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An evaluation of the use of chromic oxide, polyethylene glycol and Cr-EDTA as markers for digestive studies along the small intestine of ruminants

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Summary

In cows, fitted with a T-shaped cannula in the duodenum and with a re-entrant cannula in the ileum, ileal recoveries were determined of Cr_2O_3, impregnated onto paper, and polyethylene glycol, introduced into the duodenum. The length of the collection periods of digesta showed to be of importance. The mean transit time of both markers along the small intestine did not differ.

In sheep, fitted with an infusion tube into the abomasum and with an ileal re-entrant cannula, polyethylene glycol was recovered quantitatively over 10-h collection periods of digesta.

In sheep, fitted with an infusion tube into the abomasum and with 4 T-shaped cannulas along the small intestine, flow rates of digesta in jejunum and ileum determined with polyethylene glycol and Cr-EDTA were comparable.

Introduction

Experiments with the objective to study the digestion and absorption of digesta components along the small intestinal tract can be carried out with animals surgically prepared either with re-entrant cannulas or with single T-shaped cannulas. Re-entrant cannulas make it possible to collect digesta from the intestine quantitatively. Unfortunately, with re-entrant cannulas delayed transit times of digesta along the gastrointestinal tract at the onset of the collection period have been reported. Moreover re-entrant cannulas interfere with the motility of the intestine and obstruct digesta flow (Wenham, 1979). With single T-shaped cannulas, one cannot be certain if a representative sample is collected from the cannula. However, implantation of a T-shaped cannula is less severe in comparison with re-entrant cannulas. Therefore in digestive studies the use of T-shaped cannulas in favour of re-entrant cannulas has to be considered seriously. Besides, in

order to follow the digestive process, along the small intestine several cannulas are needed, preferably in the same animal. Because there is lack of room, the number of re-entrant cannulas one can place along the small intestinal tract of small ruminants is quite limited. With T-shaped cannulas the number of places available for cannulation is certainly greater. In order to estimate the flow of particular digesta components, one has to rely then on markers, which have to fulfil several criteria, such as non-absorbability and representative behaviour regarding the digesta components studied.

In order to evaluate different aspects of the use of some intestinal markers, the following experiments were conducted. The recovery of chromic oxide (Cr_2O_3) and polyethylene glycol (PEG) from the ileum of dairy cows was determined in periods of collection of 8 and 120 h. In sheep recovery of PEG was determined from ileal digesta in periods of collection of 10 h. In dairy cows mean transit times of PEG and Cr_2O_3 in the small intestine were compared. Finally, in sheep provided with four T-shaped cannulas along the small intestine, estimates of flow rate of digesta based on the use of PEG and of Cr-EDTA as markers were compared.

Materials and methods

Animals and feeding

In the first series of experiments (Exp. 1) 3 HF dairy cows were used. They were fitted with a T-shaped cannula into the duodenum distally to the hepatic flexure. In the terminal ileum they were fitted with a re-entrant cannula (Table 1). The cannulas (22 mm inner diameter) were prepared of hard pvc. The rations consisted of hay and mixed concentrates, supplied twice a day at 08h00 and 17h00. Feed requirements were calculated according to Dutch feeding standards.

In the second series of experiments (Exp. 2) 4 Texel wethers were used, fitted with a silastic infusion tube into the abomasal fundus and with a hard pvc re-entrant cannula (12 mm inner diameter) into the terminal ileum. The sheep were fed at maintenance level. The rations consisted of hay (600 g/d) and mixed concentrates (300 g/d), supplied in 6 equal portions spread over the day.

For the third series of experiments (Exp. 3) the same 3 dairy cows were used as those of Exp. 1. The cows were kept under comparable conditions.

Table 1. Outline of the animals used in the various experiments and of the types of cannulation applied. The abomasal infusion tube is indicated by I, the T-shaped cannula and re-entrant cannula by T and R, respectively.

Exp.	abomasum	small intestine	colon
1, 3 cows		T	R
2 sheep	I		R
4 sheep	I	T T T	T

The fourth series of experiments (Exp. 4) was carried out with 4 Texel wethers. They were fitted with a silastic infusion tube into the abomasal fundus and 4 hard plastic T-shaped cannulas along the small intestine, in the proximal duodenum a few centimeters behind the pylorus (I), in the duodenum about 20 cm distally to the hepatic flexure (II), in the mid-jejunum (III) and in the terminal ileum (IV). Sheep were fed a ration quantitatively and qualitatively comparable to that of Exp. 2, supplied in 2 equal portions at 08h00 and 17h00.

