Biochem. J. (1985) **230**, 675–681
Printed in Great Britain

Decreased urea synthesis in cafeteria-diet-induced obesity in the rat

Teresa BARBER,* Juan R. VIÑA,† José VIÑA† and José CABO*
**Departamento de Bioquímica, Facultad de Farmacia, and* †*Departamento de Bioquímica y Fisiología, Facultad de Medicina, Avenida Blasco Ibañez 13 y 17, 46010 Valencia, Spain*

(*Received 11 March 1985/9 May 1985; accepted 22 May 1985*)

1. Feeding rats with a cafeteria diet resulted in increases in total body weight and in epididymal-adipose-tissue weight. Those rats excreted significantly less N than did controls. The amount of N ingested by cafeteria-diet-fed rats was kept equal to that of controls. 2. This decrease in N excretion is explained by a decrease in urinary excretion of urea. This may be due to the following facts. (i) The rate of synthesis of urea from precursors by isolated hepatocytes from cafeteria-diet-fed rats was lower than in controls. (ii) In cafeteria-diet-fed rats the activities of all the enzymes of the urea cycle are decreased. The major percentage decreases are those of carbamoyl-phosphate synthetase (EC 6.3.4.16) and of argininosuccinate synthetase (EC 6.3.4.5), the enzymes probably involved in the regulation of the overall rate of the cycle. When rats are switched to normal chow diet, the enzyme activities return to normal values. (iii) The uptake of amino acids by liver of cafeteria-diet-fed rats is lower than in controls. 3. These results contrast with those obtained previously by using other models of obesity in rat (i.e. genetic or hypothalamic), in which N excretion was increased.

Of the various procedures currently available to promote obesity in rats, the cafeteria-diet model (Sclafani & Springer, 1976) has the advantage that it is similar to the majority of human cases in that obesity is induced by voluntary hyperphagia of energy-rich food (Sclafani & Springer, 1976; Rothwell & Stock, 1979).

The aim of this work as to study the adaptive changes in N metabolism induced by feeding rats during 30 days with 'cafeteria diet'. The amount of N ingested by cafeteria-fed rats was kept equal to that of controls. N metabolism in obese rats has been studied (Karakasch *et al.*, 1980; Dunn & Hartsook, 1980). However, these authors used animals with hypothalamic or genetic obesities. Cafeteria-diet-induced obesity has the advantage that obesity is due only to overfeeding, and thus interpretation of the metabolic changes observed is easier because they are not masked by other abnormalities associated with obesity of hypothalamic or genetic origin.

Here we have found that cafeteria-diet-fed animals excrete less N than the controls, although N intake was kept the same in both groups. The decrease in N excretion is explained by a decrease in the excretion of urea in the urine. We have found that the capacity of the hepatocytes from cafeteria-fed rats to synthesize urea from precursors is decreased, and the uptake of amino acids by liver and the hepatic activity of the enzymes of the urea cycle are also decreased.

Experimental

Materials

Male rats of the Wistar strain were used. They were placed in individual cages and divided in two groups (for 4 weeks). One group of rats were fed *ad libitum* on a standard diet for rats from Prasa, Vara de Quart, Valencia (Spain). The experimental group was fed *ad libitum* with a 'cafeteria diet' (Sclafani & Springer, 1976), in which animals were offered daily a variety of palatable high-energy foods (four different types of food from a selection of ten) in addition to their normal chow diet. Since our major interest was to ensure a similar N intake in both control and cafeteria-fed groups, we measured the N content of each type of food used by the micro-Kjeldahl method (Markham, 1942). The values found were similar to those published by Paul & Southgate (1976). The obtained N contents (g/100g of food) of the various types of food appropriately mixed in our cafeteria diet were: potato crisps, 0.67%; milk chocolate, 1.33%;

chocolate cookies, 0.96%; corn flakes, 0.92%; cheese biscuits, 1.04%; puffed wheat 2.31%; Spanish-type salami, 2.51%; parmesan cheese, 5.01%; almonds, 3.26%; peanuts, 4.49%. The N content of the standard chow diet was 2.34%. Its lipid content was 3.6% and its carbohydrate content was 53%. Information about the carbohydrate and lipid contents of the types of food used for cafeteria feeding was obtained from food composition tables (Paul & Southgate, 1976).

