1 Title

2 On the Driver of Blood Circulation Beyond the Heart

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8 Abstract

9 The heart is widely acknowledged as the unique driver of blood circulation. Recently, we
10 discovered a flow-driving mechanism that can operate without imposed pressure, using
11 infrared (IR) energy to propel flow. We considered the possibility that, by exploiting this
12 mechanism, blood vessels, themselves, could propel flow. We verified the existence of
13 this driving mechanism by using a three-day-old chick-embryo model. When the heart was
14 stopped, blood continued to flow, albeit at a lower velocity. When IR was introduced,
15 flow increased, by ~300%. When IR energy was diminished under otherwise
16 physiological conditions, blood failed to flow. Hence, this IR-dependent, vessel-based
17 flow-driving mechanism may indeed operate in the circulatory system, complementing the
18 action of the heart.

20 MAIN TEXT

21 Introduction

22 Of all physiological functions, blood circulation stands as one of the most fundamental.
23 Few cells can survive without proper circulation. Indeed, the significance of vascular flow
24 is difficult to overstate (*1*).

25 The sole driver of blood flow is widely acknowledged to be pressure, mainly generated
26 from the heart (*2, 3*). This paradigm is arguably one of the best known in biology,
27 seemingly leaving little room for doubt.

28 On the other hand, blood can apparently flow without a beating heart. After the heart had
29 been arrested, postmortem blood flow was confirmed in mice, rats, dogs, and chick
30 embryos (*4-7*). The flow persisted from 15 minutes to several hours. Furthermore, some
31 amphibian larvae could live up to 15 days, and even differentiate following surgical
32 removal of the heart (*8-10*), implying an alternative means for propelling blood.

33 Continued blood flow without a beating heart raises an obvious question: could the heart
34 be the sole driver of the circulation? Puzzled by clinical and experimental evidence that
35 does not fit the current paradigm, generations of established physicians and physiologists
36 since the 19th century have repeatedly raised this question (*4, 11-15*). Among those
37 skeptics, the consensus is clear: the heart cannot be the only driver of the circulation; a
38 complementary driving mechanism must exist in blood vessels, presumably in the
39 capillaries (*4, 12-15*). However, the precise mechanism has remained unclear. Under the
40 standard pressure-driven flow paradigm, it is not obvious that how blood vessels,
41 especially capillaries, could pump blood by themselves (*4, 12-15*).

42 On the other hand, pressure is not the only way to drive fluid flow through a vessel. We
43 recently discovered that flow can also arise from a driving force involving tubular

surfaces. Surface activity can generate axial chemical concentration gradients, which can drive intratubular flow (16-18). Relative to pressure-driven flow, surface-induced flow has several distinct features, the most important of which may be its signature feature: the flow can be fueled by infrared radiation (IR) energy (18).

Here, in an avian circulatory model, we confirm several predictions arising from this flow mechanism. First, flow continues after cardiac ejection has been stopped; this implies some driving mechanism beyond ventricular ejection. Second, IR energy fuels this flow, both in the post-mortem situation and also in the normal physiological state. All of this demonstrates the existence of a second driver of blood flow, beyond the heart: the vessels, themselves.

Materials and Methods

As a model, we employed the vitelline network of the early-stage chick embryo (Fig. 1). The model has several advantages (see below) that enabled us to study the blood flow without a pressure gradient. The embryonic heart is easily accessible, and can be stopped by directly injecting 10-30 μ l of 3M KCl solution (7, 19).

The vascular network, diameter $\sim$ 3 cm, floats on the top of the yolk, forming an almost 2D-plane. The lack of any prominent 3D feature to the vascular network has the advantage of being able to exclude gravitational force as a driving factor (20). Further, the vascular network's small size facilitates the tracking of any potential vascular contractions (6). This embryonic model contains all the essential elements of the circulation: the heart, blood vessels and blood; yet, the smooth muscles are not fully functional at this early stage (21-24), which can help exclude vasomotor actions as a factor when studying the flow.

Fig.1. Experimental setup, involving three-day old chick embryo, showing the vitelline vascular network. The egg was situated in an onstage incubator. The shell's

blunt end and the associated membrane were removed. The embryo lies on the top-middle of the egg yolk; vitelline arteries and veins grow out of the embryo, and connect to the marginal vein, whose approximate location is marked with the dashed red circle. The marginal vein forms the external boundary of the network. Anterior and posterior vitelline veins connect the marginal vein with the embryo. Because of the veins' non-branching nature and relatively uniform diameter, the midpoints of either the anterior or posterior vitelline vein, marked with yellow circles, were selected as regions of interest (ROI) for quantitative blood flow measurements. Pilot experiments showed that the blood flow velocities were similar in the two veins; hence, vein selection was based primarily on accessibility.