Collection of digesta

In Exp. 1, ileal digesta were collected from the re-entrant cannula via a pvc flexible tubing in a vial kept at 40 °C. Per 30 min the contents of this vial were weighed, and after vigorous manual mixing a 2 % sample was withdrawn. The remaining part was returned through the distal cannula directly after sampling, without addition of donor digesta. Ileal digesta were collected following two time schedules, continuously during 5 days (Exp. 1.1) or for 5 intermittent periods of 8 h on 5 consecutive days (Exp. 1.2). Per day (Exp. 1.1) or per sampling period of 8 h (Exp. 1.2) digesta samples were pooled. They were kept at −20 °C until analyses were carried out.

Both experiments were repeated once, so that in total digesta were sampled for 6 periods of 120 h and 30 periods of 8 h with 3 cows.

In Exp. 2, a comparable collection procedure was followed, except that a 5% sample was withdrawn and that vial contents were weighed, sampled and returned after each 15-min time interval. Ileal digesta were collected for 10 h periods. In total digesta were sampled for 64 of such periods with 4 sheep (4 × 16). Per collection period samples were pooled and kept at -20 °C.

In Exp. 3, ileal digesta were collected for 4 periods of 8 h on 4 consecutive days. With intervals of 30 min digesta collected in the vial were sampled (10 %) and the remainder returned through the distal cannula, without addition of donor digesta. Samples were stored individually at −20 °C.

In Exp. 4, digesta samples were withdrawn from the T-shaped cannulas inserted into the mid-jejunum (III) and terminal ileum (IV). Per sampling day per cannula with intervals of about 2 h, 4 samples were withdrawn, each of about 20 g. These 4 samples were pooled and kept at −20 °C. The amount of digesta sampled was equivalent to about 3% of the digesta passing along cannula III and about 4% of the digesta passing along cannula IV. In total digesta were sampled for 64 sampling periods with 4 sheep (4 × 16).

Marker infusion

In Exp. 1, paper impregnated with Cr_2O_3 was ground with a hammermill through a 1-mm sieve and then suspended (2 g Cr_2O_3/kg) in water containing carboxymethylcellulose (10 g/l). The marker infusate contained also PEG (150 g/kg) and was infused continuously into the duodenal cannula at a rate of about 30 g/h. Infusion of the marker was started about 16 h before collection of the ileal digesta was started, so that at the onset of the collection period a steady-

state marker concentration at the site of digesta collection had been obtained (see results, Exp. 3).

In Exp. 2, an aqueous solution of PEG was infused continuously into the abomasum of sheep at a rate of about 1.85 g PEG/h. Collection of ileal digesta started 10 h after the onset of the PEG infusion. In comparison with Exp. 1 marker infusion was started later, but mean retention time of the liquid phase of digesta in abomasum plus small intestine is considerably lower than 10 h and thus a steady state marker concentration in the ileum at the onset of digesta collection was to be expected.

In Exp. 3, 35 g of a marker infusate containing about 450 g PEG/kg and 5 g Cr_2O_3/kg (impregnated onto paper and ground as in Exp. 1) was injected into the duodenal cannula of dairy cows at 07h00, 1 h before the collection period of 8 h began.

In Exp. 4, the marker infusate contained 90 g PEG/kg and 3 g Cr as Cr-EDTA/kg, infused at a rate of 20 g/h into the abomasum. In comparison with Exp. 2 marker infusion was started earlier, more than 24 h before withdrawing of samples from the single T-shaped cannula was started. The Cr-EDTA was prepared as described by Binnerts et al. (1968).

Analytical procedures

For analyses of Cr_2O_3 about 1 g of dried material was ashed and consequently dissolved with 3 ml 10 % $MnSO_4$ in 80 % phosphoric acid and 4 ml 4.5% potassium bromate until the mixture turned dark purple. Then the purple solution was transferred quantitatively into a volumetric flask, 25 ml of a calcium chloride solution (1.48 %) was added and after proper dilution Cr concentration was determined with the atomic absorption spectrophotometer (Perkin Elmer 360) at a wave length of 357.9 nm against Cr standards, prepared of $K_2Cr_2O_4$ in water and run through the same procedure (van 't Klooster et al., 1969).

