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# CHANGES IN VASCULAR AND EXTRAVASCULAR VOLUMES OF EEL MUSCLE IN RESPONSE TO CATECHOLAMINES: THE FUNCTION OF THE CAUDAL LYMPHATIC HEART

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SUMMARY

1. Vascular volume changes in an isolated saline-perfused eel tail preparation in response to catecholamines were small (< 2%) and are explicable in terms of changes in volume of pre-capillary resistance vessels.

2. Extravascular–extracellular (interstitial) volume increased less than 3% during infusion of adrenaline (AD) at concentrations of $1 \times 10^{-6}$ to $1 \times 10^{-3}$ M. Injection of doses of AD and noradrenaline (NA) between 1 nmol and 100 nmol caused maximum interstitial volume changes of less than 11%.

3. Isoprenaline caused only very small changes in vascular and interstitial volumes.

4. Caudal lymph heart frequency increased when high concentrations (> $1 \times 10^{-6}$ M) and doses (> 1 nmol) of AD and NA were administered.

5. Caudal lymph heart frequency increases were significantly correlated with changes in outflow after vascular volume adjustments. One function of the caudal lymph heart is to return interstitial fluid to the vascular system.

## INTRODUCTION

Vascular volumes of teleost fishes are small in comparison to those of other vertebrates (Holmes & Donaldson, 1970; Avtalion *et al.* 1974), and are estimated to be around three ml per 100 g body weight (3%; see Randall, 1970). Different vascular beds receive different blood flows. Not all capillaries of an individual vascular bed are perfused at all times (Mellander & Johansson, 1968), and blood flow varies with the activity of the tissue. Thus there are changes in the amount of blood actively circulating through the animal with time. In mammals, increases in the amount of blood actively circulating are accomplished in the short term by constriction of large-volume, low-pressure reservoirs such as spleen and liver, but especially by constriction of large veins (Mellander & Johansson, 1968). Previous workers (Rose *et al.* 1962; Oberg, 1967) have calculated that up to 90% of vascular capacity changes during vasoconstriction of skeletal muscle vascular beds originate from increased venous tone. Less than 10% of the capacitance changes are derived from changes in volume of resistance vessels.

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Since teleost vascular volumes are low, mobilization of pools of blood at times of increased blood flow would be necessary to avoid functional hypovolemia. As much of their vascular volume (~42%) is contained within skeletal muscle vessels (Stevens, 1968), an increase in tone of skeletal muscle veins could introduce a significant amount of blood into the actively circulating pool. Teleost veins, however, are reported to be aneural (Burnstock, 1969). Therefore capacitance changes in skeletal muscle during vasoconstriction should result principally from pre-capillary vessel diameter changes. However, the proportion of the vascular volume contained within the pre-capillary resistance vessels is less than 10% of the total regional blood volume (see Oberg, 1967). Thus, if vascular resistance increases by 400% due to pre-capillary vessel constriction, the radius of these resistance vessels decreases by about 30%. Blood volume is proportional to $r^3$, so a resistance increase of 400% represents a volume decrease of about 50%. Therefore, if teleost veins are aneural and do not respond to adrenergic agonists by constricting, the vascular volume changes caused by vasoconstriction in resistance vessels should be less than 5% (0·6 ml.kg$^{-1}$) of the vascular volume of the tissue.

Vasoconstriction reduces the capacity of blood vessels by a decrease in radius. However, at constant flow, vascular pressures increase. Pappenheimer & Soto-Rivera (1948) derived an equation for capillary pressure, *Pc* in which

$$Pc = \frac{(r_v/r_a . P_a) + P_v}{1 + r_v/r_a},$$

where $r_v/r_a$ = the ratio of venous to arterial resistance, $P_a$ = arterial pressure, $P_v$ = venous pressure. During constriction, $r_v/r_a$ is about $\frac{1}{8}$, whereas at rest it is $\frac{1}{4}$ (see Mellander & Johansson, 1968). Therefore during a vasoconstriction in which *Pa* increases twofold, *Pc* increases by $\frac{1}{10}$, provided *Pv* does not vary. Increased capillary pressures force fluid from the lumen of the vessel into the surrounding tissues (Landis & Pappenheimer, 1963). Higher tissue pressures will be transmitted through tissues to lymphatic vessels thereby increasing lymphatic pressures and flows (Granger & Taylor, 1978). Extravasation of fluid caused by elevated capillary pressures will be facilitated by the permeable capillaries of fish (Hargens, Millard & Johansen, 1972).

This report describes capacitance changes and extravasation of fluid during catecholamine-mediated vasoconstriction in an isolated eel tail preparation. Frequency of the caudal lymphatic heart is correlated with estimates of the amount of fluid extravasated in an attempt to define the function of this organ.