We recorded blood flow using a video camera outfitted with an objective lens (Fig. S1). With a 6x (N.A. = 0.18) and a 10x (N.A. = 0.25) objective lens, we observed the dynamics of postmortem blood flow. During these experiments, the chick embryo was situated in an onstage incubator, whose temperature was maintained at 37 ± 0.5 °C.

With a 32x objective lens (N.A. = 0.40), we studied the impact of IR energy on both postmortem and physiological blood flow quantitatively. Blood flow in the region of interest (ROI) was recorded (Fig. 1), and the flow velocity was quantified by using particle-image velocimetry (PIV) (25-28). After the embryo was prepared for study, the first three minutes of measurement were taken as the control. For postmortem blood-flow studies, the starting point of that control was the time that the KCl was injected into the heart. For physiological flow studies, the starting point of the control was when the embryo was suitably exposed. Immediately following the control measurement, IR from a ceramic IR lamp (Zoo Med, CE150) was shone onto the embryo for another three minutes, during which measurement continued. Then the IR source was withdrawn, and measurement continued for the final three minutes. In all experiments, the chick embryo was continuously situated in an onstage incubator, whose temperature was maintained at 37 ± 0.5 °C.

For physiological circulation experiments, we also observed the effect of IR deficiency. To carry out those experiments, a live embryo was transferred into an empty beaker, exposed to room temperature, which ranged between 19 °C and 23 °C. Observations were made 1 minute and 50 minutes, respectively, after the embryo remained at room temperature.

Results

We first observed the dynamics of postmortem blood flow. Within one or two seconds following KCl injection, the embryonic heart stopped. Blood flow, as measured in the ROI, persisted, although it slowed substantially (Movie S1). Sometimes that flow even briefly reversed (Movie S2). Following such initial stall, or reversal, venous flow slightly accelerated in the normal physiological direction, stabilizing at ~ 25 $\mu\text{m/s}$ approximately two minutes after cardiac arrest. Postmortem venous flow persisted for approximately 50 minutes (Movie S3), in a direction the same as the natural one, e.g., artery to the vein. Postmortem venous blood flow in the normal physiological direction was observed in all experiments, without exception.

In capillaries, following cardiac arrest, blood flow persisted in some beds. A qualitative impression was that less than half of the total capillary beds remained open. In those open capillaries, the flow direction was generally normal, from artery to vein (Movie S4).

In arteries, postmortem blood flow initially reversed direction: blood flowed from thinner arterial branches back to the larger arteries. In the trunk vitelline arteries adjacent to the heart, the reversal persisted for approximately 10 minutes, longer than the brief reversal sometimes observed in veins. The restoration of natural flow direction occurred first at the peripheral region of the arterial network, gradually moving upstream and ultimately reaching the heart region (Movie S5). Eventually, that flow emptied the arteries. They became pale, indicating loss of red-blood cells (Fig. 2B). Emptying occurred consistently, in all experiments.

Thus, following brief transients, the direction of postmortem blood flow was the same as physiological blood flow in all vessel beds.

Associated with the postmortem blood flow, a zone devoid of red blood cells gradually appeared just inside the vessel wall. An example can be seen at the arterial wall (Fig. 2C; Movie 5 0:01:00 - 0:01:13). This cell-free zone may correspond to the so-called “exclusion zone” (EZ), previously reported (18, 29-38).

We next studied the effect of IR. During postmortem blood flow, velocity significantly increased during IR exposure, reaching a peak more than three times the control value after approximately three minutes. Following removal of the IR source, the velocity returned near to its original value (Fig. 2D). Thus, we could confirm the positive effect of IR on postmortem blood flow.

Fig. 2. Features of postmortem blood flow. (A) Vitelline artery and vein in live chick embryo. Artery and vein overlap, the artery lying partially beneath the vein. Blue arrows indicate the direction of venous flow, red arrows, arterial flow. (B) Vitelline artery and vein in a postmortem chick embryo, 20 minutes after the heart had stopped. Blood tends to exit arteries, leaving them pale. (C) Exclusion Zone (EZ) in vitelline arteries 150 seconds after cardiac arrest. Note the erythrocyte-free region between the vessel wall and the boundary of the blood stream. The image is a snapshot from Movie S5. (D) Effect of IR exposure on venous postmortem blood flow ($n = 6$). Flow velocity increased appreciably after IR was turned on, diminishing when the IR source was removed. Error bars show standard deviations.

We also studied the effect of IR on blood flow in the physiological state, i.e., with the heart functioning. With IR application, venous flow velocity increased by ~30%, beginning almost immediately (Fig. 3A). After the IR source was removed, the venous flow diminished below the baseline. This positive effect of IR agrees with the results of previous studies (39, 40).

