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Valve-related modes of pump failure in collecting lymphatics: numerical and experimental investigation

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Abstract

Lymph is transported along collecting lymphatic vessels by intrinsic and extrinsic pumping. The walls have muscle of a type intermediate between blood-vascular smooth muscle and myocardium; a contracting segment between two valves (a lymphangion) constitutes a pump. This intrinsic mechanism is investigated *ex vivo* in isolated, spontaneously contracting, perfused segments subjected to controlled external pressures. The reaction to varying afterload is probed by slowly ramping up the outlet pressure until pumping fails. Often the failure occurs when the contraction raises intralymphangion pressure insufficiently to overcome the outlet pressure, open the outlet valve and cause ejection, but many segments fail by other means, the mechanisms of which are not clear. We here elucidate those mechanisms by resort to a numerical model. Experimental observations are paired with comparable findings from computer simulations, using a lumped-parameter model that incorporates previously measured valve properties, plus new measurements of active contractile and passive elastic properties, and the dependence of contraction frequency on transmural pressure, all taken from isobaric twitch contraction experiments in the same vessel. Surprisingly, the model predicts seven different possible modes of pump failure, each defined by a different sequence of valve events, with their occurrence depending on the parameter values and boundary conditions. Some, but not all, modes were found experimentally. Further model investigation reveals routes by which a vessel exhibiting one mode of failure might under altered circumstances exhibit another.

Keywords

lymphatic vessel; lymphatic valve; fluid-structure interaction; pump function

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⁵Where ‘diameter’ is redefined as $(4A/\pi)$, A being the vessel cross-sectional area.

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1 Introduction

The lymphatic vascular system can be broadly thought of as consisting of two distinct types of vessel: initial lymphatics and collecting lymphatics. Initial lymphatics constitute a converging manifold of passive vessels with few internal valves; they are responsible for the derivation of lymph from interstitial fluid. Collecting lymphatics are a further converging manifold of muscular vessels, subdivided by frequent internal valves into short segments called lymphangions; they receive lymph from the initial lymphatics and actively pump it onward, to lymph nodes and eventually to the subclavian veins (Zawieja et al. 2011).

Much of what we know about the detailed mechanics of collecting lymphatic vessel function comes from experiments on isolated perfused segments of rat mesentery collecting lymphatic vessel (Davis et al. 2009; Davis et al. 2011; Davis et al. 2012; Scallan et al. 2012). These experiments allow control of pressures fed to the vessel at each end, measurement of an internal pressure by micropuncture, and video-based analysis of vessel diameter and valve function, all while the vessel continues to maintain tone and mount spontaneous contractions. Most of the experiments involve a vessel segment consisting of only a single lymphangion with its inlet and outlet valves, mounted on cannulae upstream and downstream by means of the cut stubs of the lymphangions that would have been adjacent *in vivo*. A fundamental investigative approach that has been very fruitful is to examine the response of the biological preparation to challenge by progressive increase of the pressure downstream.

However, the results of such experiments can be hard to interpret, given the necessarily limited number of variables that can be measured. This is where a computer model can be useful, especially if a relatively simple model can be shown to explain apparently complex phenomena.

As reported in published papers (Bertram et al. 2011a; Jamalian et al. 2013; Bertram et al. 2014a; Bertram et al. 2016b), we have progressively developed a series of computer models of a collecting lymphatic vessel. Because the system is highly nonlinear, and the experimental data available for comparison are largely measurements of time-variables at specific locations, the models use lumped-parameter technique to describe one or several lymphangions in series. Starting with equations which represented the *in vivo* properties only rather approximately (Bertram et al. 2011a), over time we have incorporated into these models realistic (based on biological measurement) representations of the passive pressure/diameter relationship for the vessel, the length/tension relationship for the wall muscle, the relaxation period between contractions, and the hysteresis and transmural-pressure-dependent opening/closing properties of the valves.

Since we are here making comparison with experimental data for one lymphangion, we here focus on a version of the model that also involves only a single lymphangion. However, as described in the next section, the model is a further advance beyond those that we have deployed previously in how it represents the contractile properties: for the first time, the simulated contractions are quantitatively based on measured contractions, and active tension is no longer a simple pressure add-on to the passive pressure/diameter relation.

By omitting some physiological regulatory response mechanisms [such as those modelled for long time-scales by Caulk et al. (2016)], the model retains the advantage it originally possessed of conceptual simplicity, which allows confident interpretation of its findings. Nevertheless, as described in section 3, it predicts multiple qualitatively distinct patterns of possible response to outlet pressure ramping, in fact more than we have found experimentally. It thereby illuminates complex experimental observations that were difficult to interpret when first observed.

2 Methods

2.1 Mathematical model

Our published model (Bertram et al. 2014a) uses a lumped-parameter technique to combine equations of conservation of mass and of momentum, along with a constitutive relation linking transmural pressure to diameter, for each of one or more lymphangions in series. (The transmural pressure p_{tm} is defined as internal pressure minus external.) The model incorporates valves with p_{tm} -dependent bias to the open state, and hysteresis, following the lymphatic valve characteristics discovered experimentally (Davis et al. 2011).

The version of the model used here is an advance over previously published ones in that it models contraction as a time-dependent continuous transition from a passive pressure-diameter relation to a fully activated one and back again. Because fully contracted lymphatic vessels reach diameters considerably smaller than any attained (even at zero p_{tm}) in the passive state, the transition between these extremes is modelled as a pressure-dependent transition in diameter. The passive and fully activated pressure-diameter relations are curves fitted to the extremes of isotonic twitch¹ data recorded from a rat mesenteric vessel at a series of values of p_{tm} from 0 to 50 cmH₂O. Details of these experiments are given in section 2.3. Thus the ‘passive’ state for this model is actually that corresponding to the small degree of standing active tone being maintained by the vessel. This differs from the maximally relaxed pressure-diameter relation (curve marked ‘passive’ in Figure 1A) which is recorded at the end of such a twitch contraction experiment by substituting Ca²⁺-free medium.

The same experiments also yielded data on the variation of the rate of spontaneous twitch contractions with p_{tm} , and a curve least-squares-fitted to those data was used to vary the contraction frequency in the model. This was achieved by averaging the values of p_{tm} traversed in the immediately preceding contraction and using the corresponding rate taken from the fitted curve to set the duration of the period of relaxation t_r before the start of the next contraction. Thus the duration of systole remained fixed, while that of diastole varied, in accordance with what has been observed in collecting lymphatics *in vivo* (Zawieja et al. 1993). The model equations are given in the Appendix; further details of the implementation of the new arrangements for active contraction and variable contraction frequency are given in section 2.3.

