Gut, 1970, **11**, 268-270

Modification of polyethylene glycol estimation suitable for use with small mammals

J. M. BOULTER AND H. B. McMICHAEL

From the Institute of Clinical Research, Middlesex Hospital, London, W1

SUMMARY Concentration of polyethylene glycol (PEG) has been measured satisfactorily using only 0·25 ml of intestinal aspirate (containing approximately 2·5 mg/l PEG). Hyden's turbidimetric assay was modified by reading turbidity at 420 mμ. The addition of extra protein was found to facilitate filtration after protein precipitation.

Polyethylene glycol remains the first choice of unabsorbed water-soluble marker substances for use in intestinal perfusion studies (Schedl, 1966). Its gravimetric and colorimetric assays (Shaffer and Critchfield, 1947; Oliver and Preston, 1949) are tedious, and so current assays are based on the turbidimetric method of Hyden (1956). Minor modifications have been made by Corbett, Greenhalgh, Gwynn, and Walker (1958), Smith (1959), and Ishikawa (1966). In 1967 Malawer and Powell introduced the addition of gum arabic to stabilize the resultant emulsion, eliminating the necessity for precise timing in reading the turbidity.

Two problems were encountered using PEG in small mammals such as rats (Dawson and McMichael, 1968). First, using the usual perfusate PEG concentration (2·5 mg/ml) and the standard assay, at least 1 ml of aspirate is necessary for each single assay, whereas the total sample available may be less than 2 millilitres. We therefore modified the volumes used, together with the wavelength at which the turbidity is read. Secondly we found that there was insufficient protein to produce a clear solution after 'protein precipitation'.

Materials

The following solutions were used: barium chloride ($BaCl_2.2H_2O$) AR 10% w/v, zinc sulphate ($ZnSO_4.7H_2O$) AR 5% w/v, barium hydroxide ($Ba(OH)_2.8H_2O$) AR 0·3N, and PEG 4000.[1] For the gum arabic-trichloro-acetic acid (TCA) solution, 30% w/v TCA ($CCl_4.COOH$) in 5% w/v $BaCl_2$ was mixed, in a ratio of 4 parts to 1 part, with 36 mg/l gum arabic.[2] The protein solution was about 50 mg protein per litre of distilled water; and to 1 litre of distilled water was added 0·5 ml stored human plasma.

The barium chloride, zinc sulphate, and gum arabic solutions were all made up by simple addition. The barium hydroxide solution was made by dissolving $BA(OH)_2$ in distilled water rendered free of carbon dioxide, and the strength of the solution was adjusted by titration against standard hydrogen chloride. The TCA solution was also made by simple solution and was then filtered through Whatman no. 1 paper. The TCA and the gum arabic solutions have always been mixed on the day of use.

Method

Intestinal aspirate (0·25 ml) containing approximately 2·5 mg PEG/ml was pipetted, using an E-MIL micro-constriction pipette, into 10 ml of the protein solution. Then 1 ml $BaCl_2$, 2 ml $Ba(OH)_2$, and 2 ml $ZnSO_4$ were added, in that order, by automatic pipette,[3] and the final

[1]Carbowax, laboratory reagent, British Drug Houses, Poole, England.
[2]Hopkins and Williams, Chadwell Heath, Essex.
[3]Zippette, Jencons Ltd, Hemel Hempstead.

