In general, our study supports the conviction that tetracyclines in the doses normally used in antibiotic therapy [1] inhibit mitochondrial protein synthesis and not cytoplasmic protein synthesis. The experience that tetracyclines have no serious side effects notwithstanding this inhibitory action has, of course, to be explained. Most likely a decrease of up to 50 per cent of the activity of the terminal enzyme of the respiratory chain does not lead to the situation that oxidative phosphorylation becomes rate-limiting for adequate functioning of most tissues and organs.

The steady state tissue distribution studies presented in this paper show that oxytetracycline and doxycycline in doses comparable to those used in antibiotic treatment do not accumulate in most tissues. They inhibit specifically mitochondrial protein synthesis. Only at high dose does cytoplasmic protein synthesis also seem to be impaired.

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Na^+, K^+-ATPase activity and noradrenaline turnover in brown adipose tissue of rats exhibiting diet-induced thermogenesis

Recently it has been demonstrated that the hyperphagia induced by offering rats a varied and palatable 'cafeteria' diet is accompanied by large, compensatory increases in heat production that can prevent the development of obesity [1]. This diet-induced thermogenesis (DIT) is similar to the non-shivering thermogenesis (NST) exhibited by cold-adapted animals, since both involve the sympathetic noradrenergic activation of brown adipose tissue (BAT). Furthermore it can be shown that the thermogenic response of cafeteria-fed and cold-adapted rats to noradrenaline is almost entirely due to increases in BAT oxygen consumption [2, 3]. The high thermogenic capacity of BAT is due

to reduced coupling of oxidative phosphorylation which Nicholls [4] ascribes to the presence of a proton conductance pathway across the inner mitochondrial membrane. Although this pathway is increased in rats exhibiting NST and DIT [5, 6], Horowitz [7] has proposed that another mechanism involving Na^+, K^+-ATPase is implicated in NST. We therefore decided to investigate the possibility that this enzyme system is similarly involved in the diet-induced thermogenesis exhibited by cafeteria rats.

Male, Sprague–Dawley rats (120 g body wt) were maintained on either a conventional stock diet or the cafeteria diet of 10–12 days [1]. Resting oxygen consumption (VO_2) was measured in a closed-circuit respirometer for 2 hr during the day at a temperature of 29 ± 1° as previously described [8]. At sacrifice the Interscapular BAT was dissected out and homogenised (10% w/v in 0.32 M Sucrose) in a Potter-S homogeniser fitted with a Teflon pestle (2000 rpm, 7 strokes). Microsomal material was prepared by centrifugation of the homogenate at 18,000g_{av} for 20 min. The supernatant was collected and centrifuged at 100,000g_{av} for 30 min and the resulting pellet was either resuspended in 50 mM Tris–HCl or frozen at −30° prior to analysis. Sodium–potassium-activated, magnesium-dependent, adenosinetriphosphatase (EC 3.6.1.3 Na^+, K^+-ATPase) was calculated as the difference between total ATPase and Na^+-ATPase activity as described in detail by Gilbert and Wyllie [9]. Adenylate cyclase activity was measured by monitoring the rate of formation of cyclic AMP from ATP [10] and noradrenaline uptake was measured by monitoring the uptake of [^{3}H]noradrenaline [11]. Uptake 1 was defined as that component blocked by desipramine (5×10^{-6} M) while uptake 2 was considered to be the component blocked by corticosterone (4×10^{-5} M). Noradrenaline was measured fluorometrically [12]. Turnover was assessed after blocking noradrenaline synthesis with α-methyl para-tyrosine. All reagents were of 'analar' grade and glass-distilled, deionised water used. All values are presented as the mean ± S.E.M. of 4 experiments (control) or 6 experiments (cafeteria).

As in previous studies [1], measurements of VO_2 on days 5–10 revealed a 30 per cent increase in cafeteria-fed rats ($P < 0.001$) and an enhanced (2-fold) increase to the thermogenic actions of noradrenaline. Injection of the ganglion-blocking agent hexamethonium (5 mg/kg i.p.) and the β-adrenoreceptor antagonist propranolol (5 mg/kg i.p.) blocked 73 and 88 per cent of the elevated VO_2 of cafeteria rats, respectively. The α-adrenoreceptor antagonist phentolamine (5 mg/kg i.p.) caused only an 11 per cent inhibition and none of these drugs had any significant effect on the VO_2 of stock-fed rats. Diet-induced thermogenesis of cafeteria rats can therefore be largely ascribed to the sympathetic innervation acting on β-adrenoreceptors.

