

Polyethylene glycol 4000 as a continuously administered non-absorbable faecal marker for metabolic balance studies in human subjects

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SUMMARY The use of polyethylene glycol 4000 as a non-absorbable, continuously fed faecal marker is described. The intestinal handling and transit of polyethylene glycol is compared with that of chromium sesquioxide and barium sulphate. Polyethylene glycol is an acceptable non-absorbable faecal marker for calcium, phosphorus, and fatty acids, and has several features which commend its use in preference to insoluble chromium sesquioxide and barium sulphate particularly when marking water-soluble dietary constituents.

Kreula (1947) was the first to carry out continuous faecal marking of metabolic balance investigations with chromium sesquioxide (Cr_2O_3) and he has been followed by many workers including Whitby and Lang (1960) and Rose (1964). Barium sulphate (BaSO_4) has been used as a non-absorbable marker (Dick, 1967; Figueroa, Jordan, and Bassett, 1968) and more recently Dick (1969) has used cuprous thiocyanate. All these authors have shown an increase in the reproducibility of balance data due to a more accurate assessment of the faecal component using a continuously fed non-absorbable marker.

Polyethylene glycol 4000 (PEG) has been employed as a non-absorbable faecal marker in animal studies (Hyden, 1956; Corbett, Greenhalgh, Gwynn, and Walker, 1958; Manners and Kidder, 1968). Only one report of its use in man for this purpose can be found in the literature but no attempt was made to validate the use of PEG, since total faecal recoveries were not reported (Beeken, 1967).

The present paper reports the results of using PEG as a continuously fed non-absorbable faecal marker for calcium and phosphorus balances. A comparison of PEG with Cr_2O_3 and BaSO_4 is presented. The use of PEG to correct faecal fatty acid output is also described.

Procedure

All the patients were in a metabolic ward and the general principles of Reifenstein, Albright, and Wells (1945) were observed. Diets were prepared under the supervision of a dietitian and any given

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patient ate the same normal diet every day throughout the study.

The study was designed to give an equilibration period of five or six days on diet and markers followed by two consecutive seven-day periods of balance. The patients defaecated directly into stainless steel containers held in a suitable commode. The faeces passed in the equilibration period were pooled and then two seven-day faecal collections of the balance were made. Toilet paper was not collected.

Daily faecal collections were made for the two weeks of balance in one patient taking PEG. In six patients taking PEG a post-balance faecal collection was made. During this period the administration of markers was discontinued and the patients ate a free diet. Faeces were collected until the PEG was completely excreted.

Eleven patients were given both Cr_2O_3 and PEG throughout their study and another 11 patients were given both Cr_2O_3 and BaSO_4 . One additional patient took all three markers simultaneously.

In the second part of the study the recoveries of PEG in a seven-day period of balance following a seven-day period of equilibration on diet and PEG are reported. There are 165 observations on 64 patients in this part of the study.

In all cases 500 mg of the appropriate markers, in capsules, was administered three times a day with the main meals during the equilibration and balance periods.

Polyethylene glycol 4000 (Hopkin and Williams Ltd, Chadwell Heath, Essex) was powdered and filled into capsules by hand to 500 mg \pm 5 mg. For

the second part of the study a special batch of PEG capsules was obtained from Sandoz Products Ltd (London). Since PEG is not a definite chemical compound but a mixture of polymers with slightly different molecular weights, the relative proportions varying with the batch manufactured, the standards were made from the same batch of PEG that was used to prepare the patients' capsules. Care was taken over the medication of patients on balance because PEG is used in certain oral pharmaceutical formulations.

Barium sulphate BP was also hand-filled into capsules to the same tolerance limits as PEG. Chromium sesquioxide capsules were obtained from Sandoz Products Ltd (London).

Analytical Methods

Faeces were homogenized in the collection container with three times their weight of deionized distilled water using a Silverson AXR homogenizer. Whilst the homogenizer was still running aliquots of the homogenate (weighed on a Salter top-pan balance 0.01 g) were taken for the appropriate analyses.

The digestion and oxidation steps in the estimation of chromium sesquioxide were carried out, with suitable safety precautions, using the method of Daly and Anstall (1964). The resulting dichromate solution was determined colorimetrically according to the method described by Clarkson (1967).

