

Application of the Thermodilution Technique for Measuring Cardiac Output and Assessing Cardiac Stroke Volume in Crabs

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ABSTRACT A thermodilution technique is used to measure the flow of hemolymph through the heart of crabs. The technique consists of injecting a small volume of cold filtered sea water into the ventricle and monitoring temperature changes with time in an artery immediately downstream from the heart using a surgically implanted thermocouple. An experimental evaluation of the method affirms that it is a direct measure of the bulk flow of hemolymph through the heart. Values of cardiac output determined in this manner fall within the range of Fick estimates of hemolymph flow made on animals treated similarly. Since measurement of cardiac output by thermodilution can be achieved in a matter of seconds, simultaneous monitoring of heart rate permits the calculation of cardiac stroke volume. In two species of crabs examined, much of the variation in cardiac output is accounted for by changes in stroke volume rather than changes in heart rate.

The study of crustacean respiration and circulation during the past 10 years has progressed to a point where reasonable comparisons of respiratory mechanisms are now possible between this group and the vertebrates. One very important feature which distinguishes crustaceans from vertebrates is the smaller capacity of the crustacean hemolymph to hold oxygen. The presence of hemocyanin in the hemolymph never enhances its oxygen-carrying capacity more than 7 times above the amount of physically dissolved oxygen, a small amount by vertebrate standards. Such a strategy imposes the flow of larger volumes of hemolymph through the crustacean circulatory system to achieve similar rates of oxygen delivery.

The existence of high hemolymph flow rates in crustaceans has been well documented, estimating cardiac output using the Fick principle (Mangum and Weiland, '75; Taylor, '76; McMahan and Wilkens, '77; Taylor and Butler, '78; Burnett, '79; McMahan et al., '79). High hemolymph flow velocities have been measured directly in the heart, sinuses, and abdominal artery of the lobster, *Panulirus interruptus* (Belman, '75). Two studies (Blatchford, '71, Belman, '75) deal with the hemodynamics of hemolymph flow through the crustacean cir-

culatory system. Both studies detail the pressure relations which move the hemolymph from the heart, through the arteries, the infra-branchial sinuses, and the pericardium. However, the changes in both stroke volume and heart rate, which are manifested by changes in cardiac output, cannot be readily determined from these approaches. The use of the Fick principle, where

$$\text{cardiac output (ml hemolymph/kg-min)} = \frac{\text{oxygen uptake (ml O}_2\text{/kg-min)}}{\text{arterial-venous O}_2\text{ concentration difference (ml O}_2\text{/ml hemolymph)}}$$

yields values for cardiac output representing, at best, an average flow rate over at least several minutes. This method of evaluation precludes detection of short-term changes in cardiac output and cardiac stroke volume.

The present study describes the application of the thermodilution technique (Fegler, '53, '54), a modification of the classical indicator dilution method, for the direct measurement of cardiac output in decapod crustaceans. The

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technique measures total hemolymph flow in unrestrained animals over a period of several seconds, permitting an assessment of cardiac stroke volume. We describe the variation of cardiac output with time in 2 different species of crabs and the dependency of this variation on stroke volume. In addition, cardiac output measurements made using the thermodilution technique are compared to values obtained using the Fick principle.

MATERIALS AND METHODS

Cancer anthonyi (Rathbun) of both sexes (150–280 gm wet weight) were collected by diving in Mission Bay, San Diego, California. *Cancer magister* (Dana) were obtained from the Bamfield Marine Station, British Columbia, and were transported to the University of Calgary. Animals were held in facilities at the University of San Diego and the University of Calgary in well-aerated sea water for a minimum of 3 days prior to use. *C. anthonyi* was maintained at 20–23°C while *C. magister* was maintained at 10 ± 1°C. Crabs were fed fish 2–3 times each week, except 24 hours before use.

A modified thermodilution technique was used to measure cardiac output (Fegler, '53, '54). The technique is similar to the one utilized by Bourne ('74) to measure cardiac output in the pink abalone, *Haliotis corrugata*. A small volume of hemolymph or filtered sea water, cooled to a temperature lower than that of the animal, was used as an indicator and injected into the ventricle. The temperature change of the hemolymph in the antennary artery immediately downstream from the ventricle was monitored as a function of time. Temperature changes in the artery were measured using a copper-constantan thermocouple (mean 95% response time = 0.071 sec in moving water) housed in the tip of a 29-ga. hypodermic needle. The tip of the needle was inserted retrograde into the right antennary artery 5–6 mm distal to its exit from the ventricle. Approach to the artery was made through a hole drilled in the carapace. Care was taken not to occlude the vessel. The probe was immobilized, and the hole was sealed by the application of softened dental wax. The output from the implanted probe was read on a Bailey Instrument BAT8 thermometer differentially against a similar probe immersed in an ice bath. Balancing, amplification, and recording were done with a Brush-Gould 2600 oscillograph. The thermocouple was calibrated with a thermometer graduated in 0.1°C increments,

and the calibration at a particular gain was expressed as °C/mm pen deflection.

