You've Got To Have Heart... Or Do You?

Jo-Ann E. Mellish, Alan W. Pinder, and Steven C. Smith
Department of Biology,
Dalhousie University,
Halifax, Nova Scotia B3H 4J1, CANADA

Why does heartbeat begin to beat so early? During development, one of the first vertebrate organ systems to begin functioning is the cardiovascular system. In axolotl embryos, the heart begins to beat at stage 35 (see Bordzilovskaya et al., 1989, for staging), and circulation begins shortly thereafter (stages 36-37).

Traditionally, most biologists assume that the presence of a structure implies function, purpose, and importance (Ayala, 1977). Therefore, it is generally assumed by physiologists and others that the heart and circulatory system begin functioning early in development because they are necessary for the survival and continued development of the organism.

Since one of the most important functions of the circulatory system is gas exchange (Boell et al. 1963; Adolph, 1979; Burggren and Pinder, 1991; Boutilier et al. 1992; Burggren and Just, 1992), it seems reasonable to assume that this is one (if not the) major purpose for the early onset of cardiovascular function. This view is at least circumstantially supported by the observation that, in the axolotl, the gills first become recognisable, separate structures (and thus begin to greatly increase their surface area) simultaneously with the onset of heartbeat (at stage 35; Bordzilovskaya et al., 1989).

Paradoxically, embryologists have long known that amphibian embryos and larvae can survive for days or weeks without a heart (Knower, 1907; Copenhaver, 1926; Copenhaver, 1955). One obvious example of this are *cardiac-lethal* mutant (*c/c*) axolotls, which have a non-functional heart yet survive for up to 2-3 weeks post-hatching (Humphrey, 1972; data not shown).

The extended survival of *c/c* embryos and larvae has been noted by physiologists with surprise, consternation, and some excitement (Burggren and Pinder, 1991; Burggren and Just, 1992). This is partly because their survival clearly contradicts the above assumptions about the early function of the cardiovascular system, and partly because of the promise they hold for examining the relative roles of active respiration and passive diffusion during early development. However, no one has previously used *c/c* animals to critically examine the role of the early cardiovascular system in oxygen transport.

Therefore, we have begun an examination of the role of the circulatory system and diffusion in gas exchange by measuring oxygen uptake of individual embryos using small (chamber volume = 3 mL) Plexiglas respirometers fitted with Endeco T1125 pulsed dissolved oxygen electrodes (Fig. 1) set to record O_2 concentrations at 5 min. intervals.

Oxygen consumption at 18°C in 25% Holtfreter's solution was measured in sealed respirometers for 3 types of stage 39 embryos: unmanipulated wild-type control embryos (WT; n = 24), wild-type embryos treated with 5% carbon monoxide (designated CO; n = 22), and heartless wild-type embryos (HL; n = 8) from which both heart field areas were surgically removed during late neurulation (stages 18-20).

Immediately following respirometry, all subjects were anaesthetized in MS-222, wet weights were determined, and the animals were dried to determine their dry weights. Weights were the same for WT and CO embryos, but were smaller for HL embryos, presumably reflecting the mass of the heart field mesoderm removed (data not shown).

Oxygen consumptions shown do not include consumption by the electrodes, and are expressed per unit dry weight. Total oxygen conductance was calculated according to the model of Piiper et al. (1976), and both measures of respiration were plotted against environmental O_2 concentration ($[O_2]$).

We chose to study stage 39 embryos for several reasons: i) the circulatory system is well established in WT embryos, ii) HL embryos could have an extensive recovery period, iii) *c/c* embryos can be identified with absolute certainty, and iv) *c/c* and HL embryos were not yet severely edemic. Wild-type embryos were supplied by the University of Ottawa Axolotl Colony and from one spawning performed locally, while *c/c* embryos were supplied by the Indiana University Axolotl Colony.

Oxygen consumption in stage 39 embryos. For all types of embryos, oxygen consumption declined as the environmental $[O_2]$ fell ($p < 0.001$; Fig. 2). Oxygen uptake differed significantly among all three groups ($p = 0.004$).