¹A ‘twitch’ is a normal spontaneous lymphatic vessel contraction, lasting of the order of 2 s from inception to the end of decay back to the initial diameter; the term is here used to distinguish these contractions from the tetanic contractions induced pharmacologically in the same experiments for comparison.

2.2 Ramping experiments

The experimental data on ramping were obtained in experiments on isolated segments of rat mesentery lymphatic vessel conducted with the aim of investigating the vessels' contractile response to changes in afterload, but not with the specific aim of comparison with the more recently developed numerical model. Some of these data have already been published elsewhere. A detailed description of the methods employed is already available (Davis et al. 2011), so only a brief summary will be given here. All animal protocols were approved by the institutional Animal Care and Use Committee at the University of Missouri, and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Collecting lymphatic vessels of diameter 80–220 μm were dissected from the mesentery of anaesthetized male Sprague-Dawley rats. Segments of vessel containing two valves were cleaned of adherent external tissue and mounted on glass micropipettes, all whilst immersed in physiological saline solution. With the pipettes connected to reservoirs supplying an identical pressure of $\sim 13 \text{ cmH}_2\text{O}$ upstream and downstream, the distance between micropipettes was adjusted such that vessel buckling was avoided. The preparation was moved to the stage of an inverting microscope, from which vessel images were recorded continuously at 30 fr/s during the experiment by means of a 2 Mpx monochrome CCD camera (Basler A641FM), interfaced by Firewire to a computer. Inside vessel diameter at a chosen axial location was tracked continuously using a custom algorithm (Davis 2005). Control of the reservoir pressure applied to each micropipette was effected by one of two custom-made analog servo systems each utilizing an electromagnetic actuator and power amplifier. The systems allowed precise independent control according to computer-supplied signals of the pressures p_{in} and p_{out} applied to the inlet and outlet reservoirs respectively. Arbitrary combinations of step and/or ramp changes could be created for one or both pressures.

Intraluminal pressure between the two valves was measured directly by the servo-null method, using micropipettes with a tip diameter of $\sim 2 \mu\text{m}$, back-filled with 2 M NaCl and connected to a commercial servo-null system (IPM model 4A). The vessel puncture was performed after cannulation but before raising bath temperature to 37 C, to minimise trauma. This pressure, as with p_{in} and p_{out} , was transduced by a CyberSense 104 low-pressure transducer (range $\pm 60 \text{ cmH}_2\text{O}$); all three pressure signals were digitized at 60 Hz via a 16-bit A/D card. The fluid in the perfusing bath applied an external pressure of 0.5 cmH_2O , so p_{tm} was 0.5 cmH_2O less than intraluminal pressure.

During cannulation, the whole vessel was rotated so as to bring the plane of symmetry dividing the two leaflets of a valve perpendicular to the eventual image plane. This was not possible to achieve perfectly for both valves; often the valve orientations differed by 90° . Where valve orientations differed, the vessel was orientated to favour the outlet valve. In subsequent replay, the position of each valve was detected by measuring image intensity change in a narrow window centred on where the valve leaflets pressed together when closed [see Davis et al. (2011) for more detail]. The analogue output from the optical densitometer, after normalisation to compensate for varying average vessel-lumen pixel intensity with contraction, was converted to a binary trace by thresholding. Because the outlet valve orientation was favoured, the inlet valve tracking is in some cases less reliable.

2.3 Twitch contraction experiments

The above methods, but without measurement of intraluminal pressure, were also applied to the studies of spontaneous twitch contractions undertaken especially for this work. Again the vessels used were segments of rat mesentery collecting lymphatic. After the cannulated vessel had stabilised and resumed spontaneous contractions, a series of several contractions was recorded at each of a series of constant pressures, pausing for ~1 min at each level, with both p_{in} and p_{out} set to the same value. To avoid the possibility of damage to the contractile machinery through over-distension, these pressures started at 3 cmH₂O and then descended to as close to zero as possible, commensurate with the vessel continuing to contract (contractions became widely spaced in time when p_{tm} was near zero), before being progressively stepped up to a maximum of 50 cmH₂O (where contractions were frequent but the amplitude of diameter decrease was small). Subsequently the vessel was maximally activated through the addition of saline buffered to a K⁺ concentration of 140 mM and substance P (sP, concentration 1 μ M) to the vessel bath. Under these conditions a powerful tetanic contraction is maintained. Again a series of pressure steps, this time between 0 and 60 cmH₂O, was explored; the steps descended from 50 cmH₂O to 0, back up to a maximum this time of 60 cmH₂O. In some experiments a second cycle of pressure-step descent and re-ascent was performed. Finally, the vessel was allowed to equilibrate in Ca²⁺-free medium before a final series of pressure steps, down from 60 cmH₂O to 0 and back up to 20 cmH₂O was explored. Diameter was tracked automatically from the video images during the experiment, but the automated tracking was not reliable at the lowest pressures (Davis 2005), so the video data for these was later re-analysed manually. Three technically satisfactory experiments were achieved; the data used here come from the most comprehensive of these.

The twitch contractions recorded at each pressure exhibited highly reproducible initial and maximal shortening diameters, as shown in Figure 1A, where respectively all blue circles and all red circles recorded at a given pressure largely overlap. At every pressure, initial diameter was less than that recorded in Ca²⁺-free medium (“passive” in Fig. 1A), showing that twitches were superimposed on a well-maintained moderate level of standing tone. The minimum diameters achieved at each pressure by twitch contractions were far greater, i.e. the shortening was much less, than those achieved (red curve) when tetanic contraction (an abnormal state) was artificially induced by application of K⁺/sP. The twitches were reproducible, in the sense that at a given pressure each twitch started from the same maximum diameter and yielded the same minimum diameter (red circles) to a good approximation. However, the tetanic pressure-diameter path (continuous red curve) traced out by connecting the points visited in chronological order after K⁺/sP showed a lack of reproducibility, i.e. different diameters occurred when the same pressures were revisited. Thus, the degree of pharmacologically induced contraction did not remain constant during the time needed to obtain data from all pressure levels. Partly for this reason, and partly because the tetanic state is artificial, we did not use these data for the model. However the data are shown for their inherent interest, in showing how much more contractile reserve the lymphatic vessel possesses than is evident in normal conditions. The data for the fully passive vessel, without any standing tone, are equivalent to what we have previously (Bertram et al. 2014a; Bertram et al. 2016b; Bertram et al. 2016a) used in the model as the

relaxed pressure-diameter relation when a contraction was not taking place, but relate to a different vessel.

