 ± 6.8	0.6 ± 0.6
14.5	9.5 ± 4.9	4.9 ± 2.4	23.9 ± 3.2	8.0 ± 5.6	26.1 ±12.0	27 ±14
LBSM						
2.5	4.9 ± 3.0	64.5 ±10.5	27.7 ±11.5	2.9 ±2.7	*	*
6.5	3.7 ± 0.8	3.9 ± 0.9	55.1 ± 3.1	32.4 ± 3.6	4.8 ± 0.7	0.1 ± 0.1
10.5	2.9 ± 2.2	2.9 ± 1.8	36.7 ± 2.3	13.8 ± 3.2	43.1 ± 1.0	0.6 ± 0.3
14.5	15.1 ± 6.2	4.9 ± 3.3	28.1 ± 4.4	6.9 ± 3.3	37.6 ± 7.3	7.3 ± 7.3
SBSM						
2.5	3.5 ± 2.6	32.2 ±15.1	61.5 ±20.4	1.5 ± 1.5	1.2 ± 1.2	*
6.5	2.3 ± 0.2	2.4 ± 0.1	67.4 ± 2.7	20.4 ± 3.5	7.2 ± 4.5	0.3 ± 0.2
10.5	6.3 ± 6.3	1.7 ± 1.7	34.9 ± 6.7	12.6 ± 4.9	27.9 ±14.0	16.6 ±16
14.5	8.6 ± 5.1	2.5 ± 1.2	23.0 ± 4.1	3.6 ± 1.9	26.3 ±10.0	36 ±15
PSB						
2.5	14.2 ± 4.7	83.1 ± 6.4	2.6 ± 2.5	*	0.1 ± 0.1	*
6.5	5.2 ± 3.0	7.5 ± 3.7	46.0 ± 7.4	31.1 ± 6.7	10.0 ± 3.4	0.2 ± 0.1
10.5	3.5 ± 1.1	4.1 ± 2.6	56.2 ± 8.3	9.9 ± 2.8	26.3 ± 9.0	*
14.5	2.1 ± 1.5	2.5 ± 1.6	67.3 ± 2.1	10.2 ± 0.2	16.2 ± 3.4	1.6 ± 1.6

1 Mean ± SEM. n = 3 for all groups at all times.
2 Time since first offering dose.
3 Colon bisected midway along the middle colic artery.
4 LBLM = large particle bran diet, large particle marker; LBSM = large particle bran diet, small particle marker; SBSM = small particle bran diet, small particle marker; PSM = pectin supplemented diet, small particle marker.
5 No net quantity of CoEDTA detected.

Table V.4 Mean Retention Time (MRT) and Transit Times in Hours of Chromium Mordanted Bran (CrMB) and Cobalt EDTA (Co EDTA) in the Rat Gut[1].

	MRT Combined Segments				Transit Times			MRT Individual Segments			
	S	SSI	SSIC	SSICP	Δ	π	μ	S	SI	C	P
CrMB											
1	1.7	6.0	10.6	19.0	1.7	2.4	6.5	1.7	4.3	4.6	8.4
2	1.6	5.7	12.6	19.3	1.8	1.9	5.9	1.6	4.1	6.9	6.7
3	1.9	6.4	14.7	18.0	2.1	4.2	6.0	1.9	4.5	8.3	3.3
4	2.8	4.8	*2	*	2.0	*	*	2.8	2.0	*	*
Co EDTA											
1	0.7	5.3	13.7	18.8	1.8	1.4	5.7	0.7	4.6	8.4	5.1
2	0.8	5.8	13.7	20.1	1.8	1.8	6.0	0.8	5.0	7.8	6.4
3	0.7	3.2	14.8	18.4	0.8	4.0	6.0	0.7	2.5	11.6	3.6
4	1.3	6.9	*	*	2.4	*	*	1.3	5.6	*	*

1 SI, C and P refer to stomach, small intestine, cecum and proximal colon respectively. Δ, π and μ refer to mouth to cecal, proximal and distal colon transit times respectively.

