

Measurement of non-haem iron absorption in non-anaemic rats using ^{59}Fe : can the Fe content of duodenal mucosal cells cause lumen or mucosal radioisotope dilution, or both, thus resulting in the underestimation of Fe absorption?

BY ANTHONY J. A. WRIGHT, SUSAN SOUTHON
AND SUSAN J. FAIRWEATHER-TAIT

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA

(Received 28 April 1989 – Accepted 3 July 1989)

Male Wistar rats (188 g) were fed on a semi-synthetic (SS) diet (38 mg iron/kg) *ad lib.* for 7 d and then meal-fed for 1 d. After a 21 h fast each rat was given one meal (10 g) of high-Fe SS (500 mg Fe/kg; high-Fe group) or control (38 mg Fe/kg; control group) diet. After 16 h 2 ml of an ^{59}Fe -labelled ferrous sulphate solution (18 kBq ^{59}Fe ; 120 μg Fe) was administered by gavage and equal numbers of rats from each group were killed 6 or 24 h after dosing. Mucosal uptake of ^{59}Fe from the gut lumen and transfer of ^{59}Fe from mucosa into the carcass were measured. Total Fe content of the duodenum was also determined. Mucosal ^{59}Fe uptake and transfer were markedly lower in the high-Fe group compared with the control group. The Fe content of the duodenum, the major region of Fe absorption, was significantly greater in the high-Fe group than in the controls. A larger amount of Fe may thus have been released into the lumen of the high-Fe rats, via mucosal cell turnover, resulting in a greater lumen dilution of the ^{59}Fe dose in this group compared with the controls. Calculations are presented which demonstrate that such an effect could not possibly account for the observed difference in mucosal ^{59}Fe uptake between groups. Differences in rates of 'cold' Fe and ^{59}Fe loss from the duodenal mucosa during the 6–24 h interval suggested that, at the time of dosing, Fe retained in the mucosa from the previous meal had been incorporated into a non-exchangeable pool and as such would not dilute radioactive Fe entering the mucosa. It was concluded that whole-body ^{59}Fe retention from a labelled source, given orally after an overnight fast, provided an accurate estimate of Fe absorption.

Iron absorption: Mucosal Fe uptake: Rat

Extrinsic radioisotope labelling of a non-meat meal is one of the accepted methods for determining the amount of non-haem iron absorbed (i.e. that having undergone mucosal uptake and mucosal transfer) from that meal (Monsen, 1974; Hallberg, 1982; Van Campen, 1983). It is assumed that the proportion of ^{59}Fe retained by the body, at a suitable interval after administration of a labelled test meal, reflects the proportion of Fe transferred from the intestinal lumen into the carcass (Field *et al.* 1960; Flanagan *et al.* 1980).

Results from our previous studies demonstrated an inverse relationship in non-anaemic rats between short-term variations in dietary Fe intake within the physiological concentration range and subsequent whole-body ^{59}Fe retention from a test meal given after an overnight (12–16 h) fast (Fairweather-Tait & Wright, 1984). The hypothesis that Fe absorption is regulated at the mucosal cell level in response to changes in body Fe status (liver Fe stores) is well known (Hahn *et al.* 1943), but body Fe status is unlikely to be significantly altered by one 10 g meal containing Fe at physiological concentrations. The effect observed after a single meal was therefore attributed to a short-term mucosal control of Fe absorption (Fairweather-Tait *et al.* 1985) mediated via the Fe 'status' of mucosal cells *per se*.

The effect of single, large, non-physiological 'blocking' doses of Fe on mucosal Fe levels

and subsequent Fe absorption is well known, but it is important to note that the reported 'blocking' effects of these pharmacological doses have only been noted to last less than 6 h (Hahn *et al.* 1943; Brown & Rother, 1963). In this context it is interesting that our previous results (Fairweather-Tait & Wright, 1984) indicate that, in non-anaemic rats, the prefeeding of a single 10 g meal having an Fe concentration at the American Institute of Nutrition (1977) recommended level (35 μg Fe/g), or even lower, could have an inhibitory effect on Fe absorption from a subsequent meal given 12–16 h later. Clearly, the evidence of our previous work (Fairweather-Tait & Wright, 1984; Fairweather-Tait *et al.* 1985) indicates that the duodenal mucosa exerts a far more subtle day-to-day control of Fe absorption, in the absence of changes in body Fe status, than suggested by other studies.

