The extent of release of encapsulated methionine in the intestine of cattle

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(*Received* 4 *May* 1970—*Accepted* 3 *September* 1970)

1. Factors affecting the release of methionine from kaolin–saturated-fat capsules in the intestine of cattle were investigated. Bile and pancreatin were shown to be necessary for appreciable solution of the capsule.

2. The relationship between site of release and site of absorption was ascertained. One site was shown to be in the proximal duodenum; the second longer site is in the distal duodenum and proximal jejunum. Encapsulated methionine was carried past the first absorption site.

3. The results of in vitro incubations confirmed by those of in vivo studies show that 60–65 % of the methionine becomes available for absorption in the intestine.

During fermentation in the rumen, some feed constituents which are required by the animal after absorption in the intestine are hydrolysed and destroyed (Lewis & Emery, 1962; Phillipson, 1964; Clarke, Ellinger & Phillipson, 1966; Bergen, Purser & Clive, 1968). Consequently, the ruminant may suffer from nutritional deficiencies (Reis, 1967). Furthermore, oral medication of the ruminant animal is complicated because administration of drugs may lead either to an undesirable effect on the microflora or to inactivation of the drugs by the micro-organisms. For these reasons it is desirable to have a matrix which will protect nutrients and drugs against ruminal breakdown but which will allow the substances to be released into the intestine for absorption. The production of a kaolin–saturated-fat capsule for the transport of methionine into the intestine of ruminants (Sibbald, Lougheed & Linton, 1967) was reported recently. The capsule is produced by extruding a mixture of carrier and charge ingredient, resulting in the uniform distribution of the encapsulated material throughout the matrix. The carrier consisted of saturated fat having a titre of 58–60° and kaolin ($H_2Al_2Si_2O_8 . H_2O$). The charge ingredient was DL-methionine. The capsule is not wettable, and the specific gravity of the capsule is 1·18–1·20. The capsule will carry 18–22 % of a charge material. In addition to methionine, chlortetracycline has been successfully encapsulated in this matrix (Sibbald *et al.* 1967).

The effectiveness of encapsulated methionine in sheep has been demonstrated by Linton, Lougheed & Sibbald (1968). The release of methionine from the capsule in the intestine was, however, not measured quantitatively. This information is necessary to evaluate the usefulness of the capsule.

https://doi.org/10.1079/BJN19710098 Published online by Cambridge University Press

https://doi.org/10.1079/BJN19710098 Published online by Cambridge University Press

EXPERIMENTAL

Effect of bile and pancreatin on the kaolin–saturated-fat capsule in an in vitro system

The standard in vitro incubation medium consisted of 100 mg capsules and 25 ml digesta and the procedure was essentially that of Scott & Lough (1969). The capsules were supplied by Dr Sibbald from the John Labatt Co. Limited, London, Ontario, Canada. The digesta were obtained from eight regions of the intestine of the cattle immediately after slaughter. The regions were a 10 cm section including the pylorus (pyloric), a 20 cm section proximal to entry of the bile duct (proximal duodenum), a 30 cm section distal to the bile duct entry (mid-duodenum), a 30 cm section starting 1 m beyond the duct entry (distal duodenum), a 30 cm section beginning 2 m beyond the duct entry (proximal jejunum), a 30 cm section from the mid-section of jejunum (mid-jejunum), a 30 cm section 2 m from the ileal-caecal junction (distal jejunum) and a 30 cm section immediately preceding the ileal-caecal junction (distal ileum). The animals, two young 550 kg Hereford steers and two old 600 kg Ayrshire cows, received the normal meal ration, consisting of 1·25 kg oat-barley chop and 3·50 kg hay, 3 h before death. Bile and pancreatin were obtained from the same animals. Pancreatin was a low-temperature high-speed homogenate (0° for 5 min) consisting of 50 g of pancreas and 50 ml of pH 6·5 0·02 M-Krebs–Ringer buffer. To the standard incubation medium were added either 5 ml of water or 5 ml of bile or 1 ml of pancreatin and 4 ml of water or a suspension of 0·001 g pancreatic lipase (Sigma) in 5 ml of water or 4 ml of bile and 1 ml of pancreatin. A control consisted of boiled mixture of digesta, bile and pancreatin to which capsules were added after the mixture was cooled to room temperature. Incubation was at 39° under a mixture of O_2–CO_2 (95:5) in a shaker water-bath for 1 h. At termination, the medium was centrifuged at 10000 *g*. The supernatant fraction was quantitatively recovered. The reaction in the supernatant fraction was halted by heating to 100° for 5 min; that in the particulate fraction was stopped by the addition of 10 ml of a mixture of methanol: chloroform (1:1). The accumulation of methionine in the supernatant fraction and the loss of methionine from the particulate fraction were measured to estimate how much of the capsule had dissolved. Methionine was measured either colorimetrically as the sodium prusside derivative (McCarthy & Paille, 1959) or by ion-exchange resin chromatography using the Technicon (TSM) amino acid AutoAnalyzer.

