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Correlation of Chromium Sesquioxide and [^{14}C]Cellulose as Fecal Markers in Rats (40067)

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The gastrointestinal transit time in a subject can be measured by having the subject swallow a nonabsorbable marker and measuring the time it takes for the marker to appear in the feces. Laboratory animals will not voluntarily swallow a marker such as barium impregnated polythene pellets (1), so, an investigator wishing to measure transit time in a laboratory animal must either force feed the marker or add the marker to a portion of the animal's food. Chromium sesquioxide is a marker which can be added to a diet without making it unpalatable. It is not absorbed (2), and it can be accurately measured. A marker should travel at the same rate as food residues, however, direct proof that the marker and the food residue travel at the same rate is difficult to obtain (1). This experiment was undertaken to determine whether chromium sesquioxide travels at the same rate as [^{14}C]cellulose through the gastrointestinal tract of laboratory rats. Cellulose is not digestible by mammalian enzymes and is not absorbed by rats in which coprophagy has been prevented (3).

Material and methods. Twelve male Sprague–Dawley rats (469–578 g), individually caged, were used for the experiment. The basal diet consisted of 64.5% corn starch, 20% casein, 10% corn oil, 4.5% mineral mix, and 1% vitamin mix to which 2.5%, 5%, 10%, or 20% nonlabeled cellulose was added. The diets were fed *ad libitum*. The marked diet was prepared by mixing chromium sesquioxide (J. T. Baker Chem. Co.), and about 4 mg of uniformly labeled [^{14}C]cellulose (ICN Radiochemicals) to some oil-free 2.5% cellulose diet. The [^{14}C]cellulose was prepared from *Canna indica* and is free from any soluble carbohydrates, hemicelluloses, pectin, and lignin. The corn oil was added after the two markers were uniformly mixed with the diet. The completed marked diet contained 0.42% chromium sesquioxide and 86,480 disintegrations per minute (dpm) of [^{14}C] activity per g of diet. The rats were randomly assigned to one of four blocks. A block of rats was assigned to each cellulose level for 3 days. On the third day the diet was removed at 6:00 PM. The rats were presented with a 1 g pulse of marked diet at 9:40 PM and any uneaten marked diet was removed at 10:00 PM and weighed. After the marked diet was removed, the diet of the previous 2 days was again supplied *ad libitum* to the rats. An hour after the marked diet was given, the rats were tail cupped (5) to prevent coprophagy and to collect the feces. Eight hours after giving the marked diet, the tail cups were removed and all feces from each animal were collected. After 3 days on any cellulose level, the block of rats was assigned to another cellulose level, until all the rats had been on all four levels of cellulose. It has been established by other investigators (3) that 98.6% of the [^{14}C]cellulose is passed in 3 days. The 8 hr fecal collection was dried at 100° and weighed. Each animal's feces were then ground to a powder and mixed. Each fecal collection was analyzed for chromium and [^{14}C] activity. Chromium in the feces was assayed by atomic absorption spectrophotometry after digestion of the organic matter with nitric acid and oxidation of Cr_2O_3 to Cr_2O_7 with perchloric acid (4). A sample of feces from each 8-hr collection was ignited and the CO_2 collected in a Packard 306 Oxidizer. The [^{14}C] activity was measured with a liquid scintillation counter.

The total dpm of [^{14}C] and μg Cr passed in 8 hr were determined and the percentage of the ingested markers passed was calculated. The linear regression of per cent [^{14}C] passed on per cent Cr passed, the correlation coefficient, and the 95% confidence interval were calculated. Additionally the partial correlation of dpm of [^{14}C] passed on μg Cr passed was calculated to remove the effect of variation in g fecal dry matter (8).

Results. The regression of per cent [^{14}C]

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passed on per cent Cr passed yielded a correlation coefficient (r) of 0.97 (Fig. 1) which is not significantly different from 1.00 (P <0.001, d.f.=46). The 95% confidence interval for the slope (a) of this line is $0.90 \leq a \leq 1.04$ and the interval for the y-intercept is $-0.87 \leq b \leq 1.70$.