At sacrifice the mass of interscapular BAT dissected from cafeteria rats was found to be 73 per cent greater than that of controls (controls 383 ± 12, cafeteria 659 ± 14 mg, $P < 0.001$). There was a significantly increased Na^+, K^+-ATPase activity in homogenates prepared from the BAT of the two groups (control 0.23 ± 0.01, cafeteria 0.32 ± 0.02, μmoles P_i/mg protein/hr, $P < 0.001$). In addition a remarkably high correlation ($r = 0.968$, $P < 0.001$) existed between these *in vitro* values for Na^+, K^+-ATPase and the *in vivo* measurements of VO_2 made when the animals were eating their respective diets (Fig. 1). It is worth noting that in the cafeteria group, the two animals with the lowest VO_2 also had the lowest Na^+, K^+-ATPase activity. These differences in Na^+, K^+-ATPase activity were absent from BAT microsomal fractions which suggests that the activity of this enzyme is influenced by some endogenous factor that is present at higher concentration in homogenates from cafeterial fed animals, noradrenaline is the most likely and pertinent candidate.

Therefore, the effects of different concentrations of noradrenaline on the *in vitro* Na^+, K^+-ATPase activity of membrane fractions were assessed. Noradrenaline caused

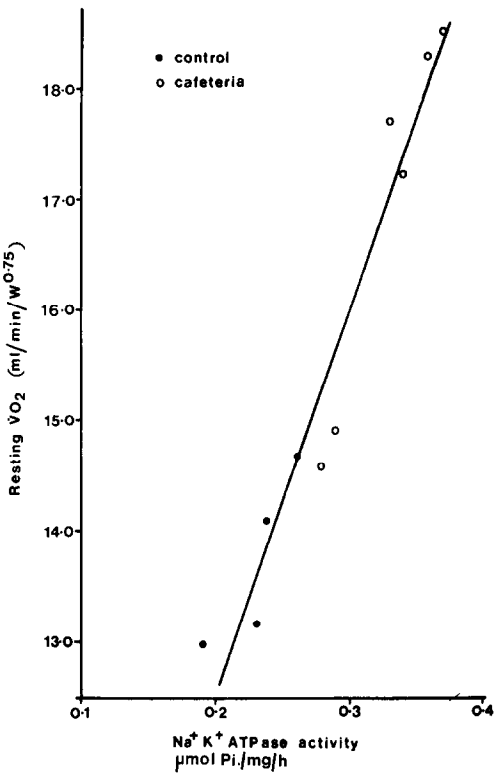

Fig. 1. Relationship between oxygen consumption and brown adipose tissue Na^+, K^+-ATPase activity in rats fed stock diet (control) or a varied and palatable diet (cafeteria).

a marked stimulation of enzyme activity and the maximal response in cafeteria rats was more than twice that of control rats (Fig. 2). The concentration of noradrenaline required to produce half-maximal stimulation was 5×10^{-6} and 1×10^{-6} M in control and cafeteria rats respectively, suggesting a change in sensitivity in the latter group. The greater response of the enzyme seen in cafeteria rats is similar to that seen in BAT of cold-adapted animals [13] and appears to involve the β-adrenoreceptor. The β-adrenoreceptor agonist isoprenaline (10^{-4} M) for example, produced increases in Na^+, K^+-ATPase activity comparable to maximal noradrenaline stimulation (control 30.9 ± 2.5%, cafeteria 72.0 ± 2.6%) but the α-adrenoreceptor agonist phenylephrine (10^{-4} M) produced increases of only 6.8 ± 2.5% and 20.8 ± 1.9% in control and cafeteria microsomal fractions, respectively. The receptor specificity of Na^+, K^+-ATPase activation is consistent with the *in vivo* effects of α- and β-adrenoreceptor antagonists on VO_2 but interestingly differs from the α-adrenoreceptor-mediated stimulatory effects of catecholamines on brain Na^+, K^+-ATPase [14].

The marked changes in Na^+, K^+-ATPase response suggest that other β-adrenoreceptor-mediated effects should be more pronounced on the BAT of cafeteria-fed rats. Adenylate cyclase activity, often associated with this receptor was unchanged in cafeteria animals and *in vitro* addition of noradrenaline (10^{-4} M) produced similar increases in activity (control 84 ± 13%, cafeteria 113 ± 23% NS). From these results, it can be concluded that cafeteria feeding had no effect on β-adrenoreceptor number per unit mass of BAT, although the hypertrophy of this tissue in cafeteria rats resulted in an apparent 2-fold increase in total

receptor number of the interscapular depot. Other workers [15] have reported increases in the total noradrenaline-stimulated adenyl cyclase activity in BAT from cold-adapted rats, even though specific activity was decreased. The greater response of the Na^+, K^+-ATPase system to β-adrenoreceptor stimulation without changes in adenyl cyclase may be due to some modification by diet and energy intake of events subsequent to the neurotransmitter–receptor interaction.

