Riboflavin deficiency, metabolic rate and brown adipose tissue function in sucking and weanling rats

By B. E. PATTERSON* and C. J. BATES†

MRC Dunn Nutritional Laboratory, Downham's Lane, Milton Road, Cambridge CB4 1XJ

1. The effects of riboflavin deficiency on growth, whole-body oxygen consumption, cytochrome *c* oxidase (*EC* 1.9.3.1) activity and GDP-binding capacity of brown adipose tissue were measured in three groups of rats: sucking pups, weanling rats, and dams. Control groups were weight-matched, pair-fed or fed *ad lib*.

2. Riboflavin deficiency reduced growth rate and increased the activation coefficient of erythrocyte glutathione reductase (NAD(P)H) (*EC* 1.6.4.2), as predicted. In sucking pups it also reduced whole-body O_2 consumption per unit body-weight, especially after noradrenaline stimulation. In weanling rats, however, it increased O_2 consumption both before and after noradrenaline stimulation.

3. Cytochrome *c* oxidase (*EC* 1.9.9.1) activity of brown adipose tissue was not consistently affected by riboflavin deficiency. Binding of [³H]GDP to the mitochondria was increased in the deficient weanling rats.

4. Weanling rats therefore, seemed better able to withstand the effects of severe depletion. Their reduced growth and increased non-shivering thermogenesis helped to counteract the unfavourable ratio of riboflavin:other tissue-building materials. The relevance for thermoregulation in riboflavin-deficient children is discussed.

In view of the widespread occurrence of riboflavin deficiency in children (Bates, 1987), and the important implications for thermoregulation (Duerden & Bates, 1985*b*), the study of riboflavin deficiency was undertaken in an animal model at different stages of early post-natal development.

An impairment of β-oxidation of fatty acids has consistently been observed in studies on riboflavin-deficient animals. The first step of the β-oxidation cycle is catalysed by a flavin-dependent dehydrogenase, comprising three (alternative) enzymes, classified by their substrate specificities as short-, medium- and long-chain fatty acyl coenzyme A dehydrogenase. The activity of these enzymes in rat-liver homogenates is depressed during riboflavin deficiency (Hoppel *et al.* 1979; Olpin & Bates, 1982*b*; Sakurai *et al.* 1982).

Brown adipose tissue (BAT) is another important site of β-oxidation, and plays an essential role in metabolic heat generation (i.e. non-shivering thermogenesis) in cold-adapted animals, hibernators and the neonate of many species including man (Hull & Hardman, 1970).

Several studies have shown that hepatic mitochondrial oxygen consumption is reduced in severely riboflavin-deficient weanling rats (Hoppel & Tandler, 1975, 1976; Taniguchi & Nakamura, 1976; Hoppel *et al.* 1979; Olpin & Bates, 1982*b*; Sakurai *et al.* 1982). Duerden & Bates (1985*b*) observed that BAT mitochondrial O_2 consumption is impaired in 15-d-old riboflavin-deficient sucking pups.

In the light of the known biochemical roles of riboflavin, it is clear that flavins play a central role in whole-body energy metabolism. This fact raises some important questions about the functional consequences of riboflavin deficiency on overall metabolic rate, particularly during the neonatal period when fatty acids are the major energy source, and when BAT thermogenesis is essential for the maintenance of thermal equilibrium.

* Present address: Pfizer Central Research, Sandwich, Kent CT13 9NJ.
† For reprints.

Table 1. *Composition of purified, riboflavin-deficient diet (g/kg)*

Casein*	210† (105)‡
Sucrose	706† (815)‡
Arachis oil	30
Briggs salt mixture§	50
Cystine	1·5
Choline chloride	0·200
Calcium pantothenate	0·020
Thiamin hydrochloride	0·004
Pyridoxine hydrochloride	0·009
Nicotinamide	0·025
Biotin	0·001
Folic acid (pteroylmonoglutamic acid)	0·001
Vitamin B_{12} (cyanocobalamin)	5×10^{-5}
Vitamin A (retinyl acetate)	0·0021 (retinol equivalents)
Vitamin D_2	$7·5 \times 10^{-6}$
Vitamin K (menadione)	0·009
Vitamin E (α-tocopherol)	0·25

* BDH, Poole, Dorset (low in vitamins).
† Dams.
‡ Weanling rats.
§ Greenfield *et al.* (1969).

