The use of a Thiry-Vella loop of jejunum to study the intestinal absorption of calcium and inorganic phosphate in the conscious pig

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1. Pigs, each surgically prepared with a Thiry-Vella jejunal loop were used to study the absorption of calcium and inorganic phosphate from the intestine.

2. The loops were perfused daily for 6–8 h with a nutrient solution and absorption from the perfusate was measured.

3. The technique employed minimized atrophy of the jejunal mucosa and enabled the measurement of hourly or daily changes in absorption rate of components of the luminal fluid.

4. No differences were observed when polyethylene glycol (PEG; molecular weight 4000), [^{14}C]PEG or ^{51}Cr-EDTA were used as markers of net water movement.

5. Increasing the concentration of Ca in the perfusate resulted in the demonstration of a two-component relationship between net absorption rate of Ca and intraluminal Ca concentration. An initial rapid absorption rate from 0 to 4 mM was found, then a slower rate from approximately 5 mM upwards which did not saturate at the highest concentration tested (25 mM).

6. Increasing the concentration of phosphate in the perfusion solution increased the net absorption of phosphate from that solution. Although a two-component system, similar to that for Ca, was not evident, net absorption of phosphate was not saturated at the highest concentration tested (50 mM).

7. The absorption of Ca was unaffected by the presence of phosphate in the solution but Ca (2·5 mM) enhanced the absorption of phosphate.

The intestinal absorption of calcium and inorganic phosphate can be studied by a wide variety of in vitro and in vivo techniques. The in vivo methods include the balance technique, injection into ligated loops of intestine and several methods using oral radio Ca. They all have their advantages and disadvantages. Measurement of net or unidirectional absorption from a defined length of the small intestine can be achieved by several methods. A method used mainly in larger animals is the perfusion of a chronically-isolated Thiry-Vella loop of intestine with or without re-entrant cannulas. The former is more acceptable in that digesta can flow through the loop when it is not being used for absorption studies so that the intestinal mucosa is maintained in a normal condition. However, this arrangement can be associated with practical difficulties caused by impaction of intestinal contents in the external cannula connexions. It also involves direct contact between the digesta and the experimental length of gut, except when actual absorption measurements are in progress. Thus, with this preparation, dietary changes and corresponding changes in composition of the digesta might induce directly alterations in the mucosal content of Ca-binding protein, as proposed by Freund & Bronner (1975), which might then lead to changes in the absorptive process for Ca independently from alterations in vitamin D metabolism. It was for these reasons that use has been made of a Thiry-Vella (Markowitz, Archibald & Downie, 1964) loop of jejunum to investigate the mechanism of absorption of Ca and inorganic phosphate in conscious, unstressed pigs.

EXPERIMENTAL

Surgical preparation of the Thiry-Vella loop

Young, immature Large-White pigs of either sex, weighing 18–25 kg, were used in the studies. The males were castrated as piglets and the females were left intact. They were transferred from the University Farm several days before surgery and were fed, twice daily, a commercial pig diet (Super Growers Pellets; Page, Tadcaster, W. Yorks.) containing approximately 12 g Ca and 6 g P/kg.

After an overnight fast the pigs were tranquillized with etorphine and acepromazine (Large Animal Immobilon; Reckitt and Colman, Ltd, Hull) (0·01 ml/kg) injected intramuscularly. The trachea was intubated and anaesthesia maintained by Halothane (ICI, Pharmaceuticals Division, Macclesfield, Cheshire) using a closed-circuit apparatus (Stephens Drawover Apparatus; Australian Anaesthetic Equipment Co. Ltd, Sydney, Australia). Under aseptic conditions a laparotomy was performed in the right flank and the length of intestine required for the Thiry-Vella loop was selected. The proximal end was usually 1·8 m distal from the pylorus and the total length 1·8 m (11–13% of the length of the small intestine). The intestine was resected at these points and continuity of the intestinal tract was restored by an end-to-end anastomosis (4/0 atraumatic Mersilk; Ethicon, Edinburgh). The free ends of the jejunal segment were brought to the surface through separate stab incisions on either side of the main incision and sutured to the skin as open stomata to facilitate catheterization. The abdominal incision was closed in layers using chromic catgut sutures (Ethicon), and the skin sutured with Mersilk. The animals were given a post-operative course of intramuscular injections of Streptopen (Glaxo, Greenford, Middx.) for 4 d and were kept in a warm pen. They were offered only glucose solution (50 g/l) for 2 d, after which the commercial pig diet was gradually re-introduced. At least 2 weeks were allowed for the animals to recover from the operation before measurements of absorption commenced.