For inclusion in the model, we fitted curves to the pre- and peak twitch data of Fig. 1A. To achieve best fit and for computational convenience, the fits were expressed non-analytically as a look up table. Plots of the resulting functions, shown as Figure 1B, indicate the extremes of each contraction, which consisted of the imposition of a time-varying pressure-diameter relation lying between these extremes. The (negative) p_{tm} -dependent diameter difference between peak twitch and pre-twitch was multiplied by a prescribed function of time varying smoothly from 0 to 1 and back to 0 (see Appendix), and the resulting function of p_{tm} and t was added to the pre-twitch p_{tm} -dependent diameters. At every instant, the actual (D, p_{tm}) -operating point was free to range up or down the instantaneously governing pressure-diameter relation according to what was needed to match the lymphangion's contraction to the external constraints represented by the valves and the currently prevailing conditions beyond. We also least-squares-fitted a curve to the fully passive data in Fig. 1A; we can thus apply the same method to the diameter differences caused by standing tone as we have to the twitch contractions. However for current purposes we simply used the pre-twitch (D, p_{tm}) -relation to describe the vessel at all times when a contraction was not in progress, i.e. we took the standing tone to be a constant of the vessel's characteristics throughout.

The averaged rate of spontaneous twitch contractions in the experiments of Fig. 1A was a strong function of the distending pressure in the range 0 to 20 cmH₂O, as shown in Figure 2. A good fit to these data ($r^2 = 0.988$) was achieved with the function $y = -1.39q^2 + 12.6q + 0.647$, where $q = \ln(1 + x)$, x being the pressure in cmH₂O and y the frequency in contractions per minute. Through the incorporation of this function into the model, the latter mimicked the contractile performance of a specific lymphatic vessel in respect of standing tone, contraction strength and contraction rate.

3 Results

3.1 Types of ramping response

Combining behaviours observed from both the modelling results and the isolated lymphatic vessel experiments, eight qualitatively distinct patterns of response behaviour were observed; see Table 1. All except behaviour 8 start from an operating point providing normal pumping, with both valves cycling between open and shut, intra-lymphangion pressure varying between inlet and outlet pressure, and time-average forward flow being generated. It is important to note that the model does not prescribe valve state; each valve state at each instant is the result of the current and past values of both trans-valvular pressure, and transmural pressure on the currently relevant side of the valve. These pressures evolve in time according to the simultaneous solution of all the model equations subject to the prevailing boundary conditions.

Of these, all except behaviour 8 are observed in the numerical model. Only behaviours 2, 7 and 8 are observed in the p_{out} -ramp experiments, but patterns of response related to the other behaviours are observed if p_{out} -step experiments are included (see Supplement). In the

following sub-sections, each behaviour will be examined separately, with respect to examples observed either *ex vivo*, or *in silico*, or both. Some of the experimental results have been published previously, as will be noted in the relevant place.

3.1.1 Behaviour 1—Behaviour 1 is defined by the inlet valve ceasing to open in diastole, as shown in Figure 3. Prior to that, lymphangion systole has been becoming progressively less effective in producing muscle shortening (and thereby diameter reduction) as the pressure beyond the outlet valve, to which the muscle is exposed during systole when the outlet valve is open, mounts. With less and less ejection, the lymphangion becomes progressively more engorged, and diastolic pressure slowly increases until it eventually exceeds the inlet pressure. After the final closure of the inlet valve, with outlet pressure continuing to increase, muscle shortening becomes even less effective, so the minimum diameter reached during systole continues to increase slightly. Secondary lymphatic valves are biased to the open position; they do not close until there is a small but significant adverse pressure difference across them (Davis et al. 2011). This behaviour is reproduced in the model (Bertram et al. 2014a); it means that closure also does not occur until there has been significant regurgitant flow through the open outlet valve, driven by the increasing adverse pressure difference that will shortly cause the valve to close. Consequently the lymphangion becomes more engorged in each successive diastole, even though the inlet valve is continuously shut. With reference to Fig. 1B, the diastolic operating point thus moves to the right along the pre-twitch curve both before and after the final inlet valve closure, such that intra-lymphangion diastolic pressure rises above the almost constant pressure beyond the inlet valve; see Figure 4. At the end of the ramp, an equilibrium is reached whereby the regurgitant flow at the very end of each systole exactly balances the ejection during the earlier part of that systole, and net outflow is zero.

3.1.2 Behaviour 2—Behaviour 2 initially develops identically to behaviour 1, i.e. V1 ceases to open, but subsequently V2 also ceases to close, as shown in Figure 5. Thereafter pressure in the lymphangion is essentially locked to p_b , except insofar as there is some ejection at the beginning of each systole, causing intra-lymphangion pressure (p_{11} , p_m and p_{12}) to rise slightly and momentarily above p_b , and an essentially equal amount of regurgitation back through V2 at the end of systole, causing intra-lymphangion pressure to dip equally briefly below p_b . Under these isobaric³ conditions, the amount of shortening (reduction of D) with each contraction actually increases relative to that which was occurring previously, when much of the active tension development went into raising the intra-lymphangion pressure from its low diastolic value.

Equivalent experimental behaviour was observed in a rat vessel where p_{in} was maintained at 4.5 cmH₂O and p_{out} was ramped up from 5.5 to 18 cmH₂O; see Figure 6. Until p_{out} reached about 15 cmH₂O, normal pumping was maintained, with the lymphangion producing more and more wall tension with every contraction in order to overcome the outlet pressure, open the outlet valve and eject. The diminishing extent of ejection is evident in the reducing extent of diameter reduction with each systole. V1 then ceased to open, leaving

³But not precisely isotonic, because of the variations in D .

lymphangion pressure isolated from p_{in} . Through a combination of end-systolic regurgitation and diastolic leakback through V2, mean D increases through the subsequent series of beats and accordingly diastolic pressure climbs past the knee of the diastolic pressure-diameter relation (arrowhead). V2 then ceases to close, pinning lymphangion pressure to p_{out} . As in Fig. 5, the extent of D -change when contraction occurs is immediately increased in this isobaric condition, relative to when V2 was still operating normally. In this particular vessel, the rate of contractions did not vary substantially during the p_{out} ramp.

3.1.3 Behaviour 3—Behaviour 3 is a variant of behaviour 2 in which V2 resumes normal operation as the ramp progresses further; see Figure 7. This late change has little obvious effect on the extent of D -reduction in systole; it is evident mainly in lymphangion pressure dropping below p_{out} when V2 is closed. However, the minimum pressure reached during each diastole is very much greater than that which pertained during the previous period when V2 was operating normally and V1 remained closed (from $t = 8$ to $t = 16$ s). The reason why is evident in Figure 8; the first phase of this pump state ('cn' in the notation introduced in Table 1) had lymphangion pressure dropping back to the below-knee part of the pre-twitch (D , p_{tm})-relation, whereas the late phase sees diastole well up on the steep above-knee part of this curve. Each V2 closure (green cross) happens with the (D , p_{tm})-operating point right on the curve; lymphangion pressure then drops, with the operating point moving down this curve. The next contraction initially raises lymphangion pressure isometrically with V2 closed; this is a vertical rise on Fig. 8, causing the subsequent V2-opening (green circle) to occur at a slightly smaller diameter than the previous closure.

