Iron status in rats fed on diets containing marginal amounts of vitamin A

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Severe vitamin A deficiency in rats is known to cause anaemia associated with growth retardation and impaired water retention. However, study of the effect of marginal vitamin A intake is of more interest because such intake may mirror the situation in humans in many developing countries. Therefore, in two experiments, the effect of marginal vitamin A deficiency on Fe status was investigated in male rats. After 28 d of feeding either low- or high-vitamin A diets (0 or 120 *v.* 1200 retinol equivalents/kg feed), body weight and feed intake were not influenced by the level of vitamin A in the diet. Liver weight was lowered by vitamin A deficiency. Water intake was not influenced in rats fed on a low-vitamin A diet. Plasma retinol concentrations were decreased in rats fed on diets low in vitamin A. Marginal vitamin A deficiency produced slightly lower blood haemoglobin concentrations; it did not systematically affect packed cell volume. The concentration of Fe in liver was significantly higher when diets low in vitamin A were fed, but hepatic Fe mass was not affected. Significantly lower Fe levels were observed in femurs of rats with vitamin A deficiency. The effects on liver and femur Fe concentrations were seen with diets adequate in Fe but not with diets deficient in Fe. The efficiency of apparent Fe absorption was significantly increased by low intakes of vitamin A, provided that the dietary Fe concentration was adequate. It is speculated that depressed uptake of Fe by bone marrow is the primary feature of altered Fe status in rats with marginal vitamin A deficiency.

Vitamin A: Iron: Anaemia: Rat

In women and children a direct correlation between plasma levels of vitamin A and blood levels of haemoglobin has been found (Hodges *et al.* 1978; Mejia *et al.* 1982; Bloem *et al.* 1989). This relationship is probably causal as experimentally induced vitamin A deficiency in humans produces anaemia (Wagner, 1940; Hodges *et al.* 1978). Intervention studies have also shown that supplementation of deficient children with vitamin A improves Fe status and increases haematopoiesis (Mohanram *et al.* 1977; Mejia & Arroyave, 1982; Bloem *et al.* 1990). Thus, vitamin A would appear to be essential for normal haematopoiesis, but its metabolic role has not been elucidated.

Vitamin A deficiency in rats causes anaemia associated with increased Fe concentrations in liver and spleen (Mejia *et al.* 1979*a*, *b*; Staab *et al.* 1984), suggesting interference with mobilization of Fe from tissue stores. Alternatively, vitamin A deficiency could inhibit Fe uptake and/or haematopoiesis by bone marrow. The depressed Fe utilization may in turn cause accumulation of Fe in storage organs. Indeed, the uptake of intravenously injected radiolabelled Fe by the liver and spleen was significantly increased in rats with vitamin A deficiency, whereas that by the femur was decreased (Mejia *et al.* 1979*b*). This implies that

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vitamin A deficiency produces decreased Fe concentrations in the femur. It could also be hypothesized that altered tissue Fe distribution as induced by vitamin A deficiency triggers changes in Fe absorption. In rats fed on diets deficient in Fe and, thus, displaying reduced Fe status, the efficiency of Fe absorption is increased (Morris, 1987).

It is questionable whether the reported studies on anaemia and Fe status in rats with vitamin A deficiency (Mejia *et al.* 1979*a*, *b*; Staab *et al.* 1984) have used a model suitable for elucidating the mechanisms involved in the anaemias in humans in developing countries where marginal vitamin A intake is a problem. In these rat studies vitamin A stores were almost completely depleted. There were clinical signs of severe vitamin A deficiency such as growth retardation and fluid loss leading to haemoconcentration, which may have even masked the degree of anaemia (Koessler *et al.* 1926; Amine *et al.* 1970; Corey & Hayes, 1972). Retarded growth can induce effects not specifically related to decreased vitamin A intake.

In the present study the major questions addressed were as follows: first, are liver Fe concentrations increased in male rats fed on diets containing marginal amounts of vitamin A? second, does marginal vitamin A deficiency influence Fe concentrations in the femur? third, does marginal vitamin A deficiency influence the efficiency of intestinal absorption of Fe?