The effect of origin of digesta within the intestine, age and sex of the animal on capsule solution was statistically determined by analysis of variance (Dixon & Massey, 1957*a*). All tests of significance were made at the 5% level of probability.

During preliminary experiments accumulation of octadecanoic acid (stearic acid) in the supernatant fraction and loss of stearic acid from the particulate fraction were also determined. Quantitative analysis of stearic acid as the methyl ester was by gas–liquid chromatography (Neudoerffer, 1967) using methyl heptadecanoate as internal standard. The correlation between the two methods of chemically determining capsule solution was linear. The correlation coefficient (linear least square correlation; Dixon & Massey, 1957*b*) for the extent of capsule solution measured as solute appearance in the supernatant fraction was $r = 0·95$. The same value based on stearic acid and

methionine residues in the particulate fraction was $r = 0{\cdot}96$. In view of the good correlation, the simpler methionine method only was used for the routine chemical determination of capsule solution. The results of this experiment, given in Table 1, were based on both the measurement of methionine release to the supernatant fraction and retention in the particulate fraction. Total recovery of methionine was $96 \pm 3{\cdot}4\%$. In this experiment the physical (microscopic, see below) measurement of capsule solution was not undertaken.

Relationship between capsule solution and sites of intestinal uptake

Four 2·5 cm gut segments were excised beginning at the pylorus at 10 cm distances from the proximal duodenal region, a single segment was removed from each of the mid and distal duodenal regions, eight segments were taken at 30 cm distances from the proximal jejunal region, and a single segment was excised from the distal ileal region. No samples were taken from the mid and distal jejunal region after preliminary results had indicated that uptake in these regions was low. The location of the intestinal regions is defined on p. 334. The intestine was obtained immediately after slaughter from two Hereford steers and one Ayrshire cow. The gut segments were everted and the adhering digesta were washed free with a cold solution of sodium chloride–potassium chloride (0·145 M:0·001 M respectively). Tissue preparations, approximately 1 cm^2 in size, consisting of lamina propia and intestinal epithelial cells were separated from the intestinal muscle layers of the gut segment and incubated with [^{14}C]methionine for 1 h at 37° in 10 ml of a mixture containing 100 mg glucose, 10 mg unlabelled methionine, 0·1 μCi [^{14}C]methionine buffered at either pH 7·76 or 3·50. The two pH optima for methionine absorption had been established in preliminary experiments using 0·02 M-citrate-phosphate buffer for the low pH range and 0·02 M-Krebs–Ringer buffer for the high pH range. At termination of incubation the tissue was recovered and freeze-dried. The dry tissue was hydrolysed in 0·5 ml of 1 N-sodium hydroxide. The hydrolysate was suspended in a Cab-o-sil–PPO–POPOP scintillation system and the number of scintillations was counted (Tye & Engel, 1965).