It has been suggested that following the uptake of Fe by the epithelial cells of the duodenal mucosa (the main site for Fe absorption: Manis & Schachter, 1962; Johnson *et al.* 1983), transfer into the body occurs in two phases: a short early phase of rapid transport (4–6 h) and a longer phase of slower transport (up to 24 h) (Wheby & Crosby, 1963; Charlton *et al.* 1965). If an oral dose of ^{59}Fe is administered before the mucosal transfer of dietary Fe from a previous meal is completed, that is within 24 h of the meal, it could be argued that differences in the amount of Fe retained by the duodenal mucosa may result in varying degrees of radioisotope dilution. Dilution may occur within the gut lumen as a consequence of normal mucosal cell turnover, or within the enterocytes themselves. Such an effect would result in a reduction in whole-body ^{59}Fe retention with increasing amounts of Fe consumed before the test meal, thereby leading to inaccurate estimates of Fe absorption. The present study was undertaken, using non-anaemic rats, to investigate the possibility of radioisotope dilution occurring in such studies and so test the validity of current techniques employed in the estimation of non-haem Fe absorption.

MATERIALS AND METHODS

Sixty immature, male Wistar rats (100–120 g) were randomly divided into four groups of fifteen and housed singly in polypropylene cages with stainless steel gridded tops and bottoms in a room at 21° having a 12 h light–dark cycle. Animal care and the regulation of scientific procedures met the criteria laid down by the United Kingdom 'Animals (Scientific Procedures) Act 1986'. All rats were given a control semi-synthetic (SS) diet (38 mg Fe/kg) with an Fe concentration similar to that recommended by the American Institute of Nutrition (1977; 35 mg Fe/kg) *ad lib.* for 7 d. On day 8 rats were given access to unrestricted amounts of food as a single meal between 15.15 and 16.45 hours. At the same time on the following day rats from groups 1 and 3 received a meal (approximately 10 g) of control diet (38 mg Fe/kg), while groups 2 and 4 received a meal of high-Fe diet (500 mg Fe/kg). The composition of control diet is shown in Table 1; high-Fe diet was made by addition of extra ferrous sulphate to the mineral mix. The next morning all rats were given 2 ml of a freshly prepared solution containing 120 μg Fe as FeSO_4 extrinsically labelled with 18 kBq ^{59}Fe (FeCl_3 in 0.1 M-hydrochloric acid, 110–740 MBq/mg Fe; Amersham International plc, Amersham, Bucks) by gavage. Immediately after intubation the exact dose of ^{59}Fe given to each animal was determined by counting the animals for 30 s in a small-animal whole-body counter (NE Technology, Beenham, Berks), as described previously (Fairweather-Tait & Wright, 1984). No food was given to the animals after dosing. Rats were killed either 6 h (groups 1 and 2; designated control–6 h and high-Fe–6 h groups) or 24 h (groups 3 and 4; designated control–24 h and high-Fe–24 h groups) after the oral dose of ^{59}Fe by intraperitoneal administration of a lethal dose of sodium pentobarbitone (1 ml \times 200 mg/ml Euthatal; May & Baker Ltd, Dagenham, Essex). One rat from each group was killed at approximately the same time. The abdomen was opened

Table 1. *Composition (g/kg) of semi-synthetic control diet*

Starch	310
Sucrose	310
Casein	200
Solkafloc	40
Mineral mix*	40
Vitamin mix†	20
DL-methionine	2
Maize oil	80

* Mineral mix (g/kg diet): CaHPO₄ 13.0, CaCO₃ 8.2, KCl 7.03, Na₂HPO₄ 7.4, MgSO₄·H₂O 4.0, MnSO₄·H₂O 0.18, ZnCO₃ 0.025, FeSO₄·7H₂O 0.144, CuSO₄·5H₂O 0.023, KIO₃ 0.001.

