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Development of a chronic canine model for measurement of absorption by substrate appearance in portal venous blood

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Summary

Research in absorption physiology requires animal models which closely resemble the *in vivo* situation. The description of a new canine model satisfying these requirements is the objective of this report. Dogs were instrumented with indwelling portal vein and carotid artery catheters, a catheter jejunostomy and an electromagnetic flow measuring probe around the portal vein enabling continuous flow recordings. Following intrajejunal infusion of nutritive substrates in the conscious animal, absorption was measured as the product of porto-arterial substrate difference and portal venous flow. The model was validated in five mongrel dogs: (1) Catheters and flow measuring device function over several months. (2) The sensitivity of the method was evaluated following intrajejunal infusion of l-glycine-l-tyrosine and its constituent amino acids. A significant portoarterial concentration difference of both amino acids enabling quantitative measurement of absorption resulted when the peptide was infused at 4 mmoles/hour (20 mM solution, 200 ml/h). (3) Infusion of complete nutritive formulas caused a significant increase in portal venous flow whereas neither saline nor the amino acids or the peptides investigated had a comparable effect. (4) A validation experiment by implantation of a second flow probe distal to the chronically implanted device provided evidence that granulomatous tissue forming around the probe does not alter the accuracy of the flow recording. – In summary, this method permits for the first time quantitative measurement of absorption by appearance rates in portal venous blood instead of by disappearance from the intestinal lumen.

Zusammenfassung

Ein neues chronisches Hundemodell zur Resorptionsmessung am wachen Tier wird beschrieben. Ein portalvenöser sowie ein arterieller Katheter, eine Katheterjejunostomie und ein portalvenöser elektromagnetischer Strömungsaufnehmer werden implantiert. Während intrajejunaler Infusion kann damit die Resorption beliebiger Substrate beim nicht sedierten Tier als das Produkt von portoarterieller Konzentrationsdifferenz und portalvenösem Blutfluß bestimmt werden. Dieses Modell wurde bei fünf Tieren validisiert: 1. Katheter und Strömungsaufnehmer wurden derart modifiziert, daß sie über mehrere Monate funktionsfähig bleiben. 2.

Acknowledgements to Prof. Dr. Kummer, Frankfurt, for advice on physics, Dr. K. Langer, Erlangen, for peptide and amino acid analyses and Mr. H. D. Stölzer for modifications of the flow probes. Further acknowledgements are due to the companies of Pfrimmer-Viggo, Erlangen, and Nestlé, Vevey, for financial support without which this work could not have been undertaken.

Die Empfindlichkeit der Resorptionsmessung wurde nach intrajejunaler Applikation des Dipeptides l-Glycin-l-Tyrosin untersucht. Bei Infusion von 4 mmol/h (20 mM Lösung, 200 ml/h) konnte eine portoarterielle Konzentrationsdifferenz und damit Resorption gemessen werden. 3. Die Infusion einer nährstoffdefinierten Diät führte zu einem raschen Anstieg des portalvenösen Blutflusses, während weder Kochsalz noch die genannte Peptidlösung einen entsprechenden Effekt hatten. 4. Nach sechswöchiger Implantation eines Strömungsaufnehmers wurde ein zweiter Aufnehmer leberfern des ersten in einem zuvor unberührten Abschnitt der Portalvene implantiert. Trotz histologisch eindeutiger narbiger Verdickung der Gefäßwand unter dem chronischen Implantat gaben beide Aufnehmer gleiche Strömungssignale. Die beschriebene Methode gestattet daher erstmals quantitative Resorptionsmessungen durch Bestimmung von portalvenösen Erscheinensraten enteral infundierter Substrate.

Key words: absorption, portal venous flow, catheter jejunostomy, canine model

Introduction

Small bowel feeding via fine bore nasointestinal tubes or by surgical access such as catheter jejunostomy is widely applied for long-term nutritional support. Nevertheless, the adequate composition of a nutritive formula for small bowel feeding is not yet well defined. High molecular formulas consisting of complete protein, complex carbohydrates and long chain triglycerides are advocated by some groups (10), whereas others still propose use of the classical chemically defined formula composed of basic amino acids, molecular carbohydrates and a small portion of medium chain triglycerides for small bowel feeding (5). Both these concepts, however, are refuted by recent evidence of superior absorption of di- and tripeptides, oligosaccharides and medium chain triglycerides (2, 3, 7, 15). Therefore, methods testing absorption under conditions comparable to the clinical situation are required. Such models should provide the following features:

1. Chronic experiments enabling day to day comparison of absorption rates in the individual animal.
2. Substrate application resembling the clinical situation with respect to site and regime of nutrient delivery.
3. Absorption measurement reflecting total uptake of the gastrointestinal tract rather than being limited to a short intestinal segment.

These requisites are met by the recently developed animal model described in this report. The presentation of results will be limited to experimental examples supporting the validity of the model and the underlying assumptions.

Methods

Principle of absorption measurements

For this model, absorption was defined as portal venous appearance of enterally infused substrates, their cleavage components or their metabolic products. Portal venous and arterial substrate concentrations as well as portal venous blood flow were measured simultaneously. The product of portoarterial concentration differ-

ence and portal flow representing substrate appearance at a given time was calculated as a measure of absorption:

$$A = (C_p - C_a) * f$$

A = absorption (mg/min or mmol/min)
C_p, C_a = portal venous and arterial substrate concentration (mg/l or mmol/l)
f = portal venous flow (l/min).