Measurements of absorption from a Thiry-Vella loop

The pigs were housed in metabolic cages several days before absorption studies were started so that they could become adjusted to their new environment. A Folatex catheter (Thackray, Leeds, or Eschmann Bros. and Walsh Ltd, Shoreham-by-Sea, Sussex) was introduced into each stoma and was held in place by inflating the balloon with approximately 5 ml distilled water. The gauge of catheter used depended on the size of the stoma. The proximal catheter was weighted to prevent onward movement by the peristaltic contractions of the loop. Plate 1 shows the arrangement of the apparatus for perfusion of the loop. At the beginning of a series of perfusion experiments which usually extended over a 'working' day, the loop was washed out with warm (37°) perfusion solution to remove any debris which had accumulated overnight. Perfusion solution, at 37°, was then pumped from the reservoir into the loop at a rate of 3 ml/min by means of a peristaltic pump (Watson-Marlow Ltd, Falmouth, Cornwall), and the effluent solution was returned to the reservoir. After a minimum period of 1 h the pump was switched off and the solution remaining in the tubing and the loop was gently blown into the reservoir and discarded. The required volume of perfusion solution was then placed in the reservoir and pumped into the loop at a rate of approximately 6 ml/min until it appeared at the distal end and returned to the reservoir. The rate was then reduced to 3 ml/min and the time at which the effluent solution appeared was recorded. This flow-rate is in the normal range found in the upper small intestine of the pig (Ivan & Farrell, 1976). At the end of the required period of time (usually 30 min or 1 h) the pump was stopped and the solution in the tubing and the loop was gently blown out into the reservoir described previously. The effluent solution was thoroughly mixed, the volume noted, and a sample

Table 1. *Comparison of the composition (/l) of the ultrafiltrate of the intestinal contents of two pigs* and the fluid used for the perfusion of Thiry-Vella jejunal loops† in pigs*

	Pig 1	Pig 2	Perfusion fluid
Sodium (mmol)	72·9	101·3	120
Potassium (mmol)	21·5	22·1	25
Magnesium (mmol)	5·23	7·80	10
Calcium (mmol)	1·83	13·8	2·5 (lactate)
Chloride (mmol)	—	—	153·4
$H_2PO_4^-$ (mmol)	—	—	1·6
Glucose (mmol)	—	—	110
Osmolarity (mosmol)	415	468	445
pH	6·7	—	6·5
Markers:			
Polyethylene glycol‡ (g)			1
[^{14}C]Polyethylene glycol‡ (μCi)			0·5
[^{51}Cr]EDTA (μCi)			3

* Ultrafiltrate (20000 *g* at 4° for 16 h) of contents of segment of small intestine (pig 1, 1·5–3·4 m from pylorus; pig 2, 2·4–4·8 m from pylorus) taken 4 h after feeding 1 kg of a 6·6 g Ca, 6·3 g P/kg diet (pig 1, 60 kg) or 3 h after feeding 600 g of the same diet (pig 2, 30 kg).

† For details of procedures, see p. 433.

‡ Molecular weight 4000.

acidified with concentrated hydrochloric acid by adding 1 ml to each 50 ml sample. Five or six measurements of absorption were usually made each day. The samples, together with an acidified sample of the original perfusion solution, were kept at 4° until they were analysed.

