Comparison of Phenol Red and Polyethyleneglycol as Nonabsorbable Markers for the Study of Intestinal Absorption in Humans

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THE USE of biologically inert marker compounds to study the functions of the human gastrointestinal tract is not new. Markers have been used as qualitative indicators for measuring the time of passage in the gastrointestinal tract for many years. More recently, markers have been used for the quantitative estimation of dilution,[1–3] transit,[4] and absorption[5, 6] of fed or infused meals or test solutions. Provided a suitable marker compound is used, quantitative estimation of volume change from the ratio of concentration of marker infused to concentration of marker recovered permits reasonably accurate calculation of absorption of a fed or infused nutrient without the necessity of complete recovery of intesinal contents. Limitations of these technics have been explored,[7–9] and it is clear that the validity of each individual technic must be established. Although the suitability of the marker may vary with each technic, certain general criteria must be met for all technics. A suitable marker compound must: (1) not be absorbed by the segment of gastrointestinal tract under study enough to modify results of the study; (2) be distributed homogeneously among the gastrointestinal contents at all points between introduction and recovery; (3) be physiologically inert with respect to the metabolic processes encountered in the study; (4) be nontoxic for the concentrations and total amounts used and (5) be easily and accurately measured in the fluids with which the study is concerned.

For perfusion studies of the absorption of nutrient from limited segments

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of human intestine, the two most commonly used markers have been phenol red (phenolsulfonphthalein, PSP) and polyethyleneglycol of molecular weight 4000 (PEG). Studies have shown that these compounds are poorly absorbed. However, although the amount of PSP absorbed by the human intestine is too small to invalidate perfusion studies of absorption from short segments of human intestine, enough is absorbed by the entire gastrointestinal tract to be easily demonstrable by measuring urinary excretion.[10] Studies of PEG absorption have shown essentially complete recovery from small intestinal contents of animals[11, 12] and man (97 ± 3%),[13] although stool recoveries usually are less than 90% of the administered amount. Although PEG 1000 and PEG 6000 are present in human urine after intravenous administration,[14] PEG 4000 is not found in urine in measurable quantities after oral administration.[15] Proof that PEG 4000 is not absorbed by the human intestine is not unequivocal, but it is clear that the amount of either PSP or PEG absorbed by a limited (15–100 cm.) segment of human small intestine is negligible in relation to the amounts used as markers.

Further validation of the use of an "unabsorbable" marker compound can be obtained by comparing the dilution or intestinal absorption indicated by each marker when two markers are used simultaneously. If each marker indicates the same proportional dilution, then (1) neither marker is absorbed to an extent great enough to modify results, or (2) each marker is absorbed or otherwise modified to exactly the same extent. When the molecular size and shape of the two marker compounds are quite different, the second possibility becomes unlikely. The present study compares apparent intestinal absorption as indicated by simultaneous use of PSP and PEG as marker compounds. It has been previously reported in abstract.[16] The current interest in this topic is indicated by similar results recently reported.[15]

MATERIALS AND METHODS

The comparisons reported in this paper were obtained during perfusion studies of absorptive capacity for dextrose in limited segments of human intestine. The studies were designed to demonstrate reproducibility of the perfusion technic and to compare results of discrete sampling with results of continuous monitoring of the effluent. Results, therefore, include more than one perfusion study for several of the patients. Included are 5 perfusions in 3 subjects with normal intestinal absorption and 3 perfusions in 2 patients with nontropical sprue. Intestinal perfusion studies were carried out using a double-lumen tube in the jejunum. The upper (infusion) opening was just beyond the ligament of Treitz and the lower (recovery) opening 15 cm. further down the jejunum. Solutions were infused at a rate of 13–20 ml./min. by means of a Harvard peristaltic pump. Infusion rate was constant for each study. Solutions infused contained dextrose concentrations ranging from 3 to 108 gm./L. in water or 3 to 54 gm./L. adjusted to 308 mosm./L. with Krebs

Henseleit solution. Each solution contained 0.03 mg./ml. of PSP and 10 mg./ml. of PEG. Results with the aqueous and saline perfusing solutions are reported separately. A total of 60 perfusion periods were studied. In each period, a single concentration of dextrose was infused for 20 min. of equilibration followed by collection of 3 samples of intestinal effluent. Sample collection time was 1.5–5 min., as required to obtain 5-ml. samples. Figures and statistical calculations are based on average absorption for each period derived by averaging apparent absorption calculated independently from each of 3 samples. Although intestinal absorptive capacity for dextrose varied greatly between normals and patients with malabsorption, preliminary computer analysis showed no evidence that the relationship between the two marker compounds differed between these groups; therefore, both groups were considered together.