Since the elevated Na^+, K^+-ATPase activity in tissue homogenates (Fig. 1) may be due to both an increase in sensitivity to noradrenaline (Fig. 2) and to increased noradrenaline levels, the BAT content of this neurotransmitter was also measured. The tissue content (μg/g BAT) was similar for both groups (control 13.80 ± 0.69, cafeteria 14.45 ± 0.74, NS) but because levels provide only limited information on metabolic activity, noradrenaline uptake and turnover were also estimated. Neuronal ($Uptake_1$) and non-neuronal ($Uptake_2$) uptake of noradrenaline in BAT of control and cafeteria-fed rats were no different but when noradrenaline synthesis was blocked with α-methyl paratyrosine, the rate of disappearance of endogenous noradrenaline stores (μg/g BAT/h) was 54 per cent faster in cafeteria (1.67 ± 0.10) than control (1.08 ± 0.09, $P < 0.01$) rats. These changes in turnover are similar to those observed *in vivo* by Young and Landsberg [16] in the heart, pancreas and liver of overfed rats but presumably the changes in BAT observed here relate more to the thermogenic effects of hyperphagia than to the cardiovascular and other responses. Preliminary experiments suggest that the increased noradrenaline turnover in cafeteria rats probably reflects an increased release of neurotransmitter from nerve terminals. Thus, not only is there a facilitation of synaptic transmission due to pre-synaptic events, but because of increased post-synaptic β-adrenoreceptor sensitivity (linked to Na^+, K^+-ATPase activity) there is a further potentiation of noradrenaline action.

In this study we have provided *in vivo* and *in vitro* evidence to support the contention that DIT results from sympathetic activation of brown adipose tissue β-adrenoreceptors and we have been able to link these metabolic effects of hyperphagia to increases in the Na^+, K^+-ATPase activity of this tissue. It is still uncertain whether this enzyme system is part of a thermogenic pathway or whether it acts to provide an intracelluar stimulus for mitochondrial thermogenesis [7] and further research is required to resolve this issue. However, we can now say that changes in both the proton conductance pathway and Na^+, K^+-ATPase activity of brown adipose tissue are consistent with the role we have proposed for this tissue in DIT and that *in vitro* measurements of the latter provide useful and convenient estimates of the level of DIT and total thermogenic capacity in the rat.

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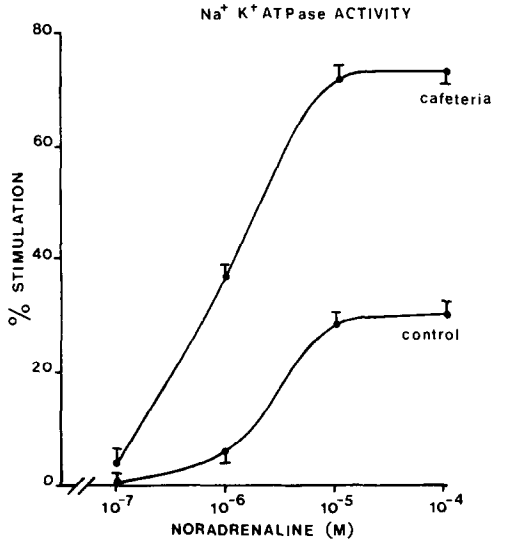

Fig. 2. Effect of concentration on the noradrenaline stimulated increase in Na^+, K^+-ATPase activity of brown adipose membrane fractions from control ($n = 4$) and cafeteria fed ($n = 6$) rats. Mean values, bar denotes S.E.M.

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Inhibition of microsomal-membrane bound and purified epoxide hydrolase by C_2–C_8 1,2-alkene oxides

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Alkene and arene oxides have been implicated as the ultimate toxic metabolites of many unsaturated hydrocarbons [1], and alkene oxides have been postulated to be obligatory intermediates in the metabolism of alkenes to glycols [2, 3]. Epoxide hydrolase (EC 4.2.1.63), a microsomal enzyme, catalyzes the conversion of epoxides to glycols and hydrodiols, which are generally less toxic than the parent compounds. Epoxide hydrolase may, therefore, be an important modulator of the toxicity of alkenes. Previous studies of the substrate [4–8] and inhibitor [9, 10] specificities of epoxide hydrolase have dealt primarily with arene oxides or substituted alkene oxides. As enzyme substrates are frequently competitive inhibitors of the activity of the enzyme, the potencies of 1,2-alkene oxides as inhibitors of epoxide hydrolase may indicate their relative susceptibilities to metabolism by this enzyme. The objective of the studies reported here was to determine the potencies of low molecular weight 1,2-alkene oxides as inhibitors of hepatic epoxide hydrolase.