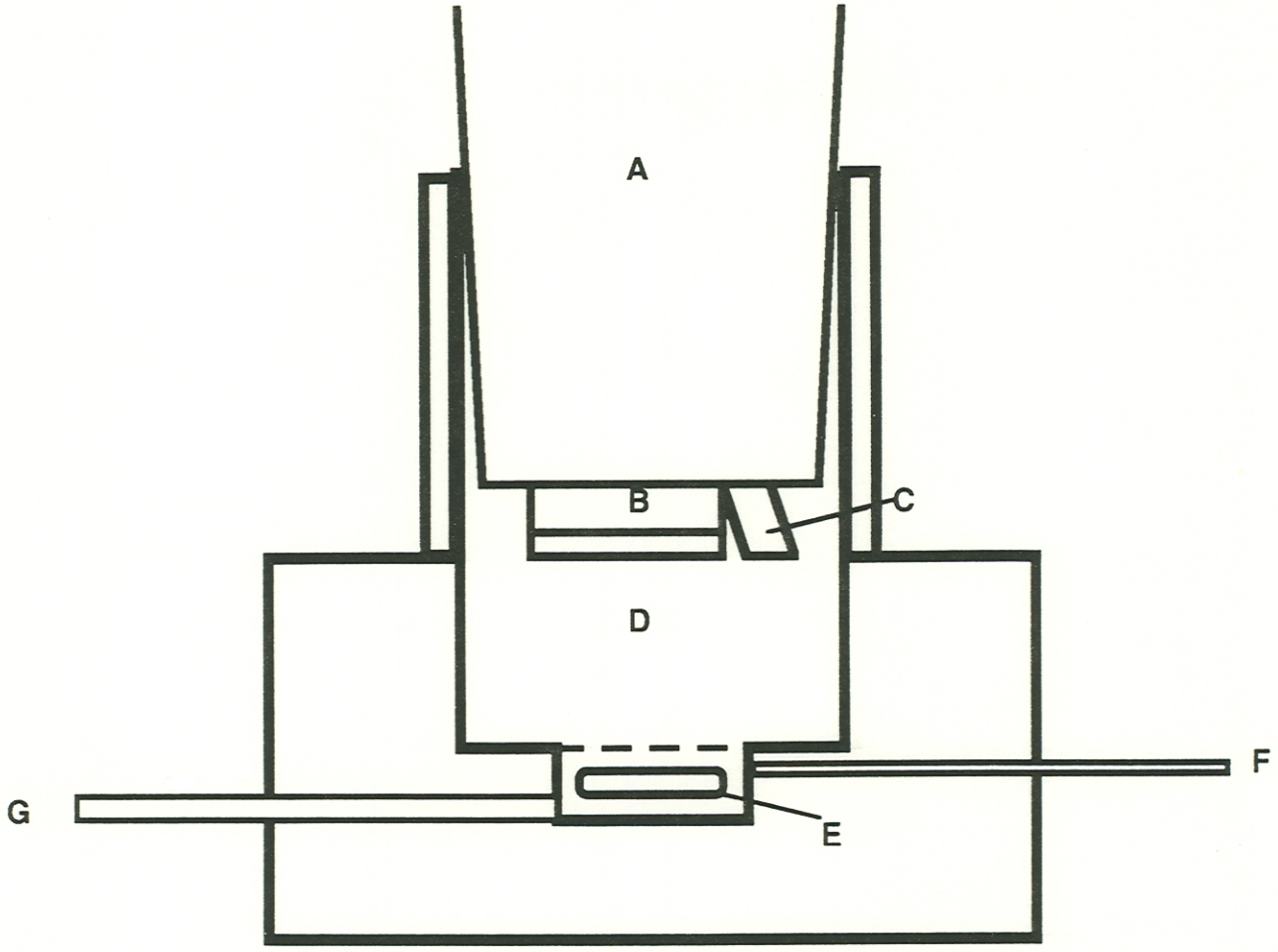

Figure 1: Schematic of the respirometer design used in this study. (A) Oxygen electrode with O_2-sensitive membrane (B) and temperature sensor (C). (D) Animal chamber with stir bar (E), and inflow (F) and outflow (G) tubes.

However, the differences appeared to be greatest at high $[O_2]$. Therefore, the oxygen consumption were also tested for differences at $[O_2] < 165\mu M$ and $> 165\mu M$. It was found that there were no significant differences among WT, CO, and HL embryos at $[O_2] < 165\mu M$ ($p = 0.064$), but that the oxygen consumption differed significantly at $[O_2] > 165\mu M$ ($p = 0.009$), with CO embryos having the lowest oxygen consumption.