Constant monitoring of the effluent by means of a four-channel Technicon Auto-Analyzer at the patient's bedside produced a continuous record of apparent absorption. Splitting of the effluent from the patient produced the discrete samples used in this analysis. Discrete samples were analyzed for PSP by Technicon Auto-Analyzer, diluting samples and standards with 0.1 M phosphate buffer pH 10.5, and reading at 560 mμ. for PSP and at 420 mμ. to correct for bile pigment (PSP = O.D. at 560 mμ.–0.065 × O.D. at 420 mμ.). It was unusual for the correction of bile pigment to exceed 0.01 O.D. units when dextrose was the nutrient. Details of factors affecting this method will be presented elsewhere.[17] PEG was analyzed turbidimetrically using the method of Hydén.[18] To obtain a colorimeter with a broad band of white light comparable to that used by Hydén, the grating was removed from a Coleman Junior spectrophotometer. A detailed discussion of limitations of analytic methods for PEG will be presented elsewhere.[19] The presence of PSP did not affect the turbidity of PEG solutions after addition of trichloracetic acid. Regression equations, computer calculated by the method of least squares, for PEG calibration curves at 10 min. in the presence of varying amounts of PSP are given in Table 1. The PSP concentration of 0.0024 mg. in the cuvet is equivalent to the effluent concentration of 0.03 mg. PSP/ml. which indicates no net water movement in our perfusion studies.

TABLE 1. REGRESSION EQUATIONS FOR PEG CALIBRATION CURVES IN THE PRESENCE OF PHENOL RED

Phenol red in cuvette (mg.)	*Equation**	*Correlation coefficient*
0.0000	$y = (.371 \pm .004)\,x + .043 \pm .014$	.995
0.0016	$y = (.452 \pm .013)\,x + .005 \pm .007$	.994
0.0024	$y = (.375 \pm .005)\,x + .047 \pm .013$	.995
0.0032	$y = (.373 \pm .008)\,x + .047 \pm .009$	.996
0.0048	$y = (.376 \pm .005)\,x + .052 \pm .013$	.995

*O.D. units are indicated by y; PEG concentration in milligrams per milliliter, by x.

RESULTS

For each period, average apparent absorption for both markers was derived by combining independent calculations of apparent absorption from 3 samples. Average apparent absorptions are shown in Fig. 1 and 2. Regression equations calculated from these data are presented in Table 2. Results were remarkably

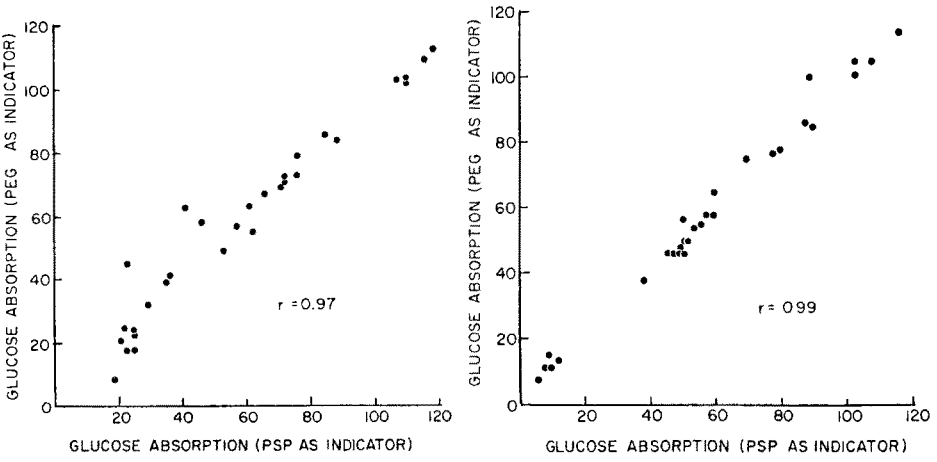

Fig. 1 and 2. Comparison of dilution indicators for measuring glucose absorption by perfusion technic (humans). **Fig. 1** (*left*). Nutrient, glucose in water. **Fig. 2** (*right*). Nutrient, glucose in saline.

similar whichever marker was used. Use of an average figure for each period, instead of individual figures for each sample, minimizes random analytical variability, but retains systematic variability such as that due to differences between the markers. No such difference was found. Results were less variable when the nutrient was dissolved in saline, but whether in water or in saline, both markers indicated essentially the same apparent dextrose absorption. In each case, the intercept of the regression line was indistinguishable from zero and its slope was indistinguishable from $y = x$.