The aim of the present study was to investigate the functional consequences of dietary riboflavin deficiency for whole-body metabolic rate and the capacity for BAT thermogenesis at different ages and stages of development.

MATERIALS AND METHODS
Animals and tissue preparation

Dams. Female DNL (Norwegian) hooded rats, whose mean body-weight was 204 g, had been reared on a standard laboratory chow diet (LAD 1; Labsure, Manea, Cambs) and were housed individually in suspended wire cages at 22° with a 12 h light–12 h dark cycle. They were assigned to the following groups: riboflavin-deficient (RD), and pair-fed (PF) and *ad lib.*-fed (AL) controls. A synthetic powdered diet (Table 1, Duerden & Bates, 1985*a*) was provided for a period of 60 d. The riboflavin-deficient diet contained 0·004 g riboflavin/kg, which was derived entirely from the casein component, described as 'low in vitamins' (BDH, Poole, Dorset) (Duerden & Bates, 1985*a*). PF and AL control animals also received this diet, containing added riboflavin, 0·015 g/kg. The PF animals were individually paired with RD animals during the depletion period before mating and pair-feeding was continued as far as possible during the rapidly increasing intakes associated with pregnancy.

The dams were mated with males which had been given the chow diet, and the presence of a vaginal plug was taken as evidence of successful mating. The dams then continued to receive the purified diets until term. Their litter numbers were each adjusted to eight (by subtraction or addition within diet groups), to ensure equal access to maternal milk, and the pups were reared to day 15 post-partum, receiving only their mother's milk. Where appropriate (see Table 3, p. 479) they received an intraperitoneal injection of noradrenaline, 800 μg/kg body-weight, 30 min before being killed. The animals were then killed by diethyl ether anaesthesia; blood was removed into heparinized containers by cardiac puncture, and

the livers were removed and stored frozen. Where appropriate, the entire interscapular BAT pad was excised, cleaned of adventitious tissues and used immediately for mitochondrial preparations and for cytochrome c oxidase (EC 1.9.3.1) assays (see p. 478).

Weanling rats. Female weanling DNL (Norwegian) hooded rats were housed individually in suspended wire cages and were maintained for 5 weeks on synthetic diets differing from those of the dams (described previously) only in the proportions of casein and sucrose (Table 1). The PF animals were individually paired with riboflavin-deficient animals, and each received the amount of food eaten by them on the previous day. The WM animals were individually weight-matched to RD animals, and received a restricted amount of food which was calculated to maintain their body-weight identical to that of the RD animals (Olpin & Bates, 1982 *a*, *b*). After approximately 5 weeks, all were killed by diethyl ether anaesthesia, and blood and liver samples were removed and stored at $-20°$ before analysis. Interscapular BAT was removed and used for mitochondrial and cytochrome c oxidase assays where appropriate.

Biochemical measurement of riboflavin status

The packed erythrocyte samples, pooled from four pups in each litter, were diluted with doubly distilled water in order to reduce precipitation of haemoglobin, before freezing at $-20°$. The activation coefficient (AC; stimulated:basal activity) of the erythrocyte glutathione reductase (NAD(P)H) (EC 1.6.4.2) (EGRAC) was then measured by a modification of the method of Prentice & Bates (1981), using a Roche Cobas centrifugal bioanalyzer. Samples were diluted to a haemoglobin concentration of 2 g/l, centrifuged for 10 min at 2000 g to remove stroma, pre-incubated at 37° for 30 min both with and without FAD (2 μM) and finally transferred to the Cobas samples cups for the enzyme rate determination and estimation of the EGRAC.

Whole-body O_2 consumption. An automated, closed-circuit O_2 consumption apparatus was used as described by Stock (1975). For the sucking pups, four pups from each litter (two males and two females) were measured simultaneously, whereas for weanling rats, individual animals were used. They were placed in a chamber on a wire grid above trays containing soda-lime (to absorb carbon dioxide) and silica gel (to absorb water), and immersed in a water-bath maintained at 33° for the sucking pups and at 29° for the weanling rats.

O_2 consumption was measured by recording the injection of air needed to maintain the chamber at a constant pressure (transducer). After 'basal' unstimulated O_2 consumption (1 h), the animals received intraperitoneal noradrenaline, 800 μg/kg body-weight, and the 'noradrenaline-stimulated' respiration rate was determined.