Solution of capsule in vitro. Capsules (100 mg) were incubated in 25 ml digesta from two Hereford steers, one having a posterior duodenal re-entrant cannula and the other having a proximal jejunal re-entrant cannula. Fresh bile and pancreatin were obtained from a slaughtered Hereford steer for each of two experiments. In one instance, capsules were incubated continuously for 6 h as described previously. In a second instance, the incubation was arrested at 15 min intervals, and fresh digesta were added to the recovered washed capsules. Capsule solution was determined by the appearance of methionine in the supernatant fraction and loss of methionine from the particulate fraction.

In a second experiment, specific numbers of capsules (size 800 ± 25 μm) were incubated successively in either duodenal or jejunal digesta. At the completion of each 15 min incubation period, the number of recoverable capsules was determined and their size was measured microscopically using an ocular micrometer (Carl Zeiss, Inc. New York; 0–1000 μm). For the successive incubations, fresh digesta containing bile and pancreatin were used.

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In a third experiment capsules (10 g) were placed in nylon bags which were subsequently suspended for 18 h in the rumen of a fistulated Hereford steer (Neathery, 1968). The size of the dry capsules was measured and the methionine content was determined on a sample of the recovered capsules. Two 1 g samples of the recovered capsules were subsequently suspended in 250 ml abomasal liquor and incubated for 30 min. Size and methionine content were again determined on the recoverable capsules. Four 100 mg samples of the recovered capsules were used for incubation for 1 h in duodenal or jejunal digesta to which bile and pancreatin had been added. Size and methionine content were again determined on the recovered capsules.

In a fourth experiment, capsules recovered from suspension in the rumen or incubation in abomasal liquor, or suspension in the rumen and incubation in abomasal liquor, were incubated for five 15 min periods in duodenal digesta containing bile and pancreatin. At the completion of each 15 min incubation period, the capsules were recovered and methionine solution was ascertained. The recovered capsules were suspended in fresh digesta containing bile and pancreatin. In all instances 500 μm capsules were selected for the first incubation in duodenal digesta. The surface of a few capsules from each treatment was examined microscopically (magnification $\times$ 1000).

Solution of the capsule in vivo. Capsules (2 g) were suspended in fresh abomasal liquor obtained through the abomasal cannula. The suspension was infused into the abomasum of the donor animal. Digesta were collected quantitatively at the re-entrant cannula for a minimum of 8 h after infusion by a procedure described by Nicholson & Sutton (1969). The re-entrant cannulas in animals B, a Hereford steer, and S, an Ayrshire heifer, were located 0·70 m from the pylorus. The cannula in animal C, also an Ayrshire heifer, was located 0·25 m from the caecum. Undissolved capsules in the 1000 g particulate fraction were recovered by flotation. Preliminary studies had shown that the recovery of capsules by flotation was $93 \pm 4\%$. The size of the recovered capsules was measured as before and the total undissolved methionine was determined. All investigations were carried out in duplicate. The three animals with re-entrant cannulas were fed on two rations to ascertain the effect of diet on capsule solution. One ration supplied a daily intake of 2 kg ground maize and 4·5 kg hay; the other supplied 4·0 kg maize and 2 kg hay. The details of the diets are given elsewhere (Neudoerffer, Leadbeater, Horney & Bayley, 1971).

RESULTS

The liberation of the encapsulated material might be brought about by either the enzymic hydrolysis of the triglyceride portion of the matrix or physical abrasion caused by peristalsis. The results shown in Fig. 1 indicate that solution of the kaolin–saturated-fat capsules is primarily a biochemical process, requiring both bile and pancreatin. The results of our in vitro experiments suggest that maximum solution begins distal to the entry of the bile and pancreatic ducts and ceases in mid-jejunum.