## METHODS

### *Perfused eel tail preparations*

The preparation used in this study has been described in detail elsewhere (Davie, 1981*a*). Briefly, it consists of the post-vent tail perfused via the caudal artery at constant flow by a peristaltic pump at 10 °C. Inflow was maintained at 0·5 ml.min$^{-1}$. 100 g$^{-1}$ of tissue and was determined gravimetrically. Outflow from the venous cannula was measured continuously by a drop counter. Input and output pressures were continuously recorded and vascular resistance was calculated by dividing the

pressure drop across the vascular bed by the flow. The perfusate was freshwater eel Ringer's solution (Rankin & Maetz, 1971).

### *Lymph heart frequency and amplitude*

Lymph heart frequency and amplitude were recorded with a Devices 4751 100 mg force displacement transducer. A glass extension was fitted to the transducer and the tip of the transducer was rested against the skin of the tail lateral to the lymph heart.

### *Calculation of capacity changes and amount of fluid extravasated*

From the introduction it is clear that during vasoconstriction there are two opposing factors which can affect outflow. Firstly, a decrease in capacity of the vessels will increase outflow by expulsion of fluid. Secondly, increased vascular pressures will cause fluid extravasation and reduce venous outflow. The time courses of these two events can be partially separated. Increased outflow will occur simultaneously with increased resistance. Slow visco-elastic or myogenic changes in vessel geometry may subsequently alter resistance. As resistance remained constant after the initial resistance rise when AD or NA was infused at constant concentrations (see Fig. 1 and Davie, 1981*a*), these changes are thought to be small.

Vascular capacity cannot change while resistance is constant because vessel radius remains the same. However, extravasation of fluid can occur during resistance increases and also after a new stable resistance level is reached. Extravasation will continue until a new 'Starling' equilibrium across capillary walls is established.

Therefore in the analysis of outflow changes during vasoconstriction, any outflow increase during resistance increases is assumed to be due to capacity reduction. The amount of fluid expelled will be an underestimate of the capacity change, since some extravasation may occur at the same time, and venous compliance may accommodate some of the capacity change. Furthermore, on average only 82% of the inflowing saline was collected from the caudal vein; the rest was lost through leakage mainly from the neural canal vessels. Therefore, estimates of capacity adjustments will be underestimates by at least 20%. In experiments to be described here, vascular volume adjustments were estimated from the sum of the outflow changes which occurred during resistance changes. By the same reasoning a decrease in outflow associated with a decrease in resistance is likely to underestimate the increase in vascular capacity. Percentage changes in vascular volume are based on a tissue vascular volume of three ml per 100 g ($3{\cdot}0 \pm 0{\cdot}2$ ml.100 $g^{-1}$; Davie, 1979).

Changes in extravascular volume are calculated by summation of the outflow changes which occurred after resistance adjustments. However, when outflow decreased during resistance increases, this could only have been due to extravasation. By the same arguments presented above, these estimates will be low by about 20%. Percentage changes in extravascular volume are made assuming an extravascular–extracellular (interstitial) volume of 10 ml.100 $g^{-1}$ (see Holmes & Donaldson, 1970; Chan *et al.* 1967; Wardle, 1971).

Fig. 1 illustrates a typical response to $1 \times 10^{-5}$ M-adrenaline. Adrenaline caused an increase in input pressure (caudal artery) which, when divided by the flow rate, gives the resistance increase of 2·78 kPa.$ml^{-1}$.min or 129%. Flow and venous pressure