3.1.4 Behaviour 4—Behaviour 4 is in one sense only a slight variation on behaviour 2, and so for the sake of brevity is not illustrated; the final situation of the valves is the same, but here the first abnormality is V2 ceasing to shut, which then immediately causes V1 to cease opening. The behaviour thus involves a different sequence of valve events, and is not simply behaviour 2 telescoped into a single transition within the duration of a contraction cycle.

3.1.5 Behaviour 5—The first abnormality here is V1 failing to open; this immediately causes V2 to cease opening, as shown in Figure 9. The immediate reaction of V2 to the V1-change is what qualitatively distinguishes this behaviour from the first transition involved in behaviour 2 (see $t = 28$ s in Fig. 5). With both valves closed, lymphangion pressure is tied neither to p_{in} during diastole nor to p_{out} during systole. There is a small amount of leakback through the closed valves; for as long as the time-average lymphangion pressure is less than $(p_{in} + p_{out})/2$, there is more leakback through V2 than through V1, and so D rises slowly, causing diastolic pressure also to rise slowly as the operating point mounts the pre-knee part of the diastolic (D , p_{tm})-relation. Because the peak-systolic (D , p_{tm})-relation is steeper at these diameters, the systolic pressure rises rather more. However the rise is insufficient to reach p_{out} and cause any further pump state transition, even when p_{out} levels off at the end of the ramp.

3.1.6 Behaviour 6—Behaviour 6 is defined by the rise instead reaching p_{out} and causing V2 to open, as shown in Figure 10; lymphangion pressure is subsequently tied to p_{out} and

V2 stays open. In terms of end pump state, behaviour 6 resembles behaviours 2 and 4, but the route taken to get there differs. This behaviour resulted from a change to the shape of the contraction activation waveform $M(t)$: a slower rate of rise and fall ($m = 1$ —see Appendix), in the process abolishing the pause at maximal activation. The reason why this causes the outlet valve to open is that the intra-lymphangion pressure varies somewhat similarly to $M(t)$. When t_r is short, lymphangion pressure is almost a square wave towards the end of Fig. 9, with a cycle-mean value that tends to rise towards the average of p_a and p_b . This value produces equal leakback through V1 and V2, and thus stabilises D (and mean intra-lymphangion pressure). In contrast, the mean lymphangion pressure late in Fig. 10 remains well below the average of p_a and p_b , inducing more leakback through V2 than V1 and thus continuing increase in D until V2 opens.

Behaviours 5 and 6 differ from all others in that they depend on a finite rate of ramping; if the rate at which the ramp increases p_b is sufficiently reduced, with all other parameters remaining unchanged, both sets of conditions give rise to behaviour 2 instead. All other simulation results shown in this paper are qualitatively unchanged if the ramp gradient is reduced.

3.1.7 Behaviour 7—The order of the numbering of the behaviour types in Table 1 finds its logic in the progression of pump states, but behaviour 7 is the response which might be thought of as the most obviously predictable outcome of ramping up p_{out} ; the lymphangion simply fails to mount sufficient tension during contractions to raise enough pressure to open the outlet valve, as shown in Figure 11. An example of the corresponding experimental outcome is shown in Figure 12. This particular vessel shows prominently the rate increase that attends the raising of lymphangion pressure. In over 30 such p_{out} ramp procedures carried out on isolated single-lymphangion segments, this was the outcome in around 75% of the cases (unpublished data). One might be tempted to see behaviours 1 and 7 as identical for a multi-lymphangion segment, as opposed to the single lymphangion here, but in fact behaviour 1 is more closely related to behaviour 5 insofar as the effect on the pump is concerned.

3.1.8 Behaviour 8—This behaviour (V1 ceases closing and V2 ceases opening) is unique to the experiments; no equivalent phenomenon has been observed in any of the simulations. Two examples from the same experiment are shown in Figure 13. Both suggest that the first abnormality is V2 failing to open, causing V1 to cease shutting either immediately (on the left) or a little time later (on the right). The result is that lymphangion pressure is tied to p_{in} , with essentially isobaric contractions causing only minor perturbations around that value as fluid is expelled through the inlet valve and then sucked back in as the positive p_{tm} re-expands the vessel. In both cases the starting condition is that the inlet valve remains open during contractions; this is not unusual, and reflects the known bias of lymphatic valves to the open position in the absence of a trans-valvular pressure difference tending to closure (Davis et al. 2011).

3.2 Connections between behaviours

The various model examples above employ differing combinations of parameter values and boundary conditions; those which varied included the inlet transmural pressure (p_{ae} , or $p_a - p_e$), the adverse pressure faced by the pump at the start of the ramp ($p_{b1} - p_a$), and the minimum valve resistance when fully open (R_{Vn}); see Table 2. Because more than one parameter is varied, and because some of these are not accessible for variation experimentally, it is not clear how the simulation results might be used experimentally to induce a vessel which initially exhibits one behaviour to exhibit another. However, as a result of further work done involving the systematic variation of only one parameter at a time, it is possible to define means by which to pass between some of the above behaviours, by changing only the pressure conditions applied to the lymphangion. These findings are experimentally testable predictions.

In every case in the following, the final ramp pressure $p_{b2} = p_{b1} + 11$ cmH₂O, and the time taken to get from p_{b1} to p_{b2} was 59 s. All results dealt with so far come from one model, termed A8e or A8h depending on the method of solution (which does not affect the results). However for this purpose we detail also results obtained in two other model variants, A7e and A8g. Model A7e is essentially that described in our previous publications; see for instance Bertram et al. (2014a). Model A8g utilizes valves which vary their switching thresholds according to prevailing diameter instead of transmural pressure—see Appendix. Thus in model A8e, with $p_{b1} = p_a + 2$ cmH₂O and $R_{Vn} = 8 \times 10^6$ (all unstated units are c.g.s.), a series of ramping runs was conducted with inlet transmural pressure p_{ae} set in the range from 0 to 2 cmH₂O. At and near the setting 0 cmH₂O, these yielded behaviour 5. At mid-range values, behaviour 1 resulted, and at and near 2 cmH₂O the outcome was behaviour 4. With the same values of $p_{b1} - p_a$ and R_{Vn} , a similar series of runs of model variant A8g yielded behaviour 7 at and near $p_{ae} = 0$ cmH₂O, and behaviour 2 at and near $p_{ae} = 1$ cmH₂O. Again with the same $p_{b1} - p_a$, and R_{Vn} , a series of runs of model⁴ A7e resulted in behaviour 7 at and near $p_{ae} = 2$ cmH₂O, and behaviour 4 at and near $p_{ae} = 4$ cmH₂O. Recall that behaviour 4 is only a slight variant of behaviour 2; thus the qualitative changing of behaviour in models A8g and A7e with higher values of p_{ae} was essentially similar, albeit the change occurred at a different value of p_{ae} .

