ELSEVIER

CBP

Lactose causes heart arrhythmia in the water flea *Daphnia pulex*

Anthony K. Campbell[a],*, Kenneth T. Wann[b], Stephanie B. Matthews[c]

[a]*Department of Medical Biochemistry and Immunology, Wales College of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK*
[b]*Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cathays Park, Cardiff, CF10 3XF, UK*
[c]*Department of Medical Biochemistry and Immunology, Llandough Hospital, Cardiff and Vale NHS Trust, Penarth, Vale of Glamorgan, CF64 2XX, UK*

Abstract

The cladoceran *Daphnia pulex* is well established as a model for ecotoxicology. Here, we show that *D. pulex* is also useful for investigating the effects of toxins on the heart in situ and the toxic effects in lactose intolerance. The mean heart rate at 10 °C was 195.9 ± 27.0 beats/min ($n=276$, range 89.2–249.2, >80% 170–230 beats/min). *D. pulex* heart responded to caffeine, isoproteronol, adrenaline, propranolol and carbachol in the bathing medium. Lactose (50–200 mM) inhibited the heart rate by 30–100% ($K_{1/2}=60$ mM) and generated severe arrhythmia within 60 min. These effects were fully reversible by 3–4 h. Sucrose (100–200 mM) also inhibited the heart rate, but glucose (100–200 mM) and galactose (100–200 mM) had no effect, suggesting that the inhibition by lactose or sucrose was not simply an osmotic effect. The potent antibiotic ampicillin did not prevent the lactose inhibition, and two diols known to be generated by bacteria under anaerobic conditions were also without effect. The lack of effect of L-ribose (2 mM), a potent inhibitor of β-galactosidase, supported the hypothesis that lactose and other disaccharides may affect directly ion channels in the heart. The results show that *D. pulex* is a novel model system for studying effects of agonists and toxins on cell signalling and ion channels in situ.
© 2004 Elsevier Inc. All rights reserved.

1. Introduction

The cladocerans *Daphnia pulex* and *Daphnia magna* have been established as useful model systems in ecotoxicology and for investigating the ecological impact of toxic substances in freshwater (Persoone and Van de Vel, 1988; Baird et al., 1989a,b; Diamantino et al., 2000; Guilhermino et al., 2000; De Coen and Janssen, 2003). *Daphnia* can be used to study nutrition and starvation responses (Tessier et al., 1983; Elendt, 1990a,b; Elendt and Storch, 1990). *Daphnia* have been reported to have a myogenic heart (Bekker and Krijgsman, 1951), though this now needs confirming using modern electrophysiological and pharmacological techniques. The *Daphnia* heart responds to a range of agonists and antagonists that affect heart rate and rhythm in humans (Viehoever and Cohen, 1937; Sollman and Webb, 1941; Postmes et al., 1973; Villegas-Navarro et al., 2003). The aim of the experiments described here was to show whether *Daphnia* could be used to investigate the effects of sugars on the heart and to establish *Daphnia* as a model for investigating the role of toxins in human disease. A long-term objective was to test our hypothesis that the gut and systemic symptoms in people with lactose intolerance were caused either by lactose itself of by bacterial toxins produced in the gut. This paper provides the first step in this objective, showing effects of lactose on *Daphnia* heart that are similar to those in humans.

Lactose intolerance is caused by an inability to digest lactose, galactose β 1,4 glucose the sugar in milk, because of an inadequate level of the gut enzyme lactase–lactase phlorizin-hydrolase, LPH, EC 3.21.23/62 (Flatz, 1987; Sahi, 1994; Carper, 1995; Campbell and Matthews, 2001; Tolston, 2000; Swallow, 2003). β-Galactosidase also

---

* Corresponding author. Tel.: +44 29 20742951; fax: +44 29 20745440.

E-mail address: campbellak@cf.ac.uk (A.K. Campbell).

cleaves lactose into galactose and glucose and is ubiquitous in pro- and eu-karyotes. Lactose intolerance causes a wide range of gut and systemic symptoms (Srinivasan and Minocha, 1998; Matthews and Campbell, 2000, 2004; Campbell and Matthews, 2001), but a particularly dramatic symptom is the occurrence of heart palpitations and arrhythmia. We propose that the symptoms in lactose intolerance are caused either by lactose itself, inappropriately absorbed, or by toxins generated by bacteria in the small intestine. These toxins include diacetyl, acetoin, 2,3 butandiol, 1,3 propandiol, D-lactate, hydrogen and peptides. In order to test this hypothesis, a model system was required that could be used to examine the effects of lactose and these putative toxins on the heart, gut and other tissues, and that could be used to image effects on intracellular signals in live cells within an intact organism. Thus, a microscope system was established to measure the effect of lactose and other agents on heart rate and rhythm when the agents were added to the water in which the *Daphnia* were swimming.

Here, we report that lactose, at concentrations found in products containing milk, caused a dramatic decrease in *Daphnia* heart rate and induced severe arrhythmia. Our results provide further evidence of *Daphnia* as a unique model system in biology and medicine.

2. Materials and methods

2.1. Materials

All chemicals were Analar grade and were obtained either from Fisher Chemicals or Sigma Chemicals (London). Lactose is not as easy to dissolve as sugars such as sucrose, glucose and galactose. It was made up as a molar stock in water or the appropriate buffer and stored frozen, when some of it came out of solution. The stock was warmed gently in a microwave to ensure that all the lactose had dissolved before aliquoting it into lower dilutions.

2.2. Daphnia

D. pulex were obtained from our pond in Pembrokeshire, Wales, UK. They were kept in 10- or 80-l tanks on the window ledge of a laboratory at approximately 22 °C. The tanks generated enough microalgae to sustain a colony of several hundred for at least 6 months. The walls of the tanks were scraped clean every 2–3 months and topped up alternatively with pond water or distilled water to replenish water lost by evaporation. The *Daphnia* were observed in a specially constructed cooled chamber, maintained in most experiments between 10 and 11 °C, using a binocular dissecting microscope, magnification 20–40×. The *D. pulex* were approximately 1–2 mm in length, with a heart 100–200 μm long and 50 μm across.