For the determination of PEG, 2 ways of analysis were applied. In Exp. 1, 2 and 3 about 10 g of ileal digesta were diluted with water (about 40 g) and kept overnight. Then the diluted ileal digesta were centrifuged (15 min, 2000 *g*) and the resulting supernatant solution used for the estimation of PEG according to the method of Hydén (1955). The turbidity was read exactly 10 min after the addition of trichloroacetic acid against standards of PEG in water run through the whole procedure. In Exp. 4, jejunal and ileal samples were centrifuged (15 min, 2000 *g*) and the resulting supernatant diluted prior to the whole analytical procedure.

In both methods, the ultimate PEG concentration of digesta was calculated taking into account the digesta dry matter content. Both analytical procedures gave comparable results. In Exp. 4, Cr-EDTA concentration in jejunal and ileal samples was determined starting from supernatants of undiluted centrifuged (15 min, 2000 *g*) digesta samples. These supernatants were properly diluted with a 2 % NH_4Cl solution and Cr concentrations were determined with the atomic absorption spectrophotometer (Perkin Elmer 360) at a wave length of 357.9 nm against Cr standards, prepared of $K_2Cr_2O_4$ in water and diluted in the same way.

Table 2. Percentage of recovery of PEG and Cr_2O_3 and flow rate of digesta in the ileum over successive periods of collection of 24 h (Exp. 1.1), of 8 h within those of 24 h (Exp. 1.1) and of intermittent 8-h periods (Exp. 1.2). Data are presented as mean ± standard error of the mean. Data within columns, not sharing one or more common letters are significantly different ($P < 0.05$). Difference of recovery from 100 % is indicated by asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

	Recovery (%)		Ileal flow of digesta (kg/h)
	PEG	Cr_2O_3	
Exp. 1.1 24 h	92.7 ± 1.2^{a}***	82.6 ± 1.6^{a}***	2.39 ± 0.06^{ab}
8 h	89.9 ± 2.7^{a}***	80.4 ± 2.4^{a}***	2.35 ± 0.07^{a}
Exp. 1.2 8 h	97.0 ± 1.0**	84.9 ± 1.5^{a}***	2.53 ± 0.05^{b}

Results

The ileal recoveries of PEG and Cr_2O_3 for Exp. 1.1 and 1.2 are given in Table 2. Since the cows were fed only twice a day (08h00 and 17h00) comparison of the marker recoveries of Exp. 1.1 with successive 24-h collection periods with those of Exp. 1.2 with 8-h collection periods is possibly not allowed. A specific flow pattern of digesta in the ileum, related to the feeding regimen, should result in variable hourly passage rates of marker, thus affecting marker recovery over specific collection periods. In order to prevent interference of such effects with the conclusions to be drawn, ileal flow patterns of digesta were analysed for both Exp. 1.1 and 1.2 (Fig. 1 and 2, respectively). For the same reason marker recoveries in Exp. 1.2 were not only compared with the recoveries over 24-h periods of Exp. 1.1 but also with the recoveries of 8-h periods of Exp. 1.1. within those of 24 h and comparable to those of 8 h of Exp. 1.2 (08h00-16h00). Since fluctuations in flow rate of digesta may affect marker recovery, in Table 2 also the mean flow rates of digesta over the respective periods of collection are given.

Marker recovery, especially of Cr_2O_3, appeared to be disappointingly low. Nevertheless some conclusions are allowed to be drawn on the relationship be-

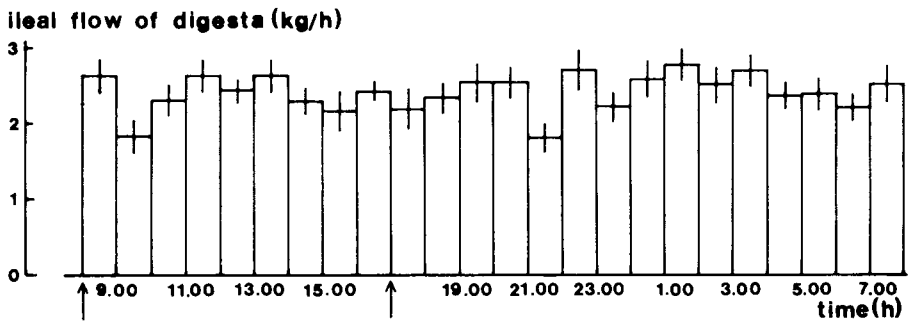

Fig. 1. Daily pattern of digesta flow in the ileum of cows over 24 h, determined in 6 joined periods of digesta collection of 5 days. The standard error of the mean is indicated by the vertical bars. Times of feeding are indicated by the arrows.