2 Not interpretable.

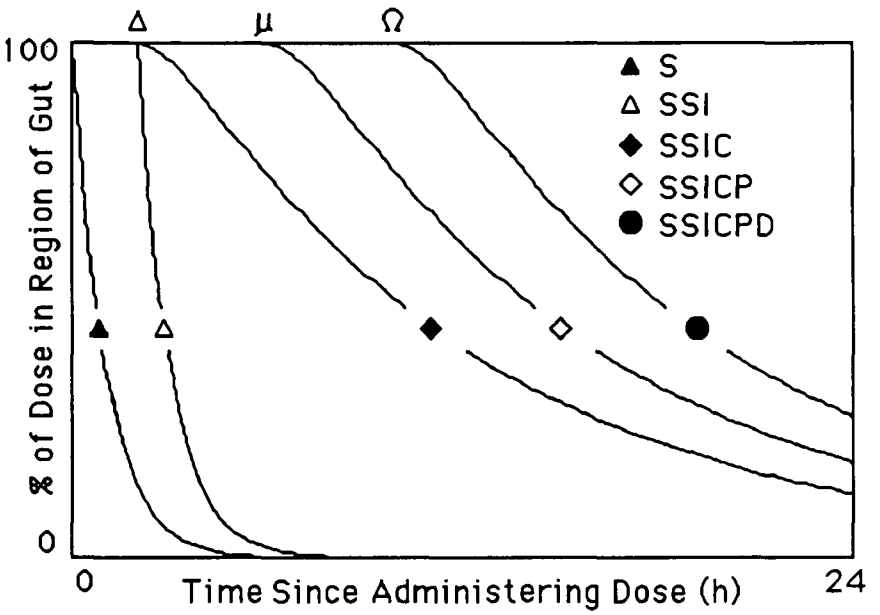

Figure V.1 Graphical representation of marker distribution in combined segments of the rat gut assuming perfect pipe flow and pool mixing. Constants set to: $k_S = 1.0$, $k_C = 0.1$, $\Delta = 2$ h, $\mu = 6$ h, $\Omega = 10$ h. The lines represent the % of total marker expected in the sequentially combined regions of the stomach (S), small intestine (SI), cecum (C), proximal (PC) and distal (DC) colon respectively.

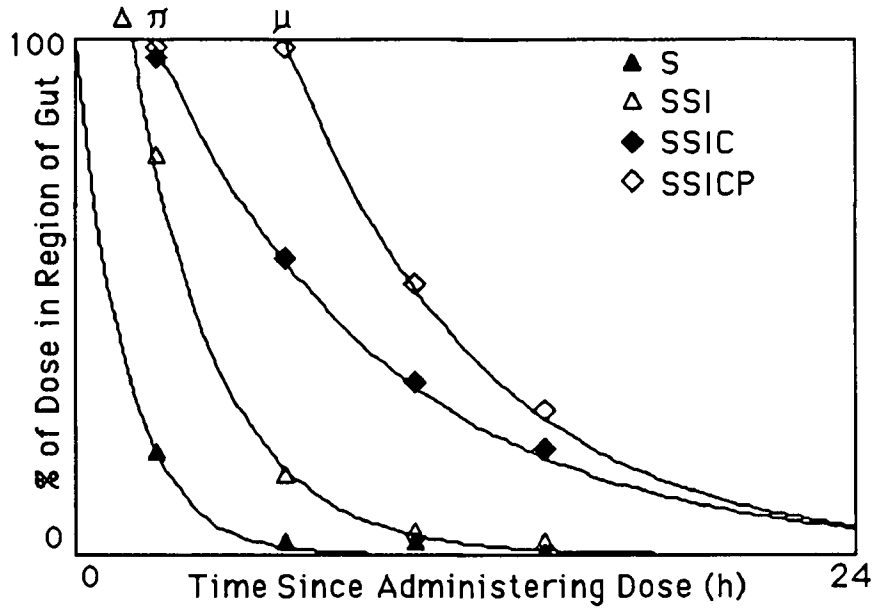

Figure V.2 Graphical representation of chromium recovery in combined gut segments of group 2 rats. Constants are $k_S = 0.66$, $k_{ssi} = 0.37$, $k_{ssic} = 0.14$, $k_{ssicp} = 0.17$, $\Delta = 1.7$ h, $\pi = 2.4$ h, $\mu = 6.5$ h.