MATERIALS AND METHODS

Two experiments were carried out; control and experimental diets were identical, apart from the amounts of vitamin A and/or Fe. The control diet (Table 1) contained 1200 retinol equivalents and 50 mg Fe/kg feed, which are the approximate requirements of young rats for these nutrients (National Research Council, 1978). In the first experiment the test diet contained no added vitamin A. In the second experiment the effect of decreased dietary vitamin A concentration (120 retinol equivalents/kg feed) was studied with dietary Fe concentrations that were either adequate or marginal (17 mg/kg feed).

Animals and housing

Male outbred Wistar rats (Cpb:WU), about 21 d old, were used. The rats were purchased from a commercial breeder (Harlan CPB, Zeist, The Netherlands). They had been fed *ad lib.* on a commercial pelleted diet (RMH-B®, Hope Farms, Woerden, The Netherlands) and tap water. The rats were kept in a room maintained at a constant temperature (20–22°) and relative humidity (40–60%), with light from 07.00 to 19.00 hours.

In the two experiments all rats were subjected to a pre-experimental period of 10 d during which the purified control diet given in Table 1 was supplied *ad lib.* The rats had free access to demineralized water. The rats were housed in groups of three or four animals in wire-topped polycarbonate cages (375 × 225 × 150 mm) with a layer of sawdust as bedding. After the pre-experimental period (day 0 of the experiment) the rats were transferred to individual metabolism cages (31400 mm^2 × 120 mm).

Experimental design

On day 0 the rats were divided into two (Expt 1) or four (Expt 2) dietary groups of equal size so that within each experiment the group body weight distributions were similar. One group in each experiment remained on the control diet and the others received a diet containing less vitamin A (Expt 1) and/or less Fe (Expt 2). The rats were fed on powdered, purified diets which were stored at 4° until feeding, and provided with demineralized water *ad lib.* Separate batches of diets were made for each experiment.

The diets during the experimental period were as follows: in Expt 1 twelve rats were fed on either the control diet (Table 1) or the same diet without added vitamin A; in Expt 2

Table 1. *Composition of the control diet (g/kg)*

Ingredients	
Casein	151
Maize oil	25
Coconut fat	25
Glucose	709·2
Cellulose	30
$CaCO_3$	12·4
$NaH_2PO_4.2H_2O$	15·1
$MgCO_3$	1·4
KCl	1·0
$KHCO_3$	7·7
Vitamin A preparation* (mg)	8
$FeSO_4.7H_2O$ (mg)	174
Vitamin premix, vitamin A-free†	12·0
Mineral premix, Fe-free‡	10·0

* Rovimix A 500®, 156·6 mg/g (F. Hoffmann-La Roche & Co. Ltd, Basle, Switzerland), consisting of retinyl acetate and retinyl palmitate; of this preparation 1200 retinol equivalents/kg feed was added.

† The vitamin A-free premix consisted of (mg): thiamin 4, riboflavin 3, nicotinic acid 20, DL-calcium pantothenate 17·8, pyridoxine 6, cyanocobalamin 50, choline chloride 2000, pteroylmonoglutamic acid 1, biotin 2, menadione 0·05, DL-α tocopheryl acetate 60, cholecalciferol 0·025, maize meal 9836·125.

‡ The mineral premix consisted of (mg): MnO_2 79, $ZnSO_4.H_2O$ 33, $NiSO_4.6H_2O$ 13, NaF 2, KI 0·2, $CuSO_4.5H_2O$ 15·7, $Na_2SeO_3.5H_2O$ 0·3, $CrCl_3.6H_2O$ 1·5, $SnCl_2.2H_2O$ 1·9, NH_4VO_3 0·2; maize meal 9853·2.

there were four dietary groups consisting of twelve rats each. One group received the control diet and another group was fed on the same diet with added $FeSO_4.7H_2O$ reduced to 17·4 mg/kg. Two further groups were fed on the control diet with vitamin A decreased to 120 retinol equivalents/kg feed and containing either 174 or 17·4 mg $FeSO_4.7H_2O$/kg. Chemical analysis (see below) showed that the high-vitamin A diets with high and low amounts of $FeSO_4.7H_2O$ contained 50 and 17 mg Fe/kg respectively. The low-vitamin A diets were found to contain 51 and 16 mg Fe/kg.