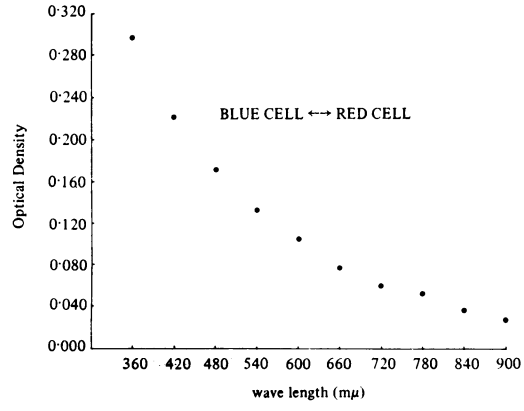

Fig. 1 *Optical density of turbidity read at different wavelengths.*

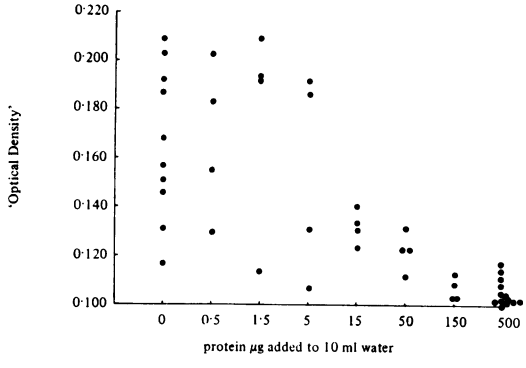

Fig. 2 *Effect of adding protein to water before 'precipitation' with $Ba(OH)_2$ and $ZnSO_4$. A low optical density indicates a clear filtrate.*

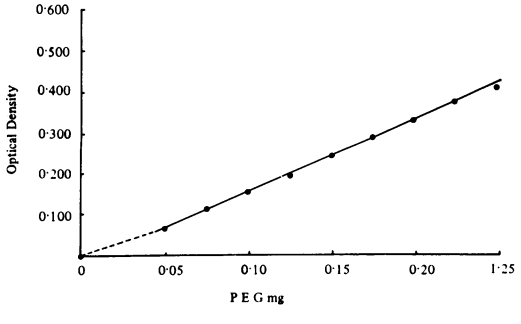

Fig. 3 *Standard*

mixture was gently everted once. The volumes of $Ba(OH)_2$ and $ZnSO_4$ had sometimes to be adjusted slightly to ensure that the two solutions were balanced, ie, that after filtration neither the addition of $Ba(OH)_2$ nor of $ZnSO_4$ would produce any further precipitate. After approximately half an hour, the flocculent precipitate settled and the supernatant was filtered through a single Whatman no. 42 paper. Three ml of each filtrate was pipetted into boiling tubes, 1·9 cm ($\frac{3}{4}$ in.) and 5 ml gum arabic-trichloro-acetic acid solution was added by automatic pipette. Standard solutions were made up to contain from 0·06 to 0·24 mg PEG/3 millilitre.

The tubes were neither shaken nor disturbed but allowed to stand for one hour. They were then read in 1 cm glass cuvettes in a Unicam SP 600 spectrophotometer against a water blank using the blue photocell and a wavelength of 420 mμ.

Results

EFFECT OF WAVELENGTH

The optical density readings given by identical samples at different wavelengths are shown in Figure 1. The readings of the samples were made at 420 mμ using the blue photocell.

ADDITION OF PROTEIN

The development of a filtrable precipitate following the mixing of barium hydroxide and zinc sulphate was dependent on the presence of a minimum amount of protein. This is clearly shown in Figure 2. The sensitivity of the instrument was increased by setting the 'water blank' to give an optical density of 0·1. The 'precipitated' solutions were filtered through Whatman no. 1 paper.The addition of greater amounts of protein did not improve the results further.

Protein content was measured in nine random intestinal aspirates taken from the small intestine of rats perfused at 0·3 ml/minute. The protein content varied from 280 μg/ml to less than 20 μg/millilitre. This is equivalent to a maximum protein addition of only 70 μg per precipitation sample, still below the optimal level.

STANDARD CURVE

This curve was linear over the range used (Figure 3). In accordance with other observers (Smith, 1959; Downes and McDonald, 1964; Ishikawa, 1966), the linear part of the curve was seen not to pass through the origin. In contrast with the results of Malawer and Powell (1967), the standard curve did not alter with a sixfold reduction in the amount of gum arabic added.

ACCURACY OF ASSAY

The standard deviation from the mean of 157 consecutive samples was 1·4%.