The values of N content found show that we used types of food either rich (i.e. peanuts, parmesan cheese) or poor (i.e. crisps, chocolate cookies) in N. Thus, by combining conveniently these types of food, we could obtain 'cafeteria diets' with various amounts of N, as described by Rothwell *et al.* (1982). Care was taken to weigh daily the amount of food really ingested by each rat and to adjust the type of highly palatable food so that the cafeteria-fed rats ingested daily the same amount of N as did controls.

Thus, the daily intake (g/24h) of rats of each group was: controls, carbohydrate 10.47 ± 1.06, lipid 0.71 ± 0.07, protein 2.91 ± 0.26; cafeteria-fed rats, carbohydrate 7.82 ± 2.05 ($P<0.01$), lipid 9.46 ± 1.90 ($P<0.001$), protein 2.75 ± 0.22 (not significant). Values are means ± S.D. ($n = 8$). Values of P refer to intakes in the cafeteria-fed group that are significantly different from controls.

After 4 weeks all rats were starved for 18–24h before the experimental assays. For the collection of urine and faeces, animals were placed during this time in individual metabolic cages. The urine samples were collected into 25ml test tubes containing 1ml of 6M-HCl. Faeces were collected and homogenized with water in a Potter–Elvehjem homogenizer and diluted to 200ml.

Reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). A physiological mixture of amino acids was prepared by mixing individual amino acids (Sigma) as described by Hensgens & Meijer (1979).

Methods

Studies in vivo. For amino acid analysis, blood was collected in heparinized syringes from the hepatic vein (inside the left hepatic lobe), the aorta and the portal vein. Protein was precipitated by mixing 1vol. of blood with 4vol. of 3.75% (w/v) sulphosalicylic acid in 0.3M-lithium citrate buffer (pH2.8); 1ml of the supernatant was collected and injected into a LKB 3201 amino acid analyser for determination of amino acids (Viña *et al.*, 1981).

The amino acids taken up by the liver were estimated by assuming that portal vein and hepatic artery represent 70% and 30% of the afferent hepatic blood flow respectively (Greenway & Stark, 1971). The hepatic percentage uptake was worked out from the equation (Rémésy *et al.*, 1978):

$$\frac{0.3(\text{aorta} - \text{hepatic vein}) + 0.7(\text{portal vein} - \text{hepatic vein})}{0.3(\text{aorta}) + 0.7(\text{portal vein})} \times 100$$

Samples of liver for amino acid analysis were obtained from rats that were not used for blood sampling. The freeze-clamped tissue (Wollenberger *et al.*, 1960) was powdered in liquid N_2 with a pestle and mortar, and a portion of frozen powder (about 1g) was extracted with 4ml of 6% (w/v) $HClO_4$ by homogenization with a motor-driven Teflon homogenizer. Supernatant (1ml) was collected and its pH adjusted to 2.2 with sulphosalicylic acid.

Studies in vitro. Hepatocytes were isolated from rat livers by the method of Berry & Friend (1969) as modified by Romero & Viña (1983). Cells (15–20mg wet wt./ml) were incubated (40min) in Krebs–Henseleit (1932) bicarbonate buffer containing 3mM-Ca^{2+}. Viability was assessed routinely by the Trypan-Blue-exclusion method as described by Baur *et al.* (1975). In all cases more than 85% of the cells excluded Trypan Blue.