During the experimental periods, lasting 28 d, feed intake and body weights were recorded. In Expt 2 water intake was also determined. During days 26–28 of Expt 2 urine and faeces of each rat were collected quantitatively. At the end of each experiment the animals were anaesthetized with diethyl ether and blood was taken by orbital puncture. The anaesthetized rats were killed by cervical dislocation. Organs were removed and frozen at −20° until analysed.

Chemical analyses

Haemoglobin concentrations in heparinized blood were determined spectrophotometrically using a test kit (Roche, Mijdrecht, The Netherlands). Packed cell volume was measured by microcentrifugation (5 min at 5000 ***g***). Plasma Fe concentrations, total Fe-binding capacity and urinary Fe were determined spectrophotometrically using a kit (Roche). Retinol in plasma was determined by a reversed-phase HPLC method modified from that of Driskell *et al.* (1982), using retinyl acetate as an internal standard. Fe in liver, spleen, femur, faeces, and feed was estimated after wet ashing with HNO_3 by flame atomic absorption spectrometry (Kreeftenberg *et al.* 1985).

Statistical analysis

Differences between the groups fed on the diets containing high and low amounts of vitamin A were evaluated statistically with two-sided Student's *t* test, and *P* values are given. Data from Expt 2 were subjected to two-way analysis of variance. The probability

Table 2. *Expt 1. Effect of dietary vitamin A on growth performance, plasma retinol concentrations and iron status**

(Values are means for twelve animals per dietary group)

Dietary vitamin A concentration (retinol equivalents/kg)...	1200	0	Statistical significance of difference: *P*	Pooled SE
Body wt (g)				
Initial (day 0)	96	96	0·921	2·1
Final (day 28)	245	229	0·032	4·9
Liver wt (g)	8·9	7·9	0·018	0·28
Feed intake (g/d)	16·7	16·0	0·236	0·38
Plasma retinol (μmol/l)	1·60	1·45	0·022	0·045
Blood haemoglobin (mmol/l)	8·5	8·2	0·076	0·09
Packed cell volume	0·44	0·44	0·830	0·002
Fe				
Plasma (μmol/l)	32·6	33·2	0·798	1·42
Liver (μmol/g)†	2·1	2·3	0·000	0·04
Liver (μmol/organ)	18·4	18·4	0·940	0·58
Spleen (μmol/g)†	4·0	4·4	0·172	0·18
Femur (μmol/g)†	1·02	0·92	0·025	0·029

* For details of dietary treatments, see Table 1 and p. 778.
† On wet weight basis.

of a type I error $< 0{\cdot}05$ was taken as the criterion of significance. Pearson correlation coefficients were calculated for anticipated relationships between selected variables.

RESULTS

Growth

Feed intakes of rats fed on either the high- or low-vitamin A diets did not differ significantly (Tables 2 and 3). In Expt 1 final body weights were slightly but significantly lower in the rats fed on the diet without added vitamin A (Table 2). In Expt 2 final body weight was not significantly influenced by the decreased intake of vitamin A (Table 3). Liver wet weight was systematically reduced by low vitamin A intake. Water intakes in Expt 2 did not differ between the dietary groups (Table 3). The dietary Fe concentrations of 17 and 50 mg/kg feed did not have a different effect on either body weight or feed intake.

Plasma retinol concentrations

Plasma retinol concentrations were significantly lower in animals fed on a low-vitamin A diet compared with those fed on an adequate amount of vitamin A (Tables 2 and 3).

