Reliability of Polyethyleneglycol as an Indicator for Digestion Studies with Swine

Part I. Rate of Passage of Polyethyleneglycol through the Digestive Tract

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Received September 22, 1965

A new method for determining a small amount of polyethyleneglycol (mol. wt. about 4,000) in feces or ingesta was described. The rates of flow and excretion along the swine digestive tract were compared between polyethyleneglycol and chromic oxide used as an indicator for digestion studies.

The former was removed from stomach more rapidly than the latter, and would be delayed in its passage along large intestine owing to absorption of water therein. There was a general agreement between the excretion variations of both indicators. Recoveries during feces collection periods were lower for polyethyleneglycol than for chromic oxide. However, there was no essential difference between both indicators in retention time in the digestive tract, calculated following the idea of Castle.

INTRODUCTION

Chromic oxide (Cr_2O_3) has been used extensively as an indicator for digestibility study. However, this substance should be used to a limited degree to study digestion and absorption along the digestive tract for its difference from nutrients in rate of flow.^{1~3)}

Sperber et al. suggested use of polyethyleneglycol of mol. wt. 4,000 (PEG) as a water soluble indicator for tracing the flow of water and solutes from the reticulo-rumen into the omasum,⁴⁾ and this technique has been used to study exchange process in the digestive tract of ruminant.^{5,6)}

The suitability of PEG as an indicator and its merits with respect to those of Cr_2O_3 with swine were investigated. This report dealt with rates of flow and excretion of PEG along the digestive tract of swine.

EXPERIMENTAL

Animals

Five Yorkshire pigs (A: sow weighing 160 kg, B, C, D, E: boars weighing 80~100 kg) were used in the experiments. They were confined in individual concrete-floored pens, after they had been accustomed to the diet.

The chemical composition and the ingredients of commercial formula feeds fed to A, B and C, and to D and E as a control diet were as follows respectively: dry matter 88.1%, crude protein 13.5%, ether extract 3.3%, N-free extract 61.1%, crude fibre 4.2%, ash 6.0% and corn, wheat bran, rice bran, soybean meal, linseed meal, fish meal, and mineral; and dry matter 85.6%, crude protein 16.9%, ether extract 3.8%, N-free extract 53.9%, crude fibre 3.9%, ash 7.1% and corn, milo, wheat bran, rice bran, alfalfa

1) F. V. Gray, A. F. Pilgrim and R. A. Weller, *Brit. J. Nutrition*, **12**, 404 (1958).

2) A. Rogerson, *ibid.*, **12**, 164 (1958).

3) S. Ishikawa, *Bulletin of Faculty of Agriculture, Mie University*, No. 10, 311 (1961).

4) I. Sperber, S. Hydén and J. Ekman, *Kungl. LantbrHögsk. Ann.*, **20**, 337 (1953).

5) R. H. Smith, *Biochem. J.*, **83**, 151 (1962).

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

soybean meal, fish meal, and mineral. Each feed was fed as a wet mash.

Flow along the Digestive Tract. The animals A, B and C were fed 1,000 g, 800 g and 800 g respectively three times daily at 6 a.m., noon and 6 p.m. for three days during which little feed was wasted and water taken ad libitum. On the fourth day, one-fifth of a single morning feed mixed with 20,000 g of PEG and 12,000 g of Cr_2O_3 for A and with 25,000 g of PEG and 14,000 g of Cr_2O_3 for each B and C were first given in a steel trough, and after its complete consumption (the trough was licked clean by the animal) the remainder of the feed was given in the same trough. And they were killed 3.5, 5.5 and 7.5 hr. respectively after the morning feeding performed in this way. Immediately after slaughter, the entire digestive tract was removed, tied off by gauze and divided into 14 sections as follows: stomach, 5 segments of equal length of small intestine, cecum and 7 segments of equal length of the remainder of large intestine. The contents of a sample thereof was collected from each section. All material, which in fluid state (stomach, small intestine and cecum) had previously been evaporated to a semi-solid on a water bath, was dried in an electric drying oven at 60–70°C for forty hours, and ground in a mortar for analysis.

Rate of passage. The animals D and E were fed 500 g and 600 g respectively three times daily at 6 a.m., noon and 6 p.m. Water was taken ad libitum.

