Letters to the Editors

Protein content of skin

Although the paper by Miwa *et al.* (1987) does demonstrate that 'Gestational protein-energy malnutrition affects the composition of developing skins of rat fetuses and their dams', aspects of their methodology and interpretation obscure the exact nature of the phenomenon that they have studied.

The fundamental problem arises from their use of the method of Lowry *et al.* (1951) to measure the protein content of their skin samples. Since Lowry's method measures only the soluble proteins it is likely to give results of variable accuracy, particularly when there are variations in the solubility of collagen. It seems likely that systematic bias was introduced by this methodology, and it may be this which accounts for the fact that so little of the dry, fat-free skin samples was protein: the value for the dams was less than 20%, and for the fetuses greater than 65% on a dry weight basis. The authors do not comment on this disparity nor do they inform us what they consider to be the remaining fraction of the skin. Their calculation of protein as a proportion of dry weight also leads to confusion, since if, as is usually done, the protein is calculated on a wet weight basis (using water contents quoted by the authors), then there is no change in the protein concentration of the fetuses with malnutrition, the converse of the authors' claim.

Further confusion is introduced by Table 3 of the paper, where the content of constituents per total skin mass (and not the concentration as the authors state in their text) of the fetuses is presented. These large and superficially impressive differences represent nothing more than the fact that the fetuses of well nourished and poorly nourished dams had different skin masses. If protein, hexosamine or hydroxyproline are calculated as a proportion of body-weight, then there are no differences. Thus the differences are not reflections of anything more fundamental than the fact that the fetuses in turn were of different size and smaller objects have smaller surfaces. In their discussion, however, the authors do not mention this, and consequently imply that these differences are reflections of a difference in composition due to dietary treatment. The reality is that the only differences in composition are very small: the differences are minor per unit dry weight (Table 2 of the paper) and even smaller on a wet weight basis (deduced from the values in Table 2).

The subject the authors address is an important one and needs experimental work, but it is likely that their paper will do little to clarify the field.

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Food iron absorption

I am extremely unhappy with the recent paper by Zhang *et al.* (1989) which in my opinion uses techniques and methods which add almost nothing to our understanding of food iron absorption in man. In my opinion the experimental design is flawed in that the diets, while formulated to contain approximately 20 mg Fe/kg from the food, on analysis actually contained nearer 30 mg Fe/kg, i.e. 10 mg contaminating Fe on the nature, availability and presence of which nothing is said. Thus the dietary intake of Fe contains approximately 33% Fe which is not from the food in question and no attempt is made to correct for this. It is therefore not surprising that some extremely confusing results were obtained because we do not know what effect the actual food had on the availability of the contaminant Fe. Even assuming that the results are correct they seem to add little to our understanding of the physiology of food Fe absorption as it occurs in man. With both foods (meat and spinach) haemoglobin regeneration efficiency and apparent Fe absorption were very similar and were very much higher than anything which has been found in man. It does not seem to me to be enough to dismiss these large differences in a statement (p. 340, paragraph 3, lines 7–9) that 'The differences in percentage Fe absorption between the animal and human studies are probably due to differences in Fe status, maturity and Fe intake relative to requirement'. It seems to me that there are major fundamental differences in the mechanisms of food Fe absorption in rats and humans at the molecular level and much more work needs to be done on these.

Finally it should be pointed out that coprophagy may have a major impact on haemoglobin regeneration studies and would well overestimate the result for different types of food (Neale, 1984).

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Neale, R. J. (1984). Coprophagy in iron-deficient rats. II.2 novel methods of prevention. *Laboratory Animals* **18**, 119–124.

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Reply to letter by Neale

We are responding to Dr Neale's letter concerning our paper (Zhang *et al.* 1989). His comment that the native iron in dietary ingredients may affect the results of our experiment was dealt with in the experimental design. We had included a group of rats fed on a basal diet containing Fe from ingredients only. These data were not included in the paper because the contribution of basal Fe was similar for all treatment groups. From the literature, twelve semipurified basal diets from various laboratories contained 3·5–22 mg native Fe/kg, averaging 9·1 mg/kg; the percentage of total Fe contributed by the ingredients ranged from 11 to 76%, averaging 28%; consistent with our paper. We recalculated haemoglobin regeneration efficiency (HRE) adjusted for Fe contributed by the basal ingredients to haemoglobin Fe gain and Fe intake (Table 1). This adjustment reduced HRE slightly, but the relationships among the treatments were not affected, consistent with the findings of Mahoney & Hendricks (1982). Thus, Fe contributed by the dietary ingredients did not cause a severe experimental flaw as Dr Neale felt.