Blood variables of Fe metabolism

In rats fed on a diet without added vitamin A (Table 2) blood haemoglobin concentrations tended to be decreased. This was also seen in Expt 2 in rats fed on the Fe-adequate diet with low vitamin A concentration (Table 3). Control rats in Expt 1 had lower blood haemoglobin concentrations than those in Expt 2; the reason for this discrepancy is not known. Packed cell volume values were decreased significantly after feeding the low-vitamin A diets in Expt 2, but only if the diet was adequate in Fe (Table 3). In Expt 1

Table 3. *Expt 2. Effect of dietary vitamin A and iron on growth performance, plasma retinol concentrations and Fe status*†

(Values are means for twelve animals per dietary group)

Dietary Fe (mg/kg)‡...	51			17						
Vitamin A (retinol equivalents/kg)...	1200	120	Statistical significance of difference §: *P*	1200	120	Statistical significance of difference §: *P*	Pooled SE	Statistical significance‖ of effect of: Vitamin A (A)	Fe	Fe × A
Body weight (g)										
Initial (day 0)	97	96	0·934	97	97	0·973	1·9	—	—	—
Final (day 28)	223	219	0·640	234	225	0·216	5·8	—	—	—
Liver wt (g)	9·2	8·6	0·236	8·8	8·3	0·178	0·30	—	—	—
Feed intake (g/d)	15·6	14·9	0·416	16·4	15·0	0·062	0·58	—	—	—
Water intake (g/d)	15·3	16·3	0·547	15·5	14·1	0·513	1·41	—	—	—
Plasma retinol (μmol/l)	1·88	1·60	0·001	1·75	1·54	0·002	0·049	*	—	—
Fe status										
Blood haemoglobin (mmol/l)	10·9	10·2	0·052	9·0	8·9	0·806	0·23	—	*	—
Packed cell volume	0·45	0·44	0·027	0·42	0·41	0·557	0·004	*	*	—
Plasma Fe (μmol/l)	29·6	30·5	0·486	25·1	27·8	0·266	1·36	—	*	—
Total Fe-binding capacity (μmol/l)	85·1	82·3	0·520	95·5	95·8	0·051	3·42	—	*	—
Transferrin saturation (%)	35	38	0·310	27	29	0·347	2·0	—	*	—
Liver Fe (μmol/g)¶	2·0	2·5	0·001	0·9	0·9	0·754	0·07	*	*	*
Liver Fe (μmol)¶	18·7	21·0	0·081	7·9	7·3	0·423	0·76	—	*	—
Spleen Fe (μmol/g)¶	4·0	4·1	0·711	2·3	2·4	0·385	0·16	—	*	—
Femur Fe (μmol/g)¶	1·13	0·97	0·002	0·74	0·70	0·229	0·025	*	*	—
Fe balance										
Intake (μmol/d)	14·0	13·3	0·383	5·5	4·7	0·006	0·41	—	*	—
Faecal output (μmol/d)	11·8	10·3	0·061	2·5	2·2	0·052	0·34	*	*	—
Retention (μmol/d)	2·2	3·0	0·125	3·0	2·6	0·068	0·30	—	—	*
Apparent absorption (%)	15	23	0·038	54	55	0·990	2·3	—	*	—

* $P < 0·05$.
† For details of dietary treatments, see Table 1 and pp. 778–779.
‡ Analysed concentrations.
§ Compared with the group fed on the same amount of Fe but adequate vitamin A.
‖ Analysis of variance.
¶ On the basis of wet weight.