Injection of indicator was made from a 1-ml glass syringe (graduated in 10-μl increments) encased within a water jacket connected to a pump driven, temperature-controlled water bath. Water jacket temperature was maintained at 10 ± 0.1°C when the ambient sea water temperature was 20°C (*C. anthonyi*) or 4 ± 0.1°C when the ambient sea water temperature was 10°C (*C. magister*). In both cases temperature equilibration of the solution in the injection syringe occurred within 3 minutes. The syringe was connected to a 20-cm-long piece of PE20 tubing tipped with the end of a 26-ga. hypodermic needle.

The needle tip was inserted into the ventricle through a 2-mm length sleeve of PE50 tubing, previously implanted in the carapace over the center of the ventricle. The volume of indicator injected for each cardiac output determination was 150 μl (300 μl for *C. magister*). Plastic spacers were fashioned to ensure that injectate volume remained constant with repeated fillings of the syringe. Pericardial pressure was monitored during most cardiac output determinations so that any effect of indicator injection on cardiac function could be seen. In a few preparations pericardial pressures were not measured to minimize disturbance to the animal. In these cases, heart rate was monitored with impedance electrodes mounted over the ventricle as described later. The first injection was made immediately following surgery to determine correct placement of the thermocouple. Hosie's ('62) equation was used to calculate cardiac output:

$$\dot{Q} = [V_i \rho_i S_i (T_i - T_h)] / (\rho_h S_h \int_{t_1}^{t_2} \Delta T_h(t) dt)$$

where \dot{Q} = cardiac output, V_i = injectate volume, ρ_i = injectate density, S_i = injectate specific heat, T_i = injectate temperature, ρ_h = hemolymph density, S_h = hemolymph specific heat, T_h = hemolymph temperature in the antennary artery.

While the difference between the density and specific heat of the injectate (when filtered sea water was used) and the hemolymph cannot be considered negligible, the terms can be ignored from the standpoint that the differences exert an opposite effect on the cardiac output calculations. The only data available on how the addition of proteins to solutions influences density and specific heat are from human blood. A comparison of the density of sea water isosmotic to human blood plasma (Smithsonian Physical Tables, '69) and the

density of human blood plasma (Phillips et al., '50) indicates an increase in the density due to the presence of proteins in the plasma. On the other hand, the specific heat of a solution decreases with the addition of proteins as illustrated by a comparison of the specific heat of sea water isosmotic to human blood plasma (Smithsonian Physical Tables, '69) with that of human blood plasma (Mendlowitz, '48). Assuming that similar relations also hold for the sea water injectate and the crab hemolymph, cardiac output is overestimated by only 2.4%. In view of this, the previous equation can be simplified to:

$$\dot{Q} = [V_i(T_i - T_h)] / \int_{t_1}^{t_2} \Delta T_h(t) dt$$

$\int_{t_1}^{t_2} \Delta T_h(t) dt$ was determined by measuring the area under the dilution curve with a planimeter. Injectate volume was small enough, and mean circulation time long enough so that recirculation of indicator did not occur during the measurement period.

The temperature of the injectate (T_i in the above equation) as it issued from the needle was found to be considerably different from water jacket temperature because of heat gain through the injection cannula wall and the unthermostatted dead space volume of the cannula. T_i was therefore determined by measuring the temperature change with a thermocouple of a rapidly stirred 1-ml volume of sea water into which indicator was injected. Conditions during these determinations were similar to experimental conditions.

In some cases we observed that a small clot had formed on the tip of the thermocouple in the intact animal. To determine if the clot has significant thermal insulating properties, we placed the thermocouple on which a clot had formed into a rapidly stirred 1-ml volume of sea water (as described previously for determination of injectate temperature) and injected a small volume of cold filtered saline into the container. We found that the measured rate of temperature change was no different from that obtained from a clean thermocouple.

Heart and scaphognathite beat frequency were also measured simultaneously with cardiac output to assess the respiratory status of each crab and to estimate cardiac output using the Fick principle. Each crab was prepared for the following measurements in a series of two or three operations, each followed by reimmersion in sea water, to minimize air exposure and stress due to handling: heart

beat frequency (f_H) and left and right scaphognathite beat frequencies (f_{scL} , f_{scR} , summed to yield f_{sc}). f_H was measured using an impedance technique similar to that of Ansell ('73), using Biocom 2991 impedance converters. Two holes were drilled in the carapace overlying the posterior pericardium so as not to penetrate the hypodermis, and two fine stainless-steel (0.0127-cm) wires were inserted above the heart. f_{sc} was measured either by using a similar impedance technique in which the wires were inserted over the anterior scaphognathite channel or by measuring branchial pressures with Statham P23BB pressure transducers or Hewlett-Packard Model 267 pressure transducers and Hewlett-Packard (Model 311A) preamplifiers. All impedance and pressure signals were displayed on a Brush-Gould 2600 recorder. Simultaneous recordings of impedance and branchial pressure confirmed the efficacy of using either technique for determining f_{sc} during forward pumping.

