Effect of Berkefeld filtration on the binding activity of human gastric juice

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SYNOPSIS The intrinsic factor content of Berkefeld-filtered human gastric juice has been studied. This appears to vary with the pH at which filtration is carried out and also between individual filters. Significant losses of intrinsic factor may result from filtration and complete loss when filtration is carried out at a low pH. The most suitable pH for filtration appears to be in the range pH 7 to 8.

It is known that filtration of human gastric juice through a Seitz filter causes it to lose intrinsic factor activity while Berkefeld filtration is said not to do this (Taylor, Castle, Heinle, and Adams, 1938; Hall, Morgan, and Campbell, 1949; Ungley, 1950) but there is little recent information on this subject (Glass, 1963). During work involving the study of the uptake of vitamin B₁₂ by microorganisms in the presence of gastric juice, the problem of sterilizing the juice was encountered. This prompted further study of the effect of Berkefeld filtration on the binding activity of gastric juice, particularly after it was noted that after filtration the binding activity of gastric juice tended to be somewhat variable.

METHODS

Cultures of *E. coli* were prepared by inoculating 1 ml of an overnight culture into 10 ml DIFCO microinoculum broth at room temperature. Cyanocobalamin was added to the cultures at room temperature in 0.1 ml water. The ⁵⁸ cobalt-labelled vitamin was obtained from the Radiochemical Centre, Amersham, as ampoules containing 3.6 µg and activity 10 µCi. To these 6.4 µg of the stable vitamin was added and the volume made up to 15 ml with sterile water. Thus 0.1 ml water would contain 1/15 µg cyanocobalamin with activity 1/15 µCi. This is approximately 12 times the amount of vitamin in 10 ml DIFCO broth. The uptake of the labelled vitamin by the microorganisms was determined after a further period of incubation for 16 hours at 37°C and at pH 6.8. The cultures were then centrifuged at 3,000 revolutions per minute for 30 minutes and the radioactivity of the supernatant was calculated using a well type scintillation counter. By comparing this with the activity of uninoculated controls the uptake of the labelled vitamin by the microorganisms was calculated.

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By combining measured volumes of human gastric juice with the cultures the uptake of vitamin B₁₂ by the organisms in the presence of gastric juice was determined. Gastric juice was obtained from subjects after augmented histamine stimulation (Kay, 1953; Card, Marks, and Sircus, 1955) and the pepsin in this inactivated by alkaline denaturation (Grassbeck, 1960). It was then kept at -20°C until required. Only juice from patients with an output of more than 20 m-equiv acid in the post-histamine hour or of known intrinsic factor concentration was used. The latter was measured by the method of Ardeman and Chanarin (1963).

Crystalline pepsin (Worthington USA) was added as 2.5 mg in 0.1 ml of water per millilitre of gastric juice. This concentration of enzyme is based on observations made on the concentration of pepsin in human gastric juice (Babkin, 1950; Reizenstein, 1959). Enzyme digestion was carried out for four hours at pH 2 and after this period the enzyme was inactivated by alkaline denaturation.

Appropriate controls were set up throughout and particular care was taken to detect bacterial contamination of the various solutions employed. All experiments were carried out in duplicate, the measure of agreement between duplicate samples being excellent.

For the filtration work six new standard filter candles (British Berkefeld Filters Ltd, No. 8) were used. They were prepared and cleaned for the experiments as instructed by the makers by boiling in water for 20 minutes. They were then autoclaved at 15 lb pressure for 10 minutes. In each filtration experiment 60 ml of juice was passed through the filters under identical conditions.

RESULTS

In Table 1 are illustrated the results of the bacterial uptake studies in the presence of increasing volumes of gastric juice which had been filtered at pH 7 compared with the uptakes in the presence of unfiltered juice. The uptakes in the presence of filtered

TABLE I

PERCENTAGE OF UPTAKE OF LABELLED CYANOCOBALAMIN BY *E. coli* IN THE PRESENCE OF INCREASING VOLUMES OF UNFILTERED GASTRIC JUICE AND OF JUICE FILTERED AT pH 7

Percentage Uptake of Radioactivity		
Volume of Gastric Juice Added (ml)	Gastric Juice Filtered at pH 7	Unfiltered Gastric Juice
0	93.0	93.0
2	18.0	12.0
6	11.0	10.0
10	10.0	7.0

juice are slightly higher, and, though the differences are small, they are reproducible. From Table II it will be seen that the binding activity of gastric juice was more susceptible to peptic digestion after it had been filtered. In Table III it will be seen from the uptake studies that the loss of binding activity becomes more pronounced as the pH at which the juice is filtered is dropped.

TABLE II

PERCENTAGE UPTAKE OF LABELLED CYANOCOBALAMIN BY *E. coli* IN THE PRESENCE OF GASTRIC JUICE EXPOSED TO PEPTIC DIGESTION BEFORE AND AFTER FILTRATION

Percentage Uptake of Radioactivity		
Volume of Gastric Juice Added (ml)	Gastric Juice Exposed to Peptic Digestion for 4 Hours before Filtration at pH 7	Gastric Juice Exposed to Peptic Digestion for 4 Hours after Filtration at pH 7
0	84.0	84.0
2	33.5	53.9
6	4.5	17.5
10	3.1	8.3

Figure 1 shows the results of an experiment in which intrinsic factor determinations were made on the gastric juice before and after filtration, the pH of the juice being altered in the range pH 6 to 10 before filtration. The intrinsic factor concentration of the juice before filtration was 65.6 ng/ml. Figure 2 illustrates the results of similar experiments, filtration being carried out at different pH values through three of the same filters used above. A different pool of gastric juice (intrinsic factor concentration 50.7 ng/ml) was employed on this occasion. These figures

FIG. 1. Effect of Berkefeld filtration of human gastric juice at pH 6-10. The intrinsic factor content of the juice was 65.6 ng/ml. The circled figures refer to the number allocated to the filter.