BAT mitochondrial function

Isolation of mitochondria. The method was derived from Cannon & Lindberg (1979). The rats were treated with noradrenaline (800 μg/kg body-weight, intraperitoneally) 30 min before the removal of BAT. All the interscapular BAT was excised, white fat, muscle and connective tissues were removed, and the BAT was homogenized in a Potter-Elvehjem homogenizer with loose-fitting pestle, with 10 ml ice-cold buffer, pH 7·2 at 0–4°. The buffer contained (mol/l): sucrose 0·25, N-2-hydroxyethyl piperazine-N-2-ethane sulphonic acid 1×10^{-3}, EDTA 2×10^{-3}. The homogenate was diluted with buffer to 15 ml, and 1 ml was removed for the determination of total cytochrome c oxidase activity (see p. 478) and protein concentration (Bensadoun & Weinstein, 1976).

The remaining homogenate was centrifuged at 12000 g for 15 min to sediment the mitochondria, nuclei and cell debris. This pellet was resuspended in buffer and centrifuged at 700 g to remove nuclei and debris. The supernatant fraction containing the mitochondria

was filtered through nylon bolting (30 μm mesh), and the mitochondria were washed twice more with buffer before resuspension at a protein concentration of about 4 mg/ml.

[8-^3H]*GDP-binding assay*. The method used was that of Goodbody & Trayhurn (1981) modified from Nicholls (1976). The mitochondria were suspended at 0·4 mg/ml in 1·0 ml buffer, pH 7·1, containing (mol/l): sucrose 0·1, choline chloride 1×10^{-2}, EDTA 1×10^{-3}, N-Tris(hydroxymethyl)methyl-2-amino-ethane-sulphonic acid 2×10^{-2}. To the mitochondrial suspension were added [U-^{14}C]sucrose (Amersham International plc, Amersham, Bucks; 0·1 μCi/ml), and [8-^{3}H]GDP (Amersham International; 10^{-5} M, 1·25 μCi/ml). After 5 min incubation at 25°, the mitochondria were sedimented by centrifugation (3 min at 25000 g in an MSE Micro-Centaur centrifuge) and were dissolved in 0·1 ml sodium hydroxide (0·75 mol/l) at 55° before addition of scintillant (Optiphase Safe; LKB). Double-label scintillation counting was used to measure the radioactivity present in both ^{3}H and ^{14}C separately, and the quantity of bound GDP was calculated after correction for the extramitochondrial fluid in the pellet, which was estimated from the [^{14}C]sucrose content.

Cytochrome c *oxidase assay*. Cytochrome c oxidase activity was measured by a modification of Yonetani & Ray (1965). The homogenate was mixed with 0·01 vol. Lubrol (Sigma), a non-ionic detergent. Reduced ferrocytochrome c (Sigma) was prepared at 50 μmol/l, in phosphate buffer (0·01 mol/l, pH 7·0). Its rate of oxidation was measured at 550 nm in the Cobas analyzer, using 10 μl BAT homogenate and 310 μl reagent. Enzyme activities from each group of four pups and from individual tissue samples from weanling rats and dams were calculated from the extinction coefficient of ferricytochrome c: 0·92 litres/mmol per cm.

Statistical analysis

Student's t test was used for intergroup comparisons, since normal distributions were observed.

RESULTS

Body-weight, relative organ weights and EGRAC (Table 2)

Dams and sucking pups. The body-weights of RD dams were significantly less than those of their PF ($P < 0.05$) and AL ($P < 0.001$) controls. The liver:body-weight ratio for RD dams was significantly elevated only in comparison with PF controls, and BAT:body-weight ratio was not significantly different between the groups. As predicted, EGRAC of the RD dams was significantly greater than that of either the PF or AL controls ($P < 0.001$).

Similar results were obtained for the RD sucking pups; body-weight was significantly less than those of the PF or AL controls (P < 0.002), and liver:body-weight ratio was significantly higher than those of PF ($P < 0.01$) or AL ($P < 0.001$) controls. EGRAC of RD sucking pups was significantly higher than those of PF or AD controls ($P < 0.001$).

Weanling rats. The body-weights of RD weanling rats were significantly lower than those of PF ($P < 0.01$) or AL ($P < 0.001$) controls. Their liver:body-weight ratio was significantly higher than those of PF ($P < 0.001$) and AL ($P < 0.01$) controls, and their BAT:body-weight ratio was higher than that of PF controls ($P < 0.01$).