The gravimetric method of Dick (1967) was used to estimate BaSO₄. Filtration of the BaSO₄, dissolved in alkaline disodium ethylenediamine tetra-acetic acid solution through two sheets of Whatman no. 115 filter paper, removed Cr₂O₃ if it was also present in the faeces.

The turbidimetric method of Malawer and Powell (1967) was modified to estimate PEG on 1.00 g aliquots of faecal homogenate. The use of Whatman no. 542 filter paper for the filtration stage after protein precipitation removed any Cr₂O₃ or BaSO₄ present in the faeces and produced a clear, colourless filtrate. Since there was no difference between the estimate of PEG standards in faeces and PEG standards in water the latter were used routinely. The removal of the fine BaSO₄ precipitate from the standard solutions after the protein precipitation stage often required the use of two filter papers.

Calcium and phosphorus were determined by AutoAnalyzer techniques on ashed aliquots of faecal homogenate taken up in acid. Total faecal fatty acids were estimated according to a modification of the method of van der Kamer, Huinik, and Weyers (1949) as reported by Varley (1962). Analyses of all markers, calcium, inorganic phosphorus, and fatty acids were the mean of duplicate estimations.

Results

RECOVERIES OF MARKERS FROM FAECES

The recoveries of Cr₂O₃, BaSO₄, and PEG added directly to marker free faeces are shown in Table I. None of the markers interfered with the recovery of any of the other two markers and zero recoveries of all three markers were obtained from six faecal samples containing no markers.

Marker	Mean Recovery (%)	SD (%)	No. of Estimations
Chromium sesquioxide	98.79	0.723	10
Barium sulphate	98.83	1.392	16
Polyethylene glycol 4000	99.60	1.612	18

Table I Recoveries of markers added directly to faeces

The error of the analytical method, for each of the three markers, is shown in Table II. If x_1 and x_2

Marker	Error of a Single Estimate of Marker	No. of Sample Estimates in Duplicate	Error of Method when Mean of Duplicate Estimations Used
Chromium sesquioxide	1.752	28	1.323
Barium sulphate	2.177	25	1.475
Polyethylene glycol 4000	1.527	25	1.236

Table II Error of marker determinations

represent duplicate estimations of the percentage of the administered dose of marker in a given faecal collection then the error of a single estimate of the marker, σ , is given by

$$\sigma = \sqrt{\frac{\sum(x_1 - x_2)^2}{2n}}$$

where n is the number of collections estimated in duplicate. The error of the marker determination when the mean of duplicate estimations is used is $\sigma/\sqrt{2}$.

RECOVERIES OF MARKERS FROM PATIENTS

Table III shows the total faecal recoveries of all the PEG given throughout the study from the six patients on whom post-balance faecal collections were made. There is a mean faecal recovery of 98.65% of the administered PEG, the range being 95.5%-101.5%. A histogram distribution of the individual seven-day faecal recoveries of PEG in the second part of the study is shown in Figure 1. The recovery of PEG is normally distributed and has a mean of 99.29% of the administered dose, which is

Patient	Percentage of Total Administered Dose of PEG Recovered from Faeces
1	95.5
2	101.5
3	99.9
4	95.06
5	100.16
6	99.8
Mean	98.65
SD	2.68

Table III Total faecal recovery of polyethylene glycol 4000 administered to patients

not significantly different from 100% ($t = 0.386$). PEG, therefore, is completely recoverable in the faeces and is being estimated accurately.

The relationship of the faecal recoveries of Cr_2O_3 and PEG for the 12 patients given these two markers should be evenly distributed around the 45° line of equality if they are excreted at the same rate. However, in the equilibration period more PEG is excreted than Cr_2O_3 the points all lying below the 45° line (Fig. 2). In week 1 the points still tend to be slightly below the expected line and it is not until week 2 that faecal Cr_2O_3 output equals faecal PEG output. Figure 3 shows the corresponding data for Cr_2O_3 and BaSO_4 . Slightly more BaSO_4 than Cr_2O_3 is excreted in the equilibration period, but probably by week 1 and definitely by week 2 the faecal outputs of Cr_2O_3 and BaSO_4 are equal.

The interrelationship of faecal output and transit time of the markers is well illustrated by the results from the patient who took all three markers simultaneously (Fig. 4). In the equilibration period more PEG is excreted than BaSO_4 , and more BaSO_4 is excreted than Cr_2O_3 . In week 2 of the balance the faecal excretions of all the markers are the same, the lines in Fig. 4 being almost parallel. Five days after discontinuing marker administration all the PEG is completely recovered. However, two days after this BaSO_4 and Cr_2O_3 are still being excreted in the faeces and have not yet been completely recovered.