Composition of perfusion solution

This was based on analyses of ultrafiltrates of the intestinal contents of two pigs (Table 1). It shows the concentrations of Na, K, Mg and Ca in the ultrafiltrates of the intestinal contents taken from the segment most closely approximating to the position of the Thiry-Vella loop (pig 1, 1·5–3·4 m; pig 2, 2·4–4·8 m from the pylorus). Sodium dihydrogen phosphate and calcium lactate were used to increase the concentrations of phosphate and of Ca in the perfusion solution and in each instance an equimolar quantity of sodium chloride was omitted to ensure constant initial osmolarity. Polyethylene glycol (PEG; molecular weight 4000) was included in the solution (1 g/l) as a non-absorbable marker of water movement, but this was subsequently replaced by ^{51}Cr-EDTA (The Radiochemical Centre, Amersham, Bucks.) or ^{14}C-labelled PEG (The Radiochemical Centre). A comparison was made between these three measurements of net water transport.

Analyses

The Ca and inorganic phosphate concentrations in the original solution and in the perfusate were measured using an AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants); for Ca, Technicon Instruments Co. Ltd (1965*a*) and for phosphate, Technicon Instruments Co. Ltd (1965*b*).

The Na, K, and Mg concentrations in the ultrafiltrates of intestinal contents were measured using a Pye Unicam SP90 atomic absorption spectrophotometer (Pye-Unicam Instruments Ltd, Cambridge).

PEG was measured by the turbidimetric method of Hydén (1955).

Osmolarity was measured by the depression of freezing point using an osmometer (Osmette; Precision Systems Inc., Newton, Massachusetts, USA).

A 1 ml sample of perfusion solution was added to 15 ml scintillation fluid (Bray, 1960) in

Table 2. *Reproducibility, over several days, of net absorption from a jejunal Thiry-Vella loop* in a pig*

(Mean values with their standard errors for six observations except day 3 when values are for three observations)

	Net absorption					
	Water (ml/h)		Calcium (mmol/h)		Phosphate (mmol/h)	
Day of experiment	Mean	SE	Mean	SE	Mean	SE
1	123	5	0·409	0·012	0·123	0·006
2	121	6	0·409	0·010	0·126	0·003
3	129	6	0·421	0·010	0·123	0·003
4	134	4	0·419	0·005	0·129	0·003
5	130	6	0·411	0·012	0·129	0·006

* For details of procedures, see pp. 432–434.

a plastic scintillation vial which minimizes adsorption of PEG to the surface (Crouthamel & Van Dyke, 1975) and the ^{14}C radioactivity content determined using an automated liquid-scintillation counter (Coru/matic 100; Tracerlab, Weybridge, Surrey).

The ^{51}Cr radioactivity content was determined using a sodium iodide crystal (Gamma-guard 150; Tracerlab).

Calculation of results

Net Ca or phosphate absorption rate (mmol/period of perfusion) was calculated from the expression $V_o[C_o-(P_o/P_x)C_x]$, where V_o (l) was the volume of the original perfusion solution, C_o and C_x were the Ca (or phosphate) concentrations (mmol/l) in the original and effluent solutions, respectively and P_o and P_x were the ^{51}Cr or [^{14}C]PEG activities or PEG concentration in the original and effluent solutions, respectively.

Statistical treatment of results

The statistical significance of differences between Ca or phosphate absorption values was calculated using Student's t test.

RESULTS

Reproducibility of absorption measurements

The net absorption of Ca and phosphate was measured five to six times daily and the results were compared to determine whether or not measurements of absorption were reproducible. Only when absorption values became reproducible on a day-to-day basis were experiments attempted. Table 2 shows that the net absorption of Ca, phosphate and water over a 'working' week (5 d) was reproducible. Fig. 1 shows that the net absorption of Ca and phosphate was reduced, not always significantly, when the Thiry-Vella loop was not perfused for 2 d. Pig 1: Ca $P<0·01$, phosphate $P<0·05$; pig 2: Ca not significant, phosphate $P<0·001$.