At 6 a.m. on the day following a preliminary five days, 8,000 g of PEG and 6,000 g of Cr_2O_3 , and 9,600 g of PEG and 7,200 g of Cr_2O_3 were simultaneously given with the diet for D and E respectively. During sixty hours after this feeding, the control diet was fed continuously and single defaecations were all separately and quantitatively collected when voided, and weighed. From 6 p.m. on one day till 6 a.m. on the next, the feeder was far away from the pens lest the animals should be disturbed in their normal sleep and excretion. Feces excreted in this interval were collected together twice at midnight and 6 a.m. An appropriate amount of feces was also collected just before the feeding of indicators for a blank test.

At 6 p.m. on the day following the sixty hour collection period (1) plus a three-day preliminary period on the control diet, the amount of which was 1,800 g/day for each animal, the same dose as for E was again given and the collection of feces was

repeated during sixty-six hours, as the time of the first appearance of Cr_2O_3 in feces is usually later for the diet fed in the evening than for that fed in the morning.⁷⁾ After the sixty-six hour collection period (2) the animals were kept on the control diet for two days.

Moreover, feces were collected three times daily for two days after each collection period. The accumulated feces were weighed and well mixed.

The known amount of each fresh feces was treated for analysis as mentioned above.

Analyses

Polyethyleneglycol. A modification of Hydén's method⁸⁾ was used to prepare the aqueous solution for PEG determination. From 1 to 3 g (the weight chosen according to PEG concentration) of air dry feces or ingesta sample was well shaken with about 20 ml of water, and allowed to stand for about twenty hours. The suspension was centrifuged, the remainder rinsed with water twice more, and the washing centrifuged. The combined supernatant fluids were made up to 100 ml. The aqueous extract, 10–20 ml (the volume chosen according to PEG concentration) was pipetted into a centrifuge tube and well shaken with 1 ml 10% (w/v) BaCl_2 and 2 ml 5% (w/v) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. After several minutes, the mixture was centrifuged at 10,000 r.p.m. (11,000 g) for ten minutes, and filtered quantitatively into a 50-ml measuring flask.

Five ml of the filtrate, containing not less than 4 mg % PEG, was mixed with 5 ml of a reagent consisting of trichloroacetic acid (30%, w/v) and BaCl_2 (5%, w/v), and the absorbance was read at 440 μ with a spectrophotometer (Shimazu QB-type Spectrophotometer). For the filtrate containing less than 4 mg % PEG, 4% (v/v) phenol was used in place of the previous reagent. The turbidity present was measured on a nephelometer (Turbidity Measuring Accessory Set attached to the same spectrophotometer), whose reading was arbitrarily set, for the concentration of 6.0 mg PEG/100 ml water as 60 and for water zero degree, using 445 μ filter. At the same time, standard aqueous solutions containing 0.6, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 16.0 mg of PEG in 100 ml respectively, were prepared, and the developments and measurements of turbidity were carried out as described above, except that the deproteinizing treatment was omitted.

7) J. H. Moore, *Brit. J. Nutrition*, **11**, 273 (1957).

8) S. Hydén, *Kungl. LantbrHögsk. Ann.*, **22**, 139 (1956).

FIG. 1. Changes with Time in the Turbidity of PEG Suspensions after Adding Trichloroacetic Acid-BaCl₂ or Phenol Solution.

Concentration of PEG (mg/100 ml) is shown for each curve.

As shown in Fig. 1, the time taken to reach a stable degree of turbidity after adding the reagent to standard solution is dependent on PEG concentration, but fifteen minutes was adopted as the average time in the present work. Consequently the measurement of turbidity was carried out soon after standing the mixture, either standard or sample PEG solution and the reagent, for fifteen minutes.

Fig. 2 shows a straight line relationship between absorbance or nephelometric reading and PEG concentration of standard solution, over the range 4.0~16.0 or 0.6~4.0 mg PEG/100 ml respectively. These findings on the stability of turbidity and the relationship between turbidity and PEG concentration did not agree with those of Hydén⁸⁾ and of Smith.⁹⁾

The mean recoveries by the turbidimetric and the nephelometric determination of known amounts of PEG added to 14 and 10 samples of air dry control feces collected for a blank test were 99.7% S.D. ± 4.1 and 103.4% S.D. ± 6.9 respectively. Those added to 10 and 10 samples of air dry PEG-free ingesta (stomach, jejunum, ileum, cecum and colon) of swine prepared for another experiment were

FIG. 2. Calibration Curves of PEG.

a: absorbance-concentration (4.0~16.0 mg)

b: nephelometric reading-concentration (0.6~4.0 mg)

105.8% S.D. ± 8.7 and 106.8% S.D. ± 8.3 respectively.

Chromic Oxide. The colorimetric method of analysis described before¹⁰⁾ was used. Feces or ingesta sample containing 5~30 mg Cr₂O₃ was digested with HNO₃—HClO₄ mixture in a Kjeldahl flask, and made up to a suitable known volume with water for the colorimetric determination.

