INTESTINAL PERFUSION IN THE STUDY OF INTESTINAL ABSORPTION

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Abstract

Several techniques for studying absorption by means of intestinal perfusion have been developed. While the principle is simple, the practice is complicated by absorption of the solvent and by excretion of fluid into the lumen. To improve reliability a "marker" is incorporated into the system; it should behave as nearly as possible like the nutrient of interest, except that it should be unabsorbable. A great many markers, including several labelled with radionuclides, have been developed for use with numerous nutrients, and perfusion methods using double or triple tubes or occlusive balloons have been tested. The perfusion technique is too complicated for routine diagnostic use, but it offers at present the only possibility of studying the function of defined sections of the small intestine in the intact human.

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Techniques such as whole-body counting, faecal balance studies, or analysis of breath, blood, or urine may give a measure of an individual's over-all ability to absorb a given nutrient. However, none of these techniques can provide kinetic information, nor do they permit the study in man of the function of one part of the small intestine separate from the rest. The one exception is surgically produced experimental models, when resection of a part of the small intestine is necessitated by some disease process.

The study of the function of isolated segments of the intestine is possible in animals both in vivo and in vitro. One of the earliest perfusion experiments in vitro may be that of Carpenter[1], performed over a hundred years ago. In man, such experiments can be performed only occasionally when surgical resection is required for other purposes. The development of techniques for intestinal intubation [2,3] has made it possible to sample intestinal contents during digestion and absorption and to start applying to man much of the experimental work previously confined to animals.

The technique of intestinal perfusion is based on the following: If a tube I - E (Fig.1), L cm long, is infused at a constant rate, R ml/min, with a solution containing substance S at a concentration of C_{SI} units/ml, if the

solvent is not absorbed or added to, and if the concentration of S at E is C_{SE} units/ml, then the amount of S absorbed (S_{Ab}) will be given by the equation -

$$S_{Ab} = R\,(C_{SI} - C_{SE}) \text{ units/min}$$

$$= R\,(C_{SI} - C_{SE}) \times \frac{1}{L} \text{ units/min.cm} \quad \ldots\ldots\ldots (1)$$

It should be noted, however, that this latter equation is only an approximation representing the mean of the whole tube and taking no account of the diminishing concentration of the solute along the length of the tube.

In vivo, a situation resembling this can be achieved by infusing fluid into a segment of the intestine through the shorter limb of a double-lumen tube and aspirating through the longer limb (Fig.2). However, there may be absorption of solvent or excretion of fluid into the lumen, and since it is not possible to collect all the effluent, a marker has to be incorporated into the system.

The ideal marker must be nonabsorbable, not be adsorbed to intestinal wall or contents, not be broken down in the intestine, not interfere with the absorptive processes, not influence motility or contractility of the intestine, mix evenly with intestinal contents, have solubility characteristics similar to those of the test substance and be easily measurable.

Various substances have been employed, such as polyethylene glycol 4000 (PEG)[4-10], phenol red[5,10,11], bromsulphthalein[9,12], iodine-labelled Rose Bengal[12], inulin-carboxyl-^{14}C[11], polyvinylpyrrolidone (PVP)[13], $^{51}CrCl_3$[14], ^{51}Cr-ethylenediaminetetra-acetic acid (^{51}Cr-EDTA)[15], ^{60}Co-vitamin B_{12}[16] and ^{131}I-labelled PVP[17]. The most widely employed marker has been PEG, and a number of the above cited studies have validated the usefulness of this substance for perfusion studies. Most investigators have measured the PEG concentration by the turbimetric method[18]. Till and Downes[19] prepared tritium-labelled PEG by the exchange reaction followed by purification on a Sephadex G25 column. They used this in studies in sheep, obtaining practically identical results with tritium counting and turbimetric estimation. More recently, ^{14}C-labelled PEG has been demonstrated to be a suitable marker in rats [20,21]. Since the ^{3}H- and ^{14}C-labelled PEG can be measured over a much greater concentration range, the usefulness of these in human studies deserves investigation. Inulin-carboxyl-^{14}C has been shown to be a suitable marker for intestinal perfusion studies in rats [11-21], but does not appear to have been used in man. ^{131}I-labelled Rose Bengal was used in dogs by Maddrey et al[12] and

