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Note

Estimation of polyethyleneglycols in human urine for studies of intestinal absorption

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In studies of human intestinal permeability, it has been customary to administer orally some probe molecule and to measure the amount of probe which reaches the bloodstream. In many cases, the probe molecules are rapidly excreted in the urine and an assessment of urinary excretion provides the advantages of non-invasive investigation. Substances used have included mannitol and cellobiose [1], lactulose and L-rhamnose [2] and polyethyleneglycols [3—5], and in all cases the molecular weights involved have not exceeded 600. Since it was desired to extend the study to include larger molecules (e.g. MW 4000), with polyethyleneglycol as the probe material of choice, it was necessary to devise a method for estimation in this wider range of molecular weights.

With polyethyleneglycols (PEG) of molecular weights of 400—500 or less, the use of gas chromatography has proved entirely acceptable [4, 5] but this method cannot readily be used with higher polymers. We have devised a method of detection and measurement of various molecular-sized PEG fractions in urine which combines separation by gel chromatography [6, 7] with differential measurement of refractive index. PEG 600 and PEG 4000 were combined in a single oral dose and the urinary excretion over 24 h was measured. It was found that the degree of absorption of PEG 4000 in healthy subjects did not exceed 2.5% of the amount ingested.

EXPERIMENTAL

Materials and equipment

Bio-Gels P-4, P-6 and P-10, all 200—400 mesh, were obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.), Polyethyleneglycols (PEG) with mean

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molecular weights (MW) 200, 300, 400 and 1540 were from Koch-Light Labs. (Colnbrook, Great Britain). Ethyleneglycol and PEG of mean MW 600, 1000, 4000 and 6000 were from BDH Chemicals (Poole, Great Britain) as were sodium azide and the non-ionic detergent Brij 35. Chromatography columns from Wright Scientific (Stonehouse, Great Britain) were used, with valves (SRV4) from Pharmacia (Uppsala, Sweden). The differential refractometer (Model 1109) and Milton-Roy pump were from Laboratory Data Control (Stone, Great Britain). An LKB (Bromma, Sweden) strip-chart recorder (Model 2210) was used. Urine for analysis was collected directly into polythene bottles containing 10 ml 25% (v/v) hydrochloric acid as preservative, and after measurement of the volume a filtered aliquot was stored at 2°C if not handled at once.

Analytical method

Two jacketed columns were used, the first (178 × 16 mm) of Bio-Gel P-4, and the second (250 × 16 mm) of Bio-Gel P-10. They were connected by two 4-port valves so that they could operate in series or with elution only through the second column while the first was back-washed under gravity from the magnetically stirred reservoir of eluent, which was water containing Brij 35 (60 mg/l) and sodium azide (20 mg/l), in a thermostat at 45°C. Water from the thermostat was circulated through the column jackets. The load-loop, also constructed from two valves as above, was of volume approximately 1.50 ml. Urine, warmed and shaken at 45°C to reduce dissolved air, was loaded with the columns in series and elution proceeded at approximately 0.7 ml/min until all PEG had passed out of the first column (25 ml). The elution was then continued in the second column while urea, etc. were back-washed off the first (30 ml), after which series-flow was resumed for a following load. Eluate from the system passed through the refractive index monitor to a 5-ml syphon delivering to waste. Each operation of the syphon was shown by an event-mark on the strip-chart of the recorder registering the output of the refractometer. Connected at the output of the pump by a stainless-steel capillary T-joint was a vertical (50 cm × 10 mm I.D.) tube of stainless steel filled with air, having at its upper end a pressure gauge; pulses were thus damped and pressures were indicated. Working pressures did not exceed 1 kg/cm^2.

Quantitation

Peaks on elution charts were reduced to equivalent triangles either by simply drawing in tangents after insertion of the baseline, or by constructing chords (a mm) at $c = 0.75\ h$ and (b mm) at $0.5\ c$ for peaks of height h mm, giving the area $A = c\ (b - \frac{a}{2})^2/(b - a)$. Areas found ($A$ mm^2) were corrected to standard chart-speed (1 mm/min). Flow-rates (F ml/min) were evaluated from the syphon volume and event-marks on the chart, and the product AF was taken as estimator of the concentration of PEG in the load applied. Values of AF corresponding to 1 mg/ml PEG in the original loads were determined by passing through the process a standard solution, usually 1.5 mg/ml PEG 600 with 0.75 mg/ml PEG 4000 dissolved in elution liquid or urine.

RESULTS

Analytical method

Typical elution patterns given by direct chromatography of urine are shown in Fig. 1. Results of recovery trials are shown in Table I. There was a linear relationship between the function AF (above) and the mass of PEG applied to the analytical system in a constant volume, and this extended well beyond the range 0—1500 μg/ml of either polymer [r = 0.999, 7DF (degrees of freedom)], whereas in analysis of urine samples the loads should not exceed 1000 μg of PEG 600 or 250 μg of PEG 4000.

