The effect of wheat bran on the absorption and accumulation of cadmium in rats

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1. The major purpose of the present study was to determine whether the addition of wheat bran to endosperm crisp bread in a composite diet given to rats for 6 weeks causes an increase in the accumulation of cadmium in the rats due to the Cd content in the bran, or whether binding factors in the bran, such as dietary fibre and phytic acid, reduce or prevent the accumulation of this Cd. A second purpose was to determine whether the accumulation of Cd can be estimated by measuring the absorption of [109]Cd given in a single meal of the diet.

2. Three groups of eight rats were fed on one of three diets. Half of each diet consisted of a basal mixture of starch, protein, oil, minerals and vitamins. The remainder consisted of crisp breads based on refined wheat flour (endosperm group), wheat flour + bran in equal amounts (bran group) and wheat flour + Cd to give a Cd content similar to that of the bran group (endosperm + Cd group). After 41 d on the diets, the rats were deprived of food but not water for 12 h and then given a 5 g test meal of their respective diets with [109]Cd added. After 3 h the remaining [109]Cd-labelled diets were replaced with the unlabelled diets for 3 h before the rats were killed.

3. The total Cd contents in the wall of the proximal small intestine, including mucus, and in the liver and kidneys were highest in the endosperm + Cd group and lowest in the endosperm group. The amounts of Cd in the intestinal wall, including mucus, and in the liver and kidneys which derived from the test meal (calculated from [109]Cd accumulation) were significantly higher in the endosperm + Cd group than in either of the other two groups. The concentration of Cd in the organs of the bran group which derived from the test meal was not significantly greater than that in the endosperm group.

4. The individual variation in Cd content in the liver and kidneys make it very difficult to demonstrate differences in Cd accumulation from the relatively low, naturally occurring Cd concentrations in the diets. Differences in the absorption of [109]Cd from test meals indicate that very little of the Cd in bran is available for absorption.

Cadmium is a potentially dangerous inorganic pollutant and is one of the major environmental problems of our time. During the last 50 years the concentration of Cd in the soil has increased by at least 0.3%/year. The decreasing pH in our environment combined with the increasing Cd concentration in the soil have led to an increased uptake of Cd in crops (Andersson, 1982). Winter wheat is the most important crop for the intake of Cd in the Swedish population (Andersson & Pettersson, 1981). The concentration of Cd is higher in wheat bran than in the endosperm (Jorhem *et al.* 1984). In bran, Cd and other minerals are probably bound to fibre and phytic acid. If bran is included in a diet as a source of dietary fibre, its ingestion may give rise to an increased Cd accumulation. However, it is also possible that binding factors in bran may prevent or reduce an increase in Cd absorption and accumulation.

While many authors have demonstrated an effect of dietary fibre or phytic acid, or both, on the intestinal absorption of essential minerals such as zinc and iron (Sandström *et al.* 1980; Simpson *et al.* 1981; Björn-Rasmussen, 1983), only a few studies have been made to determine their possible effects on the absorption of toxic minerals such as Cd. Results have been published which indicate that Cd absorption in rats may be decreased by the addition to the diet of fibre in the form of Konjac powder (Omori & Muto, 1977) or lignin (Kiyozumi *et al.* 1982). Jackl *et al.* (1985) studied the retention of [109]Cd in various organs in rats after a single dose of labelled cadmium-3-phytate and found that phytate is

responsible for a considerable decrease in the intestinal absorption of Cd. Rose & Quarterman (1984) have studied the effects of phytic acid and calcium on Cd uptake and depletion in rats. They found that the Cd contents in the liver and kidneys were unaffected by addition of phytic acid to the diet during the accumulation period or by the addition of phytate to the diet immediately after terminating exposure to diets supplemented with Cd. They also found that both the concentrations and the total organ contents of Cd in the liver and kidney were significantly increased when a Ca supplement was given alone but not when Ca was given with phytate. The test doses of Cd used in the previously described studies were either extremely high or were given in an unnatural way or form. Thus little information is available on the absorption and retention of naturally occurring Cd in the diet or the possible reduction in absorption due to the presence of dietary fibre or phytic acid, or both.