Cardiac output was also estimated according to the Fick principle in several specimens of *C. anthonyi*. Sham operations were performed so that these animals had been treated in an identical manner to animals used in thermolimitation experiments. Postbranchial hemolymph was sampled using a glass syringe and hypodermic needle from either of two holes drilled in the carapace, anterolateral to the heart, and covered with latex rubber affixed with cyanoacrylate adhesive (McDonald, '77). Prebranchial hemolymph was sampled similarly from the infrabranchial sinus at the base of the third, fourth, or fifth leg. Exhalant branchial water was sampled from a mask which had been fitted over the exhalant branchial apertures while inhalant branchial water was sampled at the base of a cheliped. Total oxygen content in both hemolymph and water samples was determined using the method described by Bridges et al. ('79). O_2 uptake (the numerator of the Fick equation) was calculated by multiplying the quantity of O_2 extracted from the inhalant branchial water by the branchial ventilation volume. f_{sc} was used to calculate ventilation volume based on the relationship for ventilation volume vs f_{sc} given by McMahan and Wilkens ('81) for a variety of crustaceans.

RESULTS

Successful thermocouple implantations were performed on five individuals of *Cancer anthonyi* and an individual *Cancer magister*.

Each thermocouple implant operation lasted 5-10 minutes with less than 1 ml of hemolymph loss. Approximately 80% of 20 crabs on which the operation was attempted survived at least 48 hours; many survived for longer periods with no apparent ill effects.

Thermodilution curves were characterized by a rapid upswing and a gradual return to the preinjection temperature as the injectate became diluted with the animal's hemolymph. A typical thermodilution curve is illustrated in Figure 1. In some preparations, transitory