The heart is situated above the brooding chamber, though the animal normally swims what appears at first glance upside down, with the feelers that move food into the mouth facing upwards. The highest magnification (40×) was required in order to see the heart beat adequately for counting. Each *D. pulex* was maintained within a 50-μl droplet throughout the experiment.

2.3. Measurement of heart rate

A wide range of artificial media have been developed to study *Daphnia* sp. (Banta, 1921; Keating, 1985; Elendt, 1989, 1990a,b; Kluttgen et al., 1994). Here, *D. pulex* were incubated in 50-μl drops of either pond water or a specially designed simple salt medium A (25 mM MOPS, 25 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $NaHCO_3$, pH 7.0) containing the agent being studied. *Daphnia* thrived in both media for several hours with no significant change in heart rate. Between six and nine droplets could be examined in each experiment on a cooled Petri dish. The *Daphnia* were free to swim around in each droplet. It was necessary to cool the *Daphnia* since the heart rate was too fast at room temperature to obtain accurate measurements. The heart rate was counted manually for three 15-s intervals and the mean converted to beats per minute. These were consistently within 5% of each other. The mean was then converted to % of time 0 for each condition. In the case of the lactose dose response (Fig. 4a,b), the results were plotted as % inhibition (i.e. the % of time 0 was subtracted from 100%) to produce a dose response similar to a conventional plot. Each condition involved three separate *Daphnia*. The temperature chosen for the majority of experiments was 10–11 °C. Great care was taken to maintain the temperature of the droplets constant. The temperature (mean±S.D.) of 276 *Daphnia* measurements was 10.6±0.3 °C (range 9.8–12.0). *Daphnia* kept in the 50-μl droplets showed no significant change in heart rate over 2 h under these conditions, where the heart rate was the same in either pond water or the simple salt medium A. In order to measure the heart rate the majority of the liquid surrounding each *Daphnia* was removed for 1–2 min, thereby preventing the animal moving about. There was no evidence visually that this affected the heart rate, the feelers that move food into the mouth, eye movement, gut motility or defecation which was observed as the animal flicked the spine at the end of its intestine. The temperature of the drops was monitored throughout each experiment using a small thermocouple and remained within ±0.5 °C of the initial temperature. This was important in view of the sensitivity of the heart rate to temperature. Although some experiments were carried out blind, it was difficult to 'blind' experiments routinely. The dramatic nature of the results, together with the unexpected timing and magnitude of many of the effects, could not be explained by bias in the measurement of heart rate.

Statistical analysis was carried out as paired *t*-tests using SPSS and the results are expressed as probability (*p*) of two values being significantly different when *p*<0.05.

3. Results

3.1. Effect of temperature on heart rate

The temperature in the pond normally varies from approximately 2–14 °C throughout the year. Thus, normal room temperature (approx. 22–24 °C) was considerably higher than that in the pond where the *Daphnia* live. However, during the year, the laboratory temperature varies from about 17 °C to over 30 °C, hopeless for reproducible heart measurements. Furthermore, the heart rate at room temperature was too high to obtain routine accurate measurements. The effect of temperature was therefore investigated (Fig. 1) in order to determine a suitable temperature for investigating the effect of lactose and other agents, and to see how rigorously this had to be controlled in order to prevent artefacts due to temperature changes when substances were added. The temperature was first reduced, leaving the *Daphnia* to stabilise at each temperature for 5 min. The heart rate decreased down to 5 °C, below which the heart usually stopped beating. The heart beat appeared regular at all temperatures with no apparent arrhythmia, even at the lowest temperature. Raising the temperature enabled the heart to start beating again. The heart rate increased at approximately 24 beats/min/°C, equivalent to approximately 10% of the heart rate at 10 °C, the temperature chosen for the remainder of the experiments. It was therefore necessary to maintain the temperature at ±0.5 °C so that changes of 5% in heart rate could be detected. No changes in temperature were observed as a result of removal
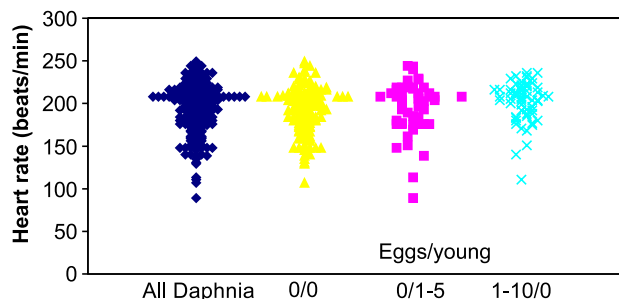

Fig. 2. Distribution of heart rate in *D. pulex*: effect of eggs (0–10) and young (0–5). The resting heart rate was measured as described in Section 2 at 10–11 °C (mean 10.6±0.3 °C in 276 separate determinations).

No. of eggs/young	Beats/min (mean)	Beats/min (% total)	Beats/min (S.D.)
0/0	192.4	98.2	2.1
0/1–5	195.8	99.9	4.3
1–10/0	203.3	103.8	3.0
Total	195.9	100	3.4

or addition of water from and to the droplets. As a result, there were no changes in heart rate when changes were made to the droplets in which the *Daphnia* were swimming. At approximately 10.5 °C in either pond water or medium A, the heart rate (mean±S.D.) of 276 separate *Daphnia* was 195.9±27.0 (range 89.2–249.2) beats/min, and in the controls with no added substances remained within 5–10% of the time 0 measurement in most experiments. There was a considerable variation in normal heart rate between individual *Daphnia* (Fig. 2), approximately 80% being between 170 and 230 beats/min, the peak in the distribution being 200–210 beats/min.