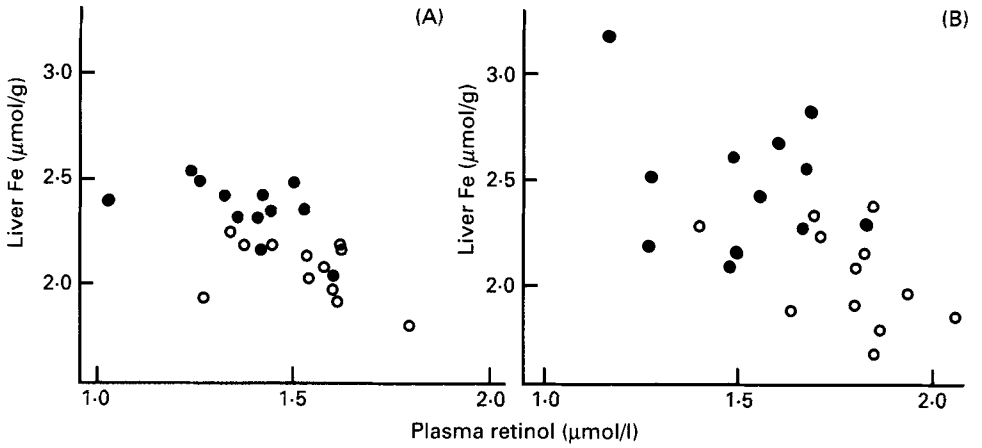

Fig. 1. Relationship between plasma retinol concentrations and liver iron concentrations in individual male rats fed on diets adequate in Fe but containing different amounts of vitamin A. (○), rats fed on an adequate amount of vitamin A ; (●), rats fed on marginal amounts of vitamin A. (A), Expt 1: $r-0{\cdot}63$, n 24, $P < 0{\cdot}001$; (B), Expt 2: $r-0{\cdot}60$, n 24, $P < 0{\cdot}001$. For details of dietary treatments, see Table 1 and p. 778–779.

packed cell volume was not affected by vitamin A intake (Table 2). Plasma Fe concentrations, total Fe-binding capacity, and saturation of plasma transferrin were not influenced significantly by vitamin A intake. In Expt 2 the effect of low intakes of iron was to decrease values for blood haemoglobin concentrations, packed cell volume, plasma Fe and percentage saturation of transferrin, whereas total Fe-binding capacity was increased (Table 3).

Iron content of organs

Liver Fe concentration was significantly increased after feeding the low-vitamin A diets, provided that the dietary Fe concentrations were adequate (Tables 2 and 3). Hepatic Fe mass was not influenced by the amount of vitamin A in the diet. Fig. 1 shows that in individual rats with varying plasma retinol concentrations there was a negative relationship between plasma retinol and liver Fe concentration.

In rats fed on diets containing marginal amounts of Fe, liver Fe concentrations were reduced by more than 50% (Table 3). In Fe-deficient rats dietary vitamin A concentration did not influence liver Fe concentrations (Table 3).

Spleen Fe concentrations were not affected by vitamin A intake. The Fe contents of femurs were significantly decreased in rats fed on the low-vitamin A diets containing an adequate Fe concentration (Tables 2 and 3). Decreased intake of Fe lowered femur Fe concentrations; low intake of vitamin A did not further lower femur Fe (Table 3).

Iron balance

Urine did not contain detectable amounts of Fe so that Fe balance was determined by Fe intake and faecal excretion only. In the rats fed on a diet adequate in Fe a decreased intake of vitamin A produced a significant ($P < 0{\cdot}05$) increase in the efficiency of apparent Fe absorption and tended to increase Fe retention (Table 3). For individual animals fed on the Fe-adequate diets but with varying amounts of vitamin A there was a negative correlation between plasma retinol concentrations and apparent Fe absorption ($r-0{\cdot}45$, n 24, $P = 0{\cdot}013$). Decreasing the intake of Fe caused an increase in the efficiency of Fe absorption associated with unchanged Fe retention. Vitamin A intake did not influence Fe balance in rats fed on the low-Fe diet.

DISCUSSION

The low-vitamin A diets which were fed for 28 d did not produce changes in body weight and feed intake while plasma retinol concentrations were only slightly decreased. On the basis of the reported relationship between plasma and liver retinol concentrations in rats (Wright & Hall, 1979) it can be estimated that liver retinol stores may have been reduced by about 80% in the rats fed on the low-vitamin A diets. However, caution is warranted when making such an inter-study comparison because experimental conditions may have varied. Nevertheless, we feel that the rats used in the present study can be considered marginally deficient in vitamin A. In contrast, other studies (Mejia *et al.* 1979*a*, *b*; Staab *et al.* 1984) on hypovitaminosis A and Fe status have used rats with severe vitamin A deficiency as evidenced by completely depleted stores of retinol in liver and growth retardation.