† Vitamin mix (mg/kg diet): nicotinic acid 60, cyanocobalamin (in mannitol) 50, calcium D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, pteroylmonoglutamic acid 10, D-biotin 1, vitamin K₁ (in lactose) 2, Rovimix E-50 150 (containing 75 mg DL- α -tocopheryl acetate), Rovimix A-500 25 (containing 3.75 mg retinol), Rovimix D₃-500 15 (containing 188 μ g cholecalciferol) (all Rovimix products from Roche), choline bitartrate 1800, starch (bulking agent) 17817.

and the small intestine (SI) was removed, rinsed externally, the lumen flushed gently with 20 ml of cold saline (9 g sodium chloride/l) and the length measured with minimal stretching. The SI was then divided into three portions: the duodenum (proximal 10% of total length), jejunum (following 40%) and ileum (remaining 50%). Each section was counted for 10 min in a Phillips PW 4580 automatic gamma counter. The carcass, minus caecum and large intestine, was counted in the small-animal whole-body counter for 30 s. An appropriate standard was used to calibrate and correlate the two counters, and due allowance was made for background correction, radioactive decay and counting efficiency. Mucosal uptake of ⁵⁹Fe can only be measured accurately shortly after ingestion of the test dose, particularly when the rate of mucosal transfer is slow, because of the possibility of loss of ⁵⁹Fe in exfoliated mucosal cells (Marx, 1979). In the present study it was assumed that 6 h was sufficient time to allow for the passage of digesta through the duodenum, which is the region of maximal Fe absorption, whilst loss of Fe via mucosal cell turnover would be minimal. Mucosal uptake was defined as the proportion of the initial dose of the label retained by the carcass plus SI at 6 h after dosing. Mucosal transfer of ⁵⁹Fe at 6 h was defined as the amount of ⁵⁹Fe in the carcass expressed as a percentage of mucosal uptake. Mucosal transfer at 24 h was defined as the amount of ⁵⁹Fe in the carcass expressed as a percentage of the mean 6 h mucosal uptake; 24 h mucosal uptake values were not used as these may be significantly underestimated due to losses of dietary Fe which, though having initially undergone mucosal uptake, are then re-released back into the lumen.

The sections of SI were oven-dried for 16 h at 85° for determination of dry weight. The duodenal tissue was then ashed in a muffle furnace for 48 h at 450°, dissolved in a minimum volume of concentrated HCl (11.7 M) and further diluted with distilled water. The Fe content was determined by atomic absorption spectroscopy using a PU 9000 (Pye Unicam, Cambridge, England).

Statistical analysis

Analysis of variance was performed on values for body-weight (at kill), food intake on the day before oral dosing, and SI length and dry weight. Since the remaining values had unequal variances, but similar coefficients of variation (i.e. similar relative standard deviations), mean values were compared using Student's unpaired *t* test.

Table 2. *The effect of previous dietary iron intake on duodenal Fe content, and the percentage of ^{59}Fe retained by small intestine (SI) and carcass of rats given an oral dose of radiolabelled ferrous sulphate**

(Value are means with their standard errors for fifteen rats)