Since *Daphnia* reproduce both asexually and sexually they often are found carrying 1–10 eggs or 1–5 young in their brooding chamber. The eggs were highly pigmented. In the experiment reported here, 56.8% of the *Daphnia* had
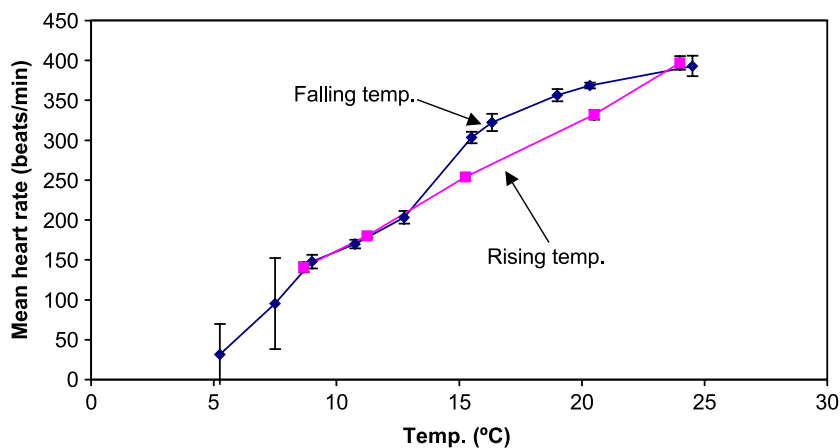

Fig. 1. Effect of temperature on *D. pulex* heart. *D. pulex* were incubated in 50-μl droplets of pond water as described in Section 2. The starting temperature was 24 °C. The temperature in the cooling bath was then lowered stepwise down to 2 °C and then raised again up to 24 °C. The heart rate was measured at each particular temperature by counting the heart for three 15-s intervals, after allowing the *D. pulex* to equilibrate for 5 min. The range for the three determinations was within 2–3 beats. The heart stopped beating below 5 °C, but started beating again as the temperature was raised. Results represent heart beats per minute and are the mean±S.E.M. of three *D. pulex*.

Table 1
Distribution of *D. pulex* with eggs and young

(a) Young with no eggs								
Eggs	0	0	0	0	0	0		
Young	0	1.0	2.0	3.0	4.0	5.0	Total no. with eggs	Overall total
No.	150.0	20.0	20.0	6.0	4.0	1.0	201.0	264.0
% no with eggs	74.6	10.0	10.0	3.0	2.0	0.5	100	
% total	56.8	7.6	7.6	2.3	1.5	0.4	76.1	

(b) Eggs with no young											
Young	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	Total no. of young
Eggs	0	0	0	0	0	0	0	0	0	0	
No.	12.0	19.0	13.0	9.0	3.0	0	4.0	1.0	0	2.0	63.0
% of total with no young	19.0	30.2	20.6	14.3	4.8	0	6.3	1.6	0	3.2	100
% overall total	4.5	7.2	4.9	3.4	1.1	0	1.5	0.4	0	0.8	23.9

D. pulex were maintained in tanks of pond water as described in Section 2. The number of eggs or young in the brood chamber was counted before the start of each experiment. In a few experiments, some young were released within the 50-μl droplet. And in two cases, the *D. pulex* shed their carapace. Results represent the data from 264 individual *D. pulex*. Care was taken to ensure that *D. pulex* was used in all experiments as the pond also contained *D. magna* that were easy to identify.

neither eggs nor young, 23.9% had eggs with no young and 19.3% had young with no eggs (Table 1). There were no significant differences in normal heart rate between these three groups.

3.2. Effect of pharmacological substances on Daphnia heart

It has been reported that *Daphnia* responds to substances that affect the rate of heart beats when added to the surrounding medium (Viehoever and Cohen, 1937; Sollman and Webb, 1941; Postmes et al., 1973; Villegas-Navarro et al., 2003). In order to establish the validity of our *Daphnia* system studied at approximately 10 °C, the effects on heart rate and rhythm of caffeine (1–10 mM), the β-adrenergic antagonist propranolol (100 μM), adrenaline (10–1000 μM), isoproteronol (100 μM) and carbachol (100 μM) were studied (Fig. 3). A total of 1–2 mM caffeine caused a small, but reproducible rise in heart rate of some 10% within 5–15 min. This was similar to the time observed for uptake of fluorescent substances such as fluorescein by *Daphnia* gut (data not shown). The heart rate remained at this elevated level for a further 15 min and then declined to approximately 50–60% of the normal heart, significantly different from the control (p=0.03). Within 30–60 min, caffeine induced an arrhythmia indicated by a highly irregular size and rate of heart beat. A wash out showed that the effect of caffeine was 50% reversible by 1 h, the heart rate returning to normal by 4–5 h. The β-adrenergic antagonist propranolol (100 μM) caused a 30–40% decrease in heart rate detectable within 5–15 min (Fig. 3), p=0.03 at 60 min, but unlike caffeine did not generate any obvious arrhythmia in the size or rate of the heart beat. Both adrenaline (100 μM) and the pure β-adrenergic receptor agonist isoproteronol (10–100 μM) only caused a small increase in the heart rate of 10–20% within 5–15 min. The cholinergic receptor agonist carbachol (100 μM) caused a 10–30% rise in heart rate detectable within 5–30 min. These data confirmed that

our *Daphnia* could respond to pharmacological agents that affect ion channels and cell signalling when added to the water in which they were swimming.

3.3. Effect of lactose and other sugars on Daphnia heart

In order to investigate whether lactose could affect the *Daphnia* heart, the effect of 2–200 mM lactose was investigated (Fig. 4a), the concentration in cow's milk being approximately 140 mM. Lactose (50–200 mM) caused a significant decrease in heart rate within 30–60 min (Fig. 4a), which appeared to be saturable. The concentration for half maximum inhibition was approximately 60 mM lactose, with a plateau at approx. 200 mM (Fig. 4b). Little or no effect of lactose (2–20 mM) was detected within 60 min. At concentrations of 100–200 mM lactose, the heart became severely arrhythmic within 30–60 min. This was a clear arrhythmia, where the heart produced
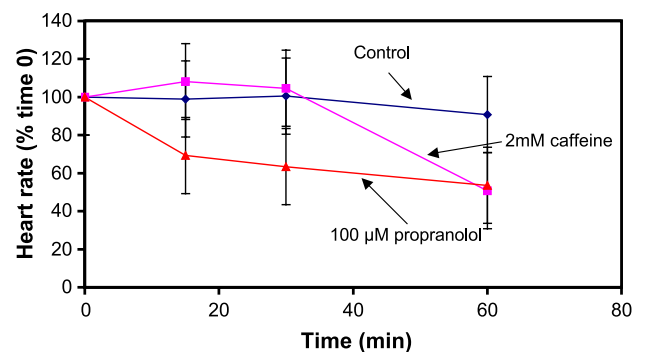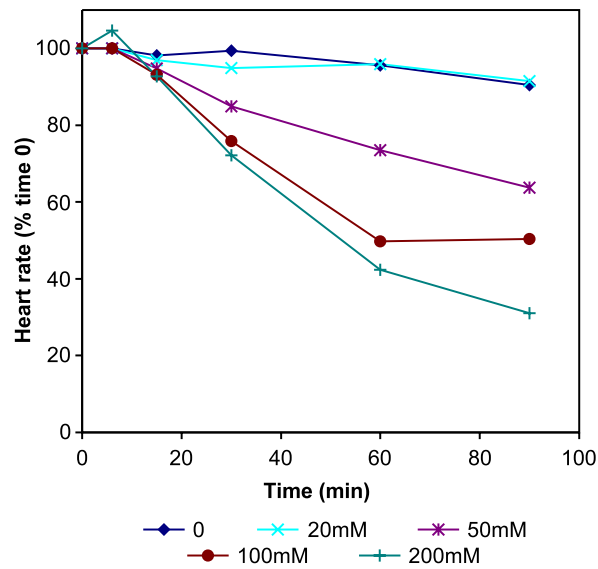