The objectives of the present study in rats were (1) to determine whether the addition of wheat bran to endosperm crisp bread in a composite diet given to rats for 6 weeks causes an increased accumulation of Cd in the rats due to the Cd content in the bran, or whether binding factors in the bran, such as dietary fibre and phytic acid, reduce or prevent the accumulation of this Cd; (2) to determine whether or not the accumulation of Cd can be estimated by measuring the absorption of ^{109}Cd given in a single meal of the diet; and (3) to examine the Cd and ^{109}Cd contents in segments of the upper small intestine shortly after the ingestion of the test meal in order to obtain an indication of where and when Cd is absorbed.

MATERIAL AND METHODS

Animals

Twenty-four male rats of the Sprague–Dawley strain (Anticimex, Sollentuna, Sweden) were used. At the start of the experiment they were 3 weeks old and weighed 66·4 (SD 3·7) g. They were assigned to one of three groups of eight rats by formal randomization and housed separately in metabolic cages of acrylic resin and stainless steel (Ehret 13–1700, Emmendingen, West Germany).

Experimental diets

Three experimental diets were used (Table 1). They were composed using one of three crisp breads, two endosperm and one bran bread. Both endosperm breads were prepared from refined wheat flour, which was not enriched with any mineral, together with water, margarine, yeast, sugar and salt. Cadmium chloride was added to the dough of one of the endosperm breads to make the concentration equal to that in the bran-enriched bread. In the bran-enriched bread, half the endosperm wheat flour by weight was replaced by bran. The breads were not raised. They were baked for 15 min at 180 ° in a hot-air oven. A basic diet, comprising potato starch, milk protein, maize oil, mineral salts and vitamins, was mixed 1:1 by dry weight with each of the breads in a blender.

The radionuclide-labelled test meals were prepared from 25 g (dry weight) of each bread to which 100 μCi ^{109}CdCl$_2$ (New England Nuclear, Boston, MA) in 1·0 ml 0·1 M-hydrochloric acid were added. After drying the breads at 90 ° for 3 d, 25 g (dry weight) of the basic diet was mixed with each bread.

Experimental procedures

Each of the three groups of rats was given one of the three diets and deionized water *ad lib.* for 41 d. The rats were then deprived of food but not water for 12 h. After the fast, the rats were given 10 μCi ^{109}Cd in a 5 g test meal. After 3 h, when all the rats had eaten at least

Table 1. *Composition of diets (/kg dry weight). Diets comprised one part basic diet and one part crisp bread**

Diet...	Endosperm†	Endosperm + Cd	Bran‡
Chief components:			
Ash (g/kg)	43	43	50
Protein (g/kg)	118	118	133
Fat (g/kg)	112	112	93
Carbohydrate (g/kg)	694	694	614
Dietary fibre (g/kg)	33	33	110
Subcomponents:			
Phytic acid (mmol/kg)	1·0	1·0	8·6
Ca (g/kg)	5·6	5·6	5·8
Zn (mg/kg)	21·5	23·0	39·8
Cd, diet (µg/kg)	16·0	33·1	31·3
Cd, test meal (µg/kg)	18·0	35·1	33·3

Vitamin fortification (/kg diet): 118 mg tocopherol, 45 mg retinol, 88 µg ergocalciferol, 14 mg thiamin chloride, 13 mg riboflavin phosphate, 7 mg pyridoxine chloride, 33 mg nicotinamide, 66 mg *myo*-inositol, 30 µg biotin, 66 mg 4-aminobenzoic acid, 33 mg calcium pantothenate, 1·3 mg folic acid, 20 µg cyanocobalamin, 0·9 g choline chloride.