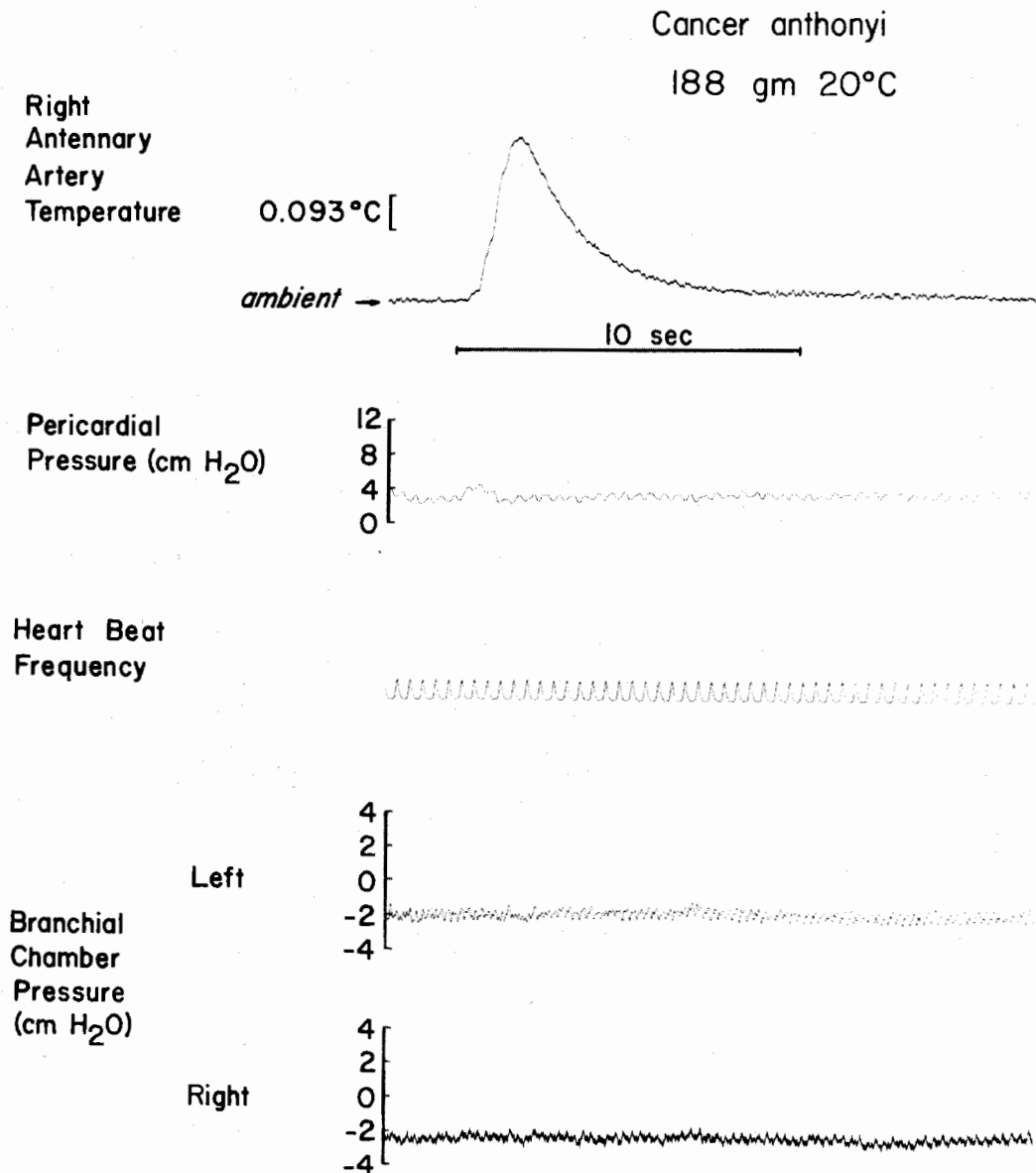


Fig. 1. A typical thermodilution curve determined during normal ventilating and cardiac activity in *Cancer anthonyi*. 150 μ l of cold filtered sea water (average temperature, 15.7°C) was injected into the ventricle causing a transitory increase in pericardial pressure and

immediately afterward a sharp decrease in right antennary artery temperature. Heart beat frequency was monitored using stainless-steel wire electrodes placed in the pericardial cavity and attached to an impedance converter.

increases in mean pericardial pressure were noted during injectate infusion (Fig. 1). These increases corresponded exactly with the duration of the injection (2-3 heart beats); after injection, mean pressure returned promptly to the preinjection level. Pericardial pressure appeared to be a good indicator of ventricular disturbance. We have evidence from several preparations, where ventricular and pericardial pressure were monitored simultaneously during an injection, that changes in mean ventricular pressure are paralleled precisely by pericardial pressure changes. In all cases, heart rate was unaffected during the injection period. Moreover, importantly, there was no change in pericardial pulse pressure during injectate infusion indicating that the ventricular volume changes associated with ventricular contraction remained essentially constant. Resting heart volumes in these crabs, estimated as being 0.3% of animal wet weight (Belman, '75; deFur and Mangum, '79), were at least 2.5 times larger than the injectate volume even in the smallest crabs and in most animals were nearly four times greater.

A preparation of *C. anthonyi* yielded several cardiac output determinations on the second day, even though all ventilatory movement had ceased (Fig. 2A). These data are interesting because of the extreme ranges measured in cardiac output. On the first day cardiac output ranged from 86 to 439 ml/kg-min while heart rate varied between 120 and 156 beats per minute. The variation in cardiac output was thus not due primarily to heart rate changes but rather to threefold changes in cardiac stroke volume (0.14-0.53 ml). On the following morning, 9 hours after the last injection, all ventilatory movements had ceased, f_H was 15-18 beats/min and cardiac output was 6-12 ml/kg-min with a stroke volume of 0.07-0.13 ml. After several cardiac output determinations, the thermocouple was removed and the crab soon died. Cardiac output also showed

considerable variation within and among other individuals of *C. anthonyi* (Fig. 2B,C,D). Much of this variation is attributed to changes in stroke volume.

Weight specific cardiac output in a large (828-gm) *C. magister* at 10°C was much lower than the smaller *C. anthonyi* at 20°C (Fig. 2E) as would be expected for a larger animal at a colder temperature (Mangum and Weiland, '75). In addition, stroke volumes were much greater, from 0.18 to 0.76 ml, reflecting the greater body size.

For comparison, cardiac output estimates determined on several restrained, sham-operated individuals of *C. anthonyi* based on the Fick principle are shown in Table 1. In each individual, postoperative heart rate was high. The Fick estimates of cardiac output fall within the range of values obtained on similarly treated crabs using the thermodilution technique.

