Vitamin B₁₂ excretion by the rat small intestine

C. A. LOEHRY AND B. CREAMER

From the Gastrointestinal Laboratory, St Thomas' Hospital, London

SUMMARY Two phases of intestinal excretion are demonstrated after a parenteral dose of ⁵⁸Co vitamin B₁₂. The first phase was maximal immediately after injection, and was related to plasma radioactivity. The second phase was delayed 36 to 48 hours and was related to labelled cells reaching the tips of the villi. The loss of vitamin B₁₂ through increased exudation and rapid cell exfoliation together with failure of reabsorption may play a part in the deficiency state seen in the coeliac syndrome.

It has been established that a proportion of a parenteral dose of vitamin B₁₂ is excreted in the faeces both in experimental animals (Okuda, Gräsbeck, and Chow, 1958; Willigan, Cronkite, Meyer, and Noto, 1958; Gräsbeck, Runeberg, and Simons, 1959; Booth and Spray, 1962) and in man (Gräsbeck, Nyberg, and Reizenstein, 1958; Adams, 1963). Although part of this excretion takes place in the bile, animals still excrete a proportion of an injected dose after ligation of the bile duct (Okuda *et al*, 1958) suggesting that the small intestinal mucosa itself plays a part in vitamin B₁₂ elimination. In a previous series of experiments (Loehry, Croft, Singh, and Creamer, 1968) we have demonstrated how iron injected parenterally may be excreted into the small intestine by two pathways. Plasma iron appears to pass directly into the lumen while iron is also taken up by the newly formed crypt epithelial cells and lost with them into the intestinal lumen after migration up to the tips of the villi. It seemed possible that the rapid cell turnover in the small intestine might be responsible for the faecal loss of a number of substances, and in the present study we have investigated the role of the small intestinal mucosa in the excretion of vitamin B₁₂.

METHODS

Male albino rats, weighing 380 to 450 g, were used for the experiments; 3 µc (1 µg) of ⁵⁸Co vitamin B₁₂ was injected intravenously or intraperitoneally and small intestinal perfusion was performed at varying time intervals after injection. A method of perfusion was used that has been previously described (Loehry *et al*, 1968) whereby a constant number of naturally desquamated epithelial cells was collected without contamination from gastric contents or bile. Perfusions were performed at the following time intervals after injection of the isotope:

10 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. Three rats were perfused at each of these times and the perfusion was maintained for three to four hours for each rat. After each experiment the rat was killed, the small intestine removed, washed, and fixed in 10% formalin. The radioactivity of the hourly perfusions and of the intestines was counted in a large well-type cintillation counter.

In the experiments where radioactivity was related to epithelial cells, the number of cells collected was varied by altering the rate of perfusion for each hour; this has been shown to produce a varying number of cells in the collected fluid proportional directly to the rate of perfusion (Loehry *et al*, 1968). The number of cells collected was estimated by the chemical DNA content of the hourly specimens (Croft and Lubran, 1965).

RESULTS

In the perfusions immediately after intravenous injection of ⁵⁸Co vitamin B₁₂ a peak radioactivity appeared in intestinal perfusions in the first hour which fell rapidly over the next three hours; a second peak was seen at the 36- to 48-hour period after injection (Fig. 1). The difference between the radioactivity in all specimens at the 36- and 48-hour period and then at the 12- and 24-hour period was statistically highly significant (*p* < 0.001). Radioactivity in the intestinal wall rose to a maximum at 12 hours and then gradually fell until at 120 hours less than 0.5% of the administered dose was present in the small intestinal wall (Fig. 2).

In order to assess whether the radioactivity present in the perfusions was related to desquamated epithelial cells a number of perfusions were performed immediately after injection and at the 36- to 48-hour period, with a varied hourly perfusion rate, thus collecting a different number of cells at each

FIG. 1. A graph showing the radioactivity in perfusion specimens at various times after parenteral administration of ⁵⁸Co vitamin B₁₂.

FIG. 2. A graph showing the radioactivity in the small intestine removed at various times after the parenteral administration of ⁵⁸Co vitamin B₁₂.