found to give satisfactory results, but it also has apparently not been used in human studies. $^{51}CrCl_3$ has been shown to be suitable as an indicator in studies of fluid in the human stomach[14], but is adsorbed by mucus and is therefore not suitable for intestinal perfusion studies[22]. ^{131}I-PVP has been shown to give results somewhat different from those with PEG, particularly in patients with sprue[17], due to binding of the ^{131}I-PVP to intestinal mucus. Labelled vitamin B_{12}[16] may be a useful marker for studies of the small intestine provided it is used in high concentrations (e.g., mg amounts), since even in the lower intestine only a small percentage will be absorbed. The different gamma-ray emitting isotopes of Co can provide a variety of easily measurable chemically identical markers.

The addition of a suitable marker to the perfusion fluid enables measurements to be made of the amount of solvent absorbed, or the amount of fluid secreted, by the segment under study. If the concentration of the marker in the fluid infused into the segment is C_{MI} and the concentration at the end of the segment is C_{ME}, then if $C_{MI} = C_{ME}$ there has been no net gain or loss of solvent and equation (1) holds. However, if C_{MI} is greater than C_{ME} there has been net absorption of solvent, and if C_{MI} is less than C_{ME} there has been net gain of fluid, i.e. secretion into the lumen. Equation (1) then becomes -

$$S_{Ab} = R \left(C_{SI} - C_{SE} \times \frac{C_{MI}}{C_{ME}}\right) \times \frac{1}{L} \text{ units/min.cm} \quad \ldots\ldots\ldots(2)$$

This forms the basis of calculations for perfusion studies performed with a double-lumen tube, where fluid is infused at R ml/min through I and withdrawn for analysis at E, L cm distal to I.

Such studies assume a number of conditions:

(1) that an ideal marker is available,

(2) that the marker concentration is uniform throughout the intestinal segment,

(3) that a steady state is achieved,

(4) that the rates at which the marker enters and leaves the segment are equal,

(5) that no fluid other than that infused enters the test segment from above, and

(6) that no reflux of the infused fluid occurs.

The uniformity of distribution of the marker in perfusion studies has usually been assumed, rather than proven. In the one published study in

rabbits[9], during perfusion with a ^{51}Cr marker a segment of duodenum was rapidly frozen and sections prepared for autoradiography. There was shown to be considerable variation in the cross sectional distribution of radioactivity. Whether the same applies in other sites and with other markers is not known.

A completely steady state is unlikely to be achieved because of uncontrollable variables such as intestinal contractions, variations in blood supply and variations in endogenous secretions. However, in order to achieve as near as possible a steady state an equilibration period is employed. Some observers use a 30 minute period, but extending this to 50 minutes has been shown to improve the reproducibility.

With a double-lumen perfusion tube no allowance can be made for endogenous secretions coming from above the test segment, nor can some degree of reflux proximal to the entry port be prevented. Some have tried to overcome this by a proximal occlusive balloon [23,24]. This technique has been shown to prevent contamination from above and to decrease significantly the variance in studies of the absorption of water and glucose in normal subjects[25].

Another method used to attempt to overcome the effects of the endogenous secretions is to employ a triple-lumen tube (Fig.3). The solution under study is infused into the intestine at a constant rate through the most proximal opening I. It then traverses a mixing segment and is sampled by aspiration at a constant rate A ml/min from the second opening (P), at which point the test segment begins. Finally the perfusate is again sampled at the opening of the third tube E, at the end of the test segment. The amount of perfusate (R_p) entering the test segment at point P will then be given by the formula -

$$R_p = R \times \frac{C_{MI}}{C_{MP}} - A$$

Equation (2) will then become

$$S_{Ab} = \left(R \times \frac{C_{MI}}{C_{MP}} - A\right)\left(C_{SP} - C_{SE} \times \frac{C_{MP}}{C_{ME}}\right) \times \frac{1}{L} \text{ units/min.cm} \quad \ldots(3)$$

Cooper et al.[7] studied the effectiveness of a 10 cm mixing segment by introducing a second marker proximal to the point of infusion and measuring the ratio of the two markers. If mixing were complete and a steady state achieved then the ratio should be constant. However, after a 30 minute period of equilibration they found a variation up to 36 % in any one 20 minute period, and if the second marker were administered as a bolus this rose to 46 %. They recommended therefore that studies should be carried on for at least three 20 minute periods and the results averaged. Whalen et al.[4] showed that a

significant reduction in the mixing error could be achieved by increasing the mixing segment from 10 to 15 cm. They also showed that errors could be further reduced by "staggering" aspirations in an attempt to study the same bolus. Therefore the sample from E was compared with the one 15 minutes earlier from P.