DISCUSSION

Analysis of postinjection temperature changes recorded from the right antennary artery indicates that once the temperature change reaches a maximum, it returns (decays) to its preinjection value according to a logarithmic function of time. Kinsman et al. ('29) and Fegler ('54) have shown empirically in model systems that the decay of a dilution curve should indeed be logarithmic. These authors used dye and temperature as the indicators in their experiments. The logarithmic decay relation for the thermodilution technique, however, may result from two quite different processes. First, the transfer of heat from the surrounding tissues to the cooled hemolymph around the thermocouple is logarithmically related to time in a static system, when hemolymph is not flowing, since the transfer of heat is a function of the declining temperature gradient itself. In a dynamic system, when hemolymph is flowing, the transfer of heat will still be logarithmic, but it

TABLE 1. Fick estimates of cardiac output in four restrained individuals of *Cancer anthonyi* at 22-23°C*

Sex	Weight (kg)	Inhalant-exhalant O ₂ difference (ml O ₂ /100 ml water)	Calculated ventilation vol (ml/min)	O ₂ uptake (ml/min)	CaO ₂ -CvO ₂ (ml O ₂ /ml hemolymph)	Cardiac output (ml/kg-min)
Male	0.257	0.08	168	0.13	0.0027	187
Female	0.201	0.08	175	0.14	0.0037	188
		0.06	182	0.109	0.0034	160
Male	0.301	0.09	108	0.097	0.0048	67
Male	0.230	0.13	193	0.25	0.0022	494
		0.18	173	0.31	0.0041	329

*Cardiac output (ml hemolymph/kg-min) = O₂ uptake (ml O₂/kg-min)/CaO₂-CvO₂(ml O₂/ml hemolymph).

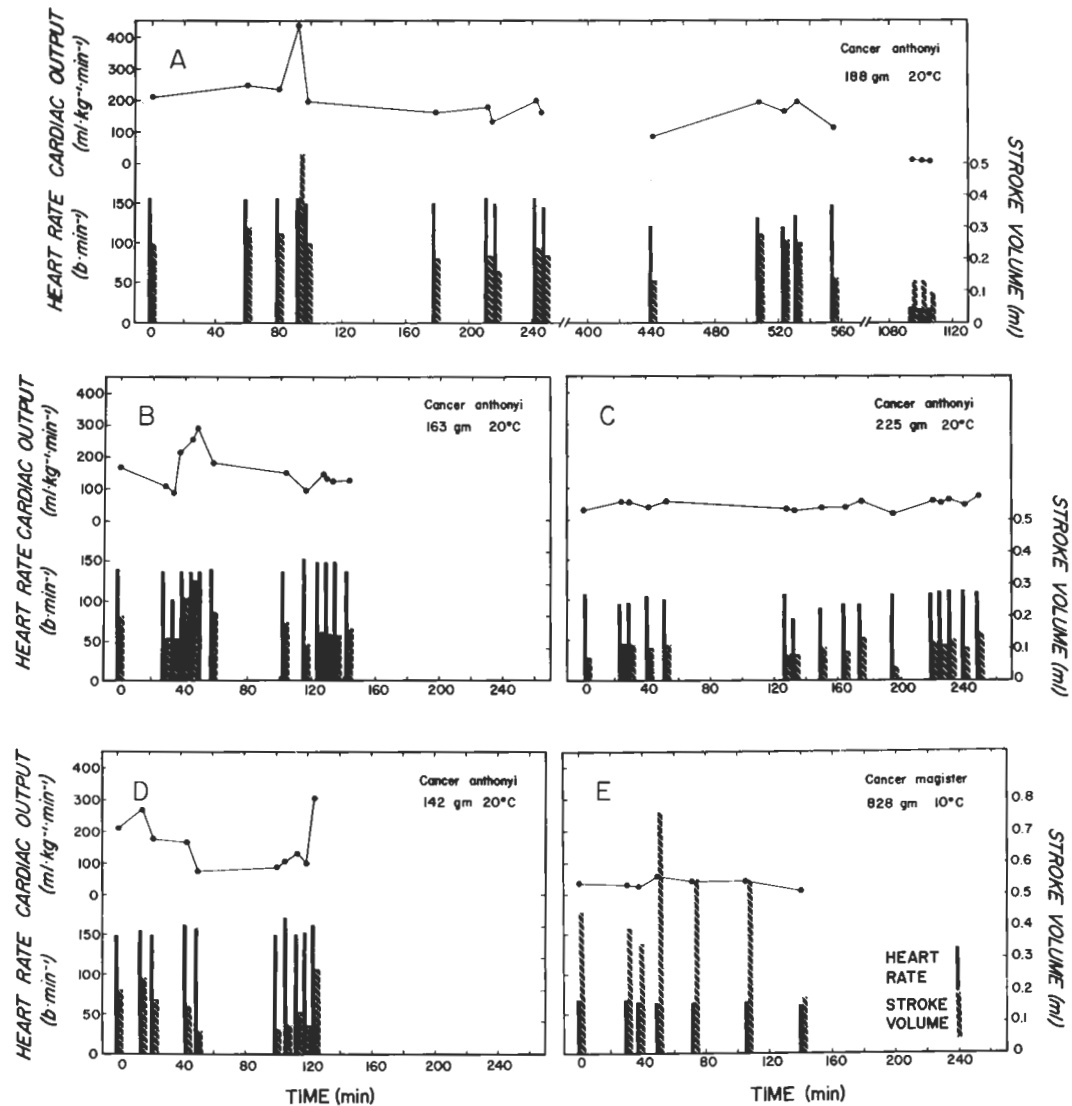


Fig. 2. Direct measurements of cardiac output and heart rate in five crabs permitted the calculation of cardiac stroke volume at various times after surgery. (A) In a preparation viable overnight, three measurements (at about 1100 min postsurgery) were obtained from a crab whose ventilatory

movements had completely ceased. (B-D) Three individuals of *Cancer anthonyi* show that variation in cardiac output is due mainly to changes in stroke volume. (E) A large *Cancer magister* at a colder temperature (10°C) shows a predictably lower cardiac output and large stroke volume.