Determination of metabolites. These were measured as previously described in the references: creatinine (Yatzidis, 1974); total liver and serum lipids (Frings *et al.*, 1972); total liver protein (Lowry *et al.* 1951); citrulline (Snodgrass & Parry, 1969); urea (Nuzum & Snodgrass, 1976), by using a standard curve designed to correct for the colour production of citrulline that had not reacted at the end of the argininosuccinate synthetase incubation period. A more sensitive assay method (Hunninghake & Grisolia, 1966) was used to measure citrulline in the absence of urea for the carbamoyl-phosphate synthetase assay, and urea in the absence of citrulline for the argininosuccinate lyase procedure.

Analysis of N content. This was done in triplicate by the micro-Kjeldahl method (Markham, 1942). Total N content of rats was assayed as described by Lin & Huang (1982).

Enzyme assays. The procedures for the assay of carbamoyl-phosphate synthetase (EC 6.3.4.16), ornithine carbamoyltransferase (EC 2.1.3.3), argininosuccinate synthetase (EC 6.3.4.5), argininosuccinate lyase (EC 4.3.2.1) and arginase (EC 3.5.3.1) were those described previously (Schimke,

1962*a*). One unit of enzyme activity is defined as the amount of enzyme which catalyses the formation of 1 μmol of product/h at 37°C.

Results

Influence of cafeteria diet on body and fat weight in rats

As expected (Rothwell & Stock, 1979), cafeteria-diet-induced hyperphagia promotes an increase in total body and epididymal-adipose-tissue weight in rats. The amounts of total lipids in liver and serum were also significantly increased. However, we did not observe changes in the liver weight of cafeteria-fed rats compared with controls (Table 1).

Nitrogen metabolites in cafeteria-diet-fed rats

Table 2 shows that N excretion was significantly lower in cafeteria-diet-fed rats than in controls. All the difference in urinary N excretion can be accounted for as decreased excretion of urea. Indeed cafeteria-fed rats excreted 3.01 mmol of urea/24 h less than did controls, i.e. 84.2 mg of urea N, which is in good agreement with the difference in N excretion found (77 mg).

The concentrations of NH_4^+ and uric acid in blood and urine were not significantly different in controls and in cafeteria-fed rats (results not shown). However, blood concentration of urea was lower in cafeteria-fed rats than in controls (Table 2). The concentration of creatinine in blood and the 24 h urine creatinine excretion were similar in controls and in cafeteria-fed rats (Table 2); therefore the renal clearance of creatinine was not affected by cafeteria feeding.

Urea synthesis in isolated hepatocytes from cafeteria-fed rats

In order to find an explanation for the decreased excretion of urea observed, we studied the capacity of hepatocytes to synthesize urea from ammonia or from a mixture of amino acids at physiological concentrations or at supra-physiological concentrations, as used by Hensgens & Meijer (1979). In all cases we found a significant decrease in the rate of urea synthesis in cafeteria-fed rats. As prevously established, the rate of urea synthesis from ammonia was increased by additions of catalytic amounts of ornithine (Krebs & Henseleit, 1932) and by lactate (Meijer & Hensgens, 1982). Lactate increased the rate of urea synthesis by about 300%

Table 1. *Physiological parameters in chow-fed and cafeteria-fed rats*

For details see the text. The results are means ± S.D. for the numbers of experiments given in parentheses. Results that are significantly different from controls are shown: *$P<0.05$; **$P<0.001$.

	Control (chow-fed)	Cafeteria-fed
Initial body wt. (g)	167 ± 7 (8)	158 ± 9 (8)
Final body wt. (g)	261 ± 34 (8)	292 ± 32 (8)*
Liver wt. (g)	7.4 ± 0.7 (7)	7.8 ± 0.9 (7)
Epididymal adipose-tissue wt. (g)	3.45 ± 0.62 (8)	5.22 ± 0.80 (8)**
Total lipids		
Liver (mg/g)	26.3 ± 3.7 (7)	43.2 ± 6.1 (8)**
Serum (mg/100 ml)	188 ± 23 (6)	259 ± 28 (8)**

Table 2. *Nitrogen balance and concentrations of major nitrogen-containing compounds in serum and urine in chow-fed and cafeteria-fed rats*

For details see the text. Rats were starved for 18–24 h, during which period N excretion was measured. The results are means ± S.D. for the numbers of experiments given in parentheses. Results that are significantly different from controls are shown: *$P<0.001$.