TABLE 2. REGRESSION EQUATIONS TO COMPARE APPARENT ABSORPTIONS CALCULATED INDEPENDENTLY WITH THE TWO MARKERS

Samples	*Equation**	*Correlation coefficient*
Dextrose in water	$y = (.920 \pm 0.44)\,x + 5.29 \pm 7.68$	.972
Dextrose in saline	$y = (.970 \pm .015)\,x + 2.03 \pm 2.40$	.997
Monitor PSP composite	$y = (1.029 \pm .030)\,x + 0.90 \pm 6.85$	.975

*Apparent absorption in milligrams per minute per segment on the x axis as indicated by PSP and on the y axis as indicated by PEG.

DISCUSSION

This agreement between apparent absorption as indicated by two independent "nonabsorbable" markers supports the evidence that either phenol red or polyethyleneglycol 4000 is an appropriate reference compound. This evidence indicates that neither is absorbed to an extent that interferes with its use as a dilution indicator in perfusion studies of dextrose absorption in limited segments of human jejunum. The present results are similar to comparisons of PEG and PSP in humans obtained by others[15, 20] and in contrast to comparisons of PEG and BSP in rabbit and dog.[7] The latter dye probably is absorbed to a greater extent and more variably than is PSP. Before applying the results of these comparisons, obtained under the specific conditions of intestinal perfusion with water-soluble nutrients, to other methods of studying intestinal function, one must consider possible limitations. PSP is unquestionably absorbed from both normal and abnormal intestine in small amounts—about 1% of an oral dose per hour from the entire gastrointestinal tract.[10] This is too small to detect in our calculations as a systematic variation within the random physiological and analytical variations present in our results. The "general tendency for slightly higher values for P.R.C. than for P.E.G." (where P.R.C. is ratio of PSP-in/PSP-out with corrections for background) noted by Schedl[15] was only 1% higher and well below the level of statistical significance. Our use of averages of 3 samples collected during infusion of each glucose concentration decreases the random biological variation present in his results, while it retains any systematic variation which might be due to unequal absorption of markers. It is reasonable to conclude that in normal subjects and in patients with intestinal malabsorption, absorption of PEG and PSP by the intestine does not interfere with their use as markers. Although absorption of PSP from the stomach does occur when contents are acid[21] and may be as high as 40% of PSP infused in a patient with Zollinger-Ellison syndrome,[22] total absorption from both stomach and intestine is usually less than 1%/hr.[10] Absorption, including absorption of PSP from the small intestine, may be modified by the presence of butyric acid[23] or EDTA.[24] The effect of such substances on the absorption of other marker compounds has not been reported. Separation of various components of intestinal contents invalidates the use of nonabsorbable markers unless marker and the nutrient act similarly. Casein and other proteins bind PSP[25] but not PEG.[7] This as well as PSP absorption from the stomach produced the dissociation between the markers PEG and PSP found by Wiggins and Dawson.[26] Other instances of marker dissociation have been noted when an insoluble marker such as Cr_2O_3 was compared with water soluble PEG; however, they may remain together and give good correlation of fecal recoveries of the two markers.[27, 28] The excellent correlation shown in this study indicates that, despite the dissimilarity in molecular structure of PSP and PEG, these two markers did not dissociate in the presence of water soluble nutrient with and without water soluble salts.

SUMMARY

When phenol red and polyethyleneglycol were used simultaneously as nonabsorbable markers in perfusion studies of the absorptive capacity of high jejunum in humans, apparent absorption was the same when calculated from either marker. This similar indication of dilution and of absorption by the two markers was found in normal subjects and in patients with nontropical sprue, whether aqueous or saline solutions of dextrose were infused. The similarity strengthens the evidence that either phenol red or polyethyleneglycol is a satisfactory "nonabsorbable" marker compound to indicate dilution in perfusion studies of dextrose and electrolyte absorption in limited segments of human intestine.

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