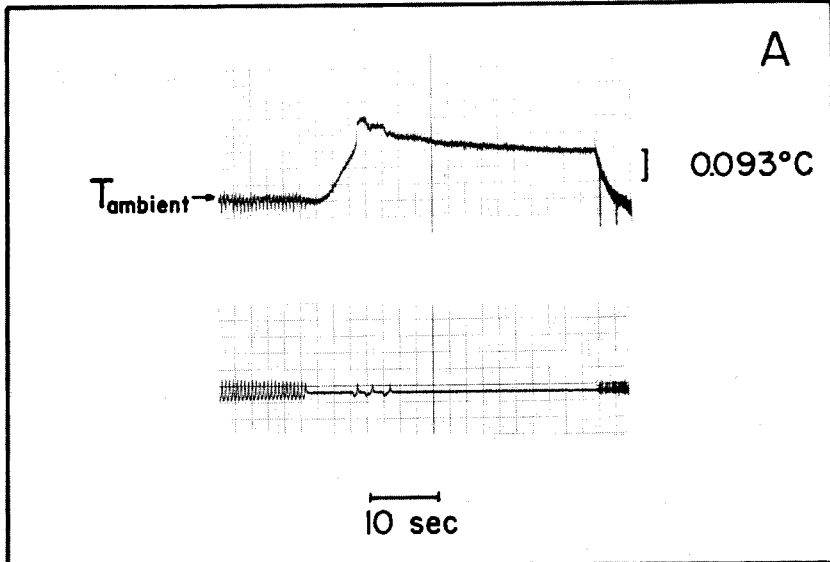
will be faster than in the static situation due to the maintenance of steeper thermal gradients. The steepest gradients occur immediately after injection and are rapidly dissipated (Fig. 1). Second, the bulk transport of hemolymph out of the ventricle carries with it smaller and smaller portions of the cold injectate with each beat of the ventricle. The proportion of the cold injectate ejected with each subsequent beat of the ventricle declines logarithmically to an in-

finitely small size providing there is adequate mixing of the residual volume of postsystolic hemolymph with hemolymph entering the ventricle from the pericardium during diastolic filling. A logarithmic relation in this case is also dependent upon the maintenance of a constant ventricular stroke volume throughout the period of measurement.

In our experiments it was likely that both processes contributed to the thermodilution

Right
Antennary
Artery
Temperature

Heart Beat
Frequency



Right
Antennary
Artery
Temperature

$$\ln \frac{T_{amb} - T(t)}{T_{amb} - T_{init}}$$

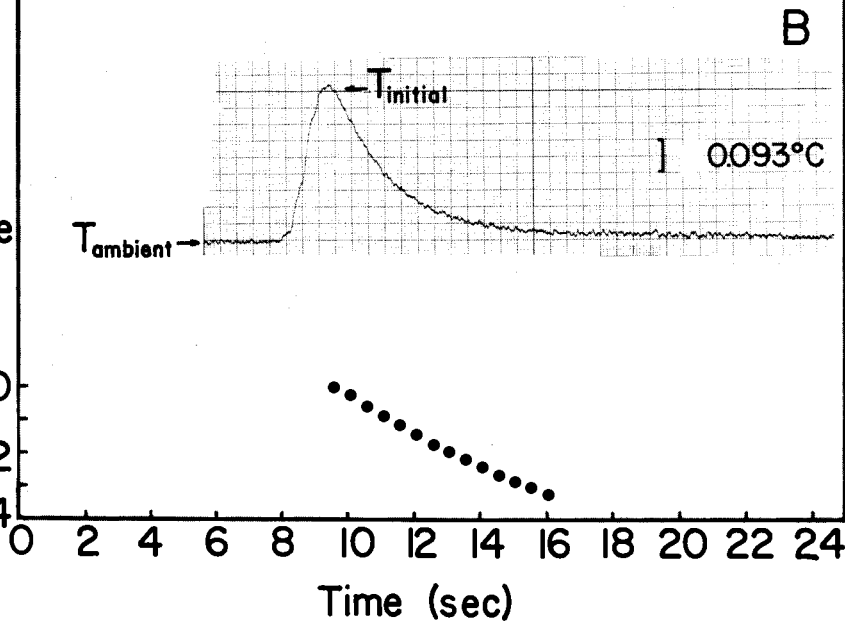


Fig. 3. Experimental verification that the thermodilution curve is due to the bulk flow of hemolymph out of the ventricle. (A) Cardiac arrest occurs during an injection in the blue crab *Callinectes sapidus*. The lack of significant temperature changes over 30 seconds during cardiac arrest indicates that heat conduction from the surrounding tissues