Mineral fortification (mg/kg diet): 21 $CuSO_4.5H_2O$, 33 KI, 1 $CoCl_2.H_2O$, 67 $ZnSO_4.7H_2O$, 167 $MnSO_2.H_2O$, 1130 $FeSO_4.7H_2O$, 4170 $MgSO_4.7H_2O$, 4960 NaCl, 7470 $NaH_2PO_4.H_2O$, 15830 $CaCO_3$, 16210 KH_2PO_4.

* For details, see p. 384.
† Prepared from refined wheat flour. For details, see p. 384.
‡ Half of the endosperm wheat flour replaced by bran.

half the test meal, the radionuclide-labelled diets were replaced with the unlabelled diets. After a further 3 h the rats were killed by exsanguination under diethylether anaesthesia.

Sample collection and analysis

Both kidneys, one-quarter of the liver which was cut into five samples, 250 mm of the proximal small intestine which was rinsed with deionized water at room temperature and cut into three segments (0–50 mm, 50–150 mm and 150–250 mm from the pylorus) as well as samples of the breads and the basic diet were taken in weighed glass tubes. The stomach, the contents of the proximal small intestine, the remainder of the small intestine, the caecum and the colon with their respective contents and the faeces were collected in separate plastic tubes. The fresh weights of the samples of liver and kidney were recorded. The samples taken in glass tubes were dried at 110 ° for 2 d and weighed again. They were then ashed for 2 d at 550 °. After being dissolved in 1·0 ml 3 M-HCl, they were evaporated to dryness at 90 ° (2 d) and then dissolved again in 2 ml 0·1 M-nitric acid.

The possible losses of Cd during dry ashing at 550 ° were studied. Two rats were injected subcutaneously with 20 µCi [109]$CdCl_2$. After 5 d the rats were killed. Four samples of kidney and five samples of liver were then collected from each rat. The [109]Cd activity in each sample was measured after drying. The samples were then ashed in the same way as the samples in the present study and the activity was measured again. The mean recovery of [109]Cd activity in the kidney and liver samples after ashing at 550 ° was 99·5%. However, 1·5% of this activity was retained in the walls of the glass tubes. Thus the true recovery was 98·0%.

Measurements and determinations

The [109]Cd activity in all samples was measured using a Packard Model 5330 gamma spectrometer with a 74 × 84 mm sodium iodide (T1) through-hole scintillation detector. 0·1 µCi [109]Cd in an appropriate volume was measured as a reference. The background

Table 2. *Instrument variables in the determination of cadmium**

Step	Temperature (°)	Ramp time (s)	Hold time (s)
Drying	120	5	20
Ashing	300	5	10
Atomization	1300	0†	3
Cleaning	2500	1	3
Cooling	50	1	9

* Non-pyrolytic graphite tubes with a platform were used. The injected volume was 20 μl. Internal gas flow was arrested during atomization. The purge gas was argon. Peak height absorbance was recorded.
† Maximum power heating.

counting rate was recorded and subtracted from the sample and reference counting rates.

The dissolved samples were further diluted with 0·1 M-HNO_3 as needed. The Cd concentrations were determined by the method of standard addition using a Perkin-Elmer Model 3030 atomic absorption spectrophotometer equipped with a deuterium background corrector, a HGA-500 graphite furnace and an AS-40 autosampler (Table 2). Known amounts of Cd in 0·1 M-HNO_3 were used as references. Four determinations were made on each sample and the mean of the four determinations was calculated. The median of the five means from each liver was used in the statistical calculations as the variation of the Cd contents of the five samples from each liver was large and skewed.

The accuracy of the method for Cd determination was assessed using US National Bureau of Standards (NBS) bovine liver (no. 1577a) and wheat flour (no. 1567) which were analysed in the same way as the samples in the present study. The ranges for the bovine liver and the wheat flour certified by NBS were 380–500 and 25–39 ng Cd/g dry weight respectively. Our mean values for the bovine liver and for the wheat flour were 460 (SD 9) and 32·3 (SD 1·4) ng Cd/g respectively.