Measurement of portal venous blood flow

An electromagnetic flow measuring technique enabling determination of zero-flow without occlusion of the portal vein (i.e. non-occlusive zero), was employed. Flow probes of differing diameters (8 to 12 mm) and the electronic measuring unit (Recomed Blutströmungsmesser) were obtained from Hellige (Freiburg, FRG). Flow was recorded either as the phasic or as the mean signal, the latter being the average output over 5 seconds.

Primary operative procedure

Antibiotic prophylaxis was instituted for 48 hours. An upper median laparotomy was performed under general anesthesia with volume-controlled ventilation in five mongrel dogs. The flow measuring probe, selected according to the diameter of the portal vein, was placed just distal to its bifurcation and calibrated as illustrated under results. The portal vein was then cannulated via a second order branch using a specially prepared polyurethane catheter: the intravascular ends of the catheters were constructed with an outer diameter of 1.8 mm, inner diameter being 1.1 mm. The remainder of the catheter was reinforced by sleeving a second polyurethane catheter over the first, the sleeve being bonded with a solvent. Resultant outer diameter was 2.6 mm. This construction was selected to provide stability and

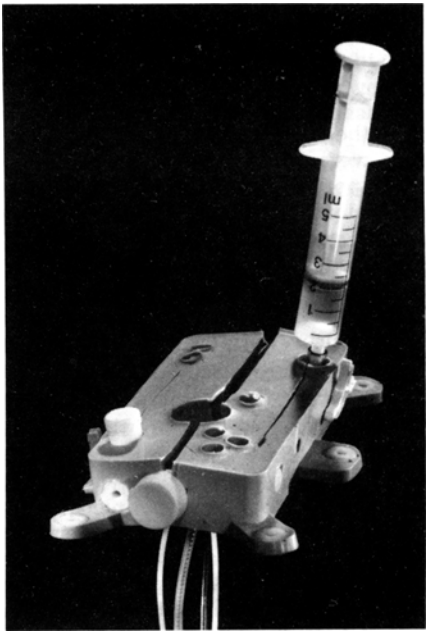

Fig. 1. Die-cast catheter port for housing the four measuring lines. The catheters can be flushed with minimal movements of the connectors.

prevent kinking of the long intra- and extraabdominal catheter. The abdominal procedure was completed by a needle catheter jejunostomy using a commercially available set (Jejunocath®, Pfrimmer & Co., Erlangen, FRG). As the next step, the carotid artery was cannulated via a cervical incision. All catheters and the flow probe cable were then tunnelled subcutaneously to a preoperatively marked point on the animal's neck. For tunnelling, connectors were topped with specially prepared blunt caps that minimize trauma, but give a safe grip with the tunnelling forceps. Topped with three-way taps, the catheters were filled with a heparin-dextran solution (5000 IU heparin/4 ml dextran 30%). All four connectors were fitted into a specially die-casted catheter port designed to keep the connectors in position and ease daily catheter flushing (Fig. 1). The catheter port was anchored by two non-absorbable sutures to the animal's skin and by four sutures to a leather harness. The harness developed is similar to that of a guide-dog, tailored to the individual animal and worn prior to the operation to accustom the dog to it.

Postoperative care and daily catheter management

Catheters were flushed every other day using a high viscosity 30% dextran-heparin solution (prepared by the hospital pharmacy) to prevent wash-out of heparin from the catheters. Daily, a portal venous flow recording was documented, the catheter port cleaned with a polyvinylpyrrolidon solution (Betadine®, Mundipharma) and the catheter exit site treated with the corresponding cream. Following the occurrence of portal venous thrombosis in two animals, we commenced anticoagulation using acetylsalicylic acid (4 mg/kg b.w.) on alternate days.

Leukocyte count, differential WBC count as well as measurement of serum-albumin, creatinine, haemoglobin and body weight were ascertained once per week. Experiments were performed providing the leukocyte count was below 20,000, albumin above 20 g/l, haemoglobin above 12 g%, and the animal consumed more than ⅔ of the daily ration and had normal stools (1). Unless these criteria were met, the dog was temporarily withdrawn from the experiment and treated accordingly.

Evaluation of the influence of nutritive substrates on portal venous flow

Prior to all experiments, the dogs were fasted for 18 hours. For the duration of the experiment, the animals were placed in a Pavlov harness. No sedatives were required. With continuous portal venous flow recording, the experiments started with an initial 30 min control period infusing isotonic saline at 200 ml/h. Thereafter, the test substrates were infused using different modes of application (e.g. continuous vs. bolus, different speeds). Those reported here are limited to infusion of saline, the peptide l-glycine-l-tyrosine, its constituent amino acids and two complete nutritive formulas. For the latter, a chemically defined diet (Peptisorb®) and a high molecular formula (Biosorb®, both products from Pfrimmer, Erlangen, FRG) were employed.