Despite the fact that our rats were only marginally deficient in vitamin A, changes in Fe metabolism could be detected. Marginal vitamin A deficiency slightly lowered group mean blood haemoglobin concentrations. In studies using rats with severe vitamin A deficiency increased haemoglobin concentrations and packed cell volume values have been found (Koessler *et al.* 1926; McLaren *et al.* 1965; Amine *et al.* 1970; Corey & Hayes, 1972). This can be explained by haemoconcentration as severe vitamin A deficiency induces impaired water retention (Mahant & Eaton, 1976). It is unlikely that the observed tendency towards decreased haemoglobin concentrations as induced by marginal vitamin A deficiency was biased by haemoconcentration. In Expt 2 water intake and urinary excretion were measured, and no effect of dietary vitamin A concentration was found. Furthermore, haemoconcentration only appears to occur when there is retardation of growth (Koessler *et al.* 1926; Corey & Hayes, 1972).

Although marginal vitamin A deficiency slightly lowered blood haemoglobin concentrations it did not influence plasma Fe concentrations, total Fe-binding capacity and transferrin saturation. Marginal Fe intake, on the other hand, lowered plasma Fe concentrations and saturation of transferrin and total Fe-binding capacity. This suggests that the mechanisms underlying the anaemias produced by deficiencies of Fe and vitamin A are different. This agrees with the observation that severe vitamin A deficiency causes hypochromic microcytic polycythaemia, whereas Fe deficiency results in hypochromic microcytic anaemia (Amine *et al.* 1970).

Further observations also indicate that vitamin A and Fe deficiencies have different effects on Fe metabolism. As observed in studies using rats with severe vitamin A deficiency (Mejia *et al.* 1979*a*, *b*; Staab *et al.* 1984), marginal vitamin A deficiency was found to elevate Fe concentrations consistently in liver. In the rats fed on Fe-adequate diets but different amounts of vitamin A there was a negative correlation between plasma retinol concentrations and liver Fe concentrations (Fig. 1). Femur Fe concentrations were slightly, but significantly, lower when marginal amounts of vitamin A were fed in diets adequate in Fe. As far as we know, effects of vitamin A status on Fe concentrations in the femur have not been reported previously.

In the rats fed on a diet adequate in Fe but with marginal vitamin A the efficiency of apparent Fe absorption was increased. It cannot be excluded that impaired Fe status as induced by vitamin A deficiency depresses the loss of endogenous Fe and that, unlike apparent absorption, true absorption of Fe was not raised. In any event, the increase in apparent Fe absorption tended to be associated with elevated Fe retention (Table 3). The inverse relationship between vitamin A intake or plasma retinol concentration and the efficiency of apparent Fe absorption may seem to be at variance with the observation that vitamin A deficiency lowered Fe status. One interpretation of these results is that vitamin