	Control (chow-fed)	Cafeteria-fed
N intake (mg/24 h)	478 ± 23 (8)	459 ± 21 (8)
N excretion (mg/24 h)		
Urinary	215 ± 19 (8)	138 ± 34 (8)*
Faecal	98 ± 12 (4)	33 ± 8 (4)*
Serum		
Urea (μmol/ml)	6.28 ± 0.54 (8)	4.37 ± 0.96 (8)*
Creatinine (μmol/ml)	0.098 ± 0.001 (4)	0.097 ± 0.001 (4)
Urine		
Urea (mmol/24 h)	7.82 ± 0.80 (8)	4.81 ± 0.76 (8)*
Creatinine (mmol/24 h)	0.12 ± 0.01 (4)	0.13 ± 0.01 (4)

both in controls and in cafeteria-fed rats. In all cases urea synthesis was linear with time during the 40 min incubation period studied. Glucagon also stimulated urea synthesis from ammonia in controls in cafeteria-fed rats. However, the rate of stimulation by glucagon was higher in controls than in cafeteria-fed rats (see Table 3). When lactate was present (together with ammonia and ornithine), glucagon had no effect on the rate of urea synthesis, as previously shown by Hensgens *et al.* (1980).

Activity of urea-cycle enzymes in cafeteria-fed rats

To find a reason for the lower capacity of isolated hepatocytes from cafeteria-fed rats to synthesize urea, we measured the activity of the enzymes of the urea cycle in whole liver homogenates and found that in livers from cafeteria-fed rats all the enzyme activities were decreased compared with controls (Table 4). Interestingly, the greater decreases were carbamoyl-phosphate synthetase and argininosuccinate synthetase, the enzymes that are implicated in the control of the overall rate of the urea cycle (Meijer & Hensgens, 1982).

When rats were fed with cafeteria diet for 30 days and then switched to normal chow diet for 2 weeks, the enzymes activities returned to normal values (Table 4).

Liver accumulation of fat is not responsible for the observed changes in enzyme activities. Indeed, when cafeteria-fed rats were treated with silymarin or choline no accumulation of fat was observed, but liver enzyme activities were still lower than in controls (Gómez *et al.*, 1985).

Amino acid concentration in arterial and portal-vein blood

Cafeteria feeding during the 30 days before the experiments caused an increase in L-aspartic acid, L-serine and L-arginine in arterial blood compared with controls, in spite of the 24 h period of starvation immediately before blood sampling. In portal vein, cafeteria feeding only induced an increase in L-aspartic acid, L-serine, L-valine and

Table 3. *Urea synthesis by isolated hepatocytes from chow-fed and cafeteria-fed rats*
For details see the text. Rats were starved for 18–24 h before the experiments. The results (μmol/min per g wet wt.) are means ± S.D. for the numbers of experiments given in parentheses. PMA is a mixture of amino acids at concentrations similar to those found in plasma from normal starved rats. Results that are significant for cafeteria-fed rats are shown: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Additions	Urea synthesis: Control (chow-fed)	Urea synthesis: Cafeteria-fed
None	0.13 ± 0.05 (8)	0.08 ± 0.04 (9)*
NH_4Cl (10 mM) + ornithine (10 mM)	0.87 ± 0.13 (9)	0.38 ± 0.07 (9)***
NH_4Cl (10 mM) + ornithine (10 mM) + lactate (10 mM)	2.78 ± 0.31 (8)	1.16 ± 0.37 (8)***
NH_4Cl (10 mM) + ornithine (10 mM) + glucagon (10 nM)	1.91 ± 0.31 (5)	0.52 ± 0.15 (6)***
NH_4Cl (10 mM) + ornithine (10 mM) + lactate (10 mM) + glucagon (10 nM)	2.73 ± 0.54 (6)	1.40 ± 0.40 (5)***
PMA	0.16 ± 0.03 (5)	0.09 ± 0.01 (8)**
5 × PMA	0.40 ± 0.09 (4)	0.17 ± 0.03 (6)***