The phytic acid concentrations were measured using the method of Ellis *et al.* (1977) as modified by Sandberg *et al.* (1982). The dietary fibre concentrations were determined using the method of Asp *et al.* (1983).

Calculations

The total consumed dose of ^{109}Cd was defined as the total amount of ^{109}Cd in the gastrointestinal tract. The total uptake in the body was assumed to be no more than twice the retention in the liver and kidneys combined (Friberg *et al.* 1974). In the present study, twice the amount of ^{109}Cd in these tissues was less than 0·2% of the total consumed dose and these values were thus excluded from the calculations. The ^{109}Cd content in the various segments of the gastrointestinal tract was normalized to the total consumed dose of ^{109}Cd in each rat.

An estimate of the relative uptake in the liver and kidneys of the ^{109}Cd from the test meal which had passed the pylorus was calculated by dividing the ^{109}Cd content in the liver or kidneys by the ^{109}Cd activity in the intestine and its contents, the latter activity being used as a measure of the ^{109}Cd available for absorption. The ^{109}Cd which had been absorbed by the body was excluded from the ^{109}Cd available for absorption as it was negligible.

The Cd content in the wall of the proximal small intestine, the liver and the kidneys which derived from 1 g of the test meal was calculated by multiplying the relative ^{109}Cd uptake in each sample by the Cd concentration in the test meal.

While it is quite likely that ^{109}Cd and dietary Cd freely exchange, no studies have yet been

reported in the literature on comparisons of the absorption of [109]Cd from extrinsically and intrinsically labelled diets. Such studies have been made on non-haem Fe (Björn-Rasmussen *et al.* 1972, 1973) and Zn (Evans *et al.* 1977) and no significant differences in the absorption of extrinsic and intrinsic radioisotopes have been demonstrated. Such studies should also be made on [109]Cd in order to provide the knowledge necessary for the correct interpretation of [109]Cd absorption studies.

Statistics

The means for a given variable in the three groups were tested using a one-way analysis of variance. If a statistically significant result was obtained, the differences between means of the groups for the variable were tested using Student's *t* test for unpaired observations. The *t* test was modified if the variances were significantly different (*F* test). Pearson product moment correlation coefficients were calculated for pairs of variables within groups and tested with Student's *t* test. In all statistical tests we have chosen to reject the null hypothesis at the 1% level ($P < 0.01$).

RESULTS

Body-weight

The animals were healthy throughout the experiment. Their body-weights increased from 66·4 (SD 3·7) to 275·6 (SD 12·1) g during the 6 weeks on the test diets. This increase in weight was slightly less than would be expected on a standard pellet diet. There were no significant differences in the gain in body-weight among the three groups.

Cd content

The Cd content per mm of the upper intestinal wall was highest in the segments 0–50 mm from the pylorus (duodenum) and lowest in the 150–250 mm segment in all three groups (Fig. 1). The Cd content decreased nearly exponentially distally in all three groups. The upper intestinal wall of the group given endosperm bread with added Cd (endosperm + Cd group) had a significantly higher Cd content than that of the group given bran bread (bran group) only in the segment 50–150 mm from pylorus. The group given endosperm bread with no added Cd (endosperm group) had significantly lower Cd contents in all three segments than those in the other two groups.

The variation in the Cd contents in the liver and kidneys among the three bread groups followed in principle the same pattern as that in the intestinal wall (Fig. 2). The only relatively certain indication of differences in Cd accumulation in these organs after 6 weeks on the three composite diets was a significant difference in the liver Cd content between the groups given endosperm bread with and without added Cd.

[109]Cd activity

The animals consumed between 50 and 99% of the radionuclide-labelled test diets. The [109]Cd content was low in the proximal and distal small intestine and high in the colon in the bran group compared with the two endosperm groups (Fig. 3). There was no [109]Cd activity in the faeces of any group.