Diet-time-interval after dose...	Control-6 h		High-Fe-6 h		Control-24 h		High-Fe-24 h	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
^{59}Fe retention (% of oral dose)								
Duodenum	3.9 ^a	0.5	6.9 ^c	0.6	1.1 ^b	0.1	1.1 ^b	0.2
Jejunum	7.8 ^a	0.5	9.2 ^a	0.7	2.0 ^b	0.1	2.1 ^b	0.2
Ileum	2.4 ^a	0.2	3.2 ^a	0.4	1.5 ^b	0.1	1.4 ^b	0.1
Carcass	48.9 ^a	3.4	9.4 ^c	0.9	59.9 ^b	2.9	17.3 ^d	1.5
Mucosal uptake (% of ^{59}Fe in carcass + SI)	62.9 ^a	3.6	28.7 ^b	2.3	64.7 ^a	3.0	21.9 ^c	1.7
% Mucosal transfer (% carcass ^{59}Fe / mucosal uptake)†	77.1 ^a	1.5	32.4 ^d	1.2	95.6 ^b	4.6	60.0 ^c	1.0
Total duodenal Fe (μg)	25.3 ^a	1.6	73.4 ^b	7.5	20.7 ^a	1.0	53.3 ^c	4.1

^{a, b, c, d} Values in any horizontal row with different superscript letters were significantly different ($P < 0.05$).

* For details of dietary treatment and oral dosing, see p. 720.

† Mucosal transfer based on mean mucosal uptake at 6 h, see p. 721.

RESULTS

Mean values for body-weight, food intake on the day before oral dosing, SI length and dry weights of duodenum, jejunum and ileum were similar for control and high-Fe groups. The overall means (pooled SEM) were: body-weight 187.6 (SE 3.2) g, food intake 10.7 (SE 0.5) g, SI length 1138 (SE 14) mm, dry weight of duodenum 168 (SE 7) mg, jejunum 476 (SE 16) mg, ileum 676 (SE 41) mg.

The percentage of ^{59}Fe retained by the SI and carcass, 6 or 24 h after the administration of the isotope, is shown in Table 2. In both the 6 and 24 h high-Fe groups the percentage of ^{59}Fe in the carcass was significantly reduced compared with their control counterparts. The amount of isotope retained in the duodenum of rats pretreated with a high-Fe meal (500 mg Fe/kg) and killed 6 h after oral dosing was significantly higher than for rats given the control meal (38 mg Fe/kg), but values for the 24 h high-Fe and control groups were similar. The percentage of ^{59}Fe associated with the jejunum and ileum at each time-point was unaffected by the dietary Fe concentration of the meal consumed before oral dosing. There was, however, a marked reduction in the amount of isotope in all sections of the SI 24 after the oral dose, compared with the 6 h value, in both the control and high-Fe groups of animals.

Mucosal uptake and mucosal transfer of ^{59}Fe were significantly lower in animals given the high-Fe meal before dosing than in controls (Table 2).

Total Fe in duodenal tissue was significantly higher in the high-Fe groups of rats (Table 2).

From values presented in Table 2 the following calculations were performed to assist with the interpretation and discussion of results (Table 3). First, the maximum rate of Fe loss into the lumen of the duodenum from exfoliated enterocytes was calculated from the

Table 3. Values derived from Table 2*; iron loss into duodenal lumen from exfoliated epithelial cells, lumen dilution of the specific activity (SA) of ^{59}Fe test dose, and transfer from duodenal tissue between 6 and 24 h after the test dose

Diet...	Control	High-Fe
Fe loss into lumen† from exfoliated duodenal epithelial cells ($\mu\text{g}/\text{h}$)	0.3	1.1
Lumen dilution‡ of oral dose of 120 μg $\text{Fe-}^{59}\text{Fe}$ (relative to original SA)	0.995	0.982
Fe disappearance (%) from duodenal tissue between 6 and 24 h	18 (30)§	27 (32)§

* For full details of calculations, see pp. 723–724.

† Calculated from the difference between the duodenal Fe content at 6 and 24 h (Table 2), assuming that no mucosal transfer is occurring during this period.

‡ Calculated on the basis that transit of the test dose through the duodenum would be completed in 2 h (i.e. that Fe loss into the lumen over this period would be 0.6 and 2.2 μg for control and high-Fe groups respectively) and that Fe from exfoliated epithelial cells would be completely and instantly available for re-absorption.