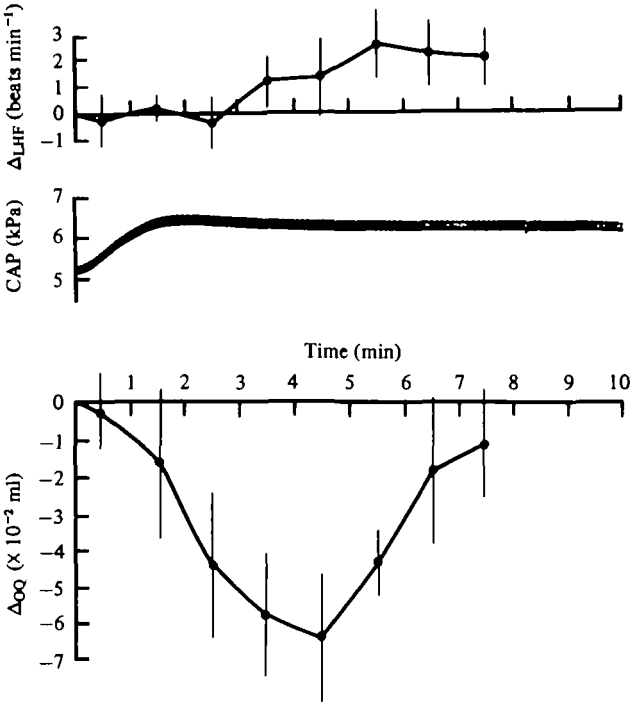


Fig. 1. Illustration of a pressure record (caudal artery pressure, CAP), mean lymph heart frequency changes (ΔLHf; n = 7), and mean outflow changes (ΔOQ) in response to infusions of $1 \times 10^{-5}$ M-AD. At constant flow, the pressure rise indicates a rise in vascular resistance of 129 %, which stabilized after 130 s. Outflow decreased to a minimum after around 5 min and lymph heart frequency increased to a peak level after 8 min. An explanation of these results is that the rise in pressure caused extravasation of perfusate from the vessels into the tissues. This fluid was drained back to the lymph heart via the lymphatic system. The lymph heart frequency increased to cope with the extra volume of lymph returning. Bars indicate ± 1 S.E.M.

were held constant while resistance increased. Graphical presentation of mean lymph heart frequency changes and mean outflow changes for all infusions of $1 \times 10^{-5}$ M-adrenaline ($n = 7$) are shown on the figure.

The fall in outflow probably results from extravasation of fluid and any decrease in vascular capacity is too small to be discernible in this record. In fact, only during infusion of $1 \times 10^{-6}$ M-adrenaline was there an increase in outflow during the pressure rise, followed by a decrease in outflow when pressure stabilized at the new elevated level.

Mean outflow before adrenaline infusion was $0{\cdot}860 \pm 0{\cdot}057$ ml.min$^{-1}$. During each minute of infusion of adrenaline the change in outflow was measured. These values are plotted in Figs. 1–3. The sum of all the outflow changes over the time of infusion of $1 \times 10^{-5}$ M-adrenaline was 0·224 ml. As the average weight of these tails was 209 g, the extravasation or amount of fluid which moved from the vessels into the tissue interstices was of the order of 1 ml.kg$^{-1}$ during the infusion period. Where an increase in outflow was observed during drug infusion, as in the case of infusion of $1 \times 10^{-6}$ M-adrenaline (see Fig. 2*b*), only the changes in outflow during the first few minutes,

Table 1. *Changes in vascular volume* ($\Delta V$) *and loss of perfusate to the interstitium* ($\Delta I$) *during infusion of adrenaline and injection of adrenaline and noradrenaline*

(Initial outflow rates, ± 1 S.E. are presented with the number of preparations exposed to the tabulated doses of adrenaline and noradrenaline.)

| Adrenaline infusion concentration (M) | $n$ | Initial outflow ($ml.min^{-1}$) | $\Delta V$ ($ml.kg^{-1}$) | $\Delta I$ ($ml.kg^{-1}$) |
|---|---|---|---|---|
| $10^{-6}$ | 7 | 0·859 ± 0·067 | 0·19 | — |
| $10^{-7}$ | 7 | 0·835 ± 0·074 | 0·34 | — |
| $10^{-6}$ | 6 | 0·865 ± 0·055 | 0·09 | 0·12 |
| $10^{-5}$ | 7 | 0·860 ± 0·057 | — | 1·07 |
| $10^{-4}$ | 9 | 0·862 ± 0·057 | — | 1·55 |
| $10^{-3}$ | 5 | 0·818 ± 0·059 | — | 0·26 |
| Adrenaline injection dose (nmoles) | | | | |
| 1 | 6 | 0·825 ± 0·212 | 0·07 | 0·26 |
| 10 | 7 | 0·753 ± 0·179 | 0·07 | 1·14 |
| 100 | 8 | 0·747 ± 0·165 | 0·27 | 3·84 |
| Noradrenaline injection dose (nmoles) | | | | |
| 1 | 7 | 0·615 ± 0·004 | 0·14 | 0·40 |
| 10 | 5 | 0·619 ± 0·041 | 0·09 | 1·19 |
| 100 | 4 | 0·600 ± 0·042 | 0·16 | 7·16 |

when pressure rose, were summed to give an estimate of vascular capacity changes. Thereafter, outflow changes are interpreted as movement of fluid into or out of the tissues.

The estimates of vascular capacity changes ($\Delta V$) and extravasation ($\Delta I$) are presented in Table 1. The standard errors of the summed $\Delta V$ values do not reflect their accuracy and are not presented. However, the standard errors of the initial outflow rates are given in Table 1 and give an idea of the precision of the outflow measurements.

Because the amount of fluid extravasated ($\Delta I$) is a derived variable, changes in lymph heart frequency are correlated with outflow changes ($\Delta$OQ) in an attempt to find a relationship between increased interstitial fluid volume and lymph heart frequency.