The Cd content per mm intestinal wall (with its mucus) which derived from 1 g of the test meal decreased nearly exponentially distally, nearly parallelling the decrease in total Cd content (Fig. 1). This Cd in the intestinal wall of the endosperm + Cd group was significantly higher than that in the other two groups but the Cd content in the bran group was not significantly higher than that in the endosperm group. Disregarding differences among the dietary groups, the proportion of the Cd content which derived from the test
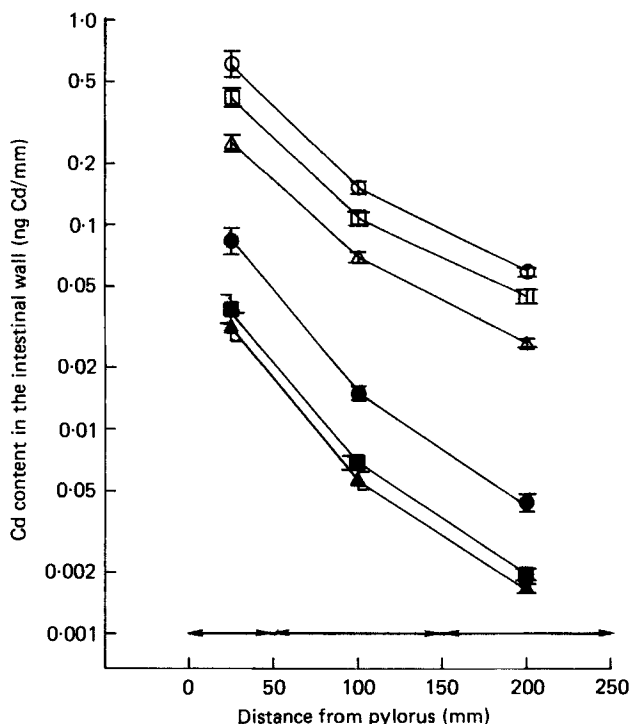
Fig. 1. The cadmium content per mm intestinal wall including mucus (0–250 mm from pylorus) from rats given the three diets (see p. 384 for details). ○, △, □, Total Cd content; ●, ▲, ■, Cd content derived from 1 g of the test meal. △, ▲, Endosperm group; ○, ●, endosperm+Cd group; □, ■, bran group. The endosperm bread was prepared from unrefined wheat flour. Half of this flour by weight was replaced by bran in the preparation of the bran-enriched bread. The values are geometric means, with their standard errors represented by vertical bars.

meal in each segment of the upper small intestine was between 4·4 and 14·3 % of its total Cd content.

The uptake of ^{109}Cd in the liver was 0·033 (SE 0·005) % of that which had passed the pylorus in the endosperm group, 0·041 (SE 0·004) % in the endosperm+Cd group and 0·019 (SE 0·001) % in the bran group. In both kidneys the uptake of ^{109}Cd was 0·0048 (SE 0·0004) % in the endosperm group, 0·0056 (SE 0·0002) % in the endosperm+Cd group and 0·0038 (SE 0·0004) % in the bran group. The ^{109}Cd uptake in the liver and kidneys in the bran group was significantly less than that in the endosperm+Cd group.

The Cd contents of the liver and kidneys which derived from 1 g of the test meal were significantly higher in the endosperm+Cd group than in either of the other two groups but the Cd contents of the liver and kidneys in the bran group were not significantly higher than those in the endosperm group (Fig. 2).