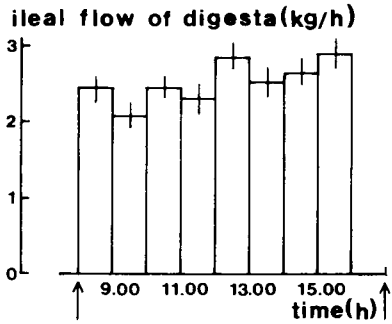

Fig. 2. Pattern of digesta flow in the ileum of cows, determined in 30 intermittent periods of digesta collection of 8 h. The standard error of the mean is indicated by the vertical bars. Times of feeding are indicated by the arrows.

tween the recovery of the markers and the method of digesta collection. In Exp. 1.1, recoveries of both markers calculated over periods of collection of 8 h did not differ significantly from those of the total collection periods of 24 h. No difference was found either between the mean hourly ileal flow rates of digesta, calculated over both periods of collection. However, when ileal digesta was collected intermittently for periods of 8 h (Exp. 1.2), a different picture was obtained. Recovery of PEG was slightly but significantly increased in comparison with the 8-h recovery of Exp. 1.1 ($P < 0.05$) and even more significantly with the 24-h recovery ($P < 0.01$). Data on Cr_2O_3 recovery pointed in the same direction, but differences were not significant. The mean hourly ileal flow rate of digesta over 8 h of Exp. 1.2 was slightly increased in comparison with the 8-h collection periods of Exp. 1.1 ($P < 0.05$). The same tended to be true in comparison with the 24-h collection periods of Exp. 1.1, however not significantly. In general, it seemed that marker recovery was related positively to mean hourly ileal flow rate of digesta.

Hourly ileal flow rate showed no specific pattern when digesta were collected during a joined period of 5 days (Exp. 1.1). However when digesta were collected during intermittent periods of 8 h (Exp. 1.2), ileal flow rate of digesta showed a steady increase with time elapsed since the start of the collection period. Possibly this is the reason why the overall hourly ileal flow rate of Exp. 1.2 was higher than of Exp. 1.1, calculated over comparable collection periods of 8 h.

Recoveries of PEG and Cr_2O_3 and mean hourly ileal flow rate of digesta calculated over the 24-h collection periods of Exp. 1.1 were arranged according to the day of collection after the start of the whole collection period of 5 days in total. The mean values ($n = 6$) are shown in Fig. 3. A dependency of all three variables on day of collection was obviously not present.

Exp. 2, carried out with sheep, showed data on PEG recovery, which were quite satisfactory; 99.2 ± 1.9 %.* However, also in this experiment, digesta were collected during intermittent periods of collection (10 h) and in this sense the results can better be compared with those of Exp. 1.2. Moreover also analysis of the ileal flow pattern of digesta over the collection period (Fig. 4) leads to

* Mean ± standard error of the mean.

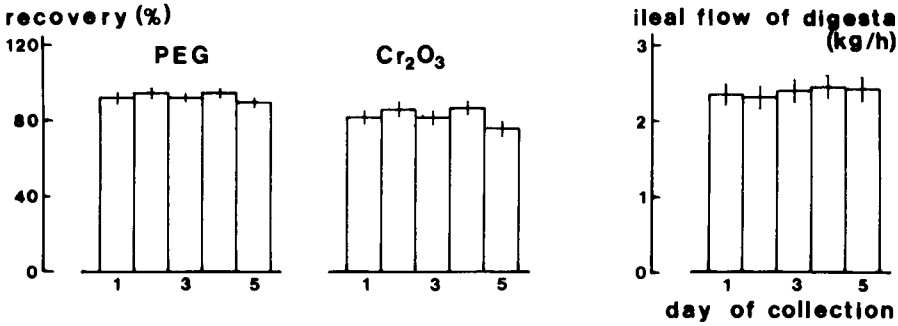

Fig. 3. Recovery of PEG and Cr_2O_3 and flow of digesta in the ileum of cows per day of joined periods of digesta collection of 5 days in dependency of time elapsed since the onset of digesta collection. The standard error of the mean is indicated by the vertical bars.

comparable conclusions. Apart from the first hour of collection, when the highest flow rate was obtained, ileal flow rate of digesta also seemed to increase steadily the following hours of collection, an increase in flow rate which is difficult to explain only as a result of the peak flow rate of the first hour of collection and the subsequent decrease thereafter.