to the right antennary artery is negligible. (B) A linearized plot of the logarithmic return of temperature in the right antennary artery of *Cancer anthonyi* (thermodilution curve from Fig. 1) to ambient temperature following ventricular injection with cold filtered sea water.

curve. The efficacy of the technique, however, depends upon the thermal dilution curve being primarily due to bulk transport of hemolymph out of the ventricle. Therefore, it was necessary to account for heat transfer from the surrounding tissues to the cooled hemolymph. We have direct evidence that the heat transfer to the hemolymph across the arterial wall is

negligible during the short period over which thermodilution curves are determined. In one set of experiments when the cold injectate was injected into the heart of a blue crab (*Callinectes sapidus* prepared similarly to *Cancer*), the heart stopped beating for about 6 seconds, beat three more times, and paused again for 30 seconds (Fig. 3A). The time lag between the

injection and the initial gradual upswing of the thermocouple trace indicates that in this particular preparation hemolymph continued to flow at very reduced rates for a short time after the heart initially stopped. This event provided an opportunity for us to observe the transfer of heat from the surrounding tissues to the hemolymph in the absence of bulk hemolymph transport. Over a period of 30 seconds, when the heart was inactive, the hemolymph in the right antennary artery was warmed by only a very small amount (0.046°C). Since the surface to volume ratio of hemolymph in this artery is large compared with the surface to volume ratio of the ventricle and since the walls of the ventricle are thicker, the relative quantity of heat conducted to hemolymph in the ventricle from the surrounding tissues must be even smaller.

As previously stated, in a dynamic system bulk hemolymph flow would serve to maintain a constant thermal gradient between the hemolymph and its surrounding tissues during an injection, resulting in a more rapid diffusive heat transfer. However, diffusive heat transfer during bulk hemolymph flow is probably not much different from the situation depicted in Figure 3A. More importantly, however, during a typical thermodilution curve determination, the maximum thermal gradient developed exists for less than about 1 second before rapidly declining. Both the data presented in Figure 3A, showing very small diffusive heat transfer, and the very short period of time over which maximum thermal gradients are developed, rule out diffusive heat gain as an important determination of the shape of the thermodilution curve.

Direct evidence that the residual volume of postsystolic hemolymph is mixed well in the ventricle with the hemolymph entering the ventricle during diastolic filling or that ventricular stroke volume remains constant throughout the period of measurement is not available. Significant changes in ventricular hemolymph mixing and stroke volume, however, would be expected to result in large deviations from the linearized plot of the logarithmic function of temperature change with time (Fig. 3B), but none was detected. Therefore, the data indicate that the thermodilution curves obtained using the procedures outlined in this paper are an accurate reflection of the bulk transport of hemolymph out of the ventricle.

By vertebrate standards the flow of hemolymph in crabs is high and this strategy

is important to offset the lower capability of crab hemolymph for transporting large amounts of oxygen (Burnett, '79). The evolution of high hemolymph flow velocities through an open circulatory system is necessary to provide a metabolically active animal with adequate supplies of oxygen. In this convective strategy, changes in hemolymph flow can be triggered by variations in the external supply of oxygen (Taylor, '76; Burnett, '79) and by variations in the demand for oxygen (Mangum and Weiland, '75; McMahan et al., '79). Mechanisms available for altering the rate of hemolymph flow include both a change in heart beat frequency and a change in cardiac stroke volume. This study presents direct evidence that changes in cardiac output in crabs can be due to adjustments of stroke volume. These data support the hypothesis of McMahan and Wilkens ('77) that changes in stroke volume, calculated using Fick estimates of cardiac output and measurements of heart rate, are evident in the period of time immediately following normal periodic ventilatory pauses in the crab *Cancer productus*. Similar results were obtained by Jorgensen et al. ('78) for the abalone, *Haliotis cracherodii*.

It is not yet known under which circumstances one or the other mechanism for changing cardiac output predominates. However, one example illustrates that perhaps both are involved in circulatory adjustments initiated by a stressful situation. The spider crab, *Libinia emarginata*, regulates its oxygen uptake when it is exposed to moderate hypoxia by increasing cardiac output to a level about 15% greater than cardiac output in normoxia (Burnett, '79). Heart rate increases by less than 10% (deFur and Mangum, '79) indicating that the higher cardiac output is achieved both by an increase in cardiac stroke volume and by an increase in heart rate.