Table 4. *Urea-cycle enzyme activities in rats fed on chow diet or cafeteria diet and re-fed on normal chow (14 days)*
For details see the text. Rats were starved for 18–24 h before the experiments. The results are expressed (units/g of liver) are means ± S.D. for the numbers of experiments given in parentheses. Results that are significantly different from those of chow-fed rats are shown: *$P<0.001$.

Enzyme	Activity: Chow-fed	Activity: Cafeteria-fed	Activity: Re-fed
Carbamoyl-phosphate synthetase	400 ± 65 (8)	152 ± 58 (8)*	365 ± 60 (4)
Ornithine carbamoyltransferase	16815 ± 2780 (8)	12057 ± 1183 (8)*	17386 ± 2800 (4)
Argininosuccinate synthetase	186 ± 18 (8)	43 ± 12 (6)*	159 ± 29 (4)
Argininosuccinate lyase	205 ± 21 (8)	126 ± 15 (8)*	171 ± 35 (4)
Arginase	50525 ± 5300 (8)	41300 ± 1263 (8)*	52319 ± 2750 (4)

L-arginine (results not shown). In a previous paper we showed that cafeteria feeding induced an increase in arterial concentration of L-glutamate, L-glutamine and L-alanine in both virgin and lactating rats (Viña & Williamson, 1981). In another study the hyperphagia induced by cafeteria feeding resulted in increases in L-threonine, L-serine, L-proline, L-citrulline and L-tyrosine, and decreases in branched-chain amino acids (Calles-Escandón *et al.*, 1984).

This may be due to different experimental conditions. Indeed, it is important to emphasize two facts of our experiments. The first is that both groups of rats had the same N intake, which was not the case in previous experiments. A second point is that the experiments were performed after 20h of food withdrawal.

Amino acid uptake by the liver

Table 5 shows that, in chow-fed rats, L-alanine was the amino acid with the highest rate of uptake, followed by L-methionine, L-arginine, L-cysteine, L-serine, L-threonine, L-aspartic acid, L-phenylalanine, L-tyrosine and L-proline. The uptakes of L-glutamate, L-glutamine and branched-chain amino acids were the lowest of all amino acids.

Cafeteria feeding induced a decrease in amino acid uptake, except for L-threonine, L-methionine, L-phenylalanine, L-lysine and branched-chain amino acids. The decrease in L-alanine uptake was very significant and was associated with a lower activity of hepatic L-alanine aminotransferase. In chow-fed rats the hepatic L-alanine aminotransferase activity was 26.1 ± 5.4 (8)μmol of substrate transformed/min per g of tissue, and in cafeteria-fed rats it was 15.8 ± 5.5 (8) ($P < 0.005$). In isolated hepatocytes from rats fed with cafeteria diet, L-alanine removal from the suspension medium was also lower than in rats fed on chow diet (Viña & Williamson, 1981).

Uptake of L-glutamine and L-glutamate by liver in the chow-fed rats was low; however, in cafeteria-fed rats, the liver released L-glutamine and L-glutamate. These two related amino acids were the only ones that were released.

The steady-state concentrations of amino acids in liver were similar in both groups, except for L-aspartic acid, L-threonine, L-serine, and L-asparagine, which were increased very significantly after the hyperphagia induced by cafeteria feeding (Table 6). The concentration of L-aspartate in liver that we obtain is an order of magnitude lower than that reported by others (Willamson *et al.*, 1967). We cannot offer an explanation for this discrepancy.