Absorption experiments

The control period (30 min, NaCl 0.9%, 200 ml/h) was followed by a 180 minutes test period (200 ml/h). Portal venous blood flow was recorded continuously, and both arterial and portal venous blood was sampled using heparinized syringes (Monovette®, Sarstedt, Nürnbrecht, FRG) at 30 min intervals. Blood loss per experiment totalled 80 ml, 5 ml each of arterial and portal venous blood being drawn at 8 points of measurement. As an example, absorption measurements following intrajejunal infusion of the dipeptide l-glycine-l-tyrosine (Bachem Feinchemikalien, Bubendorf, Switzerland) at both 2 mM and 20 mM concentrations, will be presented. As a first control, an amino acid solution containing l-glycine and l-tyrosine (Sigma, München, FRG) at concentrations of 2 mM each was used, this being the

maximal solubility of tyrosine. All substrates were adjusted to an osmolarity of 300 mosmol/kg by the addition of appropriate amounts of saline. A pH of 7.4 was maintained with 1 N NaOH. As a further control, these results were compared to those of continuous saline infusion to evaluate intestinal uptake and liberation of the respective amino acids in the postabsorptive state.

Determination of plasma amino acids and the dipeptide glycine-tyrosine

Protein-free plasma was obtained by precipitation using 5 % sulfosalicylic acid. The samples were frozen immediately at −70 °C and transported on dry ice for further processing to the Research Institute for Experimental Nutrition. Amino acids were analyzed according to Stein-Moore using an LC 5001 Biotronik automatic amino acid analyzer (Puchheim, FRG).

As for the analysis of the dipeptide, the free amino acids were removed by absorption on a copper loaded Bio-Rad column and the small peptides eluted with alcalic buffers. Following concentration and reconditioning, the samples were analyzed by the chromatography method referred to above.

Validation of long-term electromagnetic flow measurements

At relaparotomy, the position of the portal venous catheter was verified and the accuracy of the flow recording reevaluated. Non-occlusive and occlusive zero were compared, and a second flow probe implanted distal to the chronically implanted device for simultaneous flow recording. Thereafter, the animal was killed with sodium pentobarbital and the portal vein specimen removed for histological examination: the thickness of the vessel wall and its internal diameter at the site of the chronically and the acutely implanted flow probe were compared.

Calculations

Evaluation of appearance is restricted to the time of substrate infusion (3 hours) and does not include the following ebb phase, although absorption will clearly continue during this period. The experimental data obtained (portal venous flow, arterial and portal venous substrate concentration) were subjected to computer analysis. The program calculates portal venous appearance rates by multiplying portoarterial concentration differences with the corresponding portal venous flow at each point of measurement. The area under the curve of appearance rates (corrected for the appearance rate during the initial control period) is then assessed to give the total appearance during the test period (= time of substrate infusion). Finally, both actual and cumulative portal venous substrate appearance are plotted against time (examples in Figs. 8, 9 and 10).

Results

The development of this animal model is based on five dogs which participated in the experiments for an average of 9 weeks. In dogs 1 and 2 the experiments were terminated because the intracorporal part of the flow probe cable had broken (8 and 7 weeks respectively), dog 3 was withdrawn from the experiment due to portal venous thrombosis (14 weeks), and dog 4 died in consequence of sepsis due to infection of the subcutaneous tunnel (7 weeks). In the 5th dog breakage of one core of the flow probe cable also occurred, but was replaced using the improved version of the cable (see below). This dog has now been participating in the experiments for more than 7 weeks.

Examination of the *broken cables* revealed the critical breaking points to be the point of intra- to extra-abdominal exteriorization of the cable to

the subcutaneous tunnel, and at the exit site at the skin. Both are regions of stress due to continuous movement by respiration and daily manipulation at the port (flow recordings and cleaning). Furthermore, the metal cores within the cable were found to be under some external pressure because of the cable's heavy silicone insulation, further increasing the damage due to repeated bending. Therefore, the flow probe cable was modified, increasing the inner diameter of the silicone insulation and the silicone interlining between the cable's cores (outer diameter 4.5 vs. 3.1 mm).

Septic episodes were observed in all but the first dog. Blood cultures were always positive for pseudomonas and enterococci. The animals were treated accordingly (initial combination: cefoxitin and azlocillin, long-term therapy: trimethoprim- and sulfamethoxazol-containing syrup). Since all septic episodes occurred at an interval of more than two weeks postoperatively, handling of the catheters during daily catheter care seemed to be the most likely origin. Catheter care was therefore reduced and modified: on alternate days, the catheters were aspirated, flushed with saline and finally filled with a high viscous 30 % dextran solution supplemented with heparin (5000 U/ml) and Polybactrim® (polymyxin, bacitracin, neomycin). In addition, the catheter exit was treated using a highly viscous polyvinylpyrrolidon cream.

The use of silicone catheters was associated with total *portal venous thrombosis* in dog 3 and an incomplete thrombosis in dog 1. Polyurethane seemed to be less thrombogenic, as with these catheters only a thin epithelial cover was found (dogs 2 and 4) (Fig. 2). A platelet aggregation inhibiting drug was instituted as an additional thromboprophylactic measure.

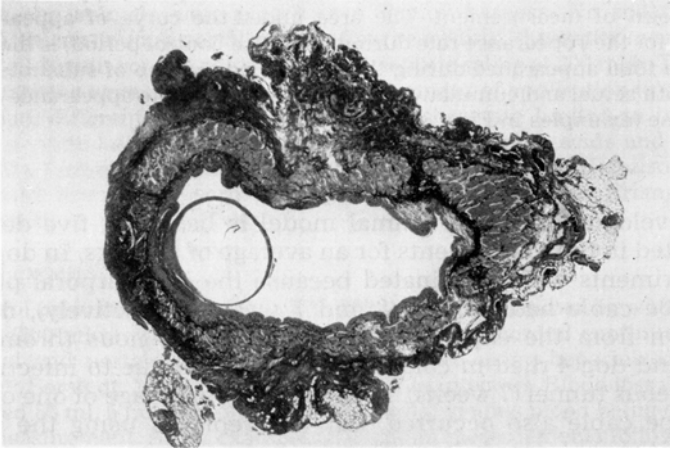

Fig. 2. Histology of portal vein specimen seven weeks after the primary operation. Following removal of the portal venous catheter, the epithelial cover remains. There is no evidence of an inflammatory reaction to the polyurethane material.