The triple-lumen technique appears to give more consistent results than the double-lumen one. There are, however, two disadvantages of the preliminary mixing segment, namely that there is no longer direct control over the composition of the fluid entering the test segment and that the solute under study may be largely absorbed in the preliminary mixing segment.

Sladen and Dawson[26], in a study of water and electrolyte absorption in man, compared the use of a double-lumen tube with that of an occlusive balloon and a triple-lumen tube, and obtained similar quantitative rates of sodium and water absorption in normal subjects with both systems. Since the double-tube system is simpler (and easier to swallow!) these authors prefer it to the triple-lumen tube, at least for studies in normal subjects - though not all would agree[27].

Rates of infusion used by different investigators have usually varied between 2 and 20 ml/min. At such rates the intestine is completely distended and to this extent the situation is unphysiological. However, at lower flow rates, which may be more physiological, the intestine will be completely distended[27] and flow will be much more variable, thus preventing the attainment of a steady state.

With this technique studies have been performed in normal subjects on the absorption of water[4,7,26,28,29], electrolytes[4,21,29,30], carbohydrates such as glucose[26,31], maltose and lactose[32], and amino acids and dipeptides[33,34].

The technique has also been used in the study of various pathological states in an attempt to define more closely abnormalities in intestinal function. This has been particularly useful in the study of the movement of water and electrolytes in various diarrhoeal states such as coeliac disease[35,36], tropical sprue[37], infantile diarrhoea[38] and cholera[39].

In conclusion, although intestinal perfusion is a laborious technique, both for the investigator and the patient, it clearly offers the only currently available method of studying the function of localised areas of the small intestine in the intact human. It may be expected to contribute much to our understanding of the physiology and pathology of intestinal absorption, but it is never likely to become a routine diagnostic procedure in clinical medicine.

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LEGENDS FOR FIGURES

Fig.1 Perfusion of a segment of a simple tube of length L.

Fig.2 Perfusion using a double-lumen tube: inlet I, exit E, perfusion length L.

Fig.3 Perfusion using a triple-lumen tube: inlet I, mixing segment I-P, sampling port P, exit E, perfusion length L.

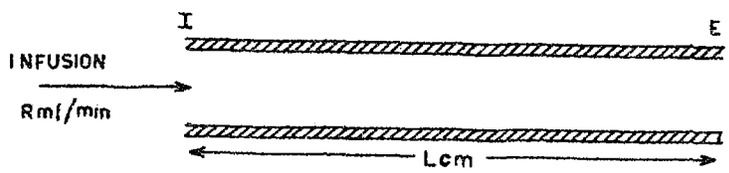

Fig. 1

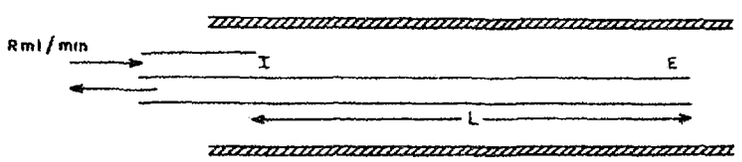

Fig. 2

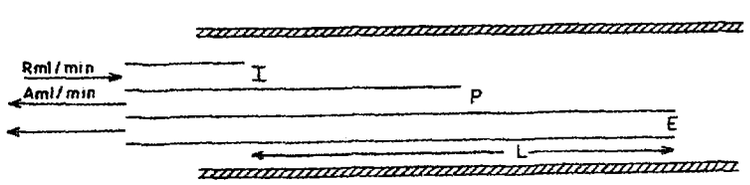

Fig. 3