Fig. 3. Effect of caffeine and propranolol on *D. pulex* heart rate. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-μl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate, 2 mM caffeine or 100 μl propranolol were added and the heart rate measured at 15, 30 and 60 min and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition.

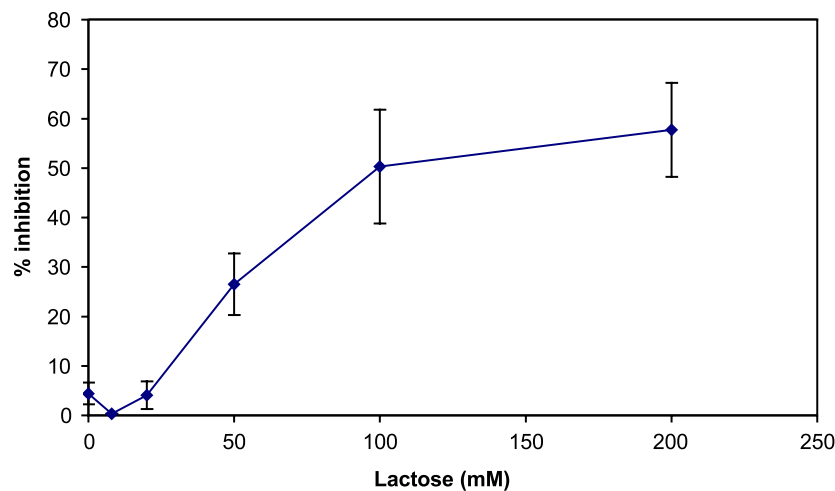(a) Dose response against time

(b) Lactose dose-response

Fig. 4. Effect of lactose on *D. pulex* heart rate. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-μl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate lactose (2, 20, 50, 100 and 200 mM) was added and the heart rate measured at 15, 30, 60 and 60 min, and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition. (a) Time course of lactose dose response. (b) Lactose dose response plotted as % inhibition against lactose concentration. $K_{1/2}$=60 mM.

1–2 very large contractions followed by 1–4 tiny contractions. In some cases, the heart stopped beating by 60 min. These results were statistically highly significant. At 60 min, p=0.026 for both 50 and 200 mM lactose relative to the controls with no added lactose. The effect of lactose was reversible (Fig. 5), the heart rate and rhythmic beating returning to normal by 3 h after removal of the lactose. When lactose was removed by a wash out, the heart rate became the same as the controls, being not significantly different within 2–3 h (p=0.38).

Sucrose (100–200 mM) also appeared to cause a decrease in heart rate and heart arrhythmia, the effect of 200 mM being greater than 100 mM (Fig. 6a). However,

glucose (100–200 mM) and galactose (100–200 mM), the sugars into which lactose is cleaved either by the human gut enzyme lactase-phlorizin hydrolase (EC 3.2.1.23/62) or β-galactosidase, had no significant effect (p=0.2 for glucose and p=0.95 for galactose at 60 min) on heart rate or rhythm over 90 min (Fig. 6b).

3.4. Did the toxic effects of lactose require metabolism?

A key question in our hypothesis is whether the effects require metabolism of lactose by intestinal bacteria or by endogenous β-galactosidase. Disaccharides such as lactose and sucrose cannot be metabolised directly by pathways
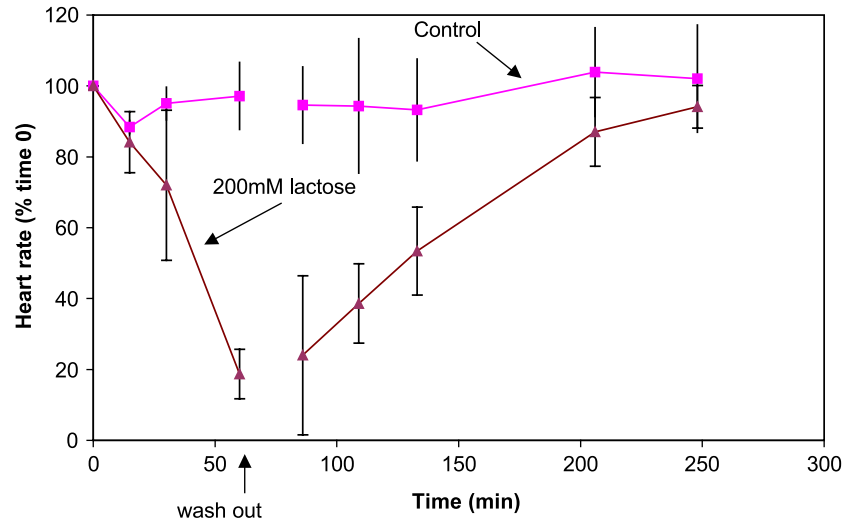
Fig. 5. Reversibility of the effect of lactose on *D. pulex* heart rate. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-μl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate, lactose (200 mM) was added and the heart rate measured at 15, 30 and 60 min, and compared with controls with no additions. The lactose was then washed out with 3×50 μl pond water, and the heart rate measured for a further 3 h, the medium being changed at each time interval. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition.