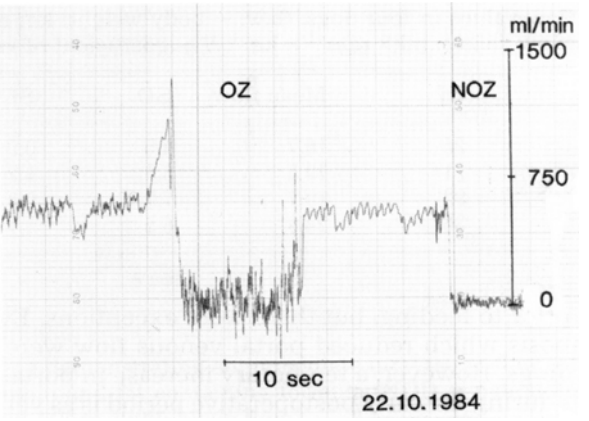

Fig. 3. Intraoperative flow recording documenting correspondence of non-occlusive zero (NOZ) and occlusive zero (OZ). (Recording from right to left. Phasic flow.)

Measurement of portal venous flow

Preoperatively, non-occlusive zero adjustment was confirmed in unstirred saline for each flow probe. Non-occlusive and occlusive zero coincided in the flow probes used for implantation.

Intraoperatively, confirmation that non-occlusive zero coincided with the indicated zero reading on manual occlusion of the portal vein (Fig. 3) and that injection of a saline bolus via the portal venous catheter elicited a corresponding flow increase (Fig. 4) was obtained.

In the postoperative period, the average portal venous flow ranged from 16.7 to 24.9 ml $\times$ min^{-1} $\times$ kg^{-1} (cf. Table 1). As a rule, portal venous flow

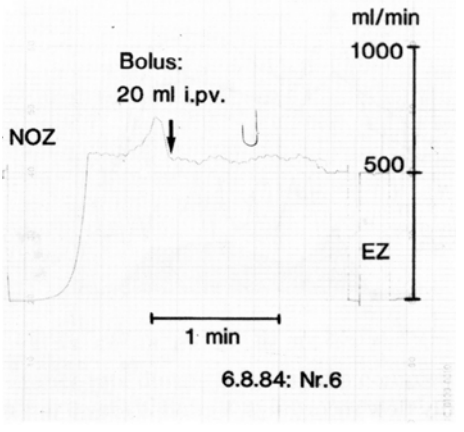

Fig. 4. Intraoperative flow recording documenting appropriate position of the portal venous catheter tip: injection of a 20 ml saline bolus is followed by a transient increase in portal venous flow. (Recording of mean flow.)

Table 1. Mean flow values in four dogs. (b.w. = body weight: kg; n = number or measurements; flow values: ml × min^{-1} × kg^{-1}; V = coefficient of variation).

Dog	Mean b.w.	n	Mean flow	SD	SEM	V
1	29	25	16.7	4.5	0.9	0.27
2	30	21	19.5	5.5	1.2	0.28
3	31	92	19.7	7.7	0.8	0.38
4	33	36	24.9	6.5	1.1	0.26

was recorded prior to feeding, but there were exceptions. Events such as venous thrombosis which reduced portal venous flow were reflected by the flow recordings. However, a temporary increase in portal venous flow seen in all dogs during the early postoperative period is as yet unexplained (cf. Fig. 5).

Both the type of *nutritive substrates* infused and the mode of application (bolus vs. continuous) were found to *affect* portal venous flow. Continuous infusion of a whole protein- and fat-containing formula (NDD, example: Figures 6 and 7) resulted in an immediate and substantial increase in flow. The same was found for bolus application of a peptide based, chemically defined diet (CDD) (Table 2). However, continuous infusion of the CDD, of amino acids (Figure 9), peptides or saline were not followed by substantial increases in flow (Table 2).

To date, the *final validation* of the electromagnetic flow measurement has been performed in one animal where a healthy state could not be restored due to sepsis. The relaparotomy was performed six weeks after the primary operation and a second flow probe placed on the portal vein at a site previously untouched (cf. Figure 10). No difference in portal venous

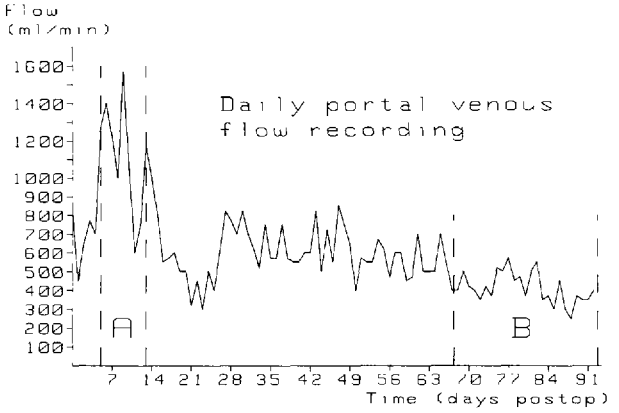

Fig. 5. Example of daily flow recordings. Period A (day 5 to 13) represents the early postoperative phase of high portal venous flow. Period B (day 68 to 92) depicts a steady decrease in flow giving rise to suspicion of portal venous thrombosis (verified at relaparotomy on day 100). On day 93 the experiment was terminated due to the broken flow probe cable.