In Exp. 3, mean transit times of PEG and Cr_2O_3 were studied in the small intestine of dairy cows. Per collection period of 30 min the marker recoveries were determined and with these recoveries the mean transit time of the markers was calculated as the weighed mean of the respective time intervals elapsed since marker introduction. For PEG a mean transit time was determined of 173.4 ± 7.0 min, and for Cr_2O_3 of 172.1 ± 6.7 min. Mean transit times of both markers along the small intestine differed not significantly, also not when the data on PEG and Cr_2O_3 were compared in pairs.

In Exp. 4, flow rates of digesta in the mid-jejunum and the terminal ileum were determined on the basis of the dilution of PEG and Cr-EDTA. With PEG flow rates were 359.0 ± 7.9 g/h and 249.7 ± 6.6 g/h for the mid-jejunum and the terminal ileum respectively. Consequently the ratio was determined of the

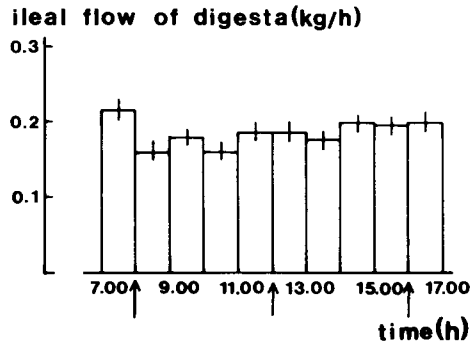

Fig. 4. Pattern of digesta flow in the ileum of sheep, determined in 64 intermittent periods of digesta collection of 10 h. The standard error of the mean is indicated by the vertical bars. Times of feeding are indicated by the arrows.

flow rate of digesta based on PEG to that based on Cr-EDTA. These ratios were 1.028 ± 0.006 for the mid-jejunum and 1.011 ± 0.007 for the terminal ileum. For the mid-jejunum this ratio differed slightly though significantly from 1 ($P < 0.001$). For the terminal ileum no difference existed between the flow rates determined with both markers.

Discussion

Collection of digesta for several successive periods of 24 h is very laborious and under conditions that no automatic equipment for collection and sampling of digesta is available, hardly realizable. Then, shorter periods of collection have to be considered, followed by extrapolation of the obtained results to 24 h results. Such an approach is only correct, however, when the collection period selected is representative for the whole period of 24 h.

Unfortunately, disconnection of a re-entrant cannula has been shown to affect flow rate of digesta. A reduction in flow rate of digesta in the duodenum and a lower recovery of PEG and Cr_2O_3 have been found in sheep (van 't Klooster et al., 1969) and cows (van 't Klooster et al., 1972). In contrast, Thompson & Lamming (1972) and Oldham & Ling (1977) found no differences in flow rate of digesta and dry matter from day to day in the duodenum of sheep, with collection periods of 72 h.

In the ileum such a flow depression at the onset of long term collection periods has also been reported. Goodall & Kay (1965) found in the ileum of sheep that flow in the first 24 h of collection was less than the average flow over 3days and that this was compensated by an increased flow during the 2nd collection period of 24 h.

It has been suggested that such depressions in digesta flow could be caused by the stress of the experimental procedure. If so, pretraining of the animals before the experiments are started is essential. In our opinion, it is evident that this condition has to be fulfilled for all types of experiment. However, it should be doubted that stress should be the only factor responsible for the depression in digesta flow at the onset of a long-term collection period. For example, some of the sheep used by Oldham & Ling (1977) were accustomed to the experimental procedure only during periods of collection of occasionally less than 1 day, but nevertheless no flow pattern of digesta in dependence of the time interval elapsed since the onset of collection was found. Unfortunately, in several other papers, the way of pre-handling the animals before the experiments is not well documented and therefore the importance of pre-training is difficult to estimate.