PEG CORRECTION OF FAECAL CALCIUM, PHOSPHORUS, AND FATTY ACID OUTPUTS

Figure 5 shows the daily faecal calcium and phosphorus outputs of the one patient on whom daily faecal collections were made throughout the two weeks of balance. Knowing the PEG content of each sample, PEG-corrected 24-hour faecal calcium and phosphorus values were calculated. In contrast to the uncorrected values the PEG-corrected daily faecal calcium and phosphorus values are virtually constant. Similarly the daily faecal fatty acid outputs of a patient on eight consecutive days of balance are

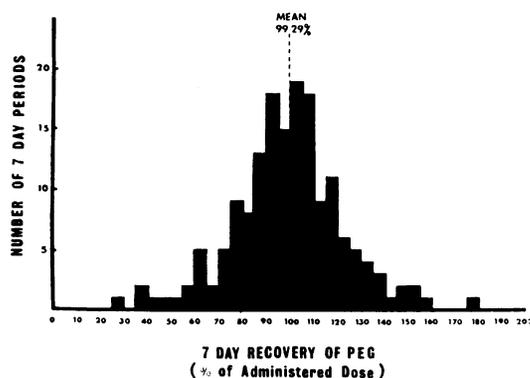


Fig. 1. Histogram showing distribution of seven-day faecal recoveries of PEG expressed as a percentage of the dose administered in seven days. The patients were equilibrated before the faecal collection was begun. Mean recovery of PEG = 99.29% ($SD = 23.57\%$, $SEM = 1.84\%$).

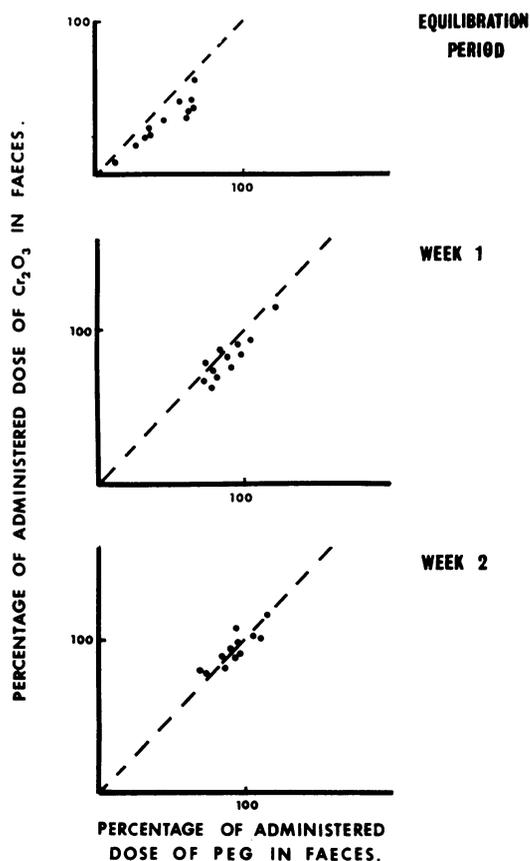


Fig. 2. Comparison of the faecal recoveries of Cr_2O_3 and PEG in the equilibration period and weeks 1 and 2 of balance. (The dotted line is the 45° line of equality.)