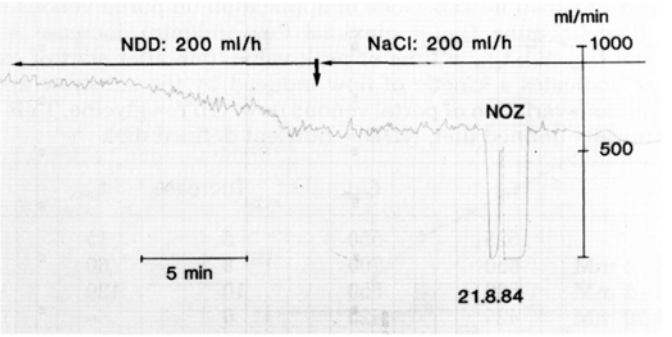

Fig. 6. Recording demonstrating an immediate increase in flow following infusion of a whole protein and fat containing formula (NDD = nährstoffdefinierte Diät). (Recording from left to right. Mean flow.)

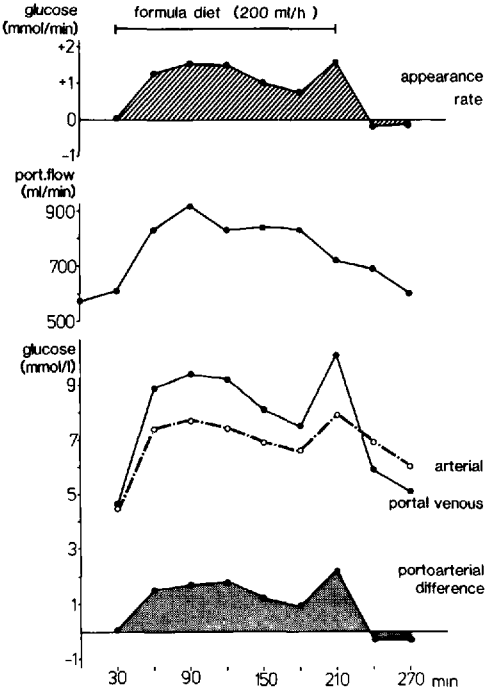

Fig. 7. Glucose appearance rate during infusion of a complete formula diet (NDD). a) Glucose appearance rate calculated from the product of portal venous flow and portoarterial concentration difference. The area under the graph represents glucose uptake during the period of investigation. b) Portal venous flow exhibiting an immediate increase after start of the enteral infusion. c) Arterial and portal venous glucose concentration as well as portoarterial concentration differences.

Table 2. Effect of substrate and mode of application on portal venous blood flow: f_i = initial flow (ml/min), f_{max} = maximal flow (ml/min), increase = difference between f_i and f_{max} (%), t_{max} = time of peak value (min after start of experiment), kinetic: "yes" indicates a kinetic of flow induced by the substrate applied, "no" indicates a random variation of portal venous flow. GLY = glycine, TYR = tyrosine, CDD = chemically defined diet, NDD = nutrient defined diet.

Dog 1	f_i	f_{max}	Increase	t_{max}	Kinetic
Saline	525	550	5	15	no
GLY+TYR 4 mM	650	700	8	60	no
GLY–TYR 2 mM	500	550	10	120	no
GLY–TYR 20 mM	425	425	0	–	no
CDD bolus	710	1000	41	50	yes
CDD continuous	600	750	25	45	yes
NDD continuous	570	920	62	90	yes
Oral food	400	550	38	120	yes

flow was detected between the first and the second flow probe, neither during unimpeded flow nor during occlusion of the vein (data not shown). Histology of the portal vein revealed a significant increase in wall thickness at the site of the chronically implanted flow probe narrowing the lumen by approximately 20 %.

Sensitivity of the model for absorption measurements

The following results were obtained on evaluation of the model's sensitivity:

Measuring the exchange of amino acids across the gastrointestinal tract in the postabsorptive state, no relevant exchange was seen for tyrosine, whereas to a small extent, glycine was released from the gastrointestinal tract to the portal vein. For alanine and glutamine, significant release and uptake respectively, was found (Table 3 and Fig. 8).

During infusion of glycine-tyrosine at a 20 mM concentration, there was a continuous portoarterial concentration difference reflecting uptake of glycine and tyrosine at an approximately 1:1 molar ratio, arterial concentrations attaining a plateau 120 min after onset of the infusion (Fig. 9).

During the 3 hour period of infusion (20 mM glycine-tyrosine, 200 ml/h) a total of 12 mmol glycine-tyrosine was infused. In this experiment, portal

Table 3. Example of amino acid exchange across the gastrointestinal tract in the postabsorptive state.