The present experiments, were carried out with well-trained animals. The cows used received their cannulas more than 3 years before these experiments were carried out. In the course of those years they were used for several other experiments, and therefore they were well accustomed to the experimental procedure. Nevertheless starting a week before the onset of the experiments several short-term collections were conducted. The sheep were surgically prepared 2-3 months before the start of the experiments. Before the start of the experiments at

least 2 weeks were available for acclimatizing to their housing and the whole experimental routine.

In Exp. 1.1 ileal digesta were collected and sampled for periods of 5 days. Data were analysed according to 5 successive days. No dependency of ileal flow of digesta, nor of the recoveries of PEG and Cr_2O_3 on day of sampling elapsed since the onset of collection could be detected (Fig. 3). This leads to the conclusion that under our experimental conditions 24 h ileal digesta collections should have been justified. In fact, it seems that even shorter collections can be practised. In comparison with the 24-h data, ileal digesta flow and recovery of PEG and Cr_2O_3 calculated over 8-h periods selected from the 24-h periods did not differ (Table 2), as was to be expected regarding the absence of a specific digesta flow pattern over 24 h (Fig. 1). Unfortunately, these 8-h data did not correspond with those determined over intermittent 8-h collections (Exp. 1.2). Then, both recovery of PEG and ileal digesta flow showed to be slightly higher. Analysis of the flow pattern of this experiment (Fig. 2) shows that digesta flow is increasing steadily with time elapsed since the start of collection. It is likely to consider that such a pattern is caused by an initial flow depression, as mentioned above, but over a shorter period. On the other hand, however, it seems that this depression in digesta flow is overcompensated during the subsequent hours, since 8-h collections result in higher flow rates of digesta in comparison with 24-h collections.

In Exp. 2, with 10 h collections about the same phenomenon is observed. Excluding the highest digesta flow of the first hour of collection, probably an initial effect of disconnecting the cannulas, digesta flow showed a steady increase the subsequent hours. It seems unlikely that such a flow pattern is related to frequency of feeding. In Exp. 1.1 (Fig. 1) and Exp. 1.2 (Fig. 2), cows were fed twice a day but only in Exp. 1.2 a steady increase in digesta flow is noticed. In Exp. 2, however, sheep were fed 6 times per day, and here also a steady increase in digesta flow appeared, which cannot be related to feeding. Such a steady increase in digesta flow the period following disconnection of the cannulas might be affected as well by the more or less continuous absence of a considerable amount of digesta from the intestine in the vial for digesta collection.

In Exp. 1.1, recovery of both PEG and Cr_2O_3 was disappointingly low, significantly lower than 100% ($P < 0.001$). Possibly for Cr_2O_3 this was to be expected more or less, since low recoveries of Cr_2O_3 have been reported by several authors (Topps et al., 1968; MacRae & Armstrong, 1969; Nicholson & Sutton, 1969; MacRae et al., 1972). It was considered that these findings reflected a depression in digesta flow, caused by the experimental procedure. In consequence, an increased amount of marker is retained in the forestomachs and the amount passing the intestines is decreased. In the present experiments, however, markers were not supplied in the forestomachs, but more distally in the abomasum (Exp. 2 and 4) or in the duodenum (Exp. 1 and 3). Also then it is possible that marker recovery at the end of the small intestine is decreased, however, only temporarily. Transit time of digesta along the small intestine of sheep and cows does not exceed a few hours, and therefore, also when digesta flow is temporarily or per-

manently depressed, marker recovery may be initially lower, but on the longer term 100 % recovery is to be expected again. So the low recovery of PEG and Cr_2O_3 cannot be caused by a depression of digesta flow only. Probably methodological errors are involved as well. In this context Kotb & Luckey (1972) stated that for PEG a specific, sensitive and accurate method of analysis is lacking, possibly the major reason why data on PEG recovery are quite frequently rather inconsistent.

With intermittent collections over shorter periods, for PEG considerably higher recoveries were found. In Exp. 1.2 with 8 h collections the recovery was still significantly lower than 100 % ($P < 0.01$), however only slightly. In Exp. 2 with 10-h collections the percentage did not differ significantly from 100 % ($P > 0.20$). For Exp. 1.1 methodological errors were considered to cause the low marker recoveries. For this reason, it is not likely, that these high recoveries found for PEG should be caused by an improvement in the method of analysis, but rather by an increased digesta flow rate in comparison with the 24-h collections of Exp. 1.1, because on a shorter term increased flow rates of digesta in the small intestine cause increased marker recoveries at the end of the small intestine.