The fact that free amino acid concentration in liver is unchanged, or even increased, in rats that show a decrease in amino acid uptake by liver suggests that this decrease is a result of a slower amino acid utilization by liver. This is in agreement with the observed decrease in the rate of urea synthesis.

Table 5. *Amino acid uptake by liver of rats fed on chow diet and cafeteria diet*

Uptake values are expressed as a percentage of the total supply of each amino acid to the liver. For details see the Experimental section. Rats were starved for 18–24h. Results are means ± S.D. for four experiments. Values that are significantly different from those of chow-fed rats are shown: *$P < 0.01$; **$P < 0.05$.

	Uptake (%)	
	Control (chow-fed)	Cafeteria-fed
L-Aspartic acid	13.7 ± 5.0	3.4 ± 2.1*
L-Threonine	13.9 ± 4.6	9.6 ± 5.9
L-Serine	14.8 ± 3.1	2.5 ± 4.9*
L-Asparagine	7.7 ± 1.3	6.5 ± 3.9
L-Glutamic acid	5.8 ± 1.7	−3.3 ± 7.9**
L-Glutamine	4.4 ± 2.6	−7.9 ± 7.9**
L-Proline	11.1 ± 3.3	3.8 ± 1.9*
Glycine	8.1 ± 4.5	2.6 ± 1.2**
L-Alanine	30.7 ± 9.7	1.8 ± 10.6*
L-Valine	4.2 ± 0.9	2.2 ± 1.8
L-Cystine	15.5 ± 5.3	6.6 ± 2.7**
L-Methionine	17.9 ± 1.9	10.4 ± 9.0
L-Isoleucine	7.5 ± 1.7	4.4 ± 3.2
L-Leucine	6.3 ± 3.9	4.8 ± 2.7
L-Tyrosine	12.0 ± 2.0	6.7 ± 0.6*
L-Phenylalanine	13.7 ± 7.0	5.1 ± 3.7
L-Lysine	0.9 ± 2.9	2.7 ± 2.9
L-Histidine	3.1 ± 1.3	1.1 ± 0.9**
L-Arginine	15.9 ± 6.3	3.5 ± 2.5**

Table 6. *Concentrations of amino acids in liver*

Values are nmol/g of liver. For details see the text. Rats were starved for 18–24h. Results are means ± S.D. for four experiments. Values that are statistically different from controls are shown: $*P<0.01$; $**P<0.05$; $***P<0.005$.

	Concn. (nmol/g)	
	Control (chow-fed)	Cafeteria-fed
L-Aspartic acid	40 ± 3	60 ± 8**
L-Threonine	207 ± 14	524 ± 96***
L-Serine	469 ± 111	1139 ± 192***
L-Asparagine	91 ± 11	237 ± 15***
L-Glutamic acid	920 ± 216	1107 ± 245
L-Glutamine	2320 ± 317	2662 ± 473
L-Proline	107 ± 6	117 ± 17
Glycine	1344 ± 93	1465 ± 164
L-Alanine	253 ± 46	365 ± 69
L-Valine	124 ± 10	154 ± 14*
L-Cystine	87 ± 10	106 ± 8*
L-Methionine	71 ± 10	76 ± 8
L-Isoleucine	67 ± 9	71 ± 9
L-Leucine	129 ± 15	127 ± 11
L-Tyrosine	69 ± 15	86 ± 15
L-Phenylalanine	36 ± 4	37 ± 2
L-Lysine	386 ± 12	457 ± 44*
L-Histidine	191 ± 35	230 ± 33
L-Arginine	234 ± 20	273 ± 18*

Discussion

We show in this paper that rats fed on cafeteria diet have a decreased N excretion. This is due to a decreased urinary excretion of urea, without changes in renal function. This contrasts with observations in other studies using other animal models of obesity in which N excretion is increased. However, this difference between hyperphagia-induced obesity (cafeteria diet) and genetic or hypothalamic obesity may be due to the marked hormonal changes which occur in these latter models. Indeed, genetically obese rats have increased concentrations of corticosteroids (Spydevold *et al.*, 1978) and hypothalamic obese rats have increased concentrations of glucagon (Karakasch *et al.*, 1970). Our results agree with those of Munro (1951, 1978), who also observed that excess energy intake resulted in a decreased N excretion in humans and rats.