Male F-344 rats (Charles River Breeding Laboratories, Wilmington, MA) were housed in hanging stainless steel cages and were allowed free access to water and food (Wayne Lab Blox) until 12 hr before being killed. Rats were pretreated by i.p. injection with 80 mg/kg sodium phenobarbital once a day for 3 days, the last dose being administered 24 hr prior to sacrifice. Rats were killed by cervical dislocation, and hepatic microsomes were prepared by the method of Dent *et al.* [11]. Microsomal epoxide hydrolase was prepared according to the method of Lu *et al.* [12]. The purified enzyme that gave one major band on sodium dodecylsulfate–polyacrylamide gel electrophoresis had a specific activity of 525 ± 15 (mean ± S.E.M., N = 10) nmoles benzo[a]pyrene 4,5-dihydrodiol, and 1715 ± 44 nmoles styrene glycol, produced per mg protein per min. Epoxide hydrolase activity was determined using [G-^{3}H]benzo[a]pyrene 4,5-oxide (Midwest Research Insitute) or [7-^{14}C]styrene 1,2-oxide (gift from Professor F. Oesch) as substrate. The assays were performed essentially as described by Oesch *et al.* [4] and Schmassman *et al.* [13]. The substrate concentrations employed were, respectively 200 μM and 2 mM for benzo[a]pyrene and styrene oxide. All assays were performed under conditions that provided linear product formation with time and protein concentration.

1,2-Alkene oxides were added to the incubation mixtures immediately prior to the addition of the substrates, except in some studies with ethylene oxide where a preincubation period of 30 min was employed. All water insoluble oxides were added as solutions in dimethylsulfoxide (DMSO); the volume of the solvent never exceeded 10 μl/ml incubation mixture. Ethylene oxide and propene oxide were added as solutions in 0.1 M Tris–HCl, pH 9.0. All solutions of the alkene oxides were prepared immediately before each experiment; any excess solution was disposed of by mixing with 5 N H_2SO_4. Solutions of ethylene oxide were prepared by bubbling the gas through 0.1 M Tris–HCl, pH 9.0. The concentration of ethylene oxide in solution was determined gravimetrically and was verified by gas chromatography at 110° on a 6-foot glass column packed with Tenax GC 60/80 mesh using a flame ionization detector with a carrier gas flow rate of 20 ml/min of N_2.

All of the alkene oxides inhibited both the microsomal and purified epoxide hydrolase (Table 1). As the chain lengths of the compounds increased their potencies as inhibitors of hydrolase activity increased, as reflected by the decreasing values of IC_{50}. The IC_{50} values obtained with octene oxide and hexene oxide were similar for both the purified and microsomally bound enzyme. With the lower molecular weight compounds (butene oxide, propene oxide and ethylene oxide), the purified enzyme was more sensitive than the microsomal enzyme to inhibition. The lower sensitivity of the microsomal enzyme probably resulted from the low molecular weight alkene oxides having reacted with microsomal proteins, effectively reducing the concentration of inhibitor that was available to the enzyme. As the chain length of the alkene oxide increased, the ratio of the IC_{50} with styrene oxide as substrate to the IC_{50} with benzo[a]pyrene 4,5-oxide as substrate (IC_{50} ratio) also increased. The IC_{50} ratio was approximately 19 for octene oxide with both the purified and the microsomal enzyme. The IC_{50} ratio decreased, as the chain length of the epoxide decreased, to a minimum of approximately 2 with ethylene oxide. The ratio of the concentration of styrene oxide to benzo[*a*]pyrene oxide in the assays was 10; the IC_{50} ratio of 19 for octene oxide indicates that this epoxide was a more effective inhibitor of the hydration of benzo[a]pyrene oxide than of styrene oxide. The decreasing IC_{50} ratio with decreasing chain length indicates that the shorter chain epoxides were relatively more efficient in inhibiting the hydration of styrene oxide than of benzo[a]pyrene oxide.

Cyclohexene oxide (CCHO) and 1,1,2-trichloropropene 2,3-oxide (TCPO) inhibited both the purified and the