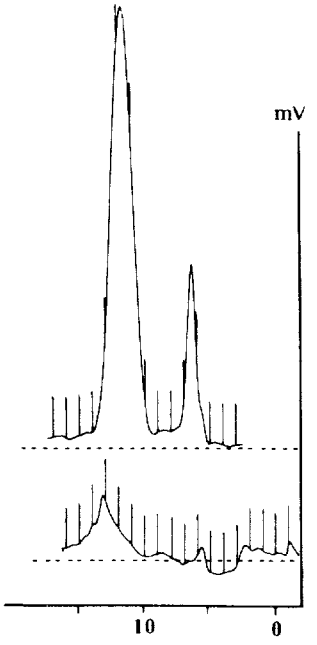

Fig. 1. Elution pattern for blank urine and urine with added PEG 600 (1500 μg/ml, tallest peak) together with PEG 4000 (300 μg/ml). Refractive index monitor attenuated × 50; 10 mV full scale. Recorder zero shown as dotted lines. Fractions were each 5.06 ml.

TABLE I

RECOVERIES FROM POSTPRANDIAL URINE OF ADDED POLYETHYLENEGLYCOLS (PEG, μg/ml)

Added		Found	
PEG 600	PEG 4000	PEG 600	PEG 4000
150	30.0	190	30.0
250	50.0	208	39.3
500	100	444	87.1
1000	200	1024	197
1500	300	1505	301
0	503	0	524
0	1005	0	1030
0	1508	0	1484

The 24-h urinary excretion of healthy adult subjects was estimated in 27 cases after an oral load containing 2.5 g PEG 600, and was found as the 95% confidence interval to be 18.7—66.3% of the load. Because of the difficulty in detecting very low levels of PEG 4000, 5 g of the higher polymer were incorporated in the solution ingested and 24-h excretions of 0.39—2.67% were obtained [8] as determining the 95% confidence interval.

DISCUSSION

In routine analysis, speed of operation and time taken over manipulations are important factors, and thus enforce a compromise between rapidity and the excellence which itself indicates the use of long columns and slow flow-rates. In the present work, the fastest flow-rates consistent with stable and low back-pressures were chosen and the columns were of minimum heights. In the preliminary treatment there is a positive exclusion of urinary solutes of lower molecular weights, but since PEG 4000 elutes near the void volume of Bio-Gel P-10, a proportion of larger molecules, e.g. proteins, may appear and in some cases a small peak, imperfectly resolved from that given by PEG 4000 on the final chromatogram, may be seen. However, this appeared not to introduce appreciable error and can be corrected for by processing a blank sample of urine voided before ingestion of the probe. For this reason, more recently introduced filtration media [9], including those capable of operating under high pressures, are being investigated. The method presented here has been used to study intestinal absorption in abnormal subjects; the results will be described elsewhere.

In preliminary work, a buffer solution (0.05 *M* phosphate, pH 7.3 with 0.1 *M* sodium chloride) was used as column eluent, but appeared to offer no advantage over the solution finally adopted. The use of a 24-h collection period was specified because excretion of PEG was always observed after the first 12 h had passed but did not continue significantly beyond 24 h after ingestion of the load. The use of increased temperature for chromatography reflects an attempt to secure maximal flow-rate without exceeding an arbitrarily set pressure in the system.

Recoveries of PEG added to normal urine to give concentrations similar to those anticipated to be found in disease were as shown in Table I. From the regression equations between prepared (x) and estimated (y) concentrations,

$y = 1.005x - 5.874$ ($r = 0.998$) for PEG 600,
$y = 0.999x - 0.224$ ($r = 0.999$) for PEG 4000,

it is seen that mean recoveries were essentially quantitative. Since the standard errors of the regression coefficients are small, variability of replicates is ascribed almost entirely to variance about the regression. This variance is such that 95% of replicates would be expected to exhibit random errors for PEG 6000 of less than 80 μg/ml, or $\pm < 10\%$ for a typical specimen. Similarly, 95% of results for PEG 4000 should be in error by $\pm < 12$ μg/ml, or $\pm < 30\%$ for normal excretions, but most estimates will of course exhibit appreciably smaller errors for both polymers.

More desirably, an internal standard such as PEG 1540 might be employed, but would require improved resolution in chromatography with subsequent increase in the time required for each estimation. If an extraction of PEG with organic solvent could be incorporated without undue prolongation of analysis, PEG 400 could be used as internal standard, as found in preliminary trials using chloroform—methanol (2:1, v/v) as extractant. It should be feasible to convert the present method into an automatic system controlled by signals from the syphon, provided piston valves were incorporated in place of those described.

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