Total oxygen conductance in stage 39 embryos. As with oxygen consumption, oxygen conductance differed significantly among all 3 groups ($p = 0.024$; Fig. 3). Again, when oxygen conductance for high and low $[O_2]$ were examined separately, there was no significant difference among WT, CO, and HL embryos at low $[O_2]$ ($< 165\mu M$, $p = 0.068$), but the differences were significant at high $[O_2]$ ($> 165\mu M$, $p = 0.023$).

Discussion

Although the importance of circulatory O_2 transport has never been measured in amphibian embryos, the circulatory system is generally believed to be important even at these early stages (Boell, 1945; Boell et al. 1963; Burggren and Pinder, 1991; Boutilier et al. 1992). This view is supported by the observation that there is a large increase in respiration with the onset of heartbeat in embryos of *Ambystoma maculatum* (Boell, 1945). One interpretation of this finding is that the functional circulatory system increases the ability of the animal to take up oxygen. Alternatively, the metabolic needs of the newly functioning heart may account for increased oxygen demand.

We have determined the oxygen consumption and total O_2 conductance in three types of embryos to determine the relative roles played by passive diffusion and cardio-

vascular O_2 transport during late embryonic development. Using wild-type embryos as controls, we have also measured O_2 consumption in wild-type embryos treated with carbon monoxide, and in embryos from which heart primordia were surgically removed early in development. Carbon monoxide was used because it greatly reduces the O_2-binding capacity of haemoglobin but allows heartbeat and circulatory function to continue. Therefore, CO embryos continued to have fully functional circulatory systems, but were largely dependent on diffusion for oxygen transport.

Heartless wild-type embryos were used to examine the effect of the total lack of circulation on O_2 consumption, and as controls for possible cryptic pleiotropic effects of the *c* gene (see Smith and Armstrong, 1993a,b). The HL embryos should not have been affected by surgical trauma since: i) surgery was performed prior to the formation of the nervous system, ii) they had an extensive recovery period (8-9 days at 18°C), and iii) early removal of the heart primordia ensured that they developed without hearts and thus had no chance to adapt to the presence of a functional circulatory system.

A priori, we had thought it possible that diffusion could compensate for the absence of circulatory O_2 transport under normoxic conditions, but that as $[O_2]$ decreased the necessity for cardiovascular O_2 transport would become apparent. However, we found that oxygen uptake and conductance was the same for WT, CO, and HL embryos at lower oxygen concentrations. Thus, even embryos without functional circulatory systems or severely reduced haemoglobin oxygen-binding capacities appear to be able to obtain sufficient O_2 through passive diffusion, even at extremely low environmental oxygen concentrations.

These results surprisingly suggest that the circulatory system is neither required nor does it appear to be a substantial contributor to the overall oxygen conductance in stage 39 axolotl embryos, despite beginning to function 4-5 days earlier. It remains unclear why O_2 consumption and conductance are higher in WT and HL than in CO embryos under normoxic conditions.

What kills c/c larvae? The presence of a functional circulatory system does not appear