In addition to the increased flow, however, there was also a change of heart rate, rising from ~100 to ~140 beats/minute. The question: does IR increase the venous blood flow by cranking up the heart rate? Or, by fueling a driving mechanism that potentially exists in the vessels? Conceivably, IR-induced increase of temperature could lead to a series of biochemical events that elevate the heart rate (41), which would in turn increase venous blood flow. On the other hand, if vessels can drive blood flow, then the direction of that flow should presumably point toward the heart. In that case, the additional IR energy should enhance venous blood flow. Enhanced venous blood flow (venous return) can, in turn, affect the heart rate and contractility via mechanisms such as Bainbridge reflex or

Frank-Starling mechanism (42, 43). Thus, the reciprocal causation between the cardiac output and venous return makes it difficult to distinguish whether the added IR impacted venous blood flow via the heart or via the blood vessels.

To further study how IR energy impacts the circulation, we investigated the effect of IR diminution. IR, above ambient, had been provided by the onstage incubator. To realize an IR deficiency, we removed the embryo from the warm onstage incubator, exposing it to the room-temperature laboratory environment.

At the lower temperature thereby achieved, the heart rate decreased appreciably, from ~100 to ~10 beats/minutes. Blood flow in the capillaries came to a complete halt, with no synchronous response to the heartbeat. Only in shunt vessels – larger blood vessels that directly connect arteries with veins – the blood flowed in a pulsed manner that corresponded to the heartbeat, providing an open passage for the circulation. This open passage indicates that the heart, albeit weak, still drives the blood flow. Yet, without sufficient IR, the heart alone is apparently insufficient to drive the blood flow through the capillaries.

Upon further observation, we found that under the diminished IR condition, the red blood cells were trapped in capillaries. Compared to the case with sufficient IR (Fig. 3B), the arteries and veins contained less blood, as indicated by their lighter color and smaller diameter (Fig. 3C). The capillaries, on the other hand, became darker in color, indicating more erythrocytes (Fig. 3C). This observation implies that blood can enter the capillaries but cannot exit, i.e., the red cells get trapped in capillaries.

The effect of deficient IR was reversible. When the embryo was returned to the incubator, the capillaries, blocked by red blood cells, gradually opened, and blood flow resumed.

Fig. 3. Effect of IR on live chick-embryo circulation. (A) Venous blood flow velocity increased appreciably when IR was turned on, and then decreased after removal ($n = 4$). Error bars show standard deviations. (B) Vitelline vascular network with embryo just removed from onstage incubator. (C) Same as left but exposed to room temperature for ~50 minutes. Compared to Fig. 3B, the arteries and veins (black dotted rectangle) appear thinner and less defined, indicating that those vessels contained less blood. Capillaries (black dashed circle), on the other hand, appear darker, indicating more blood.

The accumulation of blood in the capillaries implies that vasomotor mechanisms, which are usually used to explain the impact of temperature on circulation (44), may not be the cause of the hindered circulation. Vasomotor mechanisms regulate blood flow by changing the diameter of the arterioles, thus controlling the amount of blood entering the capillary beds. At this developmental stage, however, the smooth muscles that control arteriolar diameter are not yet fully functional. If partially functional smooth muscles were to constrict the arterioles, then the blood should have difficulty entering, but not exiting the capillary beds.

In sum, without sufficient IR energy, blood failed to exit the capillaries. This implies a driving mechanism that uses IR energy present in capillaries --- a conclusion in line with previous research (45).

Discussion

Using the vitelline vascular network of the early-stage chick embryo as a model, we confirmed that blood continued to flow, following its natural course, even when the heart ceased pumping. IR energy appears to fuel this postmortem blood flow. We could also confirm the impact of IR energy on blood flow in the normal, physiological state.

Postmortem blood flow is a long-known phenomenon, previously reported in various animal models (4-7). One feature of the postmortem flow is the tendency to deplete the arteries of blood, a finding confirmed here as well as in the older studies. Thus, when the Greeks found the arteries of the deceased devoid of blood, they considered the artery as an “air duct” (in Greek, “ἀρτηρία”, in Latin characters, “*arteria*”). We now know that the artery is by no means a duct for air. Being a misnomer, the term “artery” may in fact have reflected the existence of postmortem blood flow.

Trivial explanations?

Postmortem blood flow has been considered to be a consequence of one of two extraneous features: pressure originating from gravitational force; or, vascular contraction (6, 13, 20). As a result of these potentially trivial explanations, the phenomenon has received little attention.