Where drugs were introduced dissolved in a 0·1 ml bolus of saline, blank saline injections of the same volume were administered to each preparation and the effects of the injection in outflows recorded. More than 80% of the blank injection volume was recovered from the venous outflow within 20 s of injection. These changes in outflow were used to correct the values of outflow after drug injection. As the transit time of perfusate through the preparation was 90–150 s (Davie, 1979), the injected bolus was still in the vessels after injection. The rapid expulsion of fluid from the venous cannula associated with injection suggests that capacity changes during vasoconstriction should be quickly observed as outflow changes. All values and points on graphs are expressed as mean ± 1 S.E.M.

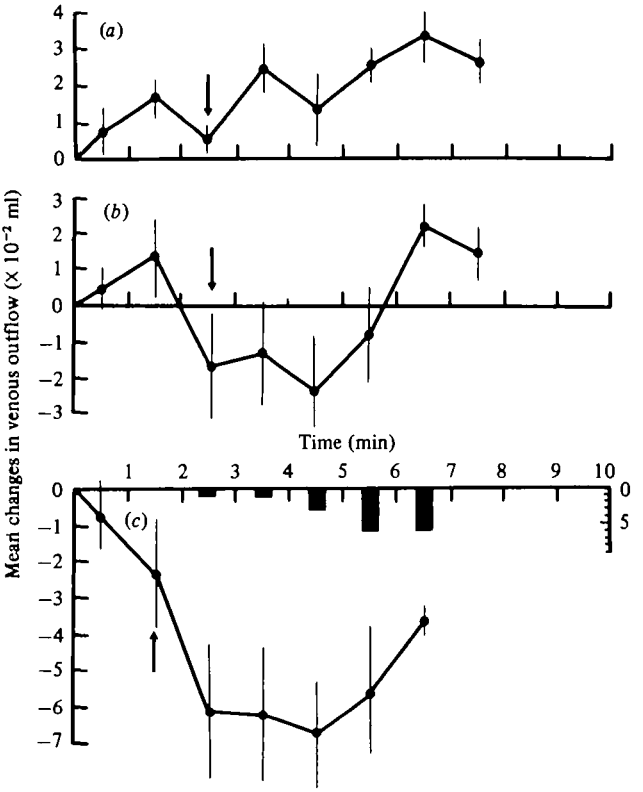


Fig. 2. Changes in venous outflow (ΔOQ) with time during infusion of AD. Arrows indicate times of stable elevated resistance levels. (*a*) $1 \times 10^{-7}$ M-AD. Resistance change $= +53 \pm 5\%$ ($n = 7$). (*b*) $1 \times 10^{-6}$ M-AD. Resistance change $= +140 \pm 29\%$ ($n = 6$). (*c*) $1 \times 10^{-4}$ M-AD. Resistance change $= +211 \pm 45\%$ ($n = 9$). Histogram on the abscissa of the graph indicates the number of preparations 'swimming' (see right-hand ordinate).

### *Drugs*

Drugs used in these experiments were L-adrenaline, free base (Sigma Chemical Co.), L-noradrenaline, free base (Sigma Chemical Co.), L-isoprenaline, bitartrate (Sigma Chemical Co.), Phentolamine, mesylate (CIBA), propranolol (ICI Ltd., Pharmaceuticals Division).

## RESULTS

### *Capacity changes*

Outflow was measured in 39 preparations. Mean changes in outflow during infusion of a peripheral vasoconstrictor, adrenaline (AD) at three concentrations are presented in Fig. 2. Arrows on graphs indicate when vascular resistance reached a new stable level. Calculated vascular volume changes are small (see Table 1). Infusion of $1 \times 10^{-7}$ M-AD caused the greatest decrease in vascular volume, which represents a decrease of about 1·4%. Mean changes in outflow during and after injection of 10 nmoles of AD and noradrenaline (NA) are illustrated in Fig. 3. Arrows on these graphs indicate when peak resistance responses occurred. Injection of 500 pmoles NA