The EGRAC of RD animals was significantly higher than those of PF ($P < 0.01$) and AL ($P < 0.02$) controls.

O_2 consumption and the effect of noradrenaline on the sucking pups and weanling rats
(Table 3)

At 10 d after birth the initial O_2 consumption was not significantly different between RD and control groups. The noradrenaline-stimulated response was significantly lower for the

Table 2. *Body-weights, organ:body-weight ratios and activation coefficient of erythrocyte glutathione reductase (EC 1.6.4.2) (EGRAC) of dams, sucking pups and weanling rats which were riboflavin-deficient, or pair-fed or* ad lib.-*fed controls*†

(Mean values with their standard errors)

Group...		Riboflavin-deficient		Pair-fed controls		*Ad lib.*-fed controls	
		Mean	SE	Mean	SE	Mean	SE
Dams and sucking pups‡							
Body-wt (g):	Dams	225	7·05	248**	6·45	269***	23·9
	Pups	86·5	4·60	105·4*	5·01	103·1*	3·92
Liver:body-wt:	Dams	0·064	0·0035	0·052*	0·002	0·063	0·0027
	Pups	0·046	0·0036	0·033**	0·0019	0·035*	0·0012
BAT:body-wt ($\times 10^3$):	Dams	0·87	0·13	0·88	0·04	0·92	0·05
	Pups	3·49	0·36	3·23	0·03	3·61	0·02
EGRAC:	Dams	1·708	0·24	1·26***	0·11	1·17***	0·08
	Pups	1·769	0·23	1·20***	0·07	1·27***	0·04
Weanling rats§							
Body-wt (g)		87	5·4	112**	4·2	181***	6·2
Liver:body-wt		0·059	0·003	0·048***	0·0016	0·044**	0·0016
BAT:body-wt ($\times 10^3$)		1·7	0·10	1·24**	0·05	1·72	0·06
EGRAC		2·10	0·21	1·37**	0·50	1·12**	0·06

Mean values were significantly different between either control group and the riboflavin-deficient group (Student's t test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$.

† At the time of death; for details of diets and procedures, see Table 1 and p. 476.

‡ Four dams and eight sets of pups (four pups in each set) in each experimental group.

§ Five rats in the riboflavin-deficient and six rats in each of the pair-fed and *ad lib.*-fed control groups.

Table 3. *Whole-body oxygen consumption (ml/min per kg body-weight) of sucking pups and weanling rats which were riboflavin-deficient, or weight-matched, pair-fed or* ad lib.-*fed controls*†

(Mean values with their standard errors)

Group...		Riboflavin-deficient		Weight-matched controls		Pair-fed controls		*Ad lib.*-fed controls	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sucking pups‡									
10-d-old	Basal§	35·3	1·0	—	—	34·1	0·5	35·0	0·3
	Stimulated‖	42·9	1·5	—	—	70·9***	0·5	68·8***	1·2
15-d-old	Basal§	28·5	1·5	—	—	35·1**	0·7	34·0**	0·2
	Stimulated‖	39·9	0·9	—	—	83·1***	2·0	74·0***	2·0
Weanling rats¶									
Basal§		35·1	0·8	—	—	28·3***	1·1	25·8***	1·0
Stimulated‖		61·0	1·4	—	—	51·5**	2·1	48·8***	1·6
Basal§		40·7	2·2	29·8***	1·0	—	—	—	—

Mean values were significantly different between either control group and the riboflavin-deficient group (Student's t test): ** $P < 0.01$, *** $P < 0.001$.

† For details of diets and procedures, see Table 1 and p. 476.

‡ Four sets, with four pups in each group.

§ Initial resting metabolic rate.

‖ Increased metabolic rate after a dose of noradrenaline (800 μg/kg intraperitoneally).

¶ Eight animals in each group.

Table 4. [³H]GDP binding and cytochrome c oxidase (EC 1.9.3.1) in brown adipose tissue of dams, suckling pups and weanling rats which were riboflavin-deficient, or weight-matched, pair-fed or ad lib.-fed controls†

(Mean values with their standard errors)