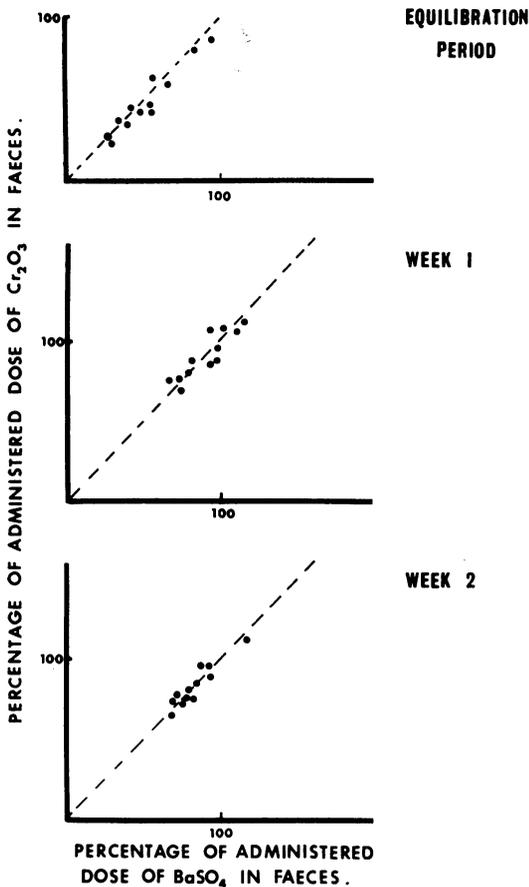


Fig. 3. Comparison of the faecal recoveries of Cr_2O_3 and BaSO_4 in the equilibration period and weeks 1 and 2 of balance. (The dotted line is the 45° line of equality.)

shown in Figure 6. There is much less variation in the PEG corrected than in the uncorrected faecal fatty acid values.

Discussion

All three markers were well tolerated by the patients and no toxic effects were observed. None of the 76 patients taking PEG showed any side effects attributable to the administration of this marker.

The mean recovery of 99.60% of PEG added directly to faeces is comparable to that of Cr_2O_3 and BaSO_4 (Table I). The errors of all the methods of analysis (Table II) are acceptable and similar to those published (Whitby and Lang, 1960).

The turbidimetric estimation of PEG is accomplished easily and accurately with commonly available equipment and there is no danger of an explosion in the estimation of PEG as there is in the analysis of Cr_2O_3 (Analytical Methods Committee, 1959). The PEG method is less laborious and exacting than the gravimetric determination of BaSO_4 , though recently Dick (1968) has reported an emission flame photometric method which reduces the time taken for BaSO_4 estimations.

Schaffer and Critchfield (1947) concluded that PEG was not absorbed from the gastrointestinal tract of rat or man and PEG is the most popular inert marker used for intubation studies (Soergel, 1968). Mean faecal recoveries of between 96.7% and 99.0% have been reported after the ingestion of large single doses of PEG by human subjects (Hyden, 1956; Borgström, Dahlqvist, Lundh, and Sjoval, 1957; Soergel and Hogan, 1967; Shields, Harris, and Davies, 1968).

The mean total faecal recovery of 98.65% of the

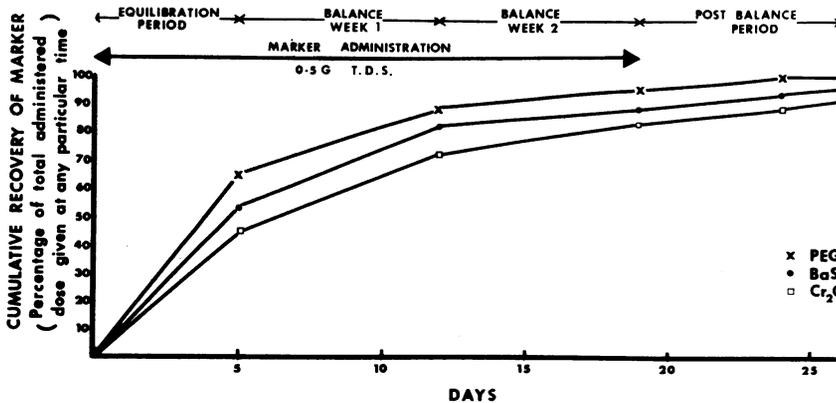


Fig. 4. Cumulative faecal recoveries of markers from the patient given all three markers simultaneously.