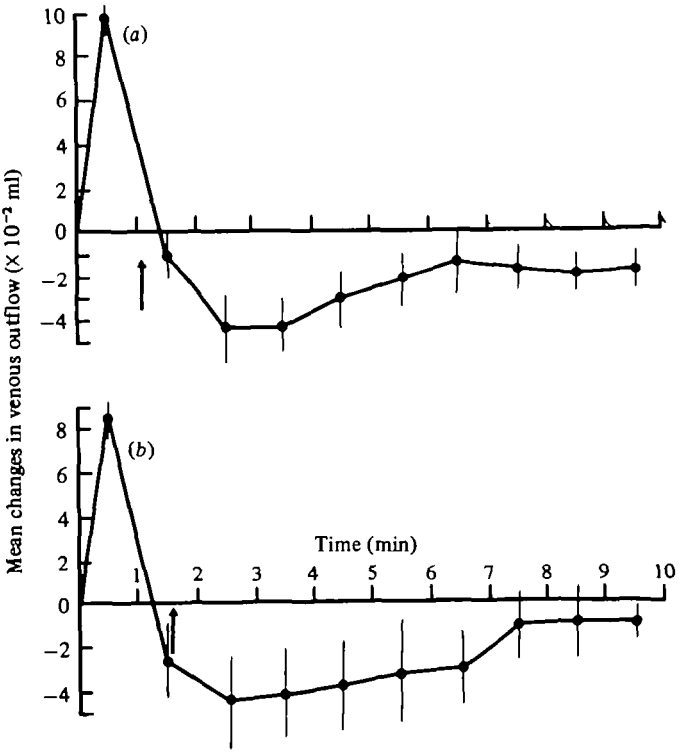


Fig. 3. Changes in venous outflow (ΔOQ) with time after injection of 10 nmoles of AD. and NA. Arrows indicate times of peak resistance responses. (*a*) 10 nmoles AD Resistance change = +105 ± 8 % (*n* = 7). (*b*) 10 nmoles NA. Resistance change = +141 ± 38 % (*n* = 5).

caused the greatest decrease in vascular volume of about 0·8 %. Isoprenaline causes vasodilation in this preparation (Davie, 1981*a*). When isoprenaline was infused into preparations at concentrations between $1 \times 10^{-10}$ M and $1 \times 10^{-5}$ M, there were small but consistent increases in vascular volume of about 0·7 %. Administration of isoprenaline as a bolus produced no clear trend towards increased or decreased vascular volume.

### *Extravascular volume changes*

Continuous infusion of $1 \times 10^{-4}$ M-AD caused an estimated increase in extravascular volume of 2·7 % (see Fig. 2) and was the largest change recorded in response to drug infusion. Steady elevated vascular resistance levels were attained within 120 s, but outflows fell to minima at 300 s in all cases, except when $1 \times 10^{-3}$ M-AD was infused. After these minima, there were steady trends back toward outflow rates recorded before drug introduction. The return toward initial outflow rates was concomitant with 'swimming' movements when $1 \times 10^{-4}$ M- and $1 \times 10^{-3}$ M-AD were administered (see Fig. 2*c*).

Injections of 1, 10, 100 nmoles of AD or NA all resulted in secondary decreases in outflow (see Fig. 3). The magnitudes of the calculated increases in extravascular volume are higher than those for infusion of drug at concentrations that produced similar resistance adjustments. Injection of 100 nmol AD caused an estimated 5·9 %

Table 2. *Mean lymph heart frequency* ± 1 S.E.M. ($n$ = 8) *immediately before the introduction of adrenaline during the course of construction of cumulative dose-response curves*

| Concentration of adrenaline (M) | Mean lymph heart frequency |
|---|---|
| $1 \times 10^{-8}$ | 62·1 ± 3·6 |
| $1 \times 10^{-7}$ | 63·8 ± 4·0 |
| $1 \times 10^{-6}$ | 63·0 ± 2·2 |
| $1 \times 10^{-5}$ | 66·5 ± 4·1 |
| $1 \times 10^{-4}$ | 68·2 ± 3·6 |
| $1 \times 10^{-3}$ | 65·6 ± 7·0 |