FIG. 2. Effect of Berkefeld filtration of human gastric juice at pH 2-7. The intrinsic factor content of the juice was 50.7 ng/ml. The circled figures refer to the number allocated to the filter.

on the whole confirm the observations made in the vitamin B₁₂ uptake experiments and indicate that the loss of binding activity noted there was due to loss of intrinsic factor activity.

DISCUSSION

The findings confirm the claim of previous workers

TABLE III

PERCENTAGE UPTAKE OF LABELLED CYANOCOBALAMIN BY *E. coli* IN THE PRESENCE OF INCREASING VOLUMES OF GASTRIC JUICE FILTERED AT DIFFERENT pH VALUES

Percentage Uptake of Radioactivity in Gastric Juice Filtered at Various pH Values

Volume of Gastric Juice Added (ml)	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
0	90.9	90.9	90.9	90.9	90.9	90.9
2	89.0	80.8	20.0	16.0	12.8	13.5
6	84.0	64.3	8.0	7.5	7.8	5.2
10	81.0	54.9	6.0	2.4	6.4	3.1

in that gastric juice which has been filtered through a Berkefeld filter still contains intrinsic factor. This, however, is true mainly of juice filtered in the pH range 7 to 8. At pH values above and below this range there tends to be some loss of intrinsic factor activity and this is complete in the lower pH range. It should be recalled here that intrinsic factor is known to be stable up to a pH of 11.5 (Glass, 1963). We have carried out numerous experiments similar to those illustrated in Figs. 1 and 2 and the results confirm the impression gained from these that there is considerable variability in the concentrations of intrinsic factor that different filters will allow through. In addition, in spite of our attempts to standardize experimental conditions, there has been some variation between results obtained after filtering the same gastric juice through the same filter on different occasions. Further work, which has included the use of other filters, leaves us in no doubt, however, that certain filters, such as no. 3 in the present study, may remove considerable quantities of intrinsic factor even if filtration is performed in the more favourable pH range.

The manner by which the filters bring about their effect is puzzling but an important clue may be contained in the observations made on the pH of the filtrate. This tends towards neutrality regardless of the pH of the juice before filtration. Thus when juice is filtered at pH 2 the pH of the effluent has changed to between 7 and 8. The application of negative pressure to the filters has not significantly affected the results obtained and it has not been possible to wash the intrinsic factor presumably left behind out of the filters through which juice has been passed at low pH with either alkali or water. Prolonging the period of filtration does not result in any significant change in the intrinsic factor concentrations of the

filtered juice though the intrinsic factor content of initial aliquots of juice even when filtered at pH 7 may be very low.

Gastric juice is sometimes used as a source of intrinsic factor in tests of vitamin B₁₂ absorption and Berkefeld filtration is a popular method of preparing it for this procedure (Taylor *et al*, 1938; Hall *et al*, 1949; Ungley, 1950). The present work suggests that occasionally this could lead to erroneous results. Thus 50 ml of gastric juice of intrinsic factor content 65.6 µg/ml if passed through the worst of our filters at pH 7 would still contain 890 ng of intrinsic factor. This would usually be enough to correct impaired absorption of vitamin B₁₂ in a patient with pernicious anaemia though more might be required in a patient with deficiency of intrinsic factor after gastric surgery (Ardeman and Chanarin, 1965). Error would of course be more likely if juice of a lower intrinsic factor concentration was being filtered. The effect of Berkefeld filtration on gastric juice in more subtle work certainly cannot be disregarded and consequently its use in the uptake studies described had to be abandoned.

REFERENCES

- Ardeman, S., and Chanarin, I. (1963). *Lancet*, 2, 1350.
 ——— (1965). *Brit. J. Haemat.*, 11, 305.
 Babkin, B. P. (1950). *Secretory Mechanism of the Digestive Glands*, 2nd ed. Hoeber, New York.
 Card, W. I., Marks, I. N., and Sircus, W. (1955). *J. Physiol. (Lond.)*, 130, 18P.
 Glass, G. B. J. (1963). *Physiol. Rev.*, 43, 529.
 Grassbeck, R. (1960). *Advanc. clin. Chem.*, 3, 299.
 Hall, B. E., Morgan, E. H., and Campbell, D. C. (1949). *Proc. Mayo Clin.*, 24, 99.
 Kay, A. W. (1953). *Brit. med. J.*, 2, 77.
 Reizenstein, P. G. (1959). *Acta med. scand.*, 165, 481.
 Taylor, F. H. L., Castle, W. B., Heinle, R. W., and Adams, M. A. (1938). *J. clin. Invest.*, 17, 335.
 Ungley, C. C. (1950). *Brit. med. J.*, 2, 908.