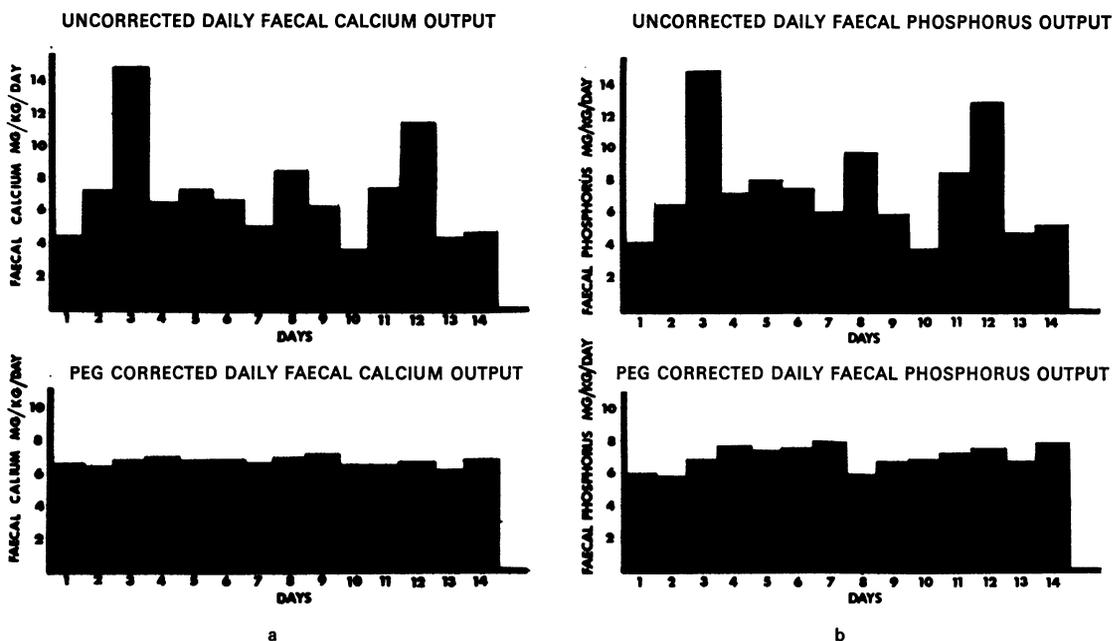


Fig. 5. Daily faecal calcium and phosphorus excretion for 14 days of balance with and without PEG correction.

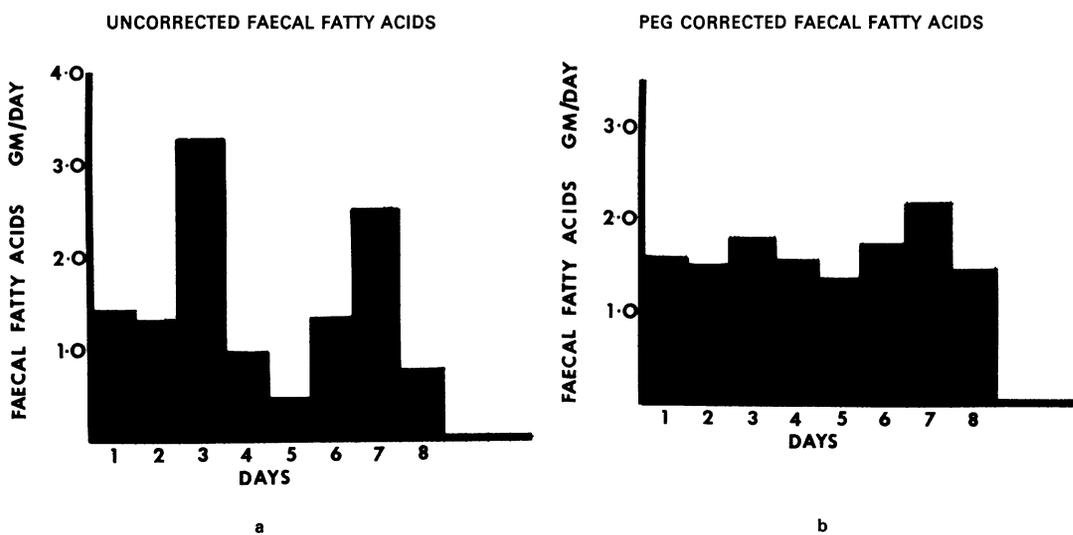


Fig. 6. Daily faecal fat excretion for eight consecutive days of balance with and without PEG correction.

administered PEG (Table III) is comparable to the total recovery of 93% of the administered Cr_2O_3 by Rose (1964) and 97% of the administered BaSO_4 by Dick (1967). In the second part of the study the normal distribution of PEG recovery has a mean of 99.29% of the administered dose (Fig. 1). These results substantiate the previous observations that PEG is not absorbed from the human gastrointestinal tract.

In view of the above findings it is perhaps surprising that Beeken (1967) reported steady-state recoveries of PEG of only 70%. Beeken suggests as one of the possible reasons for this low recovery that his subjects may have failed to take the PEG. Also some of the equilibration periods in the study were as short as three days.