The decrease in urea excreted in cafeteria-fed rats may be the result of two factors: a decrease in the uptake of amino acids by liver and/or a decrease in the capacity of the liver to form urea from precursors. Indeed, hepatocytes from cafeteria-fed rats formed urea at rates significantly lower than in controls. An important point is that the concentrations of NH_4^+ are similar in cafeteria-fed rats and in controls (results not shown). Furthermore, cafeteria-fed rats have a decreased activity of the enzymes of the urea cycle, especially carbamoyl-phosphate synthetase and argininosuccinate synthetase, which are probably involved in the regulation of the overall rate of the cycle (Meijer & Hensgens, 1982). Concerted changes in all five enzymes of the urea cycle in response to hormonal changes have been described (McLean & Gurney, 1963; Wixom *et al.*, 1972; Snodgrass *et al.*, 1978).

In this context it is important to note that, although the enzymes of the cycle are located in different cell compartments, an as yet unknown factor may exist, as postulated by Cohen (1981), to promote concerted changes in all the enzyme activities of the cycle.

Starvation (Schimke, 1962*b*) or changing the protein intake of rats (Schimke, 1962*a*) also affects N excretion. However, here we show N retention in cafeteria-fed rats that maintain the same N intake as controls. In order to investigate the possible fate of the extra N that the cafeteria-fed rats retain, we measured the total N content of these rats and found that it was significantly higher than in controls. Indeed, we found an increased N content of about 1050mg per cafeteria-fed rat. If (after 30 days of cafeteria-feeding) these rats retain 120mg of N/day more than controls, a total retention of 3600mg should be expected; we only find about 30% of this value. However, the value of 3600mg of N is obtained on the assumption that the rats retain 120mg of N more than the controls from the first day when they are submitted to cafeteria diet. It is probable that the increased N retention develops gradually after some days of

cafeteria feeding. Thus the value of 3600 mg of N is an overestimation. Lin &Huang (1982) studying N metabolism different diets, also found only 47% of the expected total body N.

The hormonal status of the animals may be responsible for the observed changes in N metabolism in cafeteria-fed rats. However, the hormonal profile of these animals is still unclear. For example Calles-Escandón *et al.* (1984) report that insulin concentrations are increased in cafeteria-fed rats. However, other reports show that the values are similar to the controls (Rolls *et al.*, 1980) or even decreased (Rothwell & Stock, 1981). These differences may be explained, at least in part, by the different experimental conditions used in these studies. However, further studies are required to clarify these points. Using cafeteria-fed rats, we show changes in N metabolism that are different from those described for other models of obesity, i.e. genetic or hypothalamic. However, the fact that in cafeteria-fed rats N excretion is decreased is in agreement with the observed decreased N excretion in humans fed on a high-energy diet (Munro, 1978). Since several cases of human obesity are induced by diet, these results may be of practical importance.

We thank Dr. I. R. Puertes for her constructive criticism and Miss Concha Garcia for her skilful technical assistance.