§ Values in parentheses estimate the percentage disappearance of Fe from duodenal tissue after deduction of the background obligatory-Fe level (10 μg) from the total duodenal Fe values listed in Table 2.

difference between duodenal Fe content at 6 and 24 h, assuming that in the worst-case no mucosal transfer of Fe into the carcass occurred during this period:

$$\begin{aligned} \text{control group } ((25.3-20.7 \mu\text{g Fe})/18 \text{ h}) &= 0.3 \mu\text{g Fe/h,} \\ \text{high-Fe group } ((73.4-53.3 \mu\text{g Fe})/18 \text{ h}) &= 1.1 \mu\text{g Fe/h.} \end{aligned}$$

Second, from the above calculation, the maximum degree of lumen dilution of the specific activity (relative to a nominal original specific activity of 1.0) of an oral dose of 120 μg $\text{Fe-}^{59}\text{Fe}$ with Fe from exfoliated duodenal cells was estimated. For this estimate it was assumed that any Fe of mucosal origin was completely and immediately made available for absorption, and that the transit time of the test dose through the duodenum is in the region of 2 h, which is the time a liquid test dose takes to clear the stomach area (Wheby & Crosby, 1963):

$$\begin{aligned} \text{control group } 120 \mu\text{g Fe}/(120 + (2 \times 0.3 \mu\text{g/h})) \mu\text{g Fe} &= \text{specific activity of } 0.995, \\ \text{high-Fe group } 120 \mu\text{g Fe}/(120 + (2 \times 1.1 \mu\text{g/h})) \mu\text{g Fe} &= \text{specific activity of } 0.982. \end{aligned}$$

Third, the percentage of the duodenal Fe that disappears between 6 and 24 h was calculated (a) before deduction of background 'obligatory' Fe:

$$\begin{aligned} \text{control group } ((25.3-20.7)/25.3 \mu\text{g Fe}) &= 18\%, \\ \text{high-Fe group } ((73.4-53.3)/73.4 \mu\text{g Fe}) &= 27\%, \end{aligned}$$

and (b) after deduction of background 'obligatory' duodenal Fe obtained from a previous study (A. J. A. Wright, unpublished results) with eight Wistar rats of mean weight of 214 (SE 3) g. These rats were raised on a control SS diet (38 mg Fe/kg) and then fed on an Fe-deficient diet (8 mg Fe/kg) for 3 d. Total duodenal Fe content at the end of this 3 d period

was 10.0 (SE 0.6) μg . It was assumed that this value was close to the obligatory background Fe content of this region of the gut:

$$\begin{aligned} \text{control group } & [((25.3 - 10) - (20.7 - 10)) / (25.3 - 10) \mu\text{g Fe}] = 30\%, \\ \text{high-Fe group } & [((73.4 - 10) - (53.3 - 10)) / (73.4 - 10) \mu\text{g Fe}] = 32\%. \end{aligned}$$

DISCUSSION

The work of Wheby & Crosby (1963) indicated that the transfer of dietary non-haem Fe from duodenal mucosal cells into the body can take up to 24 h to reach completion. This raises the possibility that estimates of Fe absorption, based on carcass radioisotope retention following an ^{59}Fe -labelled test meal, may be affected by variations in a residual pool of exchangeable mucosal Fe when the test meal is given within this time period. If this were true, measurements of Fe absorption in non-anaemic rats, showing an inverse relationship between short-term (one meal) previous dietary Fe intake and whole-body ^{59}Fe retention from a test meal given after only a 12–16 h fast (Fairweather-Tait & Wright, 1984, 1987; Fairweather-Tait *et al.* 1985), could have been significantly influenced by differences in radioisotope dilution within the enterocytes. Even in studies in which previous Fe intakes do not differ among groups, such as those in which Fe absorption is estimated from a single test meal of different foods, it could be argued that any dilution of the radiolabel by an exchangeable mucosal Fe pool would result in inaccurate estimates of absolute Fe absorption from the labelled foods. Furthermore, the common practice of estimating Fe absorption relative to that from the same dose of a highly available Fe salt, for example FeSO_4 , may be influenced by this effect, since mucosal uptake of Fe from any test food is likely to be less than that from the Fe salt and would, therefore, undergo greater dilution in any exchangeable mucosal Fe pool.