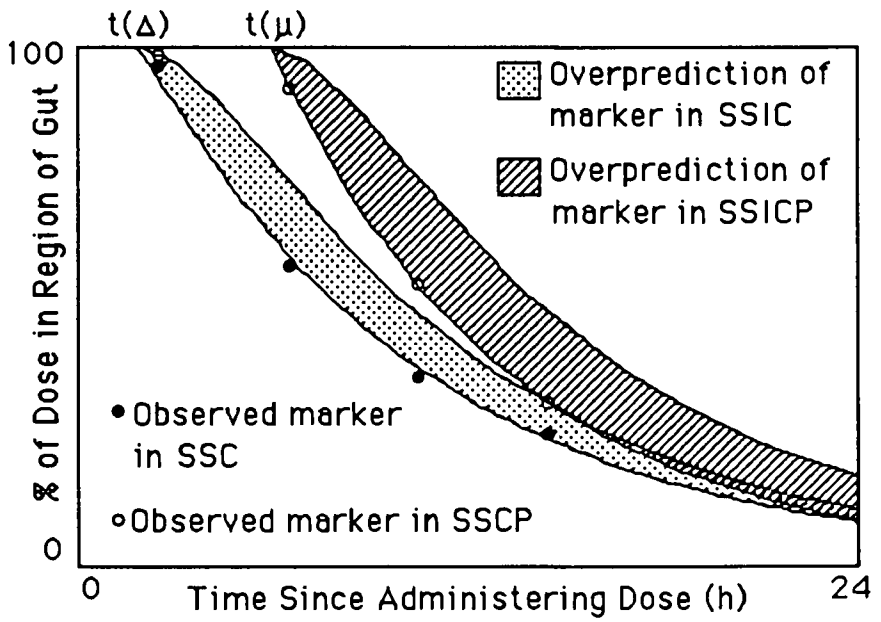

Figure V.3 Graphical representation of actual and predicted chromium recovery in combined gut segments of group 2 rats. Constants are $k_S = 0.50$, $k_{ssic} = 0.11$, $\Delta = 1.8$ h, $\mu = 5.9$ h. Single exponential regression fit to observed data is also shown.

SUMMARY

The time profile of marker loss from the rat rises abruptly from null to peak values and descends in a fashion characteristic of a first order exponential equation. This behavior is characteristic of a single pool system with constant outflow, often referred to as a single pool model, in compartmental analysis. On semi-logarithmic co-ordinates, the data points fall in a relatively linear and descending manner. A rate constant, describing the fractional rate loss of marker from the animal, can be determined from a linear regression estimate of the time profile. This approach was taken to determine if the rate loss of digestion markers given to rats was dependent on the particle size of dietary bran, and the particle size of the digestion markers themselves.

Two groups of rats were fed a large particle size dietary bran diet and were given either a large or small particle marker. The transit time and mean retention time of the markers were not significantly different. A third group of rats was fed a small particle size dietary bran diet and a small particle size marker. Once again no significant differences were detected in the parameters of rate of passage. Pectin was fed to a fourth group of rats as a model soluble dietary fiber along with a small particle size marker. In this case the transit time was not significantly affected but the mean retention time was. This indicates that the turnover time of marker in the pectin fed rats was longer. This could result from either a slower flow rate of digesta through the pectin fed animals, or from an increased pool size. In fact, the flow rates were apparently higher in the pectin fed animals indicating that the gut pool volume with these rats was much greater. Indeed, these volumes were nearly double, as estimated by the wet gut tissue weights with contents. In all cases a soluble marker was also given but in

no case was its movement significantly different from that of the particulate marker.

The rat has a stomach of a size roughly equal to the cecum and a small intestine two or three times larger. It would therefore seem likely that multiple pools would be detectable from the time profile of marker excretion. An experiment was carried out to determine if these organs do have significant pooling behavior that is somehow obscured by the movements of digesta. This was done by administering soluble and particulate markers to rats and, following sequential termination of animals over a 14 hour period, determining the quantity of marker in each region of the gut. This investigation confirmed the influence of a pectin supplemented diet on gut volume. The mean retention time was greatly extended in the cecum of pectin fed animals. By developing a mathematical model to predict the amount of marker expected in each region of the gut it was discovered that a substantial delay of marker transit occurs in the cecum. This delay could account for the adequacy of the single pool model assumed for the first experiment. The model further demonstrated the presence of a mean retention time of marker in the small intestine of bran fed animals that was longer than the transit time. This disparity indicates that significant pooling effects occur in the small intestine of the rat and that this organ does not act like a simple conduit relative to digesta flow.

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