RESULTS AND DISCUSSION

Flow of Ingesta along the Digestive Tract. The lengths of the separate portions of the intestine are tabulated in Table I.

TABLE I. LENGTH (m) OF INTESTINE

Animal	Body weight at slaughter kg	Small intestine	Cecum	Colon and rectum
A	160	18.00	0.30	5.80
B	100	18.30	0.22	4.50
C	88	17.20	0.28	5.43

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

10) S. Ishikawa, *Bulletin of Faculty of Agriculture, Mie University*, No. 18, 47 (1958).

The points on the graph of Fig. 3 represent the concentrations of PEG in each lot of the dry matter of Cr_2O_3 -free ingesta and of Cr_2O_3 in that of PEG-free ingesta, expressed as a percentage of the corresponding concentration in the diet, taken as 100, to adjust differences in indicator levels. Evidently, PEG is removed from stomach more rapidly than Cr_2O_3 , and ingesta of highest PEG content is present in the posterior portion of intestine compared

FIG. 3. Distribution of PEG and Cr_2O_3 in the Digestive Tract at Time of Slaughter.

○—○ PEG; ×—× Cr_2O_3

1. stomach

2~6. 1st~5th segment of small intestine

7. cecum

8~14. 1st~7th segment of large intestine

There is little amount of content in the 1st segment of small intestine of animal C.

with that of highest Cr_2O_3 content in each animal.

The largest amount of PEG would be dissolved in the liquid phase of gastric ingesta, the portion of which was least delayed in its passage along the digestive tract, and absorption of water would cause a gradual decrease in the rate of removal of PEG along large intestine.

Rate of Passage. Results of analyses of feces obtained during collection periods are presented in Table II. The excretion variation and the rate of excretion of PEG and Cr_2O_3 are shown in Figs. 4 and 5.

The points on the curves of convex shape represent the ratios of the concentrations of PEG in each lot of the dry matter of Cr_2O_3 -free feces, and of Cr_2O_3 in that of PEG-free feces to the corresponding concentrations in the diet, to adjust differences in indicator levels.

Both indicators were first detected in the feces at the same time for each animal in each period, except that the first PEG was excreted 2.5 hr. sooner (i.e. twelve hours) than the first Cr_2O_3 with E in the period 2. But these indicator levels were so low that the reliability of analyses became doubtfully. In general outline the graphs in Fig. 4 reveal a satisfactory degree of similarity between both indicators.

TABLE II. TOTAL DRY MATTER, PEG AND Cr_2O_3 EXCRETED IN FECES, AND RETENTION TIME

Animal	Period 1(60hr.)		Period 2(66hr.)	
	D	E	D	E
Dry matter excreted (g)*	818.8	725.6	834.8	739.6
PEG excreted (g)	6.29	7.63	7.38	6.60
PEG recovered (%)	78.6	79.5	80.2	69.6
Cr_2O_3 excreted (g)	5.42	6.22	6.47	5.89
Cr_2O_3 recovered (%)	90.3	86.3	89.8	81.9
No. of defaecations	12	13	11	16
Retention time (hr.)				
PEG	32.7	27.7	29.8	27.5
Cr_2O_3	32.9	29.8	31.7	29.7

* Total weight of feces excreted together with weights of markers calculated from weights of fresh feces collected.

FIG. 4. Variations in the Percentage of PEG and Cr_2O_3 in Feces after Dosing with (a) 6 a. m. Diet and (b) 6 p. m. Diet.

○—○ PEG; ×----× Cr_2O_3

Ingesta takes much longer time to pass through large intestine than through stomach and small intestine,^{7,11,12)} and meanwhile undergoes intestinal action of mixing. Consequently the differential distribution of both indicators among ingesta (Fig. 3) would not appear as it is in their excretion patterns.

The points on the excretion curves of sigmoid shape in Fig. 5 represent the cumulative total indicator excreted, expressed as a percentage of the total ingested, during each collection period. At the early stage the rate of excretion was slow, but thereafter increased rapidly. And at the last stage the levels of both indicators in feces lowered so much that

rate of recovery slowed down. The feces samples obtained after collection periods 1¹⁾ and 2 contained only traces or none of Cr_2O_3 measured by the method used, as reported in the previous work;¹³⁾ such was the case with PEG. Consequently recoveries of indicator would be little higher, even though feces are collected for a longer period than that in the experiments. Recoveries of PEG during periods 1 and 2 expressed as a percentage of the dosage in Table II are low, averaging 79.0 and 74.9% respectively compared with those of Cr_2O_3 . The recoveries of Cr_2O_3 were in reasonable agreement with the data previously reported.¹³⁾

These results are unlikely due to incomplete intake of the dose, incomplete collection of

11) S. Ishikawa, *Bulletin of Faculty of Agriculture, Mie University*, No. 24, 311 (1961).