The high dietary Fe concentration of the pretest meal (500 mg Fe/kg) used in the present study was chosen to exaggerate any possible radioisotope dilution effect resulting from increased levels of exchangeable Fe remaining in mucosal cells at the time of oral ^{59}Fe dosing, and this was compared with a control pretest meal containing 38 mg Fe/kg.

^{59}Fe carcass retention at 24 h in the high-Fe group was reduced by 71% compared with the control group: this was partly as a result of a lower mucosal transfer, which was reduced by 37%, but mainly as a result of a lower mucosal uptake, which was reduced by 54% (Table 2). The reduced mucosal uptake might initially suggest that Fe absorption is indeed related inversely to short-term changes in dietary Fe intake, and that our previous findings were not an artifact of ^{59}Fe dilution within the mucosal cells. However, since the Fe content of the duodenum of rats fed on a high-Fe meal was found to be significantly higher than the value for control animals, the possibility of both lumen and intracellular dilution of the radioisotope needs to be considered.

Normal mucosal cell turnover in the rat releases epithelial cells back into the intestinal lumen and their Fe content may dilute the specific activity of an oral ^{59}Fe -labelled test dose. Fe is absorbed and transferred into the carcass mainly in the duodenum (Manis & Schachter, 1962; Johnson *et al.* 1983), and since the duodenal Fe content of rats previously given a high-Fe meal is substantially greater than that of those given a control meal there may be a relatively greater lumen dilution of the specific activity of the oral ^{59}Fe test dose in the high-Fe group compared with controls. Calculations presented in the results section (see also Table 3) indicate that the maximum possible rate of Fe loss back into the duodenal lumen would be approximately 0.3 and 1.1 $\mu\text{g Fe/h}$ for control and high-Fe pretreated rat groups respectively. Wheby & Crosby (1963) have shown that an oral Fe dose given in solution totally clears the stomach in under 2 h and, on the reasonable assumption that the

duodenum would also be cleared within a similar time span, the maximum Fe loss into the lumen over this period would be approximately 0.6 or 2.2 μg Fe for control and high-Fe pretreated rats respectively. The worst possible (but highly unlikely) case is that all Fe from exfoliated duodenal epithelial cells is completely and instantly made re-available for absorption in the duodenum. If this were so then the lumen dilution of the 120 μg Fe- ^{59}Fe radiolabelled test dose would have been reduced to 0.982 in the high-Fe group and to 0.995 in controls, from a nominal value of 1.0. The comparative specific activity in the high-Fe pretreated rats relative to controls would, therefore, have been not less than 98.7%. Lumen dilution of the radioisotope could, therefore, not explain the large difference in mucosal uptake between the two dietary groups.