It also proved possible in model A8e, with parameters set for behaviour 3, to obtain instead behaviour 4, simply by running the simulation with a reduced value of $p_{b1} - p_a$. In this case the unchanging parameter values were $p_{ae} = 1$ cmH₂O, and $R_{Vn} = 2 \times 10^6$; the change in behaviour occurred between $p_{b1} - p_a = 2$ and $p_{b1} - p_a = 1$ cmH₂O.

4 Discussion

Through the agency of outlet pressure ramping, this paper compares the observed behaviour of isolated rat mesentery collecting lymphatic vessels with a computer model. The model incorporates the equations of motion as they apply to a flow at small Reynolds number, the measured highly nonlinear passive and active pressure-diameter relations of a collecting lymphatic vessel, the measured dependence of contraction rate on distending pressure, and

⁴Parameter settings for the length/active-tension curve in A7e were $M_0 = 200$, and f_{M0} ; see Bertram et al. (2016b).

the measured hysteresis and transmural-pressure-dependent bias of collecting lymphatic valves. Whereas the measurement of any single variable in a microscopic lymphatic vessel is a stern challenge, a model can readily display the evolution of as many variables as one cares to define, including many which would be completely impossible to access experimentally. The purpose of this paper is to show that a great deal of the observed behaviour can be explained and illuminated on the basis of a model containing just these elements. The paper also shows that other behaviours are possible which have yet to be observed experimentally, and provides some leads as to how experimental conditions might be varied so as to encourage the exhibition of a given behaviour.

Even an isolated lymphatic vessel is an almost unimaginably complex system, which has a number of physiological regulatory mechanisms available to it. The experimental data shown in this paper bear witness to some of these. The vessel mounts twitch contractions which are superimposed on a level of standing tone that it has determined for itself (Fig. 1A). This level is neither zero nor anywhere near the maximal level of tonic constriction that can be mounted under extreme pharmacological stimulus. The twitch contractions themselves are highly repeatable as regards how much shortening is created each time, but this shortening immediately adapts in response to a change in the transmural pressure. Fig. 2 showed how the vessel also sensitively regulates its rate of spontaneous contraction depending on the prevailing transmural pressure. All these phenomena are represented in the model.

The model can in principle be adapted to the properties of individual vessels, although in practice the process is laborious; we found that no single analytical curve sufficed to fit the pre- and peak-twitch data used here, and ultimately ended up with a look-up table for each ‘curve’ which was interpolated as needed. In any case the experiments from which the ramping results emerge are not the same as those from which come the twitch contraction data. Insofar as experiment has provided the relevant measurements, all model parameter values used conform to the order of magnitude of the corresponding physiological one; in our current state of knowledge of these minute vessels, this is as precise as uncertainty in the measured data allows.

We did simulate individual difference in the time-variation of active contraction, finding that it could be responsible by itself for a qualitatively different outcome (cf. Figs. 9 and 10). In respect of the valves, we also simulated a situation where the inlet valve required more adverse pressure difference for both opening and closure than the outlet valve, and found that this changed the observed behaviour from 5 to 7. This simulation was prompted by recent experimental observations (MJD) in isolated 2-valve mouse lymphatics. One vessel repeatedly responded to p_{out} ramping from a starting value of $p_{\text{out}} = p_{\text{in}}$ by leaving the outlet valve open throughout. A valve closure test by the established method (Davis et al. 2011) was performed on the input valve at several levels of p_{in} , during which the outlet valve remained open, thus not interfering with the test. The inlet cannula was subsequently advanced through the inlet valve and a valve closure test performed on the outlet valve. As suspected, the outlet valve required ~ 0.3 cmH₂O more adverse p_V to close than the inlet valve at all levels of p_{in} . In another such vessel which exhibited behaviour 7, the inlet valve required ~ 1 cmH₂O more adverse p_V to close than the outlet valve. Recall that a depression

of only 0.3 cmH₂O in the V1 characteristics, relative to unchanged V2, was sufficient to produce behaviour 7 in Fig. 11 instead of behaviour 5. This demonstrates that biological variation in the case of collecting lymphatics is not confined to variation between animals or between vessels, but can be highly significant even at the level of the performance of adjacent secondary valves.

As far as vessel-to-vessel variation is concerned, the physical properties which are likely to vary include the valve opening/closing characteristics, the passive mechanical properties, the maximum active-tension generation, the vessel diameter, and the vessel wall thickness. Given all these, plus the demonstrated importance of the inlet, outlet and external pressure boundary conditions, it is hardly surprising that of the seven simulated behaviours, only two have been found so far in outlet-pressure ramping experiments, while the experiments show one behaviour (Fig. 13) which has not been found through computations. Among the five behaviours not so far found experimentally, two (behaviours 5 and 6) present a special case, in that they have been shown to depend on a higher rate of pressure ramping than that used in any of the experiments. An approximation to behaviour 5 has been seen in experiments utilizing an upward step in outlet pressure instead of a ramp; see the on-line Supplement to this paper.

The imposition of slow ramps of increasing p_{out} is a technique through which a great deal has been learnt about the contractile properties of the unique type of muscle (von der Weid and Zawieja 2004) which inhabits the wall of collecting lymphatic vessels, and also about the closure properties of secondary lymphatic valves (Davis et al. 2011). Lymph transport by intrinsic pumping relies on both these elements in combination. Our model, incorporating faithful representations of the measured behaviour of both the valves and the muscle, has proved capable of reproducing two of the three types of behaviour observed in isolated vessel segments in response to p_{out} ramping. In addition it has signalled the likelihood of observing five other behaviours in future experiments, and has provided in some cases routes by which to make the transition between behaviours, based on variation of pressures under the experimenter's control. The model has provided a means of systematizing a large collection of complicated and confusing experimental observations, and has illuminated and explained the detailed means whereby some of the observed changes come about. There is clearly scope for extending the model to include further physiological regulatory mechanisms, and a first attempt in this direction has already been made by others (Caulk et al. 2016).