12) E. J. Castle and M. E. Castle, *J. Agr. Sci.*, 47, 196 (1956).

13) S. Ishikawa, *J. Agr. Chem. Soc. Japan*, 36, 442 (1962).

FIG. 5. Rate of Excretion of PEG and Cr_2O_3 , Administered Simultaneously in (a) 6 a.m. Diet and (b) 6 p.m. Diet.

○—○ PEG; ×---× Cr_2O_3

feces or such faulty sampling procedures as leaching of PEG from feces during handling and destruction of PEG by heating for drying fresh feces sample. It has been shown by several groups of workers^{5,6,14-16}) that this substance was unabsorbed from the digestive tract with a few kinds of animal, and it would be certainly so with swine. Moreover, the previous work¹⁷⁾ has shown that any degradation of PEG could not be detected in its passage through the tract.

Corbett et al.⁶⁾ suggested in their experiments on the recovery of PEG with cattle

that a major part of PEG losses occurred when protein and sulphate were precipitated in the analytical procedure described by Hydén,⁸⁾ and that it was related to the quality of feces. By the analytical methods used, the known amount of PEG added to feces or ingesta prepared for another experiments, was sometimes determined with unsatisfactory recovery. It may be said that analytical procedure of PEG should be modified to give more satisfactory repeatable results. However, the experiments indicated that recoveries of PEG added to the feces samples were usually good, although it was difficult to determine the low concentrations, less than 50mg PEG/100g air dry feces, with accuracy.

On the other hand, it is possible for both

14) S. Hydén, *Kungl. LantbrHögsk. Ann.*, 22, 411 (1956).

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

16) E. D. Jacobson, D. C. Bondy, S. A. Broitman and J. S. Fordtran, *Gastroenterology*, 44, 761 (1963).

17) S. Ishikawa, *This Journal*, 29, 173 (1965).

indicators to be retained in the digestive tract as Barnicoat¹⁸⁾ pointed out, but it is improbable that retention of PEG can occur to a greater extent than that of Cr_2O_3 .

There would seem to be no reasonable explanation of the fecal low recovery of PEG ingested. Further experiments on this point are being carried out.

For comparing the entire excretion, the area to the left of excretion curve, plotted between the cumulative total indicator excreted up to any one time, expressed as a percentage of the total excreted during each collection, and time (hr.) after dosing, was calculated following the idea of Castle,¹⁹⁾ and is presented in Table II. Two typical excretion curves (animal D in period 1) are shown in Fig. 6. This value represents retention

FIG. 6. Excretion Curves of PEG and Cr_2O_3 with Animal D in Period 1.

○—○ PEG; ×---× Cr_2O_3

18) N. R. Barnicoat, *N. Z. J. Sci. Tech. A*, 27, 202 (1945).
19) E. J. Castle, *Brit. J. Nutrition*, 10, 15 (1956).

time in hours of all the indicator in the entire digestive tract. It may be said by using this expression that there is no essential difference in the entire excretion between PEG and Cr_2O_3 , although retention time of the former is a little shorter than that of the latter.

Strictly speaking, no single indicator can follow the passage of feed ingested, as its constituents tend to show individual behaviour patterns in passage and excretion.²⁰⁾ PEG appears to be a valuable indicator for tracing the flow of water and solutes through the digestive tract also with swine, as suggested with ruminant by Sperber et al.⁴⁾ and Smith.⁵⁾ The low recovery in feces of PEG, the reason for which is not clear, is considered to be unfavorable for use of this substance as an indicator.

However, further experiments, examining both the recovery and the excretion variation in feces of swine fed on the diet containing PEG at the constant rate, are necessary to elucidate the reliability of this substance for digestibility estimate.

Acknowledgements. The author wishes to express his thanks to Misses K. Fujita and Y. Hara for their technical assistance, and to Messrs. N. Iwasa, Y. Kameyama, M. Kawato and S. Furuichi for their technical assistance and the care of the animals. This work was supported in part by a Grant in Aid for Scientific Research of the Ministry of Education.

20) S. Ishikawa, *Bulletin of Faculty of Agriculture, Mie University*, No. 23, 59 (1961).