There remains, however, the possibility that some radioisotope dilution within the duodenal mucosal cells of the high-Fe group may have exaggerated the difference in carcass retention between the high-Fe and control groups. Results from the present study show that the amount of Fe consumed before the ^{59}Fe test dose significantly affected the rate of mucosal transfer of the radiolabel. The transfer of ^{59}Fe into the carcass in the control group was 77% of mucosal uptake at 6 h after dosing and 96% after 24 h and was, therefore, near completion. Mucosal transfer in the high-Fe group was lower, particularly at the earlier time-point, with only 32% of the label having entered the carcass after 6 h, rising to 60% after 24 h. Between 6 and 24 h, it was calculated that 18 and 27% of the residual Fe in the duodenum was lost from the control and high-Fe group respectively (Table 3). A previous unpublished observation (for details, see results section) with Wistar rats, fed on an Fe-deficient diet (8 mg Fe/kg) for 3 d, resulted in a mean duodenal Fe content of 10 μg . On the reasonable assumption that this represents the 'obligatory' level of Fe in duodenal mucosa, we can deduct this value from the total mucosal Fe values obtained in the present study and calculate the loss of Fe, between 6 and 24 h, which originated from the diet. This is 30% for the control group and 32% for the high-Fe group (Table 3), which is in accordance with the calculated loss of Fe over an 18 h period (25–37%) due to mucosal cell turnover; normal mucosal cell turnover time being 48–72 h in the rat (Lipkin, 1981). Loss of ^{59}Fe activity from the duodenal tissue over the 18 h period was much higher (72–84%) than the 'cold' Fe value, indicating that the radiolabel was moving independently of the bulk of mucosal Fe. This suggests that at the time of dosing most of the previous dietary Fe had been incorporated into a non-exchangeable mucosal Fe pool, possibly mucosal ferritin (Charlton *et al.* 1965; Savin & Cook, 1980), and as such would not alter the specific activity of the ^{59}Fe relative to the test dose. The estimate of expected non-obligatory Fe loss due to mucosal cell turnover (25–37%) may, however, carry a sufficient latitude of error as to leave open the possibility that up to approximately 10% of mucosal Fe may have been in an exchangeable form, and that the lower ^{59}Fe transfer in the high-Fe group was caused by an expanded pool of exchangeable Fe in the mucosal cells of these animals compared with controls. The mucosal uptake of Fe in the high-Fe-6 h group, as a percentage of the original dose, was 28.7% (Table 2), which indicates that 34.4 μg of the 120 μg radiolabelled Fe administered in the oral dose had been taken up from the lumen. The majority of this 34.4 μg radiolabelled Fe would have been taken up by the duodenum (Manis & Schachter, 1962; Johnson *et al.* 1983). To account for the difference in mucosal transfer of radiolabel between groups at 6 h (control group 77%, high-Fe group 32%), the ^{59}Fe label associated with the 34.4 μg Fe would have to be diluted in the duodenal mucosal cells of the high-Fe group by a factor of 2.4 (77/32); that is, by 48 μg exchangeable 'cold' Fe. The non-obligatory Fe in the duodenal mucosal tissue of the high-Fe group at 6 h, when corrected for 6 h of Fe loss through mucosal cell turnover (Table 3), would suggest an Fe content at the time of dosing no greater than 70 μg :

$$((73.4 - 10 \mu\text{g Fe}) + (6 \text{ h} \times 1.1 \mu\text{g Fe/h})).$$

Even if 10% of this Fe were in an exchangeable form, it is evident that the maximum absolute amount of Fe that could be in any exchangeable pool would be insufficient to dilute the specific activity of ^{59}Fe within the mucosal cells to anywhere near the extent needed to explain lower mucosal transfer in the high-Fe pretreated group of rats in terms of mucosal radioisotope dilution. It can be concluded, therefore, that radioisotope dilution within duodenal mucosal cells is minimal.

Total Fe and ^{59}Fe associated with the mucosal tissue would not have necessarily all been intracellular, and the possibility that changes in specific activity may have occurred in the intervillous space should be considered. In the high-Fe group, ^{59}Fe from the test dose may have undergone dilution within this space by dietary Fe remaining from the previous meal, and any ^{59}Fe associated with the intervillous space at the time of death could have been lost when the tissue was rinsed with saline. The gentle rinsing procedure employed in the study was designed to remove only particulate matter from the intestine and it is highly unlikely that the relatively small volume (20 ml) of saline used, and the short time required to complete the procedure (approximately 5 s) would have been sufficient to allow exchange of solutes between the intervillous space and the perfusing solution (Blackburn & Johnson, 1983).

We conclude that for non-anaemic rats of similar Fe status there is no evidence of either lumen or mucosal ^{59}Fe dilution when a test dose is administered 16 h after a meal, even when excessively high Fe intake before ^{59}Fe dosing results in a marked increase in total mucosal Fe in the duodenum.