		Riboflavin-deficient		Weight-matched controls		Pair-fed controls		Ad lib.-fed controls	
		Mean	SE (n)	Mean	SE (n)	Mean	SE (n)	Mean	SE (n)
Dams and sucking pups‡:									
[³H]GDP bound§	Dams	196	28 (4)	—	—	381	85 (4)	290	33 (4)
	Pups	684	140 (4×4)	—	—	1637***	214 (4×4)	839	180 (4×4)
Cytochrome c oxidase‖:	Dams	25	8·5 (4)	—	—	13	2 (4)	20	4·5 (4)
	Pups	33	6 (4×4)	—	—	23	2 (4×4)	33	7 (4×4)
Weanling rats									
[³H]GDP bound§		745	63 (5)	—	—	425**	48 (5)	374***	47 (6)
Cytochrome c oxidase‖		20	1·0 (5)	—	—	19	0·9 (5)	29***	0·8 (6)
[³H]GDP bound§		604	44 (7)	445*	68 (7)	—	—	—	—
Cytochrome c oxidase‖		12	0·8 (7)	11	0·8 (7)	—	—	—	—

Mean values were significantly different between the control groups and the riboflavin-deficient group (Student's t test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets and procedures, see Table 1 and p. 476.

‡ Dams and sucking pups: four dams and four sets of four 15-d-old sucking pups, in each group.

§ pmol GDP bound/mg mitochondrial protein.

‖ mol cytochrome c oxidized/min by the entire interscapular brown adipose tissue from each animal.

RD group by 40 % compared with the PF controls ($P < 0.001$) and by 38 % compared with the AL ($P < 0.001$) controls. The rise in metabolic rate in response to noradrenaline stimulation was 21 % for RD, 108 % for PF and 97 % for AL.

By 15 d after birth, the differences between the RD and control groups had increased. The initial metabolic rate for the RD group was significantly lower by 19 % ($P < 0.01$) in comparison with PF and by 16 % ($P < 0.01$) in comparison with AL controls. The noradrenaline-stimulated response was markedly lower by 52 % in comparison with PF ($P < 0.001$) and by 46 % in comparison with AL ($P < 0.001$) controls.

Weanling deficient rats, in contrast, had significantly higher O_2 consumptions than their respective controls; by 24–36 % ($P < 0.001$) before noradrenaline stimulation, and by 18–25 % ($P < 0.01$) after noradrenaline.

BAT [³H]GDP binding and cytochrome c oxidase activity (Table 4)

Sucking pups. Reduced [³H]GDP binding was observed for RD pups in comparison with the control groups, although this was significant ($P < 0.01$) only in comparison with PF controls. Total cytochrome *c* oxidase activity was not significantly different between the groups.

For the dams, a significant difference was not observed between the groups, either for [³H]GDP binding or for total cytochrome *c* oxidase activity, although there was a consistent trend towards reduced binding in the RD dams.

Weanling rats. [³H]GDP binding for RD weanlings was markedly elevated, by 36 % ($P < 0.05$) in comparison with WM, by 75 % ($P < 0.01$) in comparison with PF, and by 99 % ($P < 0.001$) in comparison with AL controls. Total cytochrome *c* oxidase activity was similar in RD, WM and PF, although that of RD was significantly less ($P < 0.001$) than that in the AL controls. The specific activity (μmol cytochrome *c* oxidized/min per mg protein) was similar between all groups, with means of 0·65 (SE 0·16) for RD, 0·48 (SE 0·049) for PF and 0·56 (SE 0·049) for AL controls.

DISCUSSION

Noradrenaline is the normal physiological activator of BAT thermogenesis, and this function can be assessed in vivo from the increase in whole-body metabolic rate (under conditions of thermoneutrality) in response to maximal stimulation with noradrenaline (Moore & Underwood, 1963). The physiological consequences of riboflavin deficiency in the sucking pups are clearly demonstrated by the observation of a marked reduction in the noradrenaline-stimulated metabolic rate for RD animals compared with controls, which confirms previous observations (Duerden & Bates, 1985b). RD sucking pups thus apparently have an impaired capacity for thermogenesis.

The effect of riboflavin deficiency during the weaning period was quite different because an elevated metabolic rate (both initial and noradrenaline-stimulated) was observed. Furthermore, the percentage increase in metabolic rate in response to noradrenaline was comparable to that of the control groups, whereas the percentage increase in deficient sucking pups was markedly lower than that in the controls.