such as glycolysis. They have first to cleaved into their constituent monosaccharides galactose and glucose or glucose and fructose respectively. These monosaccharides, but not fructose, are then be absorbed through the sugar transporter SGLUT1 and then metabolised by cells. There are only two enzymes known that cleave lactose–lactase-phlorizin hydrolase (lactase) and β-galactosidase. Lactase is only found in mammalian gut. But β-galactosidase, which has no sequence similarity to lactase, is a ubiquitous enzyme in pro- and eu-karyotic organisms, where in the latter it is found in high activity in lysosomes. However, to metabolise lactose the cell and the organelle must have a permease to allow access of β-galactosidase to lactose. In order to test whether bacterial metabolism was required for the toxic effects of lactose on *D. pulex* heart three experimental conditions were investigated: first, the effect of an antibiotic known to kill bacteria efficiently; secondly, the effect of two known metabolites of anaerobic sugar metabolism in bacteria; thirdly, the effect of L-ribose a known inhibitor of β-galactosidase in bacteria (Huber and Brockbank, 1987). Incubation of *D. pulex* with the antibiotic ampicillin (100–300 μg/ml) for 1–4 days had no effect of the resting heart rate, and no significant effect (p=0.45 after 70 min) on the decreased heart rate and arrhythmia induced by lactose (Fig. 7a). Furthermore, the diols 1,3 propandiol and 2,3 butandiol (20 mM), anaerobic metabolites of sugar metabolism in bacteria (Campbell and Matthews, 2001), had no significant effect (p=0.98 for butan 2,3 diol and p=0.42 for propan 1,3 diol at 60 min) on *D. pulex* heart rate or rhythm (Fig. 7b). Nor were there any reproducible effects of 2 mM ribose (p=0.61 for D-ribose and p=0.11 for L-ribose at 90 min) on the lactose inhibition of *D. pulex* heart rate (Fig. 8), a concentration 10 times the K_i for β-galactosidase (Huber and Brockbank, 1987).

4. Discussion

Our results show clearly that lactose can affect dramatically the rate and rhythm of the heart beat in the cladoceran *D. pulex*. The arrhythmia observed by 60 min at high lactose concentrations was seen as 1–2 very large contractions followed by several small ones (see www.uwcm.ac.uk/med_biochem/akc and then *Daphnia*). It is interesting that lowering the temperature to 5–10 °C, or the addition of propranolol, reduced the heart rate to a similar low level to that of lactose without causing an arrhythmia. Although lactose would not normally be expected to be natural dietary ingredient for *D. pulex*, our results support the data from other model systems that many biochemical and ionic mechanisms are universal across the animal kingdom. However, further work is now required to confirm that this is similar to the heart palpitations and arrhythmia we have observed in humans with lactose intolerance (Matthews and Campbell, 2000, 2004; Campbell and Matthews, 2001). The inhibitory concentration range of 50–200 mM lactose was within that for lactose found in cow's milk, and foods or drinks containing added lactose.

There are three possible mechanisms by which lactose could affect the heart rate and rhythm: (a) a direct effect of lactose on ion channels and/or gap junctions in the heart; (b) a direct effect of lactose metabolites generated by gut microflora on ion channels and/or gap junctions in the heart; (c) an indirect mechanism involving the generation of neuropeptides or other transmitters that can modulate ion channels and/or gap junctions in the heart. But the timing of the inhibition by lactose (Fig. 4), together with the fact that the reduction in heart rate and the arrhythmia caused by lactose on *D. pulex* heart took 3–4 h to be reversed, was consistent with the effect of lactose being mediated directly

(a) Effect of 100mM sucrose and 100mM glucose
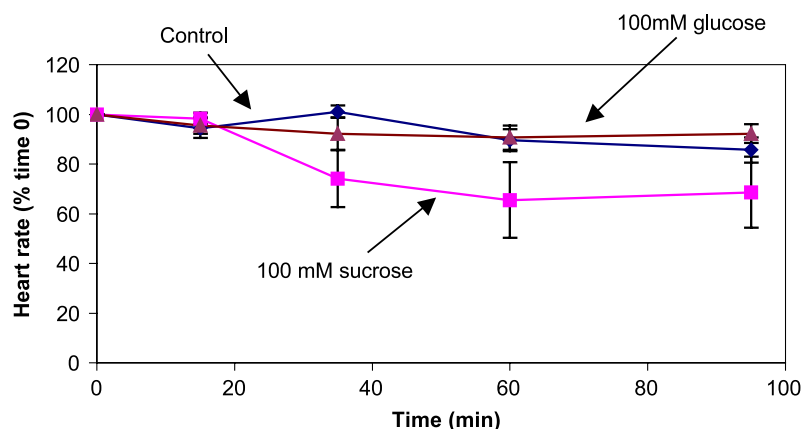
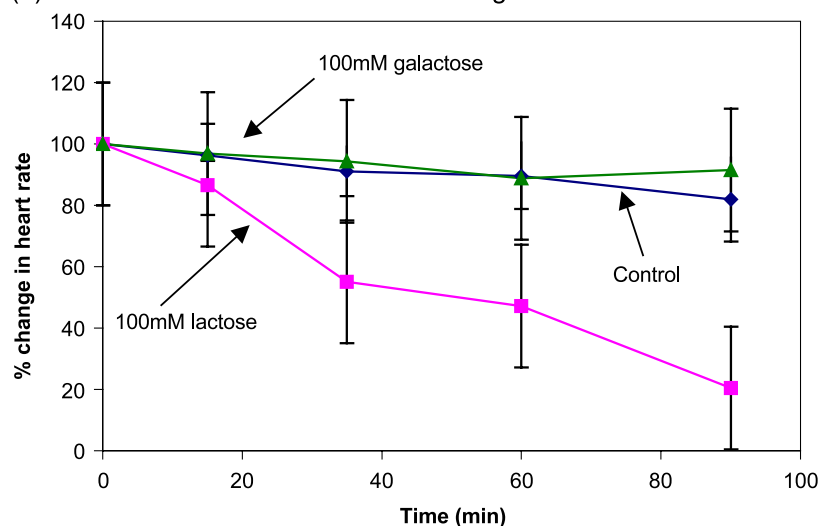
(b) Effect of 100mM lactose and 100mM galactose