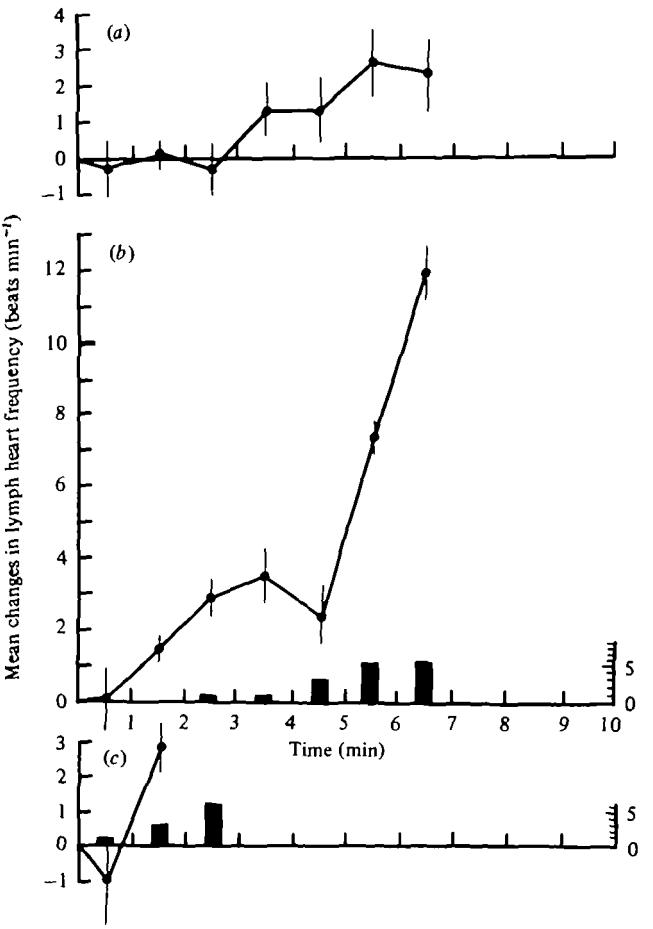


Fig. 4. Changes in lymph heart frequency (LHf) during infusion of AD at concentrations between $1 \times 10^{-8}$ M and $1 \times 10^{-3}$ M. Histograms on abscissa indicate the number of preparations 'swimming' (see right-hand ordinate). (*a*) Changes in LHf during infusion of $1 \times 10^{-8}$ M-AD. $LHf_{initial}$ = 66·5 ± 4·1 beats $min^{-1}$ ($n$ = 8). (*b*) Changes in LHf during infusion of $1 \times 10^{-4}$ M-AD. $LHf_{initial}$ = 68·2 ± 3·6 ($n$ = 9). (*c*) Changes in LHf during infusion of $1 \times 10^{-3}$ M-AD. $LHf_{initial}$ = 65·6 ± 7·1 ($n$ = 5).

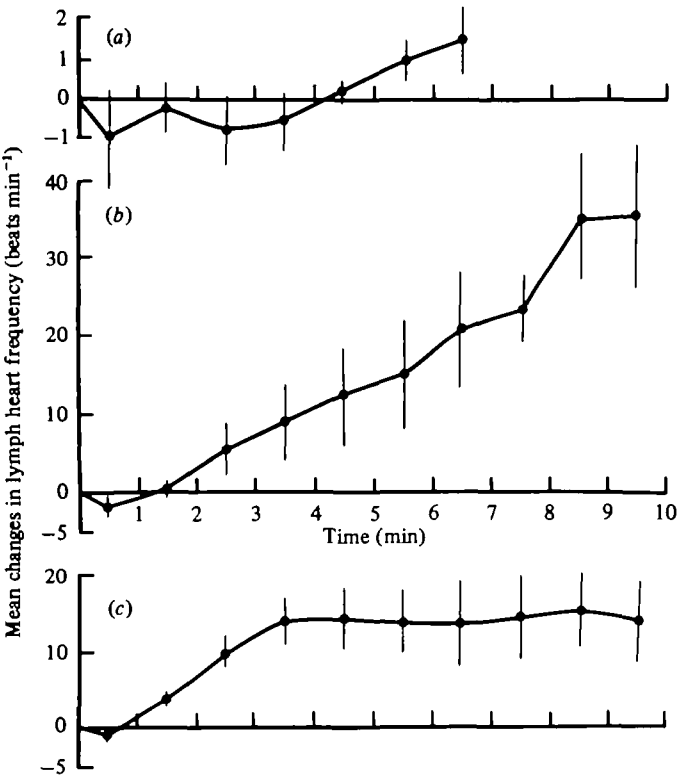


Fig. 5. Changes in lymph heart frequency (LHf) with time after injection of 1, 10 and 100 nmoles of AD into the perfusion line. LHf changed little in response to doses less than 1 nmole. (*a*) Changes in LHf after injection of 1 nmole AD. Note that the ordinate scale is five times that of the following two graphs. $LHf_{initial} = 78{\cdot}2 \pm 1{\cdot}8$ beats $min^{-1}$ ($n = 4$). (*b*) Changes in LHf after injection of 10 nmoles of AD. $LHf_{initial} = 55{\cdot}0 \pm 11{\cdot}5$ ($n = 7$). (*c*) Changes in LHf after injection of 100 nmoles of AD. $LHf_{initial} = 68{\cdot}3 \pm 6{\cdot}0$ ($n = 9$).

increase, 10 min after injection. The same dose of NA caused an estimated 10·5% increase over the same time period. In all cases of injection of AD and NA, outflow reached minima and started to increase back toward pre-injection values within 200 s. With bolus injections, drugs are continuously washed from the preparations, therefore the responses are expected to decline.

### *Effects of AD, NA and isoprenaline on lymph heart frequency*

Mean initial lymph heart frequency was $57{\cdot}68 \pm 3{\cdot}79$ beats $min^{-1}$ ($n = 80$). During the course of the experiments on individual preparations, there were no significant changes in lymph heart frequency (see Table 2). Because of different initial lymph heart frequencies, changes in lymph heart frequency ($\Delta$LHf) from the initial value are used in the analyses.