Approximately 12% of the encapsulated methionine dissolved during incubation in digesta to which no bile or pancreatin had been added. The addition of bile to the digesta doubled the extent of solution, but the effect of adding pancreatin or pan-

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creatic lipase was not so pronounced. In a mixture of bile, pancreatin and digesta, 49% of the capsules dissolved in 1 h. The negligible amount of solution observed when capsules were shaken and incubated with digesta inactivated with heat suggests that the amount of solution by abrasion is small.

The extents of capsule solution in digesta taken from various sections of the intestine were significantly different. However, the effects of sex and age were not significant,

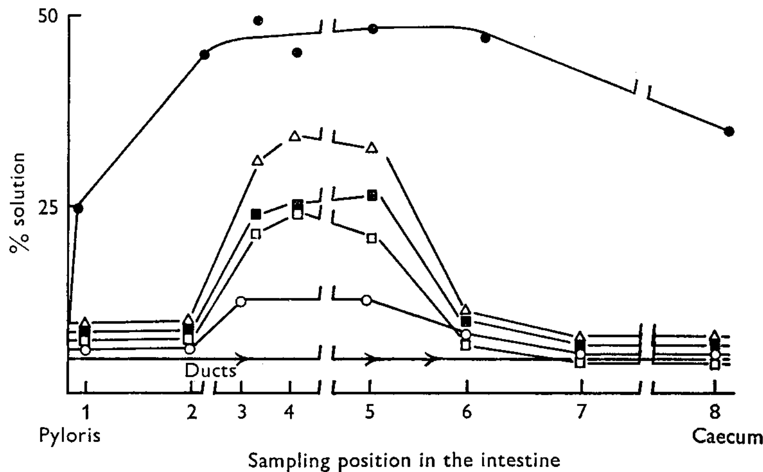

Fig. 1. Localization of capsule solution in an in vitro system of cattle digesta. Incubation media composition: ●, digesta+bile+pancreatin; △, digesta+bile; ■, digesta+pancreatin; □, digesta+pancreatic lipase; ○, unsupplemented digesta; →—, boiled digesta. Sampling position: 1, pyloric region; 2, proximal duodenal region; 3, mid-duodenal region; 4, distal duodenal region; 5, proximal jejunal region; 6, mid-jejunal region; 7, distal jejunal region; 8, distal ileal region. For definition of regions see p. 334. Abscissa not drawn to scale.

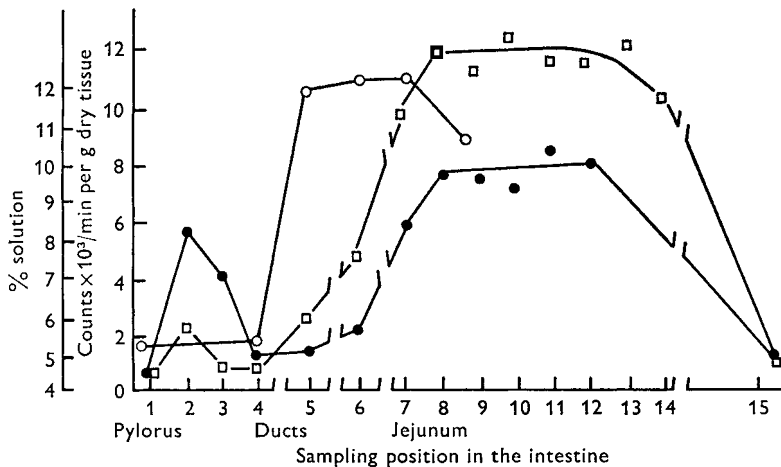

Fig. 2. Relationship between capsule solution and sites of [^{14}C]methionine uptake in the intestine of cattle. Incubation at: □, pH 7·6; ●, pH 3·5; ○, % solution (see Fig. 1). Sampling position: 1 – 4, proximal duodenal region; 5, mid-duodenal region; 6, distal duodenal region; 7 – 14, proximal jejunal region; 15, distal ileal region. For definition of regions see p. 334. Abscissa not drawn to scale.

https://doi.org/10.1079/BJN19710098 Published online by Cambridge University Press

indicating that the process of solution is independent of the nature of the animal. One cow was not lactating and the other cow was in the 310th day of lactation; thus any influence on capsule solution which may occur as a result of metabolic changes due to lactation would not have been detectable.