Finally, attention must be drawn to the fact that these determinations were performed on animals immediately following thermocouple implantation, and the high heart rates during this time are indicative of the trauma induced by this procedure. It is possible that trauma can be reduced by inserting the thermocouple directly through a small hole drilled in the carapace over the antennary artery, making it unnecessary to remove a large portion of the carapace over the artery for cannula insertion.

Although there appears to be evidence implicating adjustment of both heart rate and stroke volume in the control of cardiac output

in crustaceans, further studies using the thermodilution method or other techniques sensitive to relatively short-term changes in cardiac output are required to evaluate these two components on crabs in a variety of different situations.

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LITERATURE CITED

- Ansell, A.D. (1973) Changes in oxygen consumption, heart rate and ventilation accompanying starvation in the decapod crustacean *Cancer pagurus*. *Neth. J. Sea Res.*, 7:455-475.
- Belman, B.W. (1975) Some aspects of the circulatory physiology of the spiny lobster *Panulirus interruptus*. *Mar. Biol.*, 29:295-305.
- Blatchford, J.G. (1971) Haemodynamics of *Carcinus maenas* (L.) *Comp. Biochem. Physiol.*, 39A:193-202.
- Bourne, G.B. (1974) Hemodynamics in the pink abalone, *Haliotis corrugata* (Mollusca, Gastropoda). Ph.D. dissertation, Iowa State University, Ames, Iowa.
- Bridges, C.R., J.E.P.W. Bicudo, and G. Lykkeboe (1979) Oxygen content measurement in blood containing haemocyanin. *Comp. Biochem. Physiol.*, 62A:457-462.
- Burnett, L.E. (1979) The effects of environmental oxygen levels on the respiratory function of hemocyanin in the crabs, *Libinia emarginata* and *Ocypode quadrata*. *J. Exp. Zool.*, 210:289-300.
- deFur, P.L., and C.P. Mangum (1979) The effects of environmental variables on the heart rates of invertebrates. *Comp. Biochem. Physiol.*, 62A:283-294.
- Fegler, G. (1953) A thermocouple method of determination of heart output in anaesthetized dogs. In: Abstracts of communications, Nineteenth International Physiological Congress, Montreal, Canada, 341-343.
- Fegler, G. (1954) Measurement of cardiac output in anaesthetized animals by a thermo-dilution method. *Quart. J. Exp. Physiol.*, 39:153-164.
- Hosie, K.F. (1962) Thermal-dilution technics. *Circ. Res.*, 10:491-504.
- Jorgensen, D.D., J.R. Redmond, and S.K. Ware (1978) Circulatory changes occurring upon aerial exposure in an intertidal gastropod, *Haliotis cracherodii*. *Am. Zool.*, 18:656.
- Kinsman, J.M., J.W. Moore, and W.F. Hamilton (1929) Studies on the circulation I. Injection method: Physical and mathematical considerations. *Am. J. Physiol.*, 89:322-330.
- Mangum, C.P., and A.L. Weiland (1975) The function of hemocyanin in respiration of the blue crab *Callinectes sapidus*. *J. Exp. Zool.*, 193:257-264.
- McDonald, D.G. (1977) Respiratory physiology of the crab *Cancer magister*. Ph.D. Dissertation, Dept. of Biology, University of Calgary, Alberta, Canada.
- McMahon B.R., and J.L. Wilkens (1977) Periodic respiratory and circulatory performance in the red rock crab *Cancer productus*. *J. Exp. Zool.*, 202:363-374.
- McMahon, B.R., and J.K. Wilkens (1981) Ventilation, perfusion and oxygen uptake. In: *Biology of the Crustacea*, Academic Press, in preparation.
- McMahon B.R., D.G. McDonald, and C.M. Wood (1979) Ventilation, oxygen uptake, and hemolymph oxygen transport following enforced exhaustive activity in the Dungeness Crab *Cancer magister*. *J. Exp. Biol.*, 80:271-285.
- Mendlowitz, M. (1948) The specific heat of human blood. *Science*, 107:97-98.
- Phillips, R.A., D.D. Van Slycke, P.B. Hamilton, V.P. Dole, K. Emerson, and R.M. Archibald (1950) Measurement of specific gravities of whole blood and plasma by standard copper sulfate solutions. *J. Biol. Chem.*, 183:305-330.
- Smithsonian Physical Tables (1969) 9th Revised Edition, Smithsonian Institution Press.
- Taylor, A.C. (1976) The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. Exp. Biol.*, 65:309-322.
- Taylor, E.W., and P.J. Butler (1978) Aquatic and aerial respiration in the shore crab *Carcinus maenas* (L.), acclimated to 15°C. *J. Comp. Physiol.*, 127:315-323.