	Portal venous substrate appearance		
	mcmol/4 hrs.	mcmol/h	mcmol/h + kg b.w.
Glycine	3,641	910	30
Tyrosine	246	62	2
Alanine	9,840	2,460	82
Glutamine	−13,059	−3,265	−109

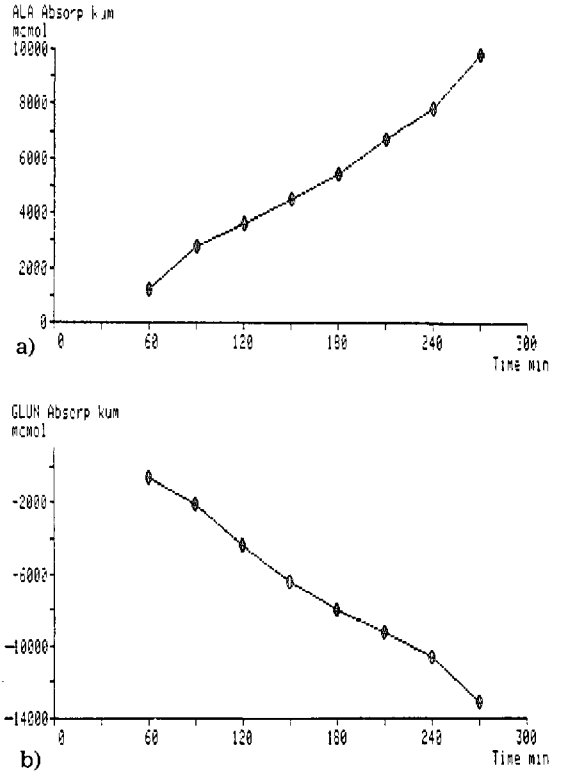

Fig. 8. Release of l-alanine (a) and uptake of l-glutamin (b) by the gastrointestinal tract during enteral infusion of saline. The cumulative appearance of both amino acids was computed. (Duration of experiment: 30 to 270 minutes.)

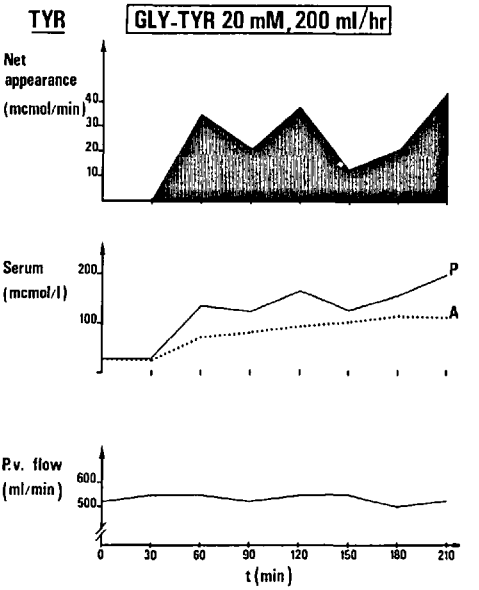

Fig. 9. Portal venous appearance following infusion of a 20 mM solution of glycine-tyrosine over 3 hours (0 to 30 min: saline control, 30 to 210 minutes: test period). Although portal venous flow remained constant (530 ± 19 ml/min) a portoarterial concentation difference developed denoting a net appearance of tyrosine and glycine (data not shown) in portal venous blood. Net appearance was calculated by subtracting the average appearance during the control period from each subsequent value of the test period. Total net appearance was obtained by evaluating the area under the graph of not appearance by computer (cf. text).

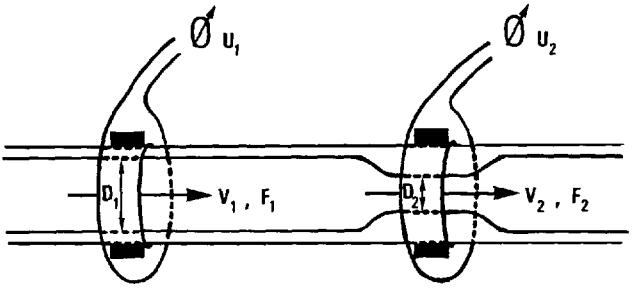

Fig. 10. Modification of blood flow measurements by changes in vessel wall thickness during chronic implantation of an electromagnetic flow measuring device. Change of voltage U (U_2 vs. U_1) consequent to a decrease in diameter D (D_2 vs. D_1) at constant values of flow ($F = F_1 = F_2$), magnetic field ($B = B_1 = B_2$), and density of the liquid (blood, $Z = Z_1 = Z_2$). Further abbreviations: R = D/2 = radius, V = velocity, A = $\pi \times R^2$ = cross section. Basic equations: (I) $U = B \times V \times 2\ R$, (II) $F = Z \times V \times A$. Substitution of V in (II) by means of (I) yields: $F = Z \times Pi \times R \times U/2B$. Since: $F_1 = F_2$. Hence: $U_1 \times R_1 = U_2 \times R_2$. Or: $U_2 = U_1 \times (D_1/D_2)$. The relation $U_2 = U_1 \times (D_1/D_2)$ may be modified by the following factors: (1) With reduction of the vessel's cross section and with increasing wall thickness, resistance between the electrodes goes down. Hence, the voltage may be *reduced* below the value given by the above equation. (2) If the magnetic field area is restricted to the inner part of the vessel (for example half the distance between the electrodes), the radius in equation (I) is smaller than in (II). Consequently U_2 might even *increase* with the reduction in cross section.

venous appearance amounted to 4.5 mmol tyrosine and 3.9 mmol glycine representing a net appearance of 38 % for tyrosine and 33 % for glycine for the three hour period of observation.