Effect of intraluminal Ca and phosphate concentration on net Ca and phosphate absorption

Fig. 2 shows the effect of increasing the concentration of Ca in the perfusate upon the net absorption of Ca and phosphate. As the intraluminal concentration of Ca increased the rate of increase of net Ca absorption was reduced. The change in the linear relationship occurred at 3–5 mM in six experiments. The slower rate was unaffected by Ca concentration in all six

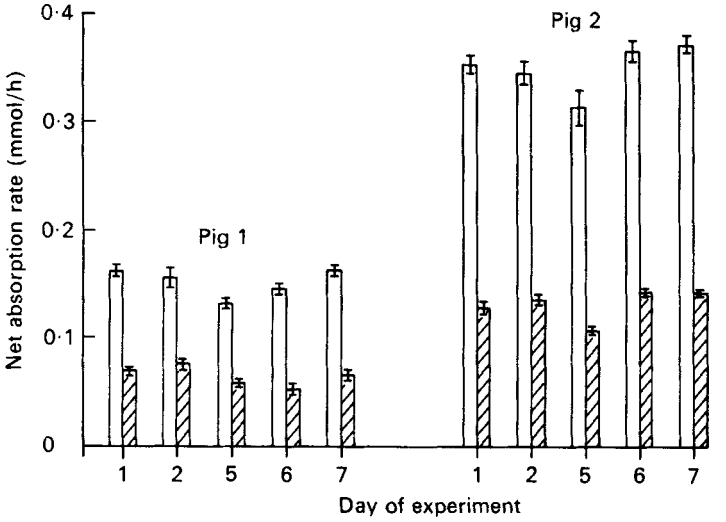

Fig. 1. The effect on net calcium (□) and phosphate (▨) absorption of failure to perfuse the Thiry-Vella loop of jejunum on days 3 and 4. Points represent mean values with their standard errors, represented by vertical bars, for five to six absorption measurements taken on each day. For details of procedures, see pp. 432–434.

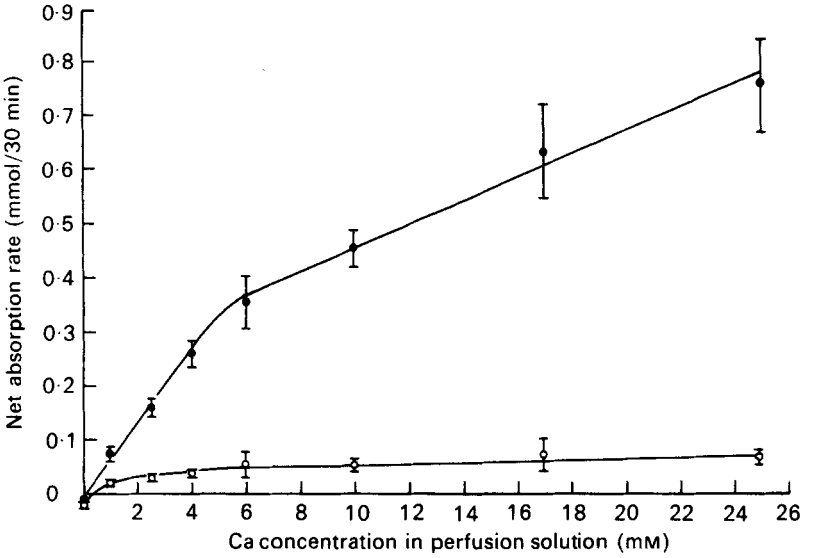

Fig. 2. The effect of increasing the concentration of calcium in the perfusion solution on the net absorption of Ca (●) and phosphate (○) (initial concentration 1·6 mM) from a Thiry-Vella loop of jejunum in a conscious pig. Points represent mean values with their standard errors, represented by vertical bars, for five 30 min perfusions at each concentration of Ca. For details of procedures, see pp. 432–434.

experiments, up to the maximum level studied (25 mM). The absorption of phosphate also increased significantly ($P < 0{\cdot}01$) when Ca was added to the solution.

The effect of increasing the concentration of phosphate in the perfusate is shown in Fig. 3. In four experiments (in one experiment, the solution did not contain Ca), increasing the concentration of phosphate in the solution caused an increase in net phosphate absorption.