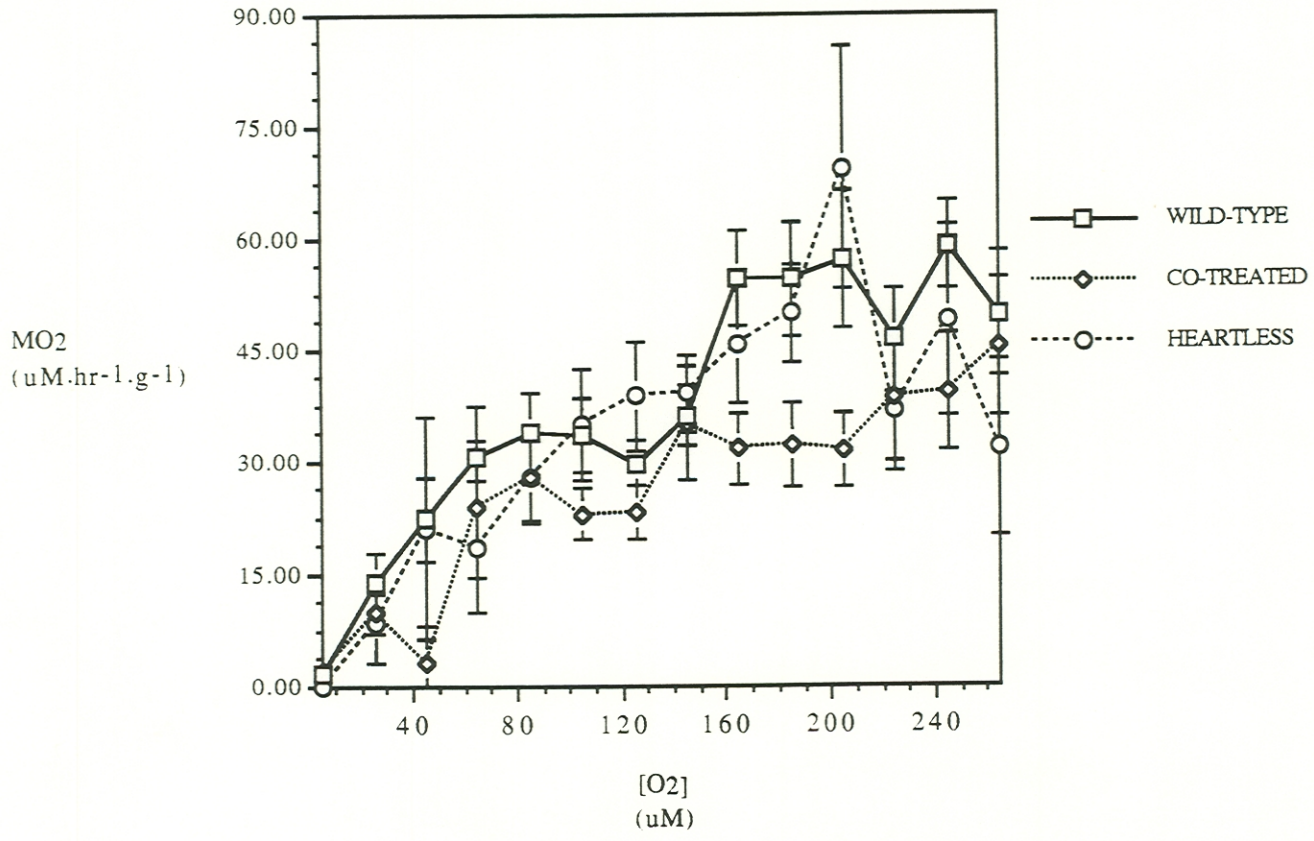

Figure 2: Oxygen consumption versus environmental oxygen concentration for stage 39 WT, CO-treated, and HL axolotl embryos. All values are ± standard error.