Following infusion of 2 mM glycine-tyrosine or its constituent amino acids, no portal venous appearance was detected (data not shown). Neither were significant amounts of the intact dipeptide glycine-tyrosine discovered under any of the above conditions in portal venous blood (data not shown).

Discussion

Enteral feeding is widely used in clinical practice today, although the mechanisms of absorption and the underlying regulatory processes are not yet completely understood. This lack of information is reflected by the arbitrary definition of formulas for enteral feeding. Further research in absorption physiology is therefore necessary using models which closely resemble the clinical situation.

A chronic canine model was therefore developed, for the first time enabling direct and quantitative absorption measurements in a conscious animal by determination of substrate appearance rates on the serosal side. The detailed description of this model including both the attendant advantages and hazards over comparative methods is the purpose of this report. Presentation of results was limited to what was deemed necessary for this purpose.

The method proposed precludes the attendant disadvantages of absorption measurements by determination of *disappearance* rates from defined intestinal segments. Occlusion by balloons may influence the intestinal motor function, increase the unstirred layer and thereby reduce the absorptive capacity of an intestinal segment (11). The same objections are valid against experimental techniques using perfusion of isolated intestinal segments such as the Thiry vella loop (9). Although these problems are exempted in non-occlusive techniques using intraluminal markers (e.g. PEG, 7), the use of any of these methods can determine the absorptive capacity for short intestinal segments only (e.g. 20 to 50 cm). Due to changes in absorption rates along the intestinal tract and some substrates exhibiting significant local specificity (13), the results obtained in a defined segment cannot be extrapolated to represent the total absorptive capacity of the intestinal tract. Monitoring the *appearance of substrates on the serosal side* is another means of measuring absorption. However, mere measurement of substrate concentrations in portal blood does not provide sufficient information, since portal venous flow varies from individual to individual (cf. Table 1) as well as from day to day (cf. Fig. 5), and may be greatly influenced by the nutritive substrates infused (cf. Table 2). Simultaneous recording of portal venous blood flow is therefore mandatory. From measurements of portal venous and arterial substrate concentrations as well as portal venous blood flow substrate appearance can be calculated at a given time.

As for the *principles of portal blood flow measurements,* direct and indirect methods are accepted: indicator dilution techniques (sulfobromophthalein, indocyanine green etc.) have frequently been used to determine total hepatic blood flow (6, 8). Although these methods can be modified to monitor portal venous flow by infusing the indicator into a mesenteric vein (8), this technique is not satisfactory for flow measurements in chronic dogs for the following reasons. (1) Indicator concentration is altered by an unknown quantity during its intestinal passage (absorption of water). Correction by simultaneous determination of hematocrit or hemoglobin concentrations in arterial and portal venous blood requires additional blood sampling. (2) Furthermore, an additional mesenteric venous catheter is necessary for indicator infusion which represents another potential source of septic and nonseptic (thrombosis, ileus) complications. (3) Indicator dilution techniques require repetitive blood sampling, and in chronic experiments blood loss should be minimized. (4) Finally, these methods do not enable continuous flow monitoring and therefore fail to detect the kinetics of flow changes following absorption. This appears to be of special relevance in view of the rapid changes in portal venous flow observed following intestinal infusion of complete nutritive formulas (cf. Fig. 6).

However, portal venous flow can be measured directly by flowmeters based on the Doppler or on the electromagnetic principle. Both methods can be applied in chronic experiments. Until recently, Doppler flowmeters had the major advantage of determination of the zero flow level without occlusion of the vessel (i.e. non-occlusive zero), but since the latest electromagnetic flowmeters also offer the non-occlusive zero adjustment, this latter method now appears the better: it does not require a large diameter

indwelling catheter, which might influence flow and act as a thrombogenic factor; it can detect reverse flow, and it is influenced to a lesser extent by variation of the flow profile (16, 17).

The principles of electromagnetic flow measurements including the non-occlusive zero adjustment, are at present generally accepted for acute experiments (17). However, to our knowledge this method has not been employed in *chronic* experiments: the few papers available giving results of such experiments neither validated the detected flow by adequate zero determinations, nor considered the influence of the "foreign body" reaction to the flow probe (11, 12).

The zero adjustment is no longer critical when using the latest generation of electromagnetic flow probes and amplifiers. However, reaction to the chronically implanted flow probe resulting in changes in vessel wall thickness remains a cause for concern during long-term flow measurements. Theoretical calculations cannot determine unequivocally the direction of alteration of flow due to thickening of the vessel wall (cf. Fig. 10). Therefore, the changes can be defined conclusively only by comparison of the flow recorded from the chronically implanted device with the signal obtained from a second acutely implanted probe at relaparotomy. This was undertaken in one animal, the flow recorded from the chronically implanted device being subsequently validated. This result, however, requires confirmation by repetition of the procedure in further animals.

Problems related to the *materials* used for the flow probe cable and for the vascular catheters could be overcome by careful selection and continuous refinement of the materials employed. In addition, an antithrombogenic prophylaxis appears indicated, preferably by the use of inhibitors of thrombocyte aggregation (salicylic acid).