Dr Neale questioned if these results add to the understanding of food Fe absorption in man. It is true that published HRE and apparent Fe absorption values are generally higher in rat studies than in human studies. However, the published Fe bioavailability results with rats are usually from very anaemic, rapidly growing animals given amounts of Fe within

Table 1. *HRE values of rats adjusted and unadjusted from Fe contributed by dietary ingredients*

	Proportion of Fe sources in diets						
Meat Fe...	100	75	50	25	0	$FeSO_4$	Basal
Spinach Fe...	0	25	50	75	100	diet	diet
Adjusted:							
Severely anaemic rats							
Trial 1	0·34	0·42	0·34	0·44	0·35	0·82	0·65
Trial 2	0·38	0·37	0·33	0·36	0·26	0·78	0·59
Mildly anaemic rats							
Trial 1	0·36	0·36	0·35	0·35	0·38	0·57	0·83
Non-anaemic rats							
Trial 2	0·40	0·36	0·35	0·39	0·24	0·40	0·62
Unadjusted:							
Severely anaemic rats							
Trial 1	0·44	0·50	0·44	0·51	0·45	0·76	0·65
Trial 2	0·45	0·44	0·41	0·44	0·37	0·71	0·59
Mildly anemic rats							
Trial 1	0·51	0·51	0·50	0·51	0·53	0·66	0·83
Non-anaemic rats							
Trial 2	0·47	0·45	0·43	0·46	0·36	0·48	0·62

their theoretical requirements. Most human studies use normal, adult male subjects who require only 1–2 mg Fe/d but are given a radioiron-labelled meal containing 3 mg Fe and then consume meals containing 12 mg Fe or more. When making comparisons, variables like Fe status, Fe intake, maturity and methodology must be controlled. How can one attribute differences in results between rats and humans only to species when so many uncontrolled factors are involved?

There is strong evidence that humans and rats absorb similar percentages of Fe when Fe statuses and Fe intakes relative to requirements are similar. When both rats and human subjects (blood donors) were in anaemic status and both were given diets with low haem-Fe, they absorbed similar percentages of dietary haem-Fe, 30–36% by rats (Buchowski *et al.* 1989) compared with 24–36% by humans (Hallberg *et al.* 1979). When high doses of haem-Fe were given to normal rats or humans, Fe absorption decreased to 3–7% in rats (Buchowski *et al.* 1989) and 1–7% in humans (Hallberg *et al.* 1979). Both Fe-deficient humans and rats utilize very similar percentages of haemoglobin and $FeSO_4$ Fe, and chelators do not affect haem-Fe absorption by humans or rats (Mahoney & Hendricks, 1984). HRE values of anaemic growing rats fed on low-Fe diets were about twelve times those of normal adult rats given high-Fe diets (77 *v.* 6%) (D. Zhang and A. W. Mahoney, unpublished results). Similarly, Fe absorption of Fe-depleted human subjects was nine times higher than that of normal subjects (Hansen *et al.* 1987). Moreover, the biochemical mechanisms of haem and non-haem-Fe absorption are similar in rats and humans (Turnbull, 1974). The mucosal enzyme systems (haem oxygenase) involved in the release of Fe from the haem ring are found in humans and rats (Raffin *et al.* 1974; Wells & Awad, 1986; Hintz *et al.* 1988). Thus, reported differences in Fe bioavailability between humans and rats may not be due to species differences but mainly due to differences in Fe status, Fe intake and maturity as we suggested (Zhang *et al.* 1989, p. 340, paragraph 3, lines 7–9).

Concerning the effect of coprophagy on Fe bioavailability, we observed decreased HRE values when coprophagy was prevented, about 25% lower in rats fed on spinach, green peas, or bran-cereal diets and about 8% lower in rats fed on $FeSO_4$-supplemented diets (D. Zhang, D. G. Hendricks and A. W. Mahoney, unpublished results). This supports the

two-non-haem-Fe-pool hypothesis suggested by Zhang *et al.* (1989). We agree that Fe bioavailability may be overestimated due to coprophagy, but it does not affect the conclusions drawn when comparing Fe bioavailabilities among rats given similar Fe sources. However, more information on effects of coprophagy on haem-Fe bioavailability is needed. Certainly, coprophagy affects utilization of many nutrients by rodents (Giovannetti, 1982).