DISCUSSION

The only relatively certain indication of differences in Cd accumulation after 6 weeks on the three composite diets was a significant difference in the liver Cd content between the groups given endosperm bread with and without added Cd. There are two probable explanations for this result. It may be that there was very little difference in the total absorption of Cd from the three diets regardless of their Cd concentrations and the presence or absence of
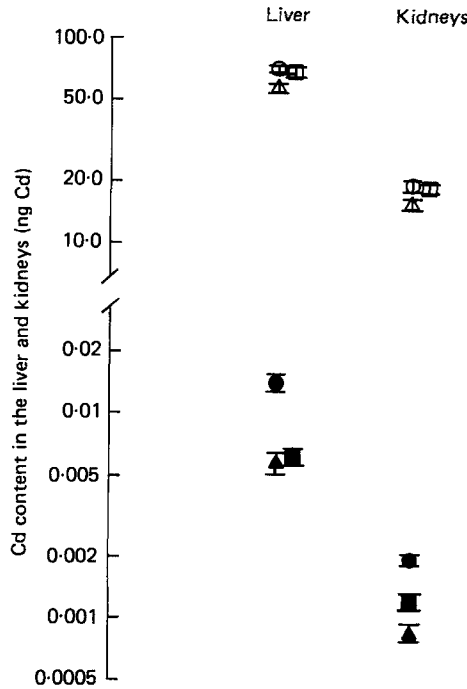
Fig. 2. The cadmium content in the whole liver and both kidneys from rats given the three diets (see p. 384 for details). ○, △, □, Total Cd content; ●, ▲, ■, Cd content derived from 1 g of the test meal. △, ▲, Endosperm group; ○, ●, endosperm + Cd group; □, ■, bran group. The endosperm bread was prepared from unrefined wheat flour. Half of this flour by weight was replaced by bran in the preparation of the bran-enriched bread. The values are geometric means, with their standard errors represented by vertical bars.
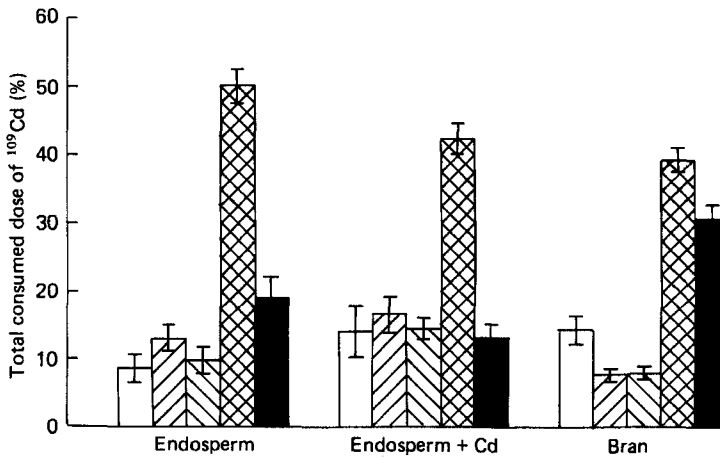
Fig. 3. The distribution of ^{109}Cd in the gastrointestinal tracts of the rats given the three diets (see p. 384 for details). □, Ventricle; ▨, proximal small intestine (0–250 mm from pylorus); ▦, distal small intestine; ⊠, caecum; ■, colon. The endosperm bread was prepared from unrefined wheat flour. Half of this flour by weight was replaced by bran in the preparation of the bran-enriched bread. The values are means, with their standard errors represented by vertical bars.

wheat bran. It is also possible that small differences in Cd accumulation from diets with relatively low, naturally occurring Cd concentrations were concealed by large initial Cd contents in the liver and kidneys and large inter-individual variations in Cd content. In another study on rats from the same source (own unpublished results), the Cd content in both kidneys in 3-week-old, weanling rats was 11·5 (SD 3·1) ng which is two-thirds to three-quarters of the Cd content in the kidneys after 6 weeks on the diets in this experiment (Fig. 2).

Even if all the Cd in both the liver and kidneys came from these diets, no more than 1 % of the Cd in any diet was accumulated in these organs (assuming that the rats ate at least 500 g during the 6-week experiment). If two-thirds to three-quarters of the Cd contents in the liver and kidneys were present at the beginning of the experiments at 3 weeks of age, then the accumulation from the diets would be less than 0·3 %. Friberg et al. (1974) have reported that the absorption of Cd in mice is as high as 2% of the given dose while Engström & Nordberg (1979) have found that the absorption of ^{109}Cd in mice was less than 1 %. However, the Cd concentrations in the diets in those studies were much higher than those in the present study and the Cd was administered in another way or form, or both.