The authors thanks Ms J. Cooke for skilled animal care.

REFERENCES

- American Institute of Nutrition (1977). Report of the American Institute of Nutrition *ad hoc* committee on standards for nutritional studies. *Journal of Nutrition* **107**, 1340–1348.
- Blackburn, N. A. & Johnson, I. T. (1983). The influence of guar gum on the movements of inulin, glucose and fluid in rat intestine. *Pflügers Archiv* **397**, 144–148.
- Brown, E. B. & Rother, M. L. (1963). Studies of the mechanisms of iron absorption. II. Influence of deficiency and other conditions on iron uptake by rats. *Journal of Laboratory and Clinical Medicine* **62**, 804–816.
- Charlton, R. W., Jacobs, R., Torrance, J. D. & Bothwell, T. H. (1965). The role of the intestinal mucosa in iron absorption. *Journal of Clinical Investigation* **44**, 543–554.
- Fairweather-Tait, S. J., Swindell, T. E. & Wright, A. J. A. (1985). Further studies in rats on the influence of previous iron intake on the estimation of bioavailability of Fe. *British Journal of Nutrition* **54**, 79–86.
- Fairweather-Tait, S. J. & Wright, A. J. A. (1984). The influence of previous iron intake on the estimation of bioavailability of Fe from a test meal given to rats. *British Journal of Nutrition* **51**, 185–191.
- Fairweather-Tait, S. J. & Wright, A. J. A. (1987). The importance of status and previous diet of animals on the estimation of bioavailability of different forms of metallic elements: studies on Fe. *Toxicological and Environmental Chemistry* **13**, 223–228.
- Field, M., Seki, M., Mitchell, M. L. & Chalmers, J. C. (1960). Studies on iron absorption. I. Determination in rats by measurement of total body radioactivity. *Journal of Laboratory and Clinical Medicine* **55**, 929–935.
- Flanagan, P. R., Haist, J. & Valberg, L. S. (1980). Comparative effects of iron deficiency induced by bleeding and a low-iron diet on the intestinal absorptive interactions of iron, cobalt, manganese, zinc, lead and calcium. *Journal of Nutrition* **110**, 1754–1763.
- Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M. & Whipple, G. H. (1943). Radioactive iron absorption by gastro-intestinal tract. Influence of anaemia, anoxia, and antecedent feeding. Distribution in growing dogs. *Journal of Experimental Medicine* **78**, 169–188.
- Hallberg, L. (1982). Iron absorption and iron deficiency. *Human Nutrition: Clinical Nutrition* **36C**, 259–278.
- Johnson, G., Jacobs, P. & Purves, L. R. (1983). Iron-binding proteins of iron-absorbing rat intestinal mucosa. *Journal of Clinical Investigation* **71**, 1467–1476.
- Lipkin, M. (1981). Proliferation and differentiation of gastrointestinal cells in normal and disease states. In *Physiology of the Gastro-intestinal Tract*, pp. 145–168 [L. R. Johnson, editor]. New York: Raven Press.
- Manis, J. G. & Schachter, D. (1962). Active transport of iron by intestine: features of the two-step mechanism. *American Journal of Physiology* **203**, 73–80.

- Marx, J. J. M. (1979). Mucosal uptake, mucosal transfer and retention of iron, measured by whole-body counting. *Scandinavian Journal of Haematology* **23**, 293–302.
- Monsen, E. R. (1974). Validation of an extrinsic iron label in monitoring absorption of nonheme food iron in normal and iron-deficient rats. *Journal of Nutrition* **104**, 1490–1495.
- Savin, M. A. & Cook J. D. (1980). Mucosal iron transport by rat small intestine. *Blood* **56**, 1029–1035.
- Van Campen, D. (1983). Iron bioavailability techniques: an overview. *Food Technology* **37**, 127–132.
- Wheby, M. S. & Crosby, W. H. (1963). The gastrointestinal tract and iron absorption. *Blood* **22**, 416–428.