Cr_2O_3 cannot be expected to be closely associated with the digesta particulate phase, and in consequence it is questionable if it behaves similarly to the different digesta solid phase components. For usage as a faecal output marker or for correction of digesta flows obtained from re-entrant cannulas, this marker is suitable, provided that the recovery equals 100 %. For measurements of transit time or for the determination of flow rates of digesta solid phase components with T-shaped cannulas, the marker used needs to be closely associated to those digesta components. For Cr_2O_3 this criterion is not fulfilled, neither for the Cr_2O_3 impregnated onto paper, as applied in the present experiments.

For Cr_2O_3 impregnated onto paper and introduced into the gastrointestinal tract distally to the forestomachs, the paper won't be broken down in the small intestine, and consequently its specific gravity will be more comparable to that of the feed particles, than of Cr_2O_3 powder as such. That the specific gravity of a marker is of importance was shown by Campling & Freer (1962). Rubber balls with a diameter of about 1.5 mm appeared to be retained longer in the gut with increasing specific gravity. Coombe & Kay (1965) compared the transit time along the small intestine of different markers. They found that PEG was retained slightly longer in the small intestine than stained straw particles, but could not give any explanation for this observation. The present results also show the same tendency, but the difference was not significant.

In the case that transit times of solid and fluid phase along the small intestine should differ considerably, samples taken from T-shaped cannulas should not be representative. The difference in transit time of both phases, however, appears to be of minor importance, and in this sense this sampling technique is probably not a source of error. In a way this is supported by the comments of Faichney (1975). He found in samples withdrawn from ileal T-shaped cannulas a ratio between the proportional concentration of the solid phase marker the phenanthroline complex of ^{103}Ru (^{103}Ru-P) to that of the fluid phase marker

^{51}Cr-EDTA close to 1 on average. MacRae (1974), however found in ileal samples, taken from a T-shaped cannula, a concentration ratio of ^{103}Ru tot ^{51}Cr, considerably lower than 1, indicating that the samples taken contained too less solid matter, probably caused by a preferential retention of water in the ileum in comparison with the solid phase and/or by sampling errors. Dry matter content of digesta passing a sampling point in the small intestine may fluctuate considerably. Probably digesta with a high dry matter content and consequently with a higher viscosity are less easily sampled resulting in a bulk sample with a dry matter content, which is too low.

With a marker of the fluid phase only an accurate determination of the amount of particles passing at the sampling point can hardly be made. MacRae (1974) reported a mean concentration ratio ^{103}RuP/^{51}Cr-EDTA of about 0.85. If only data on ^{51}Cr-EDTA had been available, the amount of dry matter should have been underestimated with less than 15%, since ^{103}Ru-P is not uniformly distributed over the solid phase. Only, if the constituent studied is contained predominantly in the phase, the particulate marker is associated to, the amount of that constituent passing at the sampling point is seriously underestimated.

For protein, it has been found that the main part in small intestinal digesta is precipitable with trichloro-acetic acid (Ben-Ghedalia et al., 1974), but this needs not to imply that this precipitable fraction is necessarily closely associated with that particulate marker. On the contrary, soluble proteins and possibly also finely suspended protein particles are expected to behave more similar to water, and thus to a water soluble marker, than to the bigger particles. Possibly, therefore, the conclusion drawn by MacRae (1974), that spotsampling procedures carried out with single markers cannot give accurate data at all, is also in view of the results of Faichney (1975), somewhat pessimistic.

If ^{103}Ru cannot be used, because of non-availability of facilities for working with isotopes, working with non-radio-active Ru has the disadvantage that it is quite costly. Therefore, we feel that under our conditions, we have to rely on markers of the liquid phase. The data determined with such a procedure, have to be interpreted with care in view of the arguments just mentioned, but with comparative experiments, systematic errors can probably be avoided for the greater part. Based on the present results, it does not matter, which of the 2 liquid phase markers is chosen for the determination of digesta flow rates in the small intestine. Flow rates determined with PEG appeared not or only slightly to differ from those determined with Cr-EDTA. The latter marker gave results with an even slightly lower coefficient of variation, probably because Cr-EDTA can be determined in a less indirect way compared with PEG.

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