If a method using the measurement of ^{109}Cd absorption from a test meal could be shown to give results which were equivalent to those from experiments in which diets are maintained for very long periods after which the Cd content of selected organs is measured, then it would be possible to measure Cd accumulation even from diets with very low Cd concentrations and to shorten experiments by many weeks or months. In the present study significant increases in Cd accumulation in the liver and kidneys, calculated from ^{109}Cd absorption, were seen in the endosperm + Cd group compared with both the endosperm and bran groups. No differences were seen between the bran group and the endosperm only group. According to these results, the addition of bran to the diet does not increase Cd accumulation. As it was not possible to demonstrate such differences in the total liver and kidney Cd contents, it is not possible to determine whether or not the two methods give similar results.

The Cd accumulation in the liver and kidneys together, calculated from the ^{109}Cd absorption and assuming that the rats ate 500 g of the diets in 42 d, is at most 9% of their total Cd content and most likely is between 10 and 25% of the presumed increase in Cd content between 3 and 9 weeks of age. This discrepancy may be due to an incomplete absorption of ^{109}Cd 3–6 h after ingestion or to a possible decrease in Cd absorption with age, or both. There is a considerable amount of ^{109}Cd activity in the wall of the upper small intestine and its mucus 3 h after the end of the 3 h ^{109}Cd ingestion period. If only 10% of the activity remaining in the upper intestine were absorbed at a later time it would suffice to account for the presumed increase in Cd accumulation in the liver and kidneys between 3 and 9 weeks of age. As between two-thirds and three-quarters of the Cd contents of these organs may have been present at 3 weeks of age, it may also be that Cd absorption decreases with age (Engström & Nordberg, 1979) and that the measurement of ^{109}Cd absorption made at 9 weeks should be lower than the average for the 6 week period on the diets.

It is possible that the ^{109}Cd did not come to a complete equilibrium with the Cd in the test meal before its entrance into the small intestine. If this was the case then the absorption of Cd from the test meals would have been overestimated when the absorption was complete. The greatest systematic error would be expected to occur in the calculation of the amount of Cd absorbed from the bran diet as binding factors in bran may make the Cd less available for exchange with ^{109}Cd. While the absorption of Cd from the bran meal as shown in Figs. 1 and 2 would be overestimated relative to that from the two endosperm meals, this would not in any way change the conclusion that factors in bran reduce the absorption of

Cd despite the higher Cd concentration in the bran diet. Further studies are necessary to determine whether or not values derived from ^{109}Cd absorption are valid measures of Cd accumulation and under which conditions they may be accurate.

Within each group for each intestinal segment the amount of Cd derived from 1 g of the test meal was positively correlated with the total amount of Cd present (r 0·55–0·92) but the correlations were significant in only two cases. Neither of these variables were correlated with the amount of test meal which had passed the pylorus (r–0·51 to 0·70). Both the total Cd content per mm intestinal wall including mucus and the Cd content calculated to have derived from 1 g of the test meal decreased nearly exponentially distally. The exponential decrease in Cd distally is an interesting observation which, together with the calculation which indicated that less than 10 % of the ^{109}Cd remaining in the mucus or intestinal wall, or both, 3 h after the test meal will be absorbed, may be evidence of Cd being bound to the intestinal mucus (Quarterman, 1984) or the epithelium. Such binding would occur most readily directly distal to the pylorus where the pH of the intestinal contents is low. The Cd transfer to the mucus and the intestinal wall would be expected to decrease distally as the pH rises (Ovesen *et al.* 1986). The loss of Cd from the wall with its mucus should then parallel the turnover of mucus or epithelium, or both. This should also be the subject of further investigation.

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