FIG. 3. The cell in perfusion specimens, measured as DNA, plotted against radioactivity at 48 hours after injection of ⁵⁸Co vitamin B₁₂, on the right at 0 to 6 hours after injection and on the left at 48 hours. Note the absence of any relationship at 0 to 6 hours as opposed to 48 hours where there is a clear relationship

hour. At the 36- to 48-hour period there was a direct relationship between DNA and radioactivity implying that the radioactivity came from intracellular ⁵⁸Co vitamin B₁₂ (Fig. 3). At the early period immediately after injection it was clear that the radioactivity was not related to desquamated cells and was probably due to plasma exudation (Fig. 3).

DISCUSSION

The experiments with ⁵⁸Co vitamin B₁₂ demonstrate two phases in the excretion of the isotope into the intestinal lumen. The first phase was maximal soon after injection, was unrelated to cell loss, and fell rapidly to a baseline after three to four hours. This period represented the time that ⁵⁸Co vitamin B₁₂ was present in the plasma in large amounts (Mollin, Pitney, Baker, and Bradley, 1956) and the intestinal loss was probably a reflection of plasma vitamin B₁₂ exuding into the lumen. The second peak at 36 to 48 hours after injection correlated with cell desquamation and this suggested that the newly formed crypt cells took up the isotope maximally in the period shortly after injection, and these labelled cells then migrated up the tips of the villi where they were shed into the lumen. A similar pattern has been demonstrated after parenteral administration of ⁵⁹Fe (Loehry *et al*, 1968) and it seems possible that several substances may be taken up into the newly formed crypt cells with their high mitotic activity, remain in them as they mature and migrate up the villi, and are finally lost with them from the extrusion zones into the intestinal lumen.

It has been estimated that in man approximately $60,000 \times 10^6$ small intestinal epithelial cells are extruded each day (Croft, unpublished observations). Iron and vitamin B₁₂ have now been shown to be lost with them and it is possible that other substances such as protein, fats, and folic acid are similarly affected. Clearly in health the normal mucosa recovers much of this loss by reabsorption, and it has been demonstrated that the net faecal loss of vitamin B₁₂ in man, after exclusion of bile is small (Adams, 1963). There are, however, situations where this loss may be of clinical significance. If the mucosa of the small intestine is in an increased turnover state a greater volume of cells will be lost and there is evidence that this situation occurs in the coeliac syndrome (Croft, Loehry, and Creamer, 1968). Here also the mucosa is abnormal and may be unable to absorb not only ingested substances but also those desquamated into the intestinal lumen. Perhaps in this state of 'exfoliative enteropathy' the small intestinal loss of vitamin B₁₂ may reach significant proportions.

REFERENCES

- Adams, J. F. (1963). The biliary and faecal loss after parenteral ^{58}Co vitamin B_{12} in man. *Scot. med. J.*, **8**, 118-121.
- Booth, M. A., and Spray, G. H. (1962). The excretion and distribution in the tissues of parenterally administered ^{58}Co vitamin B_{12} in normal and gastrectomised rats. *Brit. J. Haemat.*, **8**, 114-119.
- Croft, D. N., and Lubran, M. (1965). The estimation of deoxyribonucleic acid in the presence of sialic acid: application to analysis of human gastric washings. *Biochem. J.*, **95**, 612-620.
- , Loehry, C. A., and Creamer, B. (1968). Small-bowel cell-loss and weight-loss in the coeliac syndrome. *Lancet*, **2**, 68-70.
- Gräsbeck, R., Nyberg, W., and Reizenstein, P. (1958). Biliary and fecal vitamin B_{12} excretion in man. An isotope study. *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 780-784.
- , Runeberg, L., and Simons, K. (1959). Intrinsic factor and radio-vitamin B_{12} excretion in rats. *Acta physiol. scand.*, **47**, 370-374.
- Loehry, C. A., Croft, D. N., Singh, A. K., and Creamer, B. (1968). Cell turnover in the rat small intestinal mucosa: An appraisal of cell loss. *Gut*, **10**, 13-18.
- Mollin, D. L., Pitney, W. R., Baker, S. J., and Bradley, J. E. (1956). The plasma clearance and urinary excretion of parenterally administered ^{58}Co B_{12} . *Blood*, **11**, 31-43.
- Okuda, K., Gräsbeck, R., and Chow, B. F. (1958). Bile and vitamin B_{12} absorption. *J. Lab. clin. Med.*, **51**, 17-23.
- Willigan, D. A., Cronkite, E. P., Meyer, L. M., and Noto, S. L. (1958). Biliary excretion of Co^{60} labeled vitamin B_{12} in dogs. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 81-84.