Fig. 6. The effect of lactose, sucrose, glucose and galactose on *D. pulex* heart rate. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-µl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate, (a) sucrose (100 mM) or glucose (100 mM) and (b) lactose (100 mM) or galactose (100 mM) were added and the heart rate measured at 15, 30 and 60 min, and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition.

by a metabolite of lactose or by lactose itself on K^+, Na^+ and/or Ca^{2+} channels in the myocyte. The fact that sucrose was also capable of inducing a reduction in *D. pulex* heart rate and arrhythmia suggested that an osmotic effect of lactose, for example through stretch receptors, could not be ruled out. However, such an osmotic mechanism is not supported by the fact that neither glucose nor galactose at 100–200 mM had an effect on *D. pulex* heart, nor by the timing of the lactose inhibition and its reversal. Although by eye there was no obvious visible evidence of swelling either of the *D. pulex* as a whole, or the heart itself at times when large effects of lactose in the heart were observed, video microscopy will be needed to confirm this.

Lactose can be hydrolysed by lactase or any β-galacto-sidase. However, the lactose has no access to these enzymes, e.g. in intracellular compartments such as lysosomes, unless the cell and organelle has a lactose permease analogous to the permease, which is part of the lac operon in *Escherichia coli*. We have proposed that the effects of lactose to cause systemic symptoms in humans with lactose intolerance are caused either by lactose itself or by metabolites generated by bacteria in the large intestine, which is essentially anaerobic (Matthews and Campbell, 2000, 2004; Campbell and Matthews, 2001). However, the effects of lactose on *D. pulex* heart were unlikely to be caused by bacterial metabolites since the potent antibiotic ampicillin, expected to kill gut bacteria and thus prevent metabolite synthesis from lactose, had no effect (Fig. 7a). Furthermore, two diols known to be anaerobic metabolites of sugar metabolism in bacteria were without effect (Fig. 7b). This is perhaps not surprising since microscopic examination of the *D. pulex* showed that their guts were full of bright red fluorescent microalgae unlikely to metabolise lactose in a manner similar to the microflora in the human large intestine. Further work
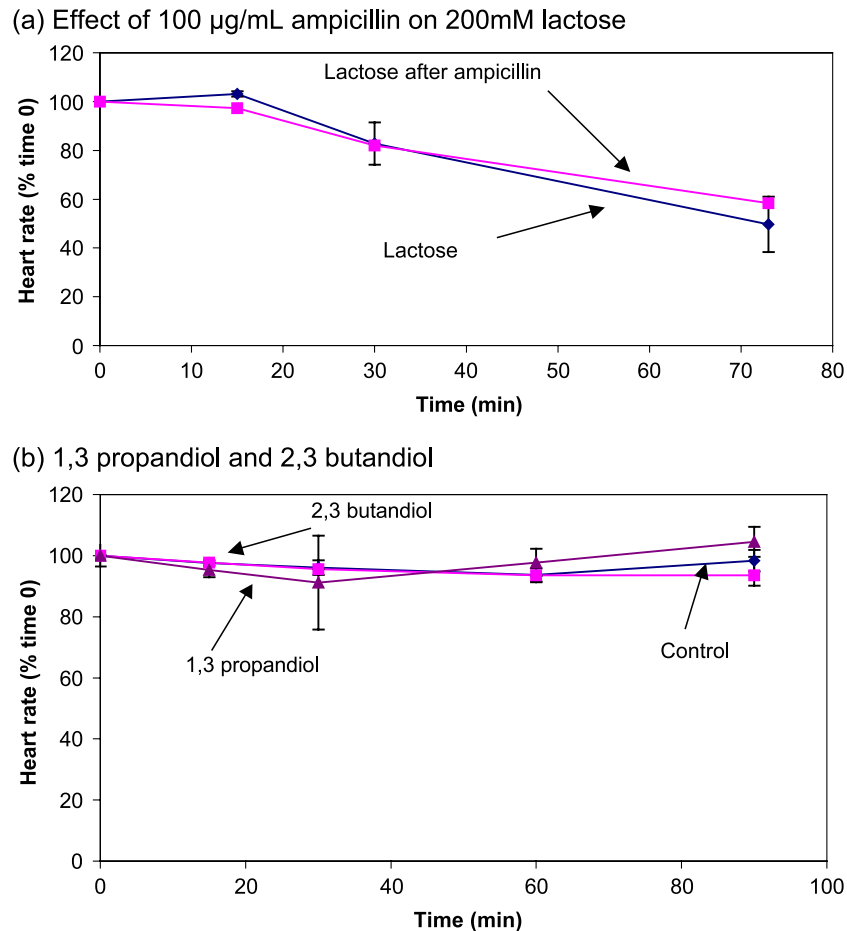
Fig. 7. The effect of ampicillin and diols on *D. pulex* heart rate. (a) Effect of ampicillin. Individual *D. pulex* were incubated in pond water at room temperature (approx. 24 °C) for up to 3 days with or without the antibiotic ampicillin (100 µg/ml). The *D. pulex* were then transferred to the incubation chamber at approx. 10.5 °C in 50-µl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate, lactose (200 mM) was added and the heart rate measured at 15, 30 and 70 min, and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition. (b) Effect of propan 1,3 diol and butan 2,3 diol. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-µl droplets and the heart rate measured as described in Section 2. After determining the resting heart rate, propan 1,3-diol (20 mM) or butan 2,3-diol (20 mM) were added and the heart rate measured at 15, 30, 60 and 90 min, and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition.

is now required to find agents that will knock out the gut flora in *D. pulex* and thus prevent the lactose effect on the heart. While the results are consistent with using *D. pulex* as a model for the toxic effects of lactose in humans, it is now necessary to confirm the claim made over 50 years ago that *D. pulex* heart, like the human heart, is myogenic (Bekker and Krijgsman, 1951). We have so far found no evidence for innervation into the heart. A key question now is what is the pacemaker, and how does it become arrhythmic, for example when the animal is exposed to lactose? This will require identification of the role of K^+, Na^+ and Ca^{2+} channels in the action potential and heart beat.