The usefulness of a capsule depends on its ability to carry the charge only as far as the site of absorption. Fig. 2 shows that one site of methionine absorption is the section of the intestine from the pylorus to the entry of the ducts. Since the capsule appears to require both pancreatin and bile for solution, the methionine would be carried past this initial site of absorption. This lack of solution may not be important since the capacity of the second, seemingly longer, site to render the capsule soluble

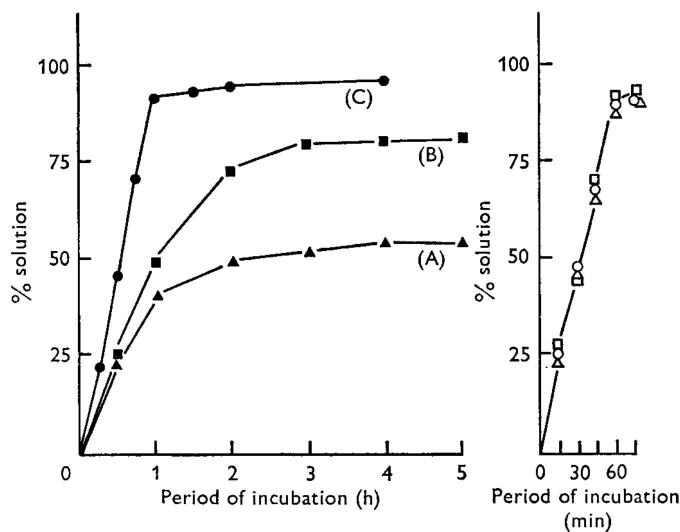

Fig. 3 Fig. 4

Fig. 3. Extent of capsule solution in an in vitro system of cattle digesta. ●, duodenal digesta (fresh digesta used at regular intervals); ■, jejunal digesta; ▲, duodenal digesta.

Fig. 4. Effect of previous incubation of capsules on their rate of solution in duodenal digesta from cattle. ○, previously suspended in the rumen; △, previously incubated in abomasal juice; □, previously suspended in the rumen and incubated in the abomasal juice.

appears to be adequate. Nevertheless, the importance of the first site cannot be completely dismissed since it was shown during preliminary studies that the rate of absorption in this region was about four times as great as the rate of uptake at the second site.

Maximum benefit from encapsulation requires complete solution. However, it was found that even digesta containing bile and pancreatin did not completely dissolve the capsule. Extended incubation with the mixture brought about 51% and 76% solution (Fig. 3A and B). By replacing the digesta in the mixture at regular intervals, the solution of the capsule was raised to 89%. The residual 10% of capsule material was due to the partial dissolving of all capsules rather than the disappearance of 90% of the capsules (Table 1). There appeared to be a size limit of 100 μm below which solution did not occur.

Previous incubation in rumen and abomasal liquor did not alter the apparent lower

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size limit (Table 2), even though an approximately 30% loss of methionine occurred in the rumen liquor and a further 15–18% of methionine disappeared from the capsule during incubation with abomasal liquor. Previous treatment of capsules did not alter the rate of their solution in duodenal digesta (Fig. 4). Comparison with Fig. 3C shows that the susceptibility to solution of treated capsules did not vary from that of untreated capsules. Thus susceptibility of capsules to solution is not altered

Table 1. *Number of capsules recovered and capsule size after four successive incubations in intestinal digesta of cattle*

	Capsules used		Capsules recovered after: First incubation		Second incubation		Third incubation		Fourth incubation	
Digesta	No.	Size (μm)	No.	Size (μm)	No.	Size (μm)	No.	Size (μm)	No.	Size (μm)
Duodenal	20	800	18	600	16	350	14	100–200	—	100
			19	600	19	400	14	100–200	—	~200
			16	600	16	350	12	100–200		
Jejunal	20	800	16	600	15	300				
			18	600	14	350				