Another concern is that of *septic complications*. Sepsis is an attendant hazard with the use of central vascular catheters, and is of particular significance when they cannot be replaced, as in this model. Asepsis during operation, vigilant attention to hygiene during daily catheter maintenance and experiments, immobilisation of the catheters in a port made to measure, an antiseptic supplement to the catheter contents and an antibiotic cream at the catheter exit site appear to be effective prophylactic measures against life-threatening sepsis. In addition, at the first signs of a septic complication, leucocyte count, blood cultures and an adequate antibiotic therapy is indicated.

Using such prophylactic and therapeutic measures, the animals can be kept in good health with all catheters and the flow-measuring device working over long periods of time.

In general, the data on *basal portal venous flow* presented here agree with those in the literature reporting electromagnetic portal venous flow in the fasted dog to be 13.9 ± 6.5 ml $\times$ kg^{-1} $\times$ min^{-1} (6) (cf. Table 1). Others, however, using an indicator dilution technique reported a portal venous blood flow of 37.5 ± 2.3 ml $\times$ min^{-1} $\times$ kg^{-1} in the conscious ($n = 3$) and of 29.1 ± 14 ml $\times$ min^{-1} $\times$ kg^{-1} in the anaesthesized dog (8). This discrepancy between the two methods is due to the necessary assumptions concerning absorption or dilution of the indicator during splenic passage, which renders the results of the two methods incomparable.

Following intestinal application of nutritive substrates, portal venous flow was found to be significantly influenced by both the type of nutrients infused and by the mode of application. Infusion of neither isotonic saline nor of the test peptide glycine-tyrosine or its constituent amino acids lead to an increase in portal venous flow. The relevant factors mediating the increase in flow following infusion of a complete nutritive formula have yet to be identified. The candidates include osmolarity, pH, protein, fat and carbohydrates. The model proposed appears suitable for such investigations.

Portal venous appearance of enterally infused substrates as a measure of absorption is closer to the in vivo situation than luminal disappearance rates. The latter are most valuable tools for the definition of substrate uptake along short intestinal segments (7, 9). However conclusions towards the total absorptive capacity of the intestinal tract should not be based on measurements of absorption in a short intestinal segment, because the affinity of the intestinal mucosa for different substrates varies along the gastrointestinal tract (13). In contrast, measurement of absorption by portal venous appearance includes uptake from upper and lower segments of the intestinal tract if steady state conditions are reached. Therefore, this method enables calculation of the total absorptive capacity and closely resembles the clinical situation with respect to both substrate application and measurement of nutrient uptake.

In addition, the nutrient components appearing on the portal venous side can be identified: following infusion of glycine-tyrosine no intact peptide was detected in portal venous blood, indicating the relevance of brush border and intracellular hydrolysis for the absorption of this peptide.

Compared to tracer techniques requiring frequent blood sampling for tracer determination, this model minimizes blood loss. More importantly, however, measurement of absorption by determination of portoarterial concentration differences is not restricted to the substances traced: portal venous and arterial aminograms as well as simultaneous determinations of carbohydrates and lipids can be performed. Whereas valuable information regarding uptake, distribution and metabolism of well-defined model substrates has emerged from tracer techniques (e.g. glucose, 1), the role of the proposed model will be the investigation of absorption from complex nutritive formulas. This reiterates the initial maxim, namely that the model should enable comparison of absorption from different nutritive formulas and thereby aid in developing criteria for the composition of complete formulas appropriate for small bowel feeding.

The most important prerequisite for such measurements is, however, the *sensitivity* of the model. From these pilot studies it can be concluded that the sensitivity in detecting absorption is comparable to measurements of disappearance rates. In this study, uptake could be detected when a 20 mM glycine-tyrosine solution was infused at 200 ml/h thereby delivering 4 mmol of the peptide per hour. No uptake was detected using a 2 mM solution at the same speed of infusion corresponding to a delivery of 0.4 mmol per hour. Infusion of a mixture of 12 dipeptides into a 20 cm segment of human intestine at 20 ml/min, resulted in a report of significant disappearance from the intestinal lumen for the 6 mM concentration,

whereas at 2 mM disappearance was minimal (15). This corresponds to the delivery of 7.2 and 2.4 mmol per hour to the small intestine and thus demonstrates the sensitivity of both methods to be in the same range. Moreover, the exchange of amino acids across the gastrointestinal tract during enteral infusion of saline also agreed with comparative literature data reporting release of alanine between 48 and 118 mcmol/h × kg and uptake of glutamine between 45 and 78 mcmol/h × kg (14). This is in corroboration of data obtained with the model described here.

In addition, this model provides a wide range of potential extensions which could be valuable in absorption physiology. The site of substrate application can be varied between gastric, duodenal and jejunal infusion, the immediate postoperative absorptive capacity may be compared with absorption during the later postoperative course, additional hepatic vein cannulation would permit measurement of transhepatic substrate flux, additional gastric and/or pancreatic fistula would enable investigation of feeding effects on these organs, and additional measurement of hormones would allow the identification of mediators between absorption and secondary effects on portal venous blood flow, and pancreatic and gastric secretion.

In summary, this model enables chronic experiments with substrate application resembling the clinical situation and measurement of absorption which reflects total uptake along the gastrointestinal tract. Although not without hazards, the problems associated appear to have been overcome and the results are reliable. The method seems especially appropriate for the evaluation of absorption from complex nutritive mixtures and will therefore help to define criteria for an optimal composition of enteral feeding formulas. Because it also offers a wide range of potential extensions, this animal model is of both clinical and physiological relevance.

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