There is also scope for extending the methodology of this paper to the comparison of model and experiment under other challenges, such as the variation of preload via p_{in} . Experiments have established (Scallan et al. 2012) that lymphatic vessels exhibit a positive Frank-Starling mechanism (increased output with increased filling) up to a filling pressure of 3 cmH₂O. Our earlier method of incorporating the length/active-tension properties of lymphatic muscle (Bertram et al. 2014a) involved an explicit function relating these two properties, which was increased monotonically over the range of diameters allowed by the passive pressure-diameter relationship. The method adopted here (Fig. 1B) is superior in that it can encompass data involving contracted diameters smaller than the relaxed diameter at $p_{\text{tm}} =$

0, but whether it leads to Frank-Starling response to varied filling is not tested under the circumstances investigated here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix – equations of the model

The basic equations are as follows.

$$\text{Mass conservation: } \frac{dD}{dt} = \frac{2(Q_1 - Q_2)}{\pi L D}. \quad (1)$$

$$\text{Momentum conservation: } p_a - p_0 = R_a Q_1; p_0 - p_1 = R_{v1} Q_1; p_1 - p_m = \frac{64}{\pi} \frac{\mu L Q_1}{D^4}; \quad (2)$$

$$p_m - p_2 = \frac{64}{\pi} \frac{\mu L Q_2}{D^4}; p_2 - p_p = R_{v2} Q_2; p_p - p_b = R_b Q_2; \quad (3)$$

where p_1 , p_m , and p_2 = pressure at the upstream end, centre, and downstream end of the lymphangion, p_a and p_b are the upstream and downstream reservoir pressures, p_0 is the pressure upstream of the inlet valve but downstream of the inlet cannula, and p_p is the pressure downstream of the outlet valve but upstream of the outlet cannula; Q_1 and Q_2 are the flow-rates into and out of the lymphangion, and D is the lymphangion diameter. These are the principal time-variables of the problem, but R_{v1} and R_{v2} , the resistances of the inlet and outlet valves, also both vary with time as a result of their dependence on the local transvalvular pressure p_v and the local transmural pressure, as

$$R_{v1} = R_{vn} + \frac{R_{vx}}{1 + \exp[-s_o(\Delta p_{v1} - \Delta p_{o1})]} \text{ and } R_{v2} = R_{vn} + \frac{R_{vx}}{1 + \exp[-s_o(\Delta p_{v2} - \Delta p_{o2})]}, \quad (4)$$

where $p_{v1} = p_0 - p_1$, $p_{v2} = p_2 - p_p$, the open/close threshold p_{o1} depends on either $p_0 - p_e$ or $p_1 - p_e$ according to the current valve state, and p_{o2} depends on either $p_2 - p_e$ or $p_p -$

p_e ; see Bertram et al. (2014b). The constants in eqns. 1–4 are the lymphangion length L (0.3 cm), the lymph viscosity μ (1 cP), the resistances of the inlet and outlet cannulae (R_a and R_b respectively), the minimum and maximum resistance of a valve (R_{Vn} and $R_{Vn} + R_{Vx}$ respectively), a parameter s_0 which governs the steepness of the smooth transition between minimum and maximum valve resistance, and the external pressure p_e . Values of R_{Vn} , R_{Vx} and s_0 used here are chosen to limit closing regurgitation and closed-valve leakage to reasonable amounts; R_{Vn} has been estimated experimentally and numerically, but further numerical work (not yet published) suggests that the earlier methods may have underestimated true values. The model can be modified to make p_e vary with time as a representation of extrinsic pumping, but this was not done here. Each valve resistance function $R_V(p_V)$ also includes a term which provides for loss of valve resistance by prolapse (Bertram et al. 2011b) if a very large adverse p_V is exceeded, but this is not approached here.

The outlet pressure p_b is raised in a linear ramp according to the following equations.

$$p_b(t) = p_{b1}, t \leq t_{b1} \quad (5a)$$

$$p_b(t) = p_{b1} + (p_{b2} - p_{b1}) \frac{t - t_{b1}}{t_{b2} - t_{b1}}, t_{b1} < t < t_{b2} \quad (5b)$$

$$p_b(t) = p_{b2}, t \geq t_{b2}, \quad (5c)$$

where the ramp begins at pressure p_{b1} and time t_{b1} and ends at pressure p_{b2} and time t_{b2} .

Time-course of active contraction: the waveform of contraction activation $M(t)$ is a smooth (continuously differentiable) curve formed by application of the following equations between the times $t = t_c$ and $t = t_c + 1/f$, where t_c is the beginning of a contraction and $1/f$ is its duration. $M(t)$ is calculated in two steps; in the first,

$$M(t) = 0.5 \{1 - \cos[2\pi f_m(t - t_c)]\}, \text{ with } f_m = mf, \text{ and } m = 1 \text{ or } 2; \quad (6a)$$

in parallel a function $M_j(t)$ is calculated and used to modify the preliminary $M(t)$ to its final form.

$$M_j(t) = m_g \{1 - \cos[2\pi f(t - t_c)]\}, \text{ with } m_g = 1 / (1 - \cos \frac{\pi}{m}); \text{ if } M_j(t) > 1, M(t) = 1. \quad (6b)$$

See fig. 3 of Bertram et al. (2016b) which shows how the resulting waveform of $M(t)$ changes with m .

The constant m is a multiplier for the rate of onset and decay of the active state; with $m = 1$, $M(t)$ increases sinusoidally and starts a similar decline as soon as it peaks; with $m = 2$, the onset and decay phases occur in half the time, and peak activation is maintained in between.

The duration of the period of relaxation t_r that starts at $t = t_c + 1/f$ is

$$t_r = \frac{1}{f_{tw}} - \frac{1}{f}, \quad (7)$$

where $f_{tw} = 60y$, with y as defined in the text relating to Fig. 2. To evaluate y , the average transmural pressure over the immediately preceding systole is defined as

$$\overline{\Delta p_{tm}} = \int_{t_c}^{t_c + 1/f} \Delta p_{tm} dt, \quad (8)$$

where $p_{tm} = p_m - p_e$ (in cmH₂O).

Constitutive relation: the two fitted curves shown in Fig. 1B can be described as

$$D_{act} = f_{act}(\Delta p_{tm}); D_{psv} = f_{psv}(\Delta p_{tm}), \quad (9)$$

where the subscripts act and psv indicate the peak-twitch and pre-twitch states respectively; see section 2.3 for details of the derivation of the curves. Then

$$\Delta D_{act} = D_{psv} - D_{act}, \text{ and } D = D_{psv} - M(t) \Delta D_{act}.$$

Thus we have the curve family $D = f(t, p_{tm})$, or, inversely, $p_{tm} = f^{-1}(D, t)$, where the inverse can be found either iteratively or by converting the function to a look-up table which is interpolated as needed.

Solution procedure: we combine eqns. 1–3 to eliminate Q_1 and Q_2 and obtain

$$\frac{dD}{dt} = \frac{D^3}{32\mu L^2} (p_{11} - 2p_m + p_{12}). \quad (10)$$

At each time-step we integrate this first-order ordinary differential equation (ODE) using a 4th-order Runge-Kutta scheme. The rest of the equations collectively provide algebraic constraints on the ODE, together making up a differential-algebraic equation (DAE) system. However, the system cannot be solved with conventional DAE solvers, because of the solution discontinuities arising from the valve hysteresis; see Bertram et al. (2014b).