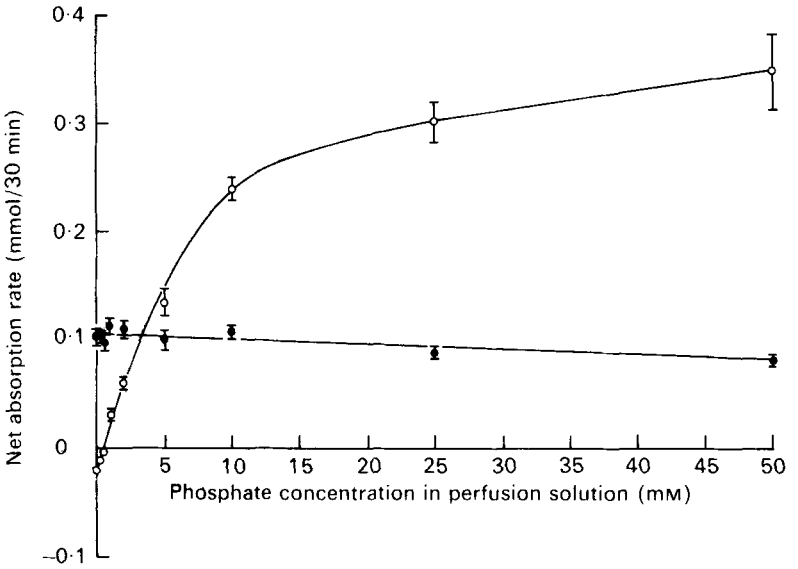

Fig. 3. The effect of increasing the concentration of phosphate in the perfusion solution on the net absorption of phosphate (○) and calcium (●) (initial concentration, 2·5 mM) from a Thiry-Vella loop of jejunum in a conscious pig. Points represent the mean values with their standard errors, represented by vertical bars, for net absorption for five 30 min perfusions at each concentration of phosphate. For details of procedures, see pp. 432–434.

Although the rate of increase in phosphate absorption became less as the phosphate concentration increased, the net absorption of phosphate was not saturated at the highest concentration tested (50 mM) in any experiment. In the three experiments in which 2·5 mM-Ca was included in the solution, the concentration of phosphate at which no net absorption or secretion of phosphate occurred was 0·5–0·6 mM, while in the experiment in which Ca was omitted from the solution no net absorption occurred below 1·3 mM-phosphate. That is, the presence of 2·5 mM-Ca in the perfusate appeared to increase net phosphate absorption. Except in one experiment (Fig. 3) during which the absorption of Ca was significantly ($P < 0.02$) reduced at high phosphate concentration (50 mM), the addition of phosphate to the perfusion solution had no consistent effect on the absorption of Ca, in contrast to the effect of Ca on phosphate absorption (Fig. 2).

Comparison between PEG 4000, [^{14}C]PEG and ^{51}Cr-EDTA as markers of net water movement

Table 3 shows the mean net water absorption rate measured during 2 weeks using three different markers of water absorption. There was excellent agreement between results obtained by the three methods.

DISCUSSION

The jejunum was chosen for study because it has been shown to be the major site of calcium absorption in the pig (Moore & Tyler, 1955). The Thiry-Vella loop technique for the study of Ca absorption has been used extensively in dogs by Cramer (1968) and has several important advantages over other methods of measuring Ca and phosphate absorption. It can be used in the conscious animal, it is more precise quantitatively, so long as water-tight junctions with the loop ends can be made, and it can be used to answer questions about mechanisms of absorption and the influence of various factors and pharmacological agents

Table 3. *A comparison between polyethylene glycol (PEG 4000)*, [^{14}C]PEG and ^{51}Cr-EDTA as markers of net water absorption from a jejunal Thiry-Vella loop† in a pig*

(Mean values with their standard errors; no. of observations in parentheses)

Day of experiment	Absorption (ml/h) PEG 4000 Mean	SE	[^{14}C]PEG Mean	SE	Day of experiment	Absorption (ml/h) PEG 4000 Mean	SE	^{51}Cr-EDTA Mean	SE
1 (5)	112	3	113	6	1 (6)	114	6	109	6
2 (6)	125	6	115	4	2 (6)	129	5	131	6
3 (6)	123	5	118	6	3 (11)	143	3	147	4
4 (5)	127	6	116	8	4 (5)	159	6	153	9
5 (6)	142	4	139	5	5 (6)	127	5	132	5
Over all (28)	126	3	121	3	Over all (34)	135	3	136	4

* Molecular weight 4000.