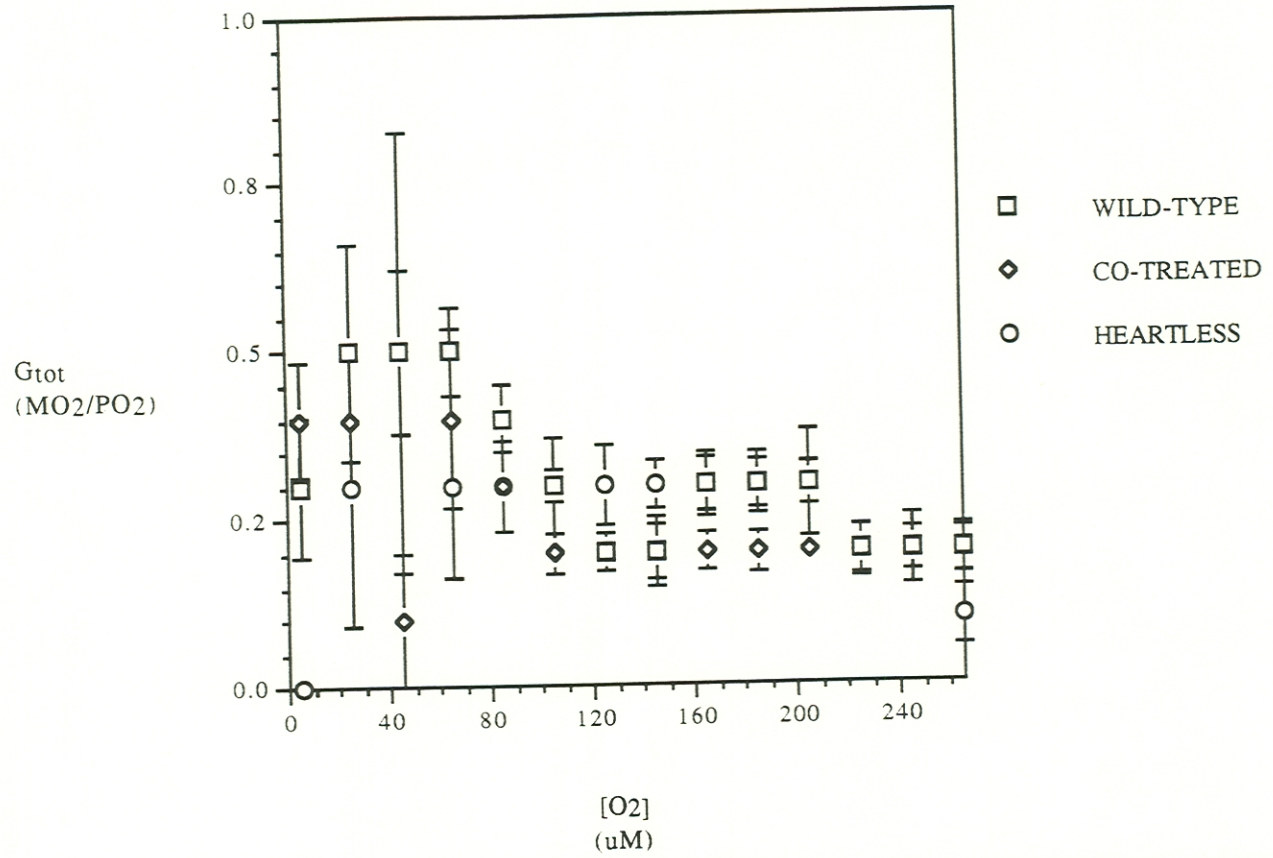

Figure 3: Total oxygen conductance versus environmental oxygen concentration for stage 39 WT, CO-treated, and HL axolotl embryos. All values are ± standard error.

to be necessary for oxygen transport in early embryos. This suggests that *c/c* embryos (and their HL phenocopies) may not be dying from anoxia, particularly when maintained under normoxic conditions. However, both *c/c* and HL embryos do eventually die. This raises the question of what kills them. One possibility is that edema eventually causes a sufficient increase in size, and thus diffusion distance, to prevent adequate oxygen supply through diffusion. (As well, some tantalising preliminary data suggest that this problem may be exacerbated by further decrease in O_2 uptake by *c/c* embryos which is not present in HL embryos; data not shown).

Alternatively, the absence of some other function of the circulatory system may ultimately prove lethal. This could include the inability to excrete nitrogenous wastes, and/or maintain ionic and water balance, due to the absence of renal function.

A further possibility is that circulation is not necessary (at least until the animals are much larger), and that *c/c* embryos eventually die of something unrelated to its absence. Since the inability of *c/c* larvae to feed does not appear to be linked to the lack of circulation (Smith and Armstrong, 1993a, b), it may be simply that *cardiac-lethal* larvae eventually starve to death. Even if they could feed, the lack of circulation might prevent efficient nutrient distribution, and the larvae might still die from the effects of starvation. We are presently examining these possibilities.

Why does the circulatory system begin to function so early? As mentioned earlier, most of us commonly assume that if a structure is present, it has a purpose. However, the survival of *c/c* and HL embryos for weeks in the absence of circulatory function suggests that the cardiovascular system begins to function well in advance of being required. Our demonstration that oxygen transport is the same in WT, CO, and HL embryos even at low [O_2] supports this conclusion. But if true, why does the circulatory system begin to function at such an early stage?

The answer to this question must, for the moment, remain unclear. However, one conceivable explanation is that the system begins to function for purposes other than O_2 transport, such as for the removal of nitrogenous wastes, and/or to maintain ionic and water balance. A second possibility is that hemodynamic pressure is required to promote angio-

genesis (i.e. see Chapman, 1918; Clark, 1918; Copenhaver, 1955). Thus, it may be that early cardiovascular function is only necessary to ensure proper development of the circulatory system.

As a final alternative, one could take the heretical view that the circulatory system begins to function so early *for no reason at all*! In taking this view, it could be suggested that if there were no selective *dis*advantage to heartbeat beginning at earlier stages than required, the heart could begin to beat at early stages simply because it always has.

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