It is apparent from Figs. 2, 3, and 4 that PEG has a more rapid intestinal transit time than BaSO_4 which in turn has a slightly faster transit time than Cr_2O_3 . These differences are most easily observed in the equilibration and post-balance collection periods. However, in week 2 of the balance the excretions of PEG, Cr_2O_3 , and BaSO_4 are the same. The results could be explained if there is a larger dead space in the intestinal tract for Cr_2O_3 and BaSO_4 than for PEG that must be filled before steady-state excretion is attained.

The difference between dead spaces may be due to the fact that PEG is water soluble whereas BaSO_4 and Cr_2O_3 are insoluble and considerably more dense than food (the density of Cr_2O_3 was 5.21 and of BaSO_4 4.50). PEG will move with the water-soluble phase of food and this has an advantage in marking calcium and phosphorus since both are water soluble. However, PEG may be labelling a water-soluble component of the dead space which is smaller than the true dead space for food in the intestine. Alternatively the dead space for Cr_2O_3 and BaSO_4 may be greater than the true dead space for food. The very fine, dense, insoluble powders of Cr_2O_3 and BaSO_4 tend to adhere to any surface on which they are spread. The large surface area and many small villous folds of the intestinal tract may trap the insoluble markers producing a larger dead space for Cr_2O_3 and BaSO_4 than for either food or water-soluble PEG.

Davignon, Simmonds, and Ahrens (1968) have shown that 20% of their patients on prolonged balance studies failed to attain ideal steady-state excretion of Cr_2O_3 . They demonstrated that some of these patients had sequestration and accumulation of Cr_2O_3 in the large bowel, supporting the hypothesis that Cr_2O_3 is not always handled in the same way as food residues by the intestine.

Fifteen of 52 balances reported by Whitby and Lang (1960) were not acceptable because the patients

had not reached steady state excretion with respect to Cr_2O_3 . Whitby and Lang also warned of the possibility of streaming of the intestinal contents occurring and under these conditions the density of Cr_2O_3 might cause it to sediment and be carried irregularly along the intestinal lumen. Sequestration is less liable to occur with PEG than with insoluble Cr_2O_3 or BaSO_4 and streaming would not cause water-soluble PEG to sediment.

The wide variations of consecutive daily faecal calcium and phosphorus outputs are virtually eliminated when corrected by their respective PEG content (Fig. 5). Since the calcium to PEG and the phosphorus to PEG ratio in these faecal samples is constant then PEG is evenly distributed in the faeces with respect to calcium and phosphorus.

Wiggins and Dawson (1961) showed that PEG being water soluble emptied more quickly from the stomach in the water-soluble phase than did the fat of a test meal. Vavřínková and Krontl (1965) reported that PEG, at concentrations of 1 mg/ml or greater, inhibited lipase from hog pancreas and human duodenal juice *in vitro* and therefore PEG may not be suitable for the investigation of fat absorption. No increase in the faecal fatty acid excretion of patients taking PEG has been observed and after a suitable equilibration period PEG can be used to correct daily faecal fatty acid output with some success (Fig. 6). Thus PEG, although water soluble, is relatively evenly distributed in the faeces with respect to fatty acids.

To reduce any possible effect on lipase activity *in vivo* the dose of PEG can be decreased to 250 mg three times a day. The lower concentration of PEG can be estimated by reading the turbidity at 420 μm as suggested by Boulter and McMichael (1970).

The criteria for the ideal non-absorbable marker stated by Whitby and Lang (1960) and Fordtran (1966) are fulfilled equally well by PEG as by the established markers Cr_2O_3 and BaSO_4 . In fact PEG has features which commend its use in certain circumstances. In contrast to the estimation of Cr_2O_3 the estimation of PEG is not hazardous. Because of its smaller intestinal dead space equilibration is achieved more quickly with PEG than with the two insoluble markers and this allows a more rapid assessment of the balance. Being water soluble PEG more closely resembles calcium, inorganic phosphate, and other ions than do insoluble Cr_2O_3 and BaSO_4 .

The large spread of the seven-day faecal PEG outputs in Fig. 1 again emphasizes the absolute necessity for continuous faecal marking in metabolic balance investigations. The 95% limits for a seven-day timed faecal collection vary from the equivalent of 3.7 days to 10.3 days excretion of food residue. PEG is recommended as a non-absorbable con-

tinuously fed faecal marker particularly for dietary constituents which are water soluble.

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