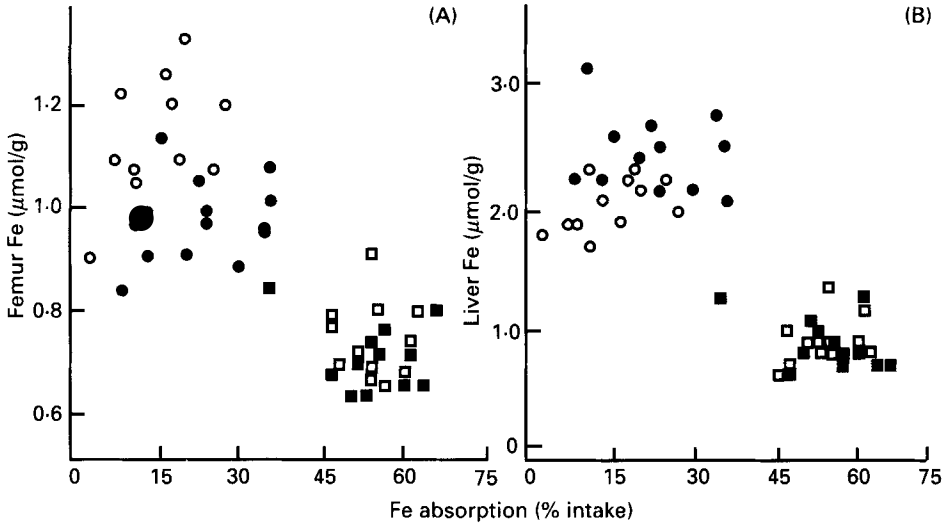

Fig. 2. Relationships between apparent iron absorption and either (A) femur Fe concentration (r −0·78, n 48, $P < 0·001$) or (B) hepatic Fe concentration (r −0·81, n 48, $P < 0·001$) for all individual animals of Expt 2. (○, □), Rats fed on an adequate amount of vitamin A; (●, ■), rats fed on a marginal amount of vitamin A; (○, ●), rats fed on an adequate amount of Fe; (□, ■), rats fed on a marginal amount of Fe. For details of dietary treatments, see Table 1 and pp. 778–779.

A reduces Fe status, which in turn triggers compensatory mechanisms, such as raised Fe absorption, in an attempt to prevent further lowering of Fe status. Marginal Fe deficiency also increased the efficiency of Fe absorption, a phenomenon that is well known (Morris, 1987). For the individual animals in Expt 2 the efficiency of Fe absorption was inversely related with Fe concentrations in the femur (Fig. 2), suggesting that femur Fe status regulates Fe absorption. Hepatic Fe concentrations were also negatively associated with apparent Fe absorption, but the correlation was mainly caused by clustering of the Fe-adequate and Fe-deficient groups at the extreme ends of the scale (Fig. 2). For rats given Fe-adequate diets with either an adequate or marginal amount of vitamin A there was no significant relationship (r 0·29, n 24, $P = 0·162$). Thus, Fe concentration of femur may be more important in determining Fe absorption than that of liver.

Mejia *et al.* (1979*b*) determined turnover of intragastrically- or intraperitoneally-administered ^{59}Fe in vitamin A-deficient and pair-fed control rats, and calculated that vitamin A deficiency increased the efficiency of true Fe absorption. This outcome indicates that the observed increase in apparent Fe absorption in rats with marginal vitamin A deficiency may not be biased by changes in faecal excretion of endogenous Fe. However, it should be noted that in the study of Mejia *et al.* (1979*b*) there was no difference in Fe absorption between *ad lib.*-fed controls and the vitamin A-deficient rats which showed decreased feed intakes. This suggests that feed intake also affects Fe absorption. In our study feed intake of vitamin A-adequate and marginally deficient animals was similar. Thus, the observed increase in apparent Fe absorption in deficient rats cannot have been affected by feed intake.

As to the metabolic basis for the tendency towards anaemia in male rats with marginal vitamin A deficiency fed on diets adequate in Fe, we can only speculate. Possibly, the primary feature is decreased uptake of plasma Fe by bone marrow. Vitamin A deficiency in rats has been reported to cause impaired uptake of intravenously injected ^{59}Fe by the femur (Mejia *et al.* 1979*b*). This could lead to the observed decreased concentrations of Fe

in the femur. Impaired uptake of Fe by the femur could also explain the reported inhibition of incorporation of ^{59}Fe into erythrocytes of rats with vitamin A deficiency (Mejia *et al.* 1979*b*). The decreased utilization of Fe by bone marrow may cause the observed accumulation of Fe in the liver. Possibly, the former effect triggers the uptake of Fe by liver rather than inhibiting Fe mobilization from this tissue. Indeed, the uptake by the liver of circulating radiolabelled Fe is enhanced in rats with vitamin A deficiency (Mejia *et al.* 1979*b*). Alternatively, enhanced Fe uptake by the liver could be related to the increased efficiency of apparent Fe absorption in rats with marginal vitamin A deficiency. The observation that marginal amounts of vitamin A in a diet deficient in Fe did not influence tissue Fe concentrations and blood haemoglobin could imply that under this condition it is the Fe rather than vitamin A provision that determines uptake of Fe by tissues.

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