The partial correlation of [^{14}C] on Cr removes the effect of variation in fecal dry matter from the correlation of [^{14}C] and Cr. This partial correlation yielded an r of 0.97 which is not significantly different from 1.00 ($P < 0.001$) (Fig. 2). The range in marker excretion appeared to be due primarily to individual animal differences and not necessarily related to the cellulose content of the diet.

Discussion. These results demonstrate that the passage of [^{14}C] from cellulose in the feces was well correlated with the passage of Cr from chromium sesquioxide. The correlation between these two fecal markers was independent from their correlation to the passage of fecal dry matter (Fig. 2).

If per cent Cr passed is a good estimate of per cent [^{14}C]cellulose passed, the true regression line should pass through the origin with a slope equal to 1.00. Such a regression line is included in the 95% confidence limits calculated from these data indicating that per cent Cr passed is a good estimate of per cent [^{14}C] passed in the feces. If cellulose were broken down in the gut some of the [^{14}C] could have been expired resulting in a low fecal recovery. However about 98% of [^{14}C] labeled cellulose can be recovered from the

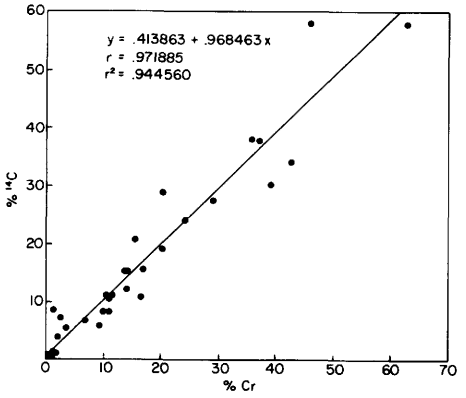

FIG. 1. Percent of dietary dose of chromium sesquioxide and of dpm of [^{14}C] from [^{14}C]cellulose passed in feces of tail-cupped rats during 8 h (n=48).

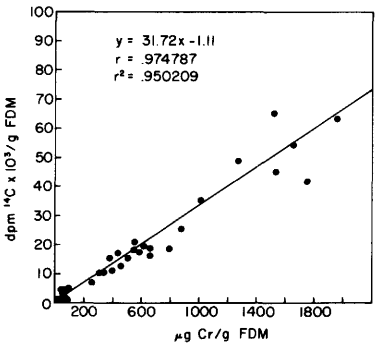

FIG. 2. Micrograms of Cr versus dpm of [^{14}C] from [^{14}C]cellulose passed in feces of tail-cupped rats during 8 h (n=48). Values are expressed per g fecal dry matter (FDM) passed in the 8-hr period.

feces in tail-cupped rats (3). The specific gravity of chromium sesquioxide is 5.21 which is higher than the specific gravity of cellulose (1.27–1.61). Despite this difference our results indicate that chromium sesquioxide can be used to mark the portion of the gut contents in rats which contain cellulose. This is in contrast to the work of Kirwan and Smith (6) who found that increasing the specific gravity of a 10 mm by 4 mm capsule from 1.1 to 1.35 accelerated its passage through the gastrointestinal tract of humans, suggesting that the specific gravity of the marker would alter its rate of transit. Additionally, this study does not provide evidence that chromium sesquioxide had a tendency to settle out relative to the cellulose residue in the gut as suggested by Mitchell and Eastwood (7).

Summary. Tail-cupped rats were fed diets of varying cellulose contents. At the end of a 3-day period rats were given a pulse dose of a diet labeled with [^{14}C]cellulose and chromium sesquioxide. The results demonstrate that passage of chromium sesquioxide was highly correlated with passage of [^{14}C]cellulose and this correlation was independent of either marker's correlation with fecal dry matter. These results indicate that use of chromium sesquioxide as a fecal marker is a good estimate of cellulose passage in the tail-cupped rat.

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