With the chick embryo as a model, we were able to exclude these two extraneous factors. As for the gravitational hypothesis, the model’s architecture would seem incompatible. The embryonic vitelline vascular network lies essentially in a 2D plane floating atop the yolk, with the venous network lying immediately above the arterial network. Hence, reaching the veins requires the arterial blood to flow *against* gravity, rendering the gravitational hypothesis unlikely. As for the vascular contraction explanation, if that were to drive the blood flow, then the contraction would need to take place in the arterial system, in order to drive the blood forward. At the beginning stage of postmortem flow, moderate vascular contraction was indeed observed in the main artery leading from the heart; at later stages, however, it ceased, the width of the blood stream remaining constant, implying that the arteries had stopped contracting (Movie 5, 0:02:03-0:02:17); yet, flow persisted.

Thus, the potential artifacts that might underlie the presence of postmortem flow appear inadequate to account for the observation. Postmortem flow appears to be a genuine feature of the cardiovascular system, revealed only after the heart stops beating.

Mechanism

In deducing the mechanism of postmortem flow, a critical feature is the role of IR energy. We found that IR enhanced postmortem flow. The energizing role of IR is a signature feature of the “surface-induced flow” phenomenon mentioned in the Introduction (18). Hence, that phenomenon may well have relevance for propelling the flow.

Surface-induced flow is driven by chemical concentration gradients originating from physico-chemical reactions that occur at the tubular or vascular surface (16-18). The vascular surface may release substances into the vessel, or remove them from the vessel, thereby altering the local intravascular concentration. Once an axial concentration gradient forms, flow is induced along that gradient (18). Chemical potential energy is thus converted into kinetic energy.

We recently confirmed that intravascular gradients may form in two ways (18): (i) material exchange across the vascular wall; or (ii) water-surface interaction. As for (i), solutes and solvents naturally enter/exit capillaries through the vascular walls; this is a fundamental feature of capillary function. Regarding water-surface interaction (ii), water interacts with many hydrophilic surfaces. The signature feature of such interaction is the formation of an ‘exclusion zone’ (EZ), an interfacial region of water that excludes particles and solutes (30, 32, 46, 47). The EZ is generally negatively charged, releasing complementary protons to the region beyond the EZ as it forms (30, 32, 46). In tubes made from EZ-forming materials, the released protons will accumulate in the core of the tube, creating an axial proton gradient, which can then drive flow (16-18). External electromagnetic radiation fuels EZ formation, with IR being the most effective wavelength (17, 18, 48). For surface-induced flow of both kinds, we confirmed that IR has a positive effect on flow *in vitro* (18).

Water-surface interaction in the vascular system?

The presence of EZ has been confirmed next to a wide range of materials including proteins and polysaccharides (18, 30, 35, 37). These are major components of blood vessels and their inner surface linings (49, 50).

Indeed, in the physiological state, an annular “cell-free layer” just inside the vessel wall has long been known. This region is devoid of red blood cells (51, 52). Given the ability of blood-vessel components (49, 50) to nucleate the growth of EZ (18), and the known ability of EZs to exclude particles, we hypothesize that the origin of that cell-free layer may lie in the presence of an exclusion zone just inside the vessel wall. We observed what appears to be an EZ immediately inside the vascular wall (Fig. 2C): red blood cells were excluded. Hence, evidence indicates that water-surface interaction exists in the vascular system.

Thus, postmortem blood flow may arise essentially from a surface-induced flow, driven by water-surface interaction. Material exchange, the companion mechanism, is unlikely to play a role in post-mortem capillaries, since the pressure required to drive that exchange (53) is largely absent post-mortem. Hence, the water-surface interaction remains as the more likely mechanism operating at the capillary level.

In the absence of any alternative post-mortem driving mechanism that we can identify, we argue that this water-surface interaction may well be the dominating driver of blood flow

in situations in which the heart has stopped beating. In the physiological situation, when the heart continues to beat, the surface-water phenomenon may merely augment the heart-driven flow (Fig. 3A).

Flow Direction: Model Considerations

The expected direction of any surface-induced flow would seem unclear *a priori*. If such a flow mechanism is to play a functional role in the vascular system, then it must propel flow in the natural direction, from arteries to veins.

To appreciate the direction expected in the vascular system, we first consider two representative examples that help identify the determining factors. We then examine those factors in the context of the entire vascular system, where we analyze the predicted direction and compare it with the observed direction.

Fig. 4A shows the first of the two examples, involving a tube with uniform physico-chemical surface activity along its length, but with some taper. Suppose this surface activity results in substances entering the tube at the same rate per unit surface area throughout the length of the tube (green arrows). Compared to the wider region of the tube, the narrower region has a higher surface-to-volume ratio, and therefore, a higher concentration. Thus, a concentration gradient will form within the tube, pointing from the narrower to the wider region. The natural tendency for concentrations to even out should drive flow toward the wider region.

On the other hand, a surface activity that removes molecules from the tube instead of introducing them into the tube should drive flow in the opposite direction, i.e., towards the narrower region.

Fig.4B shows the second example, a uniform tube with nonuniform surface activity along its length. Suppose, for example, that a portion of the tubular surface