Discussion

Shaffer and Critchfield (1947) and Oliver and Preston (1949) measured PEG by colorimetry and gravimetry, but the methods are tedious. Some success was achieved using tritium-labelled PEG (Till and Downes, 1965), but the usual method is still that based on the turbidimetric assay of Hyden (1955), who noted that extinction of light by turbid solutions was greater at shorter wavelengths (cf sunsets are red—blue light has been more readily absorbed) but made no use of this property. Ishikawa (1966) used a wavelength of 440 mμ without explanation, although he further modified the procedure for low concentrations of PEG by adding phenol instead of trichloroacetic acid, and taking nephelometer readings. Most workers have evidently used the original white light, or no filter, recommended by Hyden (1956). The use of a short wavelength appears to have no disadvantage, and greatly increases the sensitivity of the assay.

While assaying a large number of control perfusate samples, one of us observed that attempted protein precipitation in the protein-free solutions frequently resulted in a very fine precipitate which was very difficult to remove by filtration. The addition of small amounts of protein has been clearly shown (Fig. 2) to improve filtration. Aspirates from rat intestine contain suboptimal amounts of protein, whereas the addition of excess protein makes precipitation easy. Filtration through a single fine filter paper has subsequently been totally satisfactory. While low protein concentration may be a problem specific to small mammals, it has not been investigated in conditions using polyethylene glycol more conventionally.

The overall accuracy of our results compares well with those of Hyden (1955) and Malawer and Powell (1967). The use of automatic pipettes saves much time in the assay of large numbers of samples, and their use would appear to be justified by the accuracy of the results.

The use of higher concentrations of PEG in the perfusate is contraindicated by its known effects on digestion (Vavřinková and Krondl, 1965).

Facilities for this work were kindly provided by the Clinical Research Committee of the Middlesex Hospital and Medical School. We thank Dr G. D. Hadley for his encouragement. The generous financial assistance of the British Nutrition Foundation and the Wellcome Trust is gratefully acknowledged.

References

Corbett, J. L., Greenhalgh, J. F. D., Gwynn, P. E., and Walker, D. (1958). Excretion of chromium sesquioxide and polyethyleneglycol by dairy cows. *Brit. J. Nutr.*, **12**, 266-276.

Dawson, A. M., and McMichael, H. B. (1968). The effect of flow rate on glucose absorption demonstrated by perfusion studies in rat jejunum *in vivo*. *J. Physiol. (Lond.)*, **196**, 32-33P.

Downes, A. M., and McDonald, I. W. (1964). The chromium-51 complex of ethylenediamine tetraacetic acid as a soluble rumen marker. *Brit. J. Nutr.*, **18**, 153-162.

Hyden, S. (1956). A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *K. LantbrHögsk. Annlr.*, **22**, 139-145.

Ishikawa, S. (1966). Reliability of polyethyleneglycol as an indicator for digestion studies with swine. I. Rate of passage of polyethyleneglycol through the digestive tract. *Agric. biol. Chem.*, **30**, 278-284.

Malawer, S. J., and Powell, D. W. (1967). An improved turbidimetric analysis of polyethylene glycol utilizing an emulsifier. *Gastroenterology*, **53**, 250-256.

Oliver, J., and Preston, C. (1949). Estimation of non-ionic detergents. *Nature (Lond.)*, **164**, 242-243.

Shaffer, C. B., and Critchfield, F. H. (1947). Solid polyethylene glycols (Carbowax compounds): quantitative determination in biological materials. *Analyt. Chem.*, **19**, 32-34.

Schedl, H. P. (1966). Use of polyethylene glycol and phenol red as unabsorbed indicators for intestinal absorption studies in man. *Gut*, **7**, 159-163.

Smith, R. H. (1959). The development and function of the rumen in milk-fed calves. *J. agric. Sci.*, **52**, 72-78.

Till, A. R., and Downes, A. M. (1965). The preparation of tritium-labelled polyethylene glycol and its use as a soluble rumen marker. *Brit. J. Nutr.*, **19**, 435-442.

Vavřinková, H., and Krondl, A. (1965). Use of polyethylene glycol in investigations of absorption of fat. *Nature (Lond.)*, **208**, 293-294.