The increase in initial metabolic rate seen in RD weanling rats contrasts with the observations of Nolte *et al.* (1972). Here, an RD group exhibited a markedly reduced metabolic rate, as demonstrated by a decreased O_2 consumption, which was attributed to a reduction in thyroid hormone status. In the present authors' experience, however, the reduction in thyroid hormone concentration in RD animals is due to inanition and not to riboflavin deficiency *per se* (Patterson, 1987) and, therefore, cannot explain the specific metabolic rate changes caused by riboflavin deficiency. In Nolte's study (Nolte *et al.* 1972)

the rats were much older and heavier at the outset, and were fed on a riboflavin-deficient diet for 96 d, the controls being fed on a commercial rat diet *ad lib*.

The changes seen in the present study may help provide an explanation for one of the features of riboflavin deficiency in weanling rats: their inefficient utilization of energy for growth, and hence markedly reduced weight gain per unit of food consumed (Olpin & Bates, 1982*a*; Duerden & Bates, 1985*a*). For sucking pups, it is possible that the reduced metabolic rate may be accompanied by a reduced energy intake, with no net change in weight gain.

The GDP-binding assay has been widely used as a measure of the thermogenic capacity of BAT (Goodbody & Trayhurn, 1981; Sundin *et al.* 1981) and was used in the present study, together with cytochrome *c* oxidase, as an index of total thermogenic activity. For the weanling rats, the enhanced O_2 consumption of the RD group was accompanied by a reduction in BAT mitochondrial GDP binding. For the sucking pups, the GDP-binding result was less consistent, being significantly lower in the RD than in the PF controls, but not significantly different from the *ad lib.*-fed controls. In an earlier study (Duerden & Bates, 1985*b*), GDP binding was unaffected by riboflavin deficiency in sucking pups, although their BAT mitochondrial respiration, after treatment with noradrenaline, was depressed.

In the present study, the markedly elevated GDP binding observed for RD weanlings suggests an increase in the activity of the proton conductance pathway, and hence in the thermogenic capacity of BAT. The increased total cytochrome *c* oxidase activity observed in AL weanlings showed that their total thermogenic capacity was greater. However, this presumably was a reflection of their significantly greater BAT mass, in comparison with the RD and PF groups, because the cytochrome *c* oxidase specific activity was similar between all the groups.

In conclusion, it is clear that different responses to riboflavin deficiency arise during the sucking and weanling periods respectively. The intake of fat from milk during sucking is, of course, much higher than that during the weanling period, and this probably constitutes a further factor in the sequence of changes between sucking and weaning, which determines the apparent change in sensitivity to riboflavin deficiency with age. Thus, the stage of development at which deficiency is induced is an extremely important variable, and changes in the capacity for BAT thermogenesis appear to determine how the animal will cope with this nutritional insult.

Riboflavin deficiency is one of the commonest water-soluble vitamin deficiencies of human societies in developing countries, where diets lack meat, fish and dairy products. Moreover, the newborn and young babies can exhibit marked biochemical riboflavin deficiency (Bates *et al.* 1982, 1984). Failure to achieve the genetically programmed growth potential in these societies can have many causes, of which chronic riboflavin deficiency may be one. In addition, thermoregulatory performance is stressed, especially during the cooler seasons, and by traditional practices such as that of washing babies in cold water, including the newborn. Clearly, therefore, a better understanding of the implications of riboflavin deficiency for energy expenditure and thermoregulation is highly relevant to the developmental biology of many human societies. It thus seems that the prevention of riboflavin deficiency during the neonatal period warrants renewed concern.

REFERENCES

Bates, C. J. (1987). Human riboflavin requirements and metabolic consequences of deficiency in men and animals. *World Review of Nutrition and Dietetics* **50**, 215–265.

Bates, C. J., Prentice, A. M., Paul, A. A., Prentice, A., Sutcliffe, B. A. & Whitehead, R. G. (1982). Riboflavin status in infants born in rural Gambia, and the effect of a weaning food supplement. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 253–258.

Bates, C. J., Prentice, A. M., Watkinson, M., Morell, P., Foord, F. A. & Watkinson, A. (1984). Efficacy of a food supplement in correcting riboflavin deficiency in pregnant Gambian women. *Human Nutrition: Clinical Nutrition* **38C**, 363–374.

Bensadoun, A. & Weinstein, D. (1976). Assays of proteins in the presence of interfering materials. *Analytical Biochemistry* **70**, 241–250.

Cannon, B. & Lindberg, O. (1979). Mitochondria from brown adipose tissue; isolation and properties. *Methods in Enzymology* **55**, 65–78.

Duerden, J. M. & Bates, C. J. (1985a). Effect of riboflavin deficiency on reproductive performance and on biochemical indices of riboflavin status in the rat. *British Journal of Nutrition* **53**, 97–105.