The following describes solution procedure in models A7e, A8e and A8g. After advancing by one time-step we know p_{tm}^{old} , D^{curr} , and t^{curr} ; the new value of p_{tm} is then found iteratively as $p_{tm}^{new} = f^{-1}(D^{curr}, t^{curr})$, using p_{tm}^{old} as a starting value. Then $p_{tm}^{new} = p_{tm}^{new} + p_e$, and the new values of p_1 and p_2 are again found iteratively as $p_1^{new} = f(p_1^{old}, p_{tm}^{new}, D^{curr})$ and $p_2^{new} = f(p_2^{old}, p_{tm}^{new}, D^{curr})$.

In more detail for that last step, we iterate to find $p_0 - p_1 - R_{V1}Q_1 = 0$, where

$$Q_1 = \frac{\pi D^4}{64\mu L} (p_1 - p_{tm}), \text{ and } p_0 = p_a - R_a Q_1. \text{ Similarly, we iterate to find } p_2 - p_p - R_{V2}Q_2 = 0,$$

where $Q_2 = \frac{\pi D^4}{64\mu L} (p_{tm} - p_2)$ and $p_p = p_b + R_b Q_2$. In model A8h the step $p_{tm}^{new} = f^{-1}(D^{curr}, t^{curr})$ is handled differently. The function to be solved has at most three segments if sigmoidal, or one if not. Each segment is separately interpolated from the lookup table to find $p_{tm} = f^{-1}(D, t)$, then the solution closest to p_{tm}^{old} is chosen.

Section 3.2 details how some behaviours in response to ramping can be transformed to others, by running the ramp simulation with a different value of one of the boundary pressures. Some of these results were obtained from model variants other than the main one (A8e/h). Model A8g differs from A8e/h in having valves with open/close p_V -thresholds set by the current diameter instead of the local transmural pressure. The curves which constitute the two $p_V(D)$ relations are fitted to the valve-switching data of Davis et al. (2011) shown in fig. 4 of Bertram et al. (2014a), with the closing data modified as therein, utilizing the nonlinear relation $D_{psv} = f_{psv}(p_{tm})$ detailed in the appendix of Bertram et al. (2014a) to convert from transmural pressure to diameter; see Figure A1. In order to give each valve an individual characteristic, a diameter is also calculated for the stub part-lymphangion beyond the outlet valve, based on the transmural pressure at that location and the same $D_{psv}(p_{tm})$. Model A7e is that described previously (Bertram et al. 2014a).

Figure A1.

Opening and closure thresholds for the diameter-operated valves used in model A8g.

Figure 1.

A: experimental data. Passive (blue dots) and pharmacologically activated (red dots), pre-twitch and peak-twitch p/D data-points (black dots), at a series of steady pressures. The red/blue lines show the sequence of data-points visited. Twitch amplitude: horizontal black lines. Pre-twitch and peak-twitch p/D data-points are also circled (blue and red respectively); only these data were subsequently used in the model.

B: curve fits to the data. Blue circles, vessel diameter immediately prior to each spontaneous twitch contraction; red circles, diameter at the peak of each twitch (data repeated from A). Several contractions were recorded at each of 14 different values of transmural pressure (only those up to 20 cmH₂O are shown here), and the data show good reproducibility from one contraction to the next. Although the data did not extend to $p_{tm} < 0$, it is necessary to complete the curves to avoid undefined states arising in the model. The curve fitted to the pre-twitch data was extrapolated to negative values of p_{tm} , taking into account the inevitable vessel collapse² (Bertram et al. 2011a). The curve fitted to the peak twitch data was folded back to join the pre-twitch curve at a small negative p_{tm} , on the basis that shortening would rapidly become impossible in the collapse regime.

²Where 'diameter' is redefined as $(4A/\pi)$, A being the vessel cross-sectional area.

Figure 2.

The average rate of spontaneous twitch contractions, as a function of transmural pressure. The fitted curve was used in the model to determine the duration of the relaxation before the next contraction.

Figure 3.

Model schematic, and traces from a simulation run under conditions giving rise to behaviour 1. A: pressures p_a and p_b (thin black lines: $p_{a,b}$ in legend), and intravascular pressures p_{02} (magenta, upstream of V1), p_{11} (blue, downstream of V1), p_m (red, mid-lymphangion), p_{12} (green, upstream of V2), and p_{p1} (beige, downstream of V2). Traces are plotted in the order shown in the legend; earlier-plotted traces may be largely over-written by nearly identical ones plotted later. Thus here p_{02} completely over-writes p_a , and p_{p1} completely over-writes p_b . The three intra-lymphangion pressures p_{11} , p_m and p_{12} are almost identical; only during the first beats when p_b is low are p_{11} and p_m briefly visible in the rapid filling and rapid ejection phases respectively. Flow-rate traces are here omitted, but the extent of the transient dip of p_{11} , p_m and p_{12} below p_a in the filling phase, and of their transient peaking above p_b in the ejection phase, is indicative of the high flow-rates achieved initially, before ramping starts.

B: the prescribed waveform $M(t)$ controlling the movement between pre- and peak twitch states (cyan, scale 0 to 1 covering the plot height), and the lymphangion diameter (blue). During the time shown, the duration of the relaxation period between contractions shortens in response to increasing mean p_m from 2.22 to 0.50 s.

C: valve state for V1 (blue) and V2 (green) – 1 is open, 0 is closed (the two traces are slightly offset vertically for clarity). Closure and opening are both smooth gradual transitions in valve resistance until a threshold which varies according to p_m and the current valve state is passed; see Bertram et al. (2014b) for details. See the Appendix for explanation of the parameters for which values are shown (in c.g.s. units unless otherwise indicated) above the relevant panel.

Figure 4.