All pro- and eu-karyotic cells use D-ribose in their nucleotides and nucleic acid. However, it has been reported (Huber and Brockbank, 1987) that the enantiomer L-ribose is structurally very similar to the shape of galactose within lactose predicted when it binds to β-galactosidase. L-ribose is thus a potent inhibitor of β-galactosidase in bacteria and

lysosomes with a K_i of approximately 0.2 mM and can be transported into lysosomes by the sugar transporter. Neither L-ribose nor D-ribose (2 mM) had reproducible effects on the potency of lactose to reduce *D. pulex* heart rate and induce arrhythmia (Fig. 8). This supported the hypothesis that cleavage of lactose by β-galactosidase was not required and that lactose itself was likely to be the toxic agent. The K_i for D-ribose inhibiting β-galactosidase in bacteria is some 100 times that of L-ribose, though the K_i for D-ribose on eukaryotic β-galactosidase may be lower. Thus, a role for β-galactosidase in the toxic effects of lactose on *D. pulex* heart cannot be completely ruled out. Although it is clear that pharmacological substances such as caffeine, β-adrenergic agonists and antagonists, and acetyl choline added to the external fluid affect the heart rate of *D. pulex*, some of the effects are not identical to the effects of these substances on human heart. But the increased inhibition by the β-adrenergic antagonist propra-
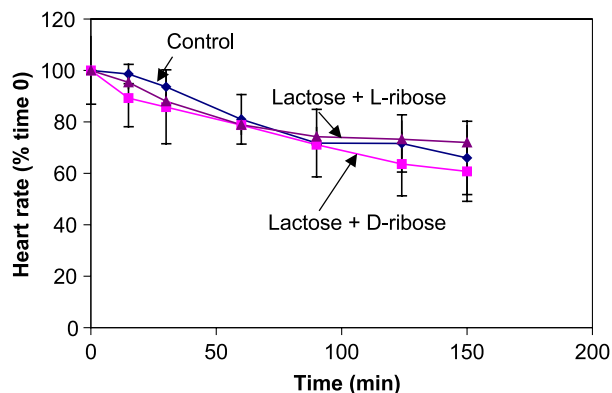
Fig. 8. The effect of L- or D-ribose on the inhibition of *D. pulex* heart rate by lactose. Individual *D. pulex* were incubated at approx. 10.5 °C in 50-μl droplets and the heart rate was measured as described in Section 2. After determining the resting heart rate, lactose (50 mM) with or without L- or D-ribose (2 mM) were added and the heart rate measured at 15, 30 and 60 min, and compared with controls with no additions. Each heart rate was the mean of three 15-s determinations. Results were plotted as % heart rate at time 0 and represent the mean±S.E.M. of three separate *D. pulex* under each condition.

nolol with lactose supports the hypothesis that the effect of lactose involved signalling and ion channels.

D. pulex is about 1–2 mm long and its heart appears to be a single chamber of approximately 50 by 150 μm controlled by a single layer of myocytes with large mitochondria (Stein et al., 1966; von Ruland, Newman and Campbell, unpublished). Unlike other arthropod hearts, it has been reported that, like the mammalian heart, the action potential is generated myogenically (Bekker and Krijgsman, 1951). This claim now needs re-examination using modern electrophysiological and pharmacological techniques. Although there is a fine membrane visible in the light microscope around the waist of the heart of *D. pulex*, the heart appeared to be made up of one compartment. The ultrastructure of *D. pulex* has not been extensively studied, but the organism contains several organs and cell types such as the multi-lens eye, muscle, gut and secretory and phago-/endocytic cells (Stein et al., 1966; Rieder, 1987; Elendt, 1989), and has agonists that regulate intracellular free Ca^{2+} and cyclic nucleotides. This suggests that *Daphnia* could also be a model system for studying cell signalling and intra- and inter-cellular communication in situ. At present, the precise number of cells making up one *Daphnia* is not known, but the size compared with the nematode worm suggest it will be <1000. *Daphnia* is well established as a model for ecotoxicity studies (Persoone and Van de Vel, 1988; Baird et al., 1989a,b; Diamantino et al., 2000; Guilhermino et al., 2000; De Coen and Janssen, 2003). Our results support previous reports that the heart of *Daphnia* responds to pharmacological substances that affect the rate and rhythm of the mammalian heart (Viehoever and Cohen, 1937; Sollman and Webb, 1941; Postmes et al., 1973; Villegas-Navarro et al., 2003). This has lead to *Daphnia* being used

widely as an educational tool. They are relatively easy to grow in a simple medium and usually reproduce asexually. When stressed they produce males. *Daphnia* is thus an in situ model for the regulation of the heart beat, heart development, gut motility and digestion, endo- and phago-cytosis, gene regulation of haem proteins, Ca^{2+} signalling and ion channel regulation. Initial experiments have shown that the eggs have a resting potential of about −30 mV (Wann and Campbell, unpublished). The sequence of the mitochondrial genome of *D. pulex* has been published (Crease, 1999) and an international consortium aims to have the full genome from its 12 chromosomes available by 2005 (Colbourne, 2004). *Daphnia* are transparent and easy to immobilise and are also smaller than many of the classic model systems in biomedicine such as *Drosophila*. *Daphnia* also has a haemoglobin that is induced by oxygen stress (Baumer et al., 2002) in a manner similar to the haemoglobin found in mammalian brain. This and our results support the argument for *Daphnia* as a model system (www.sciencemag.org/feature/plus/sfg/resources/res_model.shlml) for investigating the mechanisms and pathology of ion channels and cell signalling in a live organism, and as an important potential test system for drug discovery thereby reducing the use of mice and other mammals. The wide variation in heart rate in the *D. pulex* population (Fig. 1) is similar to the large variation in many physiological and biochemical parameters in humans and other organisms (Williams, 1998). Consistent and reproducible conclusions can be drawn by using each individual as its own control. It is understanding the mechanisms and evolutionary significance of these hitherto often ignored variations in the molecular biodiversity of natural populations that provides a challenge for contemporary biology and medicine (Campbell, 2003).

References

Baird, D.J., Barber, I., Bradley, M., Calow, P., Soares, A.M.V.M., 1989a. The *Daphnia* bioassay: a critique. Hydrobiologia 188/189, 403–406.