Table 2. *Capsule size and retention of methionine in undissolved capsule after 18 h suspension of capsules in nylon bags in the rumen of a fistulated Hereford steer, followed by successive in vitro incubation of recovered capsules in abomasal juice and either duodenal or jejunal digesta*

Original size (μm)	After suspensions in rumen*: Size (μm)	Methionine retained (%)	After abomasal incubation: Size (μm)	Methionine retained† (%)	After duodenal incubation: Size (μm)	Methionine retained† (%)	After jejunal incubation: Size (μm)	Methionine retained† (%)
800	650±50	69±4	550	52	250	18	200	15
500	400±50	72±5	300	57	200–100	11	200–100	9

* Mean values with their standard errors for four determinations.
† Mean of two analyses.

by treatment. On microscopic examination, the surface of the capsules was found to be smooth; no pitting could be detected. Leaching of the methionine from undissolved matrix would therefore not appear to be a significant process in the liberation of capsule charge.

The results of the in vivo study (Table 3) support the results of the in vitro incubations; 41 and 54% of the encapsulated methionine infused into the abomasum reached the mid-duodenum undissolved. Since the position of the re-entrant cannula in animals B and S was similar, the difference in the extent of solution may have been

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due to the greater digesta flow-rate in animal S (Neudoerffer *et al.* 1971). At the ileum 13% of the original encapsulated methionine was still associated with the particulate fraction, and undissolved capsules of 100–200 μm diameter were recovered. Capsules of similar size were recovered also from the faeces when the digesta were not removed at the re-entrant cannula after infusion.

No clear trend in the extent of solution was associated with a change in diet. Animals B and C were able to increase solution, whereas solution decreased in animal S as maize intake was increased.

Table 3. *Amounts of encapsulated methionine passing re-entrant cannula expressed as a percentage of that infused into the abomasum, in a steer (B) and two heifers (S and C) given a low-maize diet**

Animal	Location of cannula	Low-maize diet	High-maize diet
B	0·70 m from pylorus	41 ± 4†	43‡
S	0·70 m from pylorus	54‡	51‡
C	0·25 m from caecum	13 ± 3·5†	15‡

* Neudoerffer *et al.* (1971).
† Mean value with its standard error for three runs.
‡ Mean of two runs.

DISCUSSION

Treatment with formalin has been shown to improve the nutritional value of protein for the ruminant animal (Ferguson, Hemsley & Reis, 1967); also the hydroxy analogue of methionine has been shown to stimulate milk production by dairy cows (Griel, Patton, McCarthy & Chandler, 1968). In both experiments it was assumed that chemically modified nutrients escape hydrolysis in the rumen. Both methods are, however, specific for the protection of a single compound or group of compounds and are of limited value in protecting nutrients generally from degradation in the rumen. A chemically inert matrix, capable of carrying substances irrespective of their chemical nature, would be a desirable alternative for the protection of nutrients and drugs against ruminal breakdown.

On the basis of our results, 60–65% of orally administered methionine encapsulated in a kaolin–saturated-fat capsule will become available for post-ruminal absorption. The capsule is not totally resistant to ruminal breakdown (i.e. approx. 30% breakdown occurs) and the core of the capsule, containing approx. 10% of the encapsulated material, appears resistant to solution for reasons yet unknown. A 60% recovery only of encapsulated material may at this time make the kaolin–saturated-fat capsule economically unattractive, but further modifications may lead to a greater release of material in the small intestine.

The authors thank Mr R. Emslie for valued assistance with the animal experiments and Mrs R. Dunn for laboratory analyses. The financial support of the Ontario Department of Agriculture and Food is gratefully acknowledged.

https://doi.org/10.1079/BJN19710098 Published online by Cambridge University Press

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Printed in Great Britain

https://doi.org/10.1079/BJN19710098 Published online by Cambridge University Press