Infusion of AD and NA at concentrations greater than $1 \times 10^{-6}$ M caused consistent increases in LHf and amplitude. When applied topically to isolated lymph hearts AD and NA increased lymph heart amplitude, but not frequency (unpublished observations). As absolute amplitude could not be measured in this preparation,

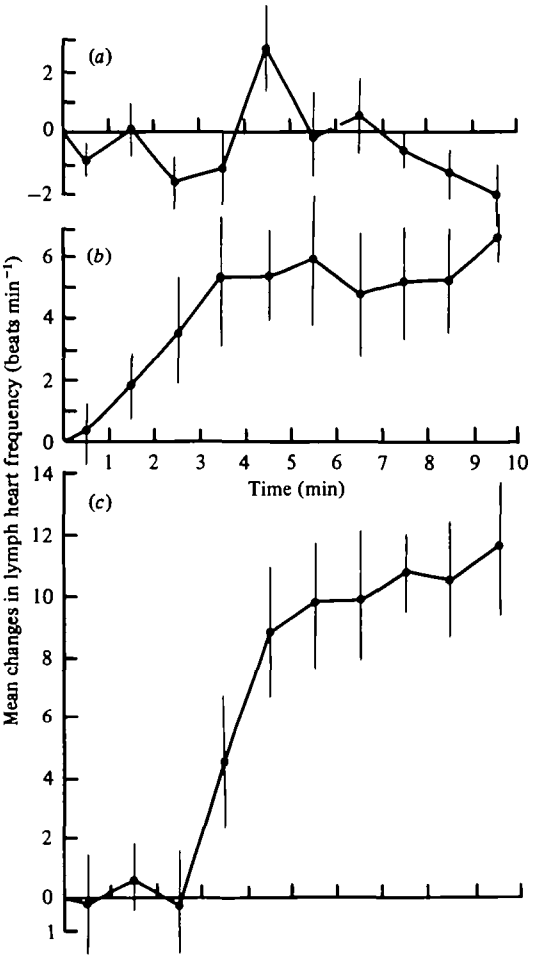


Fig. 6. Changes in lymph heart frequency (LHf) with time after injection of 1, 10 and 100 nmoles NA into the perfusion line. LHf changed little in response to doses less than 1 nmole. (*a*) Changes in LHf after injection of 1 nmole NA. $LHf_{initial} = 35{\cdot}5 \pm 5{\cdot}5$ ($n = 7$). (*b*) Changes in LHf after injection of 10 nmoles NA. $LHf_{initial} = 41{\cdot}0 \pm 3{\cdot}0$ ($n = 5$). (*c*) Changes in LHf after injection of 100 nmoles NA. $LHf_{initial} = 39{\cdot}2 \pm 7{\cdot}5$ ($n = 6$).

amplitude responses will not be discussed further. Fig. 4 illustrates the frequency changes in response to three concentrations of AD. Similarly, injection of AD and NA into the perfusion line in doses greater than 500 pmoles increased lymph heart frequency (see Figs. 5, 6).

Infusion or injection of isoprenaline at concentrations less than $1 \times 10^{-4}$ M and 10 nmoles caused small and unpredictable changes in lymph heart frequency. Higher concentrations of isoprenaline caused small increases in lymph heart frequency. However, at high concentrations of isoprenaline, vasoconstriction rather than vasodilation occurs (Davie, 1981*a*), therefore these results can be interpreted in terms of resistance increases, rather than responses to isoprenaline.

Infusion or injection of the $\alpha$-adrenergic antagonist phentolamine or the $\beta$-adrenergic antagonist propranolol at concentrations or doses sufficient to block $\alpha$ or $\beta$

vascular responses (Davie, 1979) both reduced lymph heart amplitude slightly, but had no effects on frequency.

## DISCUSSION

### *Capacity changes*

The results show that an increase in vascular resistance of an isolated eel tail preparation is accompanied by a decrease in vascular volume. The small changes are explicable in terms of radius changes of the resistance vessels, and venous tone changes need not be postulated. Although the swimming muscles contain approximately 40% of the total vascular volume of an eel, it is clear that this blood pool is not readily mobilized by circulating catecholamines.

### *Extravascular volume changes*

Administration of high concentrations of AD ($> 1 \times 10^{-6}$ M) and injection of high doses of AD and NA (> 1 nmole) produced small capacity changes followed by extravasation of perfusate. The different time course and direction of capacity and extravascular volume changes during administration of NA and AD allow separate estimates of both variables. The lag time between peak arterial pressure (peak resistance) and minimum outflow was 60–120 s. A similar lag time of 90 s between peak arterial pressure and interstitial fluid formation was measured by Poole (1977) in isolated perfused dogfish gill arches in response to alloxan, a drug which increases capillary permeability (Goetzman & Visscher, 1969).