Pre-twitch (beige) and peak twitch (grey) curves as in Fig. 1B, but here the motion of the (D, p_{tm}) -operating point (red, with the second and last full cycles of contraction and subsequent relaxation over-printed in black) is superimposed as the model executes the cycles shown in Fig. 3. Each cycle of pumping would in the absence of p_b -change be an identical anti-clockwise loop tangent to the pre- and peak twitch curves. The simulation starting point is where V1 opens (blue circle) at $p_{tm} < 0$; this point is set by the prescribed initial conditions and has no significance; the model rapidly moves to (D, p_{tm}) -coordinates which are in better equilibrium with the external conditions, before the first contraction begins. Contraction causes closure of V1 (blue cross, $D = 142 \mu\text{m}$) and almost immediately afterwards opening of V2 (green circle, $D = 142 \mu\text{m}$). That ejection ends with closure of V2 (green cross, $D = 85 \mu\text{m}$), after brief regurgitation through V2, visible here in the small increase in D just before V2 closes. As can be seen in Fig. 3, that first contraction occurs without complete filling; later cycles start from $D \approx 159 \mu\text{m}$. The first and second cycles follow the same end-systolic path. Once the p_b -ramp starts, cycles achieve tangency with the peak-twitch curve further up and to the right, but all pass through the same V1-closure location (superimposed blue crosses, $D = 159 \mu\text{m}$) until V1 ceases to open. Thereafter, contractions achieve less than nothing; the phases of isometric pressure development and isometric pressure decay (vertical red lines) are almost superimposed, but it can be seen that diameter is slightly greater at V2 closure (green cross) than at V2 opening (green circle). However each successive cycle is then further shifted to the right during diastole, as a result of leakage back through the closed V2, and in consequence the starting point for each contraction climbs up the pre-twitch curve, i.e. both D and p_{tm} increase. Throughout the ramp procedure, the extent of regurgitant flow through V2 before closure also increases, as seen in the growing end-ejection increases in D before V2 closes.

Figure 5.

Simulation run under conditions giving rise to behaviour 2 (permanent closure of V1, then later permanent opening of V2). During the 74 s of simulated time shown here, the relaxation period between contractions decreased from 1.26 to 0.36 s. See caption to Fig. 3 for details of panel content and identification of individual traces.

Figure 6.

Experiment on an isolated perfused vessel consisting of one complete lymphangion and (beyond each valve) the stump of another lymphangion, used to mount and secure the segment on a cannula. The vessel is here subjected to constant inlet-reservoir pressure p_{in} (blue) and a ramp increase of outlet-reservoir pressure p_{out} (red); these correspond to p_a and p_b respectively in the model. The intra-lymphangion pressure p_L (black) is measured by direct puncture of the vessel wall with a micropipette, and servo-null methodology. Diameter at a site downstream of the puncturing micropipette but upstream of the outlet valve was tracked by an optical method applied to the video cine recording of the vessel image as seen by inverted light microscope. The state (open or closed) of each valve (V1 = blue, V2 = red) was likewise assessed by an optical method from the video recording [see Davis et al. (2011) for details]. The result of the application of the p_{out} ramp is first that V1 ceases opening, then (in this case almost coincident with the end of the ramp) that V2 ceases closing; this is behaviour 2. Modified from fig. 10 of Scallan et al. (2013).

Figure 7.

Simulation run under conditions giving rise to behaviour 3 ($V1$ not opening, then later $V2$ not closing, then still later $V2$ operating normally). During the 74 s of simulated time shown here, the relaxation period between contractions decreased from 1.35 to 0.37 s. See caption to Fig. 3 for details of panel content and identification of individual traces.

Figure 8.

The path (red, with selected cycles overprinted in black) of the instantaneous (D , p_{tm})-operating point during a p_{out} ramp giving rise to behaviour 3 (same run as shown in Fig. 7). See caption to Fig. 4 for further detail of the plotted symbols and curves.

Figure 9.

Behaviour 5 ($V1$ stays closed, $V2$ immediately follows suit). During the 94 s of simulated time shown here, the relaxation period between contractions decreased from 1.95 to 0.69 s. See caption to Fig. 3 for details of panel content and identification of individual traces.

Figure 10.

Behaviour 6 (as 5, but V2 eventually reopens and stays open). For this run, the parameter m which multiplies the rate of contraction activation rise and fall was reduced from 2 to 1. During the 84 s of simulated time shown here, t_r decreased from 2.88 to 0.41 s. See caption to Fig. 3 for details of panel content and identification of individual traces.

Figure 11.

A simulation of behaviour 7 ($V2$ ceases to open). For this run, the two curves governing $V1$ opening and closure were depressed by 0.3 cmH₂O. During the 62 s of simulated time shown here, the relaxation period between contractions decreased from 3.49 to 1.49 s. See caption to Fig. 3 for details of panel content and identification of individual traces.

Figure 12.

Starting at $p_{\text{out}} = p_{\text{in}} = 1 \text{ cmH}_2\text{O}$, p_{out} is ramped up. As shown by the intra-lymphangion pressure (black), contractions develop the required systolic pressure up to a certain maximum (at $\sim 203.9 \text{ min}$), beyond which the outlet valve V2 (red) no longer opens; this is behaviour 7. Modified from fig. 1 of Davis et al. (2011).

Figure 13.

Two p_{out} ramps applied to the same vessel, the first starting from $p_{in} = p_{out} = 2$ cmH₂O, the second from $p_{in} = p_{out} = 1.5$ cmH₂O. The result is here termed behaviour 8.

Table 1

Qualitatively distinct behaviours in response to ramp increase of outlet pressure. V1 is the lymphangion inlet valve; V2 is the outlet valve. Behaviours involve transitions between states; pump state is described by a two-letter code referring to what the inlet then the outlet valve is doing (n = normal operation, c = stays closed, o = stays open). The table covers all possible final failure states, i.e. where at least one valve stays closed.

no.	description	state
1	V1 ceases to open	nn→cn
2	V1 ceases to open, and later V2 ceases to shut	nn→cn→co
3	V1 ceases to open; later V2 ceases to shut; still later V2 resumes normal operation	nn→cn→co→cn
4	V2 ceases to shut, causing V1 to cease opening	nn→co
5	V1 and V2 both cease to open	nn→cc
6	V1 and V2 both cease to open, but later V2 stays open	nn→cc→co
7	V2 ceases to open	nn→nc
8	V1 ceases to shut, causing V2 to cease opening	on→oc

Model parameters and boundary conditions which varied across the examples shown in the figures. An example of conditions giving rise to behaviour 4 is also given. Parameter m multiplies the rate of onset and decay of the active state, $p_{ae} = p_a - p_e$, where p_a is inlet pressure and p_e is external pressure, p_{b1} is initial outlet pressure, R_{Vn} is minimum (open-state) valve resistance, and V1 offset is the depression of the two curves of $p_V(\cdot, p_{m})$ defining the V1 opening and closure thresholds (Bertram et al. 2014b). See Appendix for other model parameter values.

behaviour	Fig.	m	p_{ae} (cmH ₂ O)	$p_{\text{b1}} - p_{\text{a}}$ (cmH ₂ O)	R_{Vn} (dyn s cm ⁻⁵)	V1 offset (cmH ₂ O)
1	3	2	1	2	8×10^6	0
2	5	2	1	5	8×10^6	0
3	7	2	0.5	5	2×10^6	0
4	-	2	2.5	1.5	8×10^6	0
5	9	2	0	5	8×10^6	0
6	10	1	0	4	8×10^6	0
7	11	2	0	2	8×10^6	-0.3