† For details of procedures, see pp. 432–434.

on absorption from this region. Hourly and daily variations in absorption can also be measured. Its main disadvantage is that disuse atrophy can occur as a result of the surgical isolation of the loop from the rest of the intestinal tract because the mucosal cells are supplied with nutrients not only from the blood but also from the luminal contents (Levin, 1969).

During the 2–3-week recovery period after surgery, some atrophy of the mucosa may have occurred from lack of exogenous nutrients and may account for the gradual increase in absorption noted during the first 2–4 weeks after the commencement of experimental perfusions. A reason for the lack of reproducibility in absorption seen with a freshly-prepared loop is associated leakage from the new stomata before healing was complete. In other pigs, the recovery period has been reduced to 7–10 d and this has been associated with higher initial levels of Ca and phosphate absorption.

Usually 6 weeks after the preparation of the Thiry-Vella loop, reproducibility of absorption measurements from day to day was excellent (Table 2). To ensure uniform absorption measurements from week to week, it was essential to recirculate the loop daily for 6–8 h. If this was done the preparation could be used for long periods (11 months). If the loop was not perfused for several days, absorption on the first and second days after the lapse was usually reduced although not always significantly (Fig. 1). Similarly, the first absorption rate measured in the morning of each day was generally lower than those obtained during the rest of the day; consequently, the loops were recirculated for at least an hour each morning before measurements of absorption began.

The composition of the perfusion solution was based on the analysis of the intestinal contents of two pigs. Glucose was included in the solution because it has been shown (Scott, 1965) that glucose is necessary in an artificial solution to maintain a normal potential difference between the mucosa and the blood. Glucose also induces a significant increase in villus height when compared with galactose or Ringer solution in jejunal blind loops in rats (Menge, Werner, Lorenz-Meyer & Riecken, 1975).

Increasing the concentration of Ca in the perfusion solution showed that the absorption of Ca has at least two components, a result also obtained by Wasserman & Kallfelz (1962) in chicks and by Walling & Rothman (1969) in rats. The first component saturates at approximately 5 mM-Ca, a result similar to that obtained by Wasserman & Kallfelz (1962). The process is considered to be primarily active because Ca is absorbed at this stage against a concentration gradient. The second component presumably represents what is primarily a

passive component and did not show any tendency to saturate up to the highest concentration tested (25 mM).

Since the secretion rate of Ca into the loop was constant and very small (0·01 mmol/30 min perfusion period) the Ca absorption rate from the loop fluid can be expressed (Papworth & Patrick, 1970) by the equation:

$$J = \frac{J_m[\text{Ca}]}{K_t + [\text{Ca}]} + P[\text{Ca}],$$

where J is the Ca absorption rate, [Ca] is the Ca concentration in the perfusion solution, and J_m, K_t and P are constants. J_m is the maximum value of J achieved by the saturable first component of the equation, K_t is the [Ca] which corresponds to $J_m/2$ and P is the permeability constant of the second component of the equation. With results from the present study, this equation becomes:

$$J = \frac{0{\cdot}246\ [\text{Ca}]}{[\text{Ca}] + 2{\cdot}42} + 0{\cdot}022\ [\text{Ca}].$$

The first component predominates at normal luminal Ca concentrations and has been shown by Papworth & Patrick (1970) to represent mainly influx across the luminal surface of the mucosal cells. They showed it to be reduced substantially by metabolic inhibitors in contrast to their effect on the second component, thus indicating the active nature of the first component. It is suggested that active transport of Ca across the intestinal mucosal cell is probably the rate-limiting step in the absorption of Ca from lumen to plasma and plays an important role in the adaptation in the efficiency of intestinal Ca absorption in response to a reduction in dietary Ca intake.