Baird, D.J., Soares, A.M.V.M., Girling, A., Barber, I., Bradley, M.C., Calow, P., 1989b. The long-term maintenance of *Daphnia magna* Straus form use I ecotoxicity tests: problems and prospects. Proc. First European Conference on Ecotoxicology, pp. 144–148.

Banta, A.M., 1921. A convenient culture medium for daphnids. Science 53, 557–558.

Baumer, C., Pirow, R., Paul, R.J., 2002. Circulatory oxygen transport in the water flea *Daphnia magna*. J. Comp. Physiol., B 172, 275–285.

Bekker, J.M., Krijgsman, B.J., 1951. Myogenic heart in *Daphnia*. J. Physiol. 115, 249.

Campbell, AK., 2003. Save those molecules! Molecular biodiversity and life. J. Appl. Ecol. 40, 193–203.

Campbell, A.K., Matthews, S.B., 2001. Lactose intolerance and the MATHS syndrome: what are the and how can I cope. Pembrokeshire, Welston Press, ISBN: 0-9540866-0-0.

Carper, S., 1995. Milk is Not for Everybody. Facts On File, New York, ISBN: 0-8160-3127-4.

Colbourne, J., 2004. http://daphnia.cgb.indiana.edu/.

Crease, T.J., 1999. The complete sequence of the mitochondrial genome of *Daphnia pulex*. Gene 233, 89–99.

De Coen, W.M., Janssen, F., 2003. A multivariate biomarker-based model predicts population-level responses of *Daphnia magna*. Environ. Toxicol. Chem. 22, 2195–2201.

Diamantino, T.C., Guilheerminjo, L., Almeida, E., Soares, A.M.V.M., 2000. Toxicity of sodium molydate and sodium dicohromate to *Daphnia magna* Straus evaluated in acute, chronic and acetyl cholinesterase inhibition tests. Ecotoxicol. Environ. Saf. 45, 253–259.

Elendt, B.-P., 1989. On the ultrastructure of the intestine of *Daphnia magna*. Zool. Anz. 223, 186–197.

Elendt, B.-P., 1990a. Nutritional quality of a microencapsulated diet for *Daphnia magna*. Effects on reproduction, fatty acid composition, and midgut ultrastructure. Arch. Hydrobiol. 118, 461–475.

Elendt, B.-P., 1990b. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on lie history parameters of *D. magna*. Water Res. 24, 1157–1167.

Elendt, B.P., Storch, V., 1990. Starvation-induced alteration of the ultrastructure of the midgut of *Daphnia magna* Straus, 1820 (Cladocera). J. Crustac. Biol. 10, 79–86.

Flatz, G., 1987. Genetics of lactose digestion in humans. Adv. Hum. Genet. 16, 1–77.

Guilhermino, L., Lacerda, M.N., Nogueira, A.J.A., Soares, A.M.V.M., 2000. In vitro and in vivo inhibition of *Daphnia magna* acetyl cholinesterase by surfactants: possible implications for contaminating biomonitoring. Sci. Total Environ. 247, 137–141.

Huber, R.E., Brockbank, R.L., 1987. Strong inhibitory effect of furanoses and sugar lactones on β-galactosidase in *Escherichia coli*. Biochemistry 26, 1526–1531.

Keating, K.I., 1985. A system of defined (sensu stricto) media for *Daphnid* (Cladocera) culture. Arch. Hydrobiol. 3, 321–327.

Kluttgen, B., Dulmer, U., Engels, M., Ratte, H.T., 1994. AdaM, an artificial freshwater for culture of zooplankton. Water Res. 28, 743–746.

Matthews, S.B., Campbell, A.K., 2000. When sugar is not so sweet. Lancet 355, 1309.

Matthews, S.B., Campbell, A.K., 2004. Lactose intolerance in the young: a new perspective. Welsh Paediatr. J. 20, 56–66.

Persoone, G., Van de Vel, A., 1988. Cost-analysis of five current aquatic toxicity test. EC Report EUR/11432/EN. Commission of the European Communities, Brussels.

Postmes, Th.J., Nacken, G., Nelissen, R.G., 1973. An electronic method for measuring the heart frequency of the water flea: *Daphnia pulex*. Experientia 30, 1478–1480.

Rieder, N., 1987. The ultrastructure of the so-called olefactory setae on the antennula of *Daphnia magna* Straus (Crustacea Cladocera). Hydrobiologia 145, 175–181.

Sahi, T., 1994. Genetics and epidemiology of adult-type hypolactasia. Scand. J. Gastroenterol. 29 (Suppl.) (202), 7–20.

Sollman, T., Webb, W.J., 1941. Response of *Daphnia* to cardiac active drugs. Quoted in Stein et al.. J. Pharmacol. Exp. Ther. 71, 261.

Srinivasan, D., Minocha, A., 1998. When to suspect lactose in tolerance. Symptomatic, ethnic, and laboratory clues. Postgrad. Med. 104, 109–123.

Stein, R.J., Richter, W.R., Zussman, R.A., Brynjolfsson, G., 1966. Ultrastructural Characterization of *Daphnia* Heart Muscle. J. Cell. Biol. 29, 168–170.

Swallow, D.M., 2003. Genetics of lactase persistence and lactose intolerance. Annu. Rev. Genet. 37, 197–219.

Tessier, A.J.L.L., Henry, C.E., Goulden, C.E., Durand, M.W., 1983. Starvation of *Daphnia*: energy reserves and reproductive allocation. Limnol. Oceanogr. 28, 667–676.

Tolston, L.G., 2000. Adult-type lactase deficiency. Nutr. Today 35, 134–138.

Viehoever, A., Cohen, I., 1937. Response of *Daphnia* to cardiac active drugs. Quoted in Stein et al. Am. J. Pharm. 109, 285.

Villegas-Navarro, A., Rosas, L.E., Reyes, J.L., 2003. The heart of *Daphnia magna*: effects of four cardioactive drugs. Comp. Biochem. Physiol. 136C, 127–134.

Williams, R.J., 1998. Biochemical individuality: the basis for the genotrophic concept. Keats Publishing, Connecticut. Reprinted from the original 1956 edition.