The significant aspects of our paper are: (*a*) effects of meat on total Fe and not just non-haem-Fe bioavailability was evaluated, (*b*) a meat-only control group was included for comparison, (*c*) spinach Fe can be well-utilized when the subject's Fe requirement is high, (*d*) meat did not enhance total Fe bioavailability when spinach Fe was utilized maximally, (*e*) relative Fe bioavailability was affected by Fe status because $FeSO_4$ Fe appears to be in a different non-haem-Fe pool from food non-haem-Fe, (*f*) Fe bioavailability was determined using animals at three Fe statuses to correlate Fe status with HRE. Because of this design, it was possible to observe different patterns of Fe utilization between $FeSO_4$ and spinach and to suggest a new hypothesis that food non-haem-Fe complexes are in a different gastrointestinal pool from highly soluble inorganic Fe salts.

Finally, we thank Dr Neale for raising these issues about our paper.

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Recommended dietary amounts of nutrients for the United Kingdom

The current review of the recommended dietary amounts (RDA) of nutrients for the UK by the Committee on Medical Aspects of Food Policy (COMA) is introducing many new concepts. Amongst the most welcome is the recent publication in the *British Journal of Nutrition* of the conceptual approach to the task (Whitehead, 1989), and the request from Dr Whitehead for a response from the nutrient sector of the food industry through its Committee for Responsible Nutrition.

The nutritional-supplements sector of the food industry is principally concerned with the manufacture, marketing and sale of nutrients in a concentrated form and often in combination in 'balanced' proportions. The supplements are conceived and consumed as

foods, but in limited amounts, in a manner comparable to the use of salt, sucrose and artificial sweeteners.

The inclusion, for the first time, in a review of RDA of nutrients which hitherto have been included only in recommendations on dietary guidelines is particularly welcome. It indicates a major advance in the approach to the role of RDA by recognizing their potential for developing food habits as an integral part of a lifestyle to promote health instead of merely preventing disease.

Major problems faced by the RDA panel, and identified by Dr Whitehead, are the lack of fundamental data, the requirement for skilled judgements and the need to retain 'openness' to allow for a continuing process of revision as new data come to hand. Moreover, these problems have to be resolved against a background of increasing regulatory complexity and a growing desire from the public for information on which it can form its own views and act upon them without unreasonable legislative constraint.

Amongst the sources of data for assessing the average intake of micronutrients amongst the population of the UK is the theoretical analysis of the food consumptions recorded in the national food surveys. This approach continues to provide an invaluable perspective of the patterns of consumption of macronutrients; but in respect of micronutrients one may question whether the data so produced are any more than an inaccurate record precisely stated. For example, a survey carried out in December 1988 (British Marketing Research Bureau, unpublished results) showed that nutritional supplements were bought by 20% of the male and 30% of the female UK population. It is unlikely that any of this will have been recorded in the data of the national food surveys.

For the COMA RDA panel, a major problem lies in the paucity of data identifying specific micronutrients with markers of optimal health, and this is further confused by the absence of an internationally accepted definition of optimal health for the many stages of human life in its varied environments. Against this background there is a case for considering that statements of 'over-consumption' and 'under-consumption' are subjective rather than objective. Upper limits of consumption can be recommended for many foods on the basis of safety, and often the physiological basis is known as, for example, with saturated fatty acids and heart disease. Variable consumptions below this upper limit for safety are a matter of personal choice and may be neither 'good' nor 'bad'.

The amounts of micronutrients that may be consumed at any one time vary substantially as, for example, in considering the consumption of vitamin B_{12} from a meal based on liver compared with that from one based on salad. Again the concept of an appropriate upper limit for consumption of liver will be based on safety. Further considerations must include the known variability in the content of micro-ingredients in different samples of the same food and of the substantial effect on availability in one food from the presence of other foods in a meal.

The delicate judgements with which Dr Whitehead expects the COMA RDA panel to conclude its work will, it is hoped, form the basis for international research and assessment in the subsequent decade. It will undoubtedly assist all involved in this field, in academia, in government and in industry, if the report of the panel expands on Dr Whitehead's conclusions on the supreme importance that all concerned recognize that the conclusions are judgements, that new data and concepts are continually being reported, that nothing is irrevocable and that constraint in the absence of contra-indications is likely to limit rather than aid the acquisition of critical information.

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