Extravasation of fluid during catecholamine-mediated vasoconstriction may be augmented by an increase in capillary permeability (Isaia, Maetz & Haywood, 1978; Reichel, 1977). Although it is not possible to separate the effects of pressure and permeability in these experiments, they both act to increase fluid extravasation. Fig. 2*a* shows that concentrations sufficient to elicit resistance and capacitance changes do not cause any measurable extravasation. This was probably because the changes in capillary pressure and permeability were too small to perturb the 'Starting' equilibrium enough for it to be measured. When $1 \times 10^{-6}$ M-AD was administered, a decrease in capacity, followed by extravasation of fluid, were both clearly visible (see Fig. 2*b*).

About five minutes after injection or infusion of AD or NA, outflow started to return toward pre-administration values. This may represent greater reabsorption of fluid into the 'venous' end of the capillaries as a result of elevated interstitial pressures (Jacobson & Kjellmer, 1964). Swimming movements of the tails associated with very high doses of AD or NA (see Figs. 2*c*, 4*b*, 4*c*) probably assisted interstitial fluid recovery by increasing lymph flow (Gnepp & Sloop, 1978). Muscular contractions increase venous return from the tail in elasmobranch fishes (Satchell, 1965; Birch, Carre & Satchell, 1969).

### *Function of the caudal lymphatic heart*

These results show that catecholamines increase interstitial volume and probably pressure (Granger & Taylor, 1978). Under these conditions more lymph will be formed. Increased lymph production should be reflected in increased activity of the caudal lymphatic heart.

Increased lymph production is a logical cause for increased lymphatic heart frequency (LHf). However, before an analysis of changes in LHf during extravasation of fluid can be made, the possibility that AD or NA directly stimulated the lymph heart must be considered.

Lymph heart frequency maxima occurred 90–300 s after outflow fell to its minimum. Should LHf have peaked at the same time or before outflow fell, then a causal relationship between extravascular fluid build-up and LHf would have to be rejected. Experiments using radio-opaque medium and X-ray photographs showed that lymph heart muscles receive perfusate within 60 s of administration. LHf peaked after 300 s of perfusion with the drug. Thus if there were any direct effects of AD and NA they were much more slowly developed than the resistance changes which peaked at about 180 s after introduction of the drug. Chan (1971) recorded maximum lymph heart frequency increases in intact Asiatic eels of 23 beats $min^{-1}$ after injection of approximately 30 nmoles of AD or NA. The response time was however 23 min, which also suggests that the responses were indirect rather than direct. As noted before, the $\alpha$ antagonist phentolamine and the $\beta$ antagonist propranolol were without effect on the lymph heart frequency at doses sufficient to eliminate the vascular effects of AD or NA. Lastly, only during resistance increases of more than approximately 100% were there any significant changes in outflow and lymph heart frequency. Lower doses and concentrations of AD and NA elicited resistance responses, but no consistent changes in lymph heart frequency were recorded. For the reasons outlined above it seems likely that the increases in LHf were the result of extravasation of fluid during elevated vascular resistance.

To examine the relationship between LHf and extravascular fluid production, cumulative decreases in outflow after the initial capacitance adjustment were calculated during each minute of exposure to AD or NA. These volumes are taken to reflect progressive increases in extravascular fluid volume. Outflow changes ($\Delta OQ$) were then plotted against LHf during each minute of drug exposure. A model II type regression (Bartlett's 'best fit' method), where both variables are measured with significant error, was used to determine whether a significant relationship exists between $\Delta OQ$ and $\Delta LHf$ (Simpson, Roe & Lewontin, 1960). When each concentration above $1 \times 10^{-7}$ M and each dose above 500 pmoles of AD and NA was analysed separately, only $1 \times 10^{-5}$ M AD, 10 nmoles AD and 100 nmol NA showed significant linear regressions ($P < 0.05$). The regression line which describes the relationship between $\Delta OQ$ and $\Delta LHf$ for all data is

$$\Delta LHf = -19.23\Delta OQ + 0.15.$$

The slopes and intercepts of the lines for each of the three groups are not significantly different. This suggests that the change in lymph heart frequency per unit change in outflow (extravasation) is independent of administration method or the drug used. The correlation coefficient for all data is 0.399 and is significant at $P < 0.01$ (77 data point pairs).

From the dimensions of the lymph heart (Davie, 1981*b*), the estimated volume of the distended lymph heart is between 0.16 $\mu$l and 0.35 $\mu$l. At an average resting frequency of 58 beats $min^{-1}$, this would result in replacement of 9–20 $\mu$l of lymph

into the caudal vein each minute, assuming that all of the volume of the lymph heart is expelled during each beat. An average-sized eel tail has an extravascular volume of about 20 ml. Although these calculations are crude, they suggest that the lymph heart could pump the entire tail interstitial fluid volume in 16–32 h, which is comparable to the times for lymph circulation in mammals (Mayerson, 1962).

The experiments described here show that one of the functions of the lymph heart in eels is to assist in the return of extravascular fluid in the face of impending oedema and return this fluid into the bloodstream.

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