Duerden, J. M. & Bates C. J. (1985b). Effect of riboflavin deficiency on lipid metabolism of liver and brown adipose tissue of suckling rat pups. *British Journal of Nutrition* **53**, 107–115.

Goodbody, A. E. & Trayhurn, P. (1981). GDP binding to brown-adipose-tissue mitochondria of diabetic-obese (*db/db*) mice). *Biochemical Journal* **194**, 1019–1022.

Greenfield, H., Briggs, G. M., Watson, R. H. J. & Yudkin, J. (1969). An improved diet for carbohydrate preference studies with rats: some criticisms of experimental diets. *Proceedings of the Nutrition Society* **28**, 43A–44A.

Hoppel, C., DiMarco, J. P. & Tandler, B. (1979). Riboflavin and rat hepatic cell structure and function. *Journal of Biological Chemistry* **254**, 4164–4170.

Hoppel, C. L. & Tandler, B. (1975). Riboflavin and mouse hepatic cell structure and function: mitochondrial oxidative metabolism in severe deficiency state. *Journal of Nutrition* **105**, 562–570.

Hoppel, C. L. & Tandler, B. (1976). Relationship between hepatic mitochondrial oxidative metabolism and morphology during riboflavin deficiency and recovery in mice. *Journal of Nutrition* **106**, 73–76.

Hull, D. & Hardman, M. J. (1970). Brown adipose tissue in newborn mammals. In *Brown Adipose Tissue*, pp. 97–115 [O. Lindberg, editor]. New York: Elsevier.

Lean, M. E. J. & James, W. P. T. (1986). Brown adipose tissue in humans. In *Brown Adipose Tissue*, pp. 339–365 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Moore, R. E. & Underwood, M. C. (1963). The thermogenic effects of noradrenaline in newborn and infant kittens and other small mammals. A possible hormonal mechanism in the control of heat production. *Journal of Physiology* **168**, 290–317.

Nicholls, D. G. (1976). Hamster brown adipose tissue mitochondria. Purine nucleotide control of the ion conductance of the inner membrane and the nature of the nucleotide binding site. *European Journal of Biochemistry* **62**, 223–228.

Nolte, J., Brdiczka, D. & Staudte, H. W. (1972). Effect of riboflavin deficiency on metabolism of the rat in hyperthyroid and euthyroid states. *Biochimica et Biophysica Acta* **268**, 611–619.

Olpin, S. E. & Bates, C. J. (1982a). Lipid metabolism in riboflavin-deficient rats. 1. Effect of dietary lipids on riboflavin status and fatty acid profiles. *British Journal of Nutrition* **47**, 577–588.

Olpin, S. E. & Bates, C. J. (1982b). Lipid metabolism in riboflavin-deficient rats. 2. Mitochondrial fatty acid oxidation and the microsomal desaturation pathway. *British Journal of Nutrition* **47**, 589–596.

Patterson, B. E. (1987). Metabolic studies on riboflavin deficiency in the rat and premature infant. PhD thesis, Cambridge University.

Prentice, A. M. & Bates, C. J. (1981). A biochemical evaluation of the erythrocyte glutathione reductase (*EC* 1.6.4.2) test for riboflavin status. 1. Rate and specificity of response in acute deficiency. *British Journal of Nutrition* **45**, 37–52.

Sakurai, T., Miyazawa, S., Furuta, S. & Hashimoto, T. (1982). Riboflavin deficiency and β-oxidative systems in rat liver. *Lipids* **17**, 598–604.

Stock, M. J. (1975). An automatic, closed-circuit oxygen consumption apparatus for small animals. *Journal of Applied Physiology* **39**, 849–850.

Sundin, U., Herron, D. & Cannon, B. (1981). Brown fat thermoregulation in developing hamsters (*Mesocricetus auratus*): a GDP-binding study. *Biology of the Neonate* **39**, 142–149.

Taniguchi, M. & Nakamura, M. (1976). Effects of riboflavin deficiency on the lipids of rat liver. *Journal of Nutritional Science and Vitaminology* **22**, 135–146.

Yonetani, T. & Ray, G. S. (1965). Studies on cytochrome oxidase. VI. Kinetics of the aerobic oxidation of ferrocytochrome C by cytochrome oxidase. *Journal of Biological Chemistry* **240**, 3392–3398.