References

Baur, H., Kasperek, S. & Pfaff, E. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 827–838

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Calles-Escandón, J., Cunningham, J. & Felig, P. (1984) *Metab. Clin. Exp.* **33**, 364–368

Cohen, P. P. (1981) *Curr. Top. Cell. Regul.* **18**, 1–19

Dunn, M. A. & Hartsook, E. W. (1980) *J. Nutr.* **110**, 1865–1879

Frings, C. S., Fendley, T. W., Dunn, R. T. & Queen, C. A. (1972) *Clin. Chem.* **18**, 673–674

Gómez, D., Barber, T., Timoneda, J. & Cabo, J. (1985) *Biochem. Soc. Trans.* **13**, 897–898

Greenway, C. V. & Stark, R. D. (1971) *Physiol. Rev.* **51**, 23–65

Hensgens, H. E. S. J. & Meijer, A. J. (1979) *Biochim. Biophys. Acta* **582**, 525–532

Hensgens, H. E. S. J., Verhoeven, A. J. & Meijer, A. J. (1980) *Eur. J. Biochem.* **107**, 197–205

Hunninghake, D. & Grisolia, S. (1966) *Anal. Biochem.* **16**, 200–205

Karakasch, C., Rohner-Jeanrenaud, F., Hustvedt, B. & Jeanrenaud, B. (1980) *Am. J. Physiol.* **238**, E32–E37

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lin, C. P. & Huang, P. (1982) *J. Nutr.* **112**, 1067–1074

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Markham, R. (1942) *Biochem. J.* **36**, 790–791

McLean, P. & Gurney, M. W. (1963) *Biochem. J.* **87**, 96–104

Meijer, A. & Hensgens, H. E. S. J. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 259–286, Academic Press, New York

Munro, H. N. (1951) *Physiol. Rev.* **31**, 449–488

Munro, H. N. (1978) *Kidney Int.* **14**, 313–316

Nuzum, C. & Snodgrass, P. (1976) in *The Urea Cycle* (Grisolia, S., Báguena, R. & Mayor, F., eds.), pp. 325–355, John Wiley and Sons, New York

Paul, A. A. & Southgate, D. A. T. (1976) *The Composition of Foods*, pp. 38–133, Elsevier/North-Holland Biomedical Press, Amsterdam

Rémésy, C., Demigné, C. & Aufrère, J. (1978) *Biochem. J.* **170**, 321–329

Rolls, B. J., Rowe, E. A. & Turner, R. C. (1980) *J. Physiol. (London)* **298**, 415–427

Romero, F. J. & Viña, J. (1983) *Biochem. Educ.* **11**, 135–136

Rothwell, N. J. & Stock, M. J. (1979) *Nature (London)* **281**, 31–35

Rothwell, N. J. & Stock, M. J. (1981) *Metab. Clin. Exp.* **30**, 673–678

Rothwell, N. J., Stock, M. J. & Tyzbir, R. S. C. (1982) *J. Nutr.* **112**, 1663–1672

Schimke, R. T. (1962*a*) *J. Biol. Chem.* **237**, 459–468

Schimke, R. T. (1962*b*) *J. Biol. Chem.* **237**, 1921–1924

Sclafani, A. & Springer, D. (1976) *Physiol. Behav.* **17**, 461–471

Snodgrass, P. J. & Parry, D. J. (1969) *J. Lab. Clin. Med.* **73**, 940–950

Snodgrass, P. J., Lin, R. C., Muller, W. A. & Aoki, T. T. (1978) *J. Biol. Chem.* **253**, 2748–2753

Spydevold, S. O., Greenbaum, A. L., Baguer, N. Z. & McLean, P. (1978) *Eur. J. Biochem.* **89**, 329–339

Viña, J. R. & Williamson, D. H. (1981) *Biochem. J.* **196**, 757–762

Viña, J., Puertes, I. R., Estrela, J. M., Viña, J. R., Galbis, J. L. (1981) *Biochem. J.* **194**, 99–102

Williamson, D. H., Lopes-Vieira, O. & Walker, B. (1967) *Biochem. J.* **104**, 497–502

Wixom, R. L., Reddy, M. K. & Cohen, P. P. (1972) *J. Biol. Chem.* **247**, 3684–3692

Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflugers Arch.* **270**, 399–412

Yatzidis, H. (1974) *Clin. Chem.* **20**, 1131–1134