Increasing the concentration of phosphate in the perfusion solution yielded similar results to those of Wasserman & Taylor (1973) in chick ileum. However, no clear evidence of a two-component system, similar to that for Ca, was evident. The reduced rate of increase in phosphate absorption with increasing phosphate concentration indicates a saturation of some component in the absorption of phosphate from the jejunum. In contrast to our findings, Wasserman & Taylor (1973) were unable to demonstrate any effect of Ca on phosphate absorption in chicks. This may be due to the segment of intestine used in their study as Chen, Castillo, Korycka-Dahl & DeLuca (1974) showed that, in rats, Ca greatly enhanced phosphate absorption in the upper duodenum but had less effect in the jejunum. The addition of phosphate to the perfusion solution had no effect on Ca absorption except in one experiment when there was a significant reduction at high phosphate concentration which may have been caused by precipitation of calcium phosphate.

No differences were observed between measurements of water absorption using three non-absorbable markers of water movement, PEG, [^{14}C]PEG and ^{51}Cr-EDTA. This confirmed the findings of Wingate, Sandberg & Phillips (1972) who found that in human jejunum [^{14}C]PEG yielded similar estimates of water movement compared with stable PEG, and Downes & McDonald (1964) who showed similar results between PEG and ^{51}Cr-EDTA as water markers in the rumen of sheep. ^{51}Cr-EDTA was chosen as the usual marker because of its ease of measurement.

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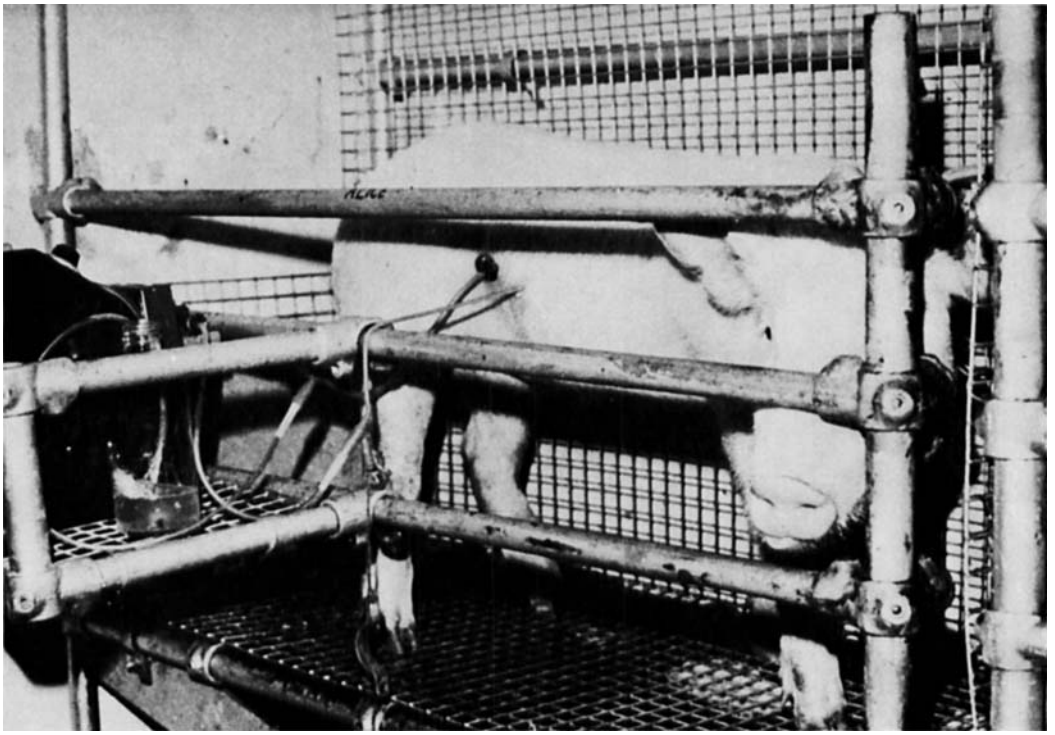

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EXPLANATION OF PLATE

Perfusion of a Thiry-Vella loop of jejunum in a conscious pig surgically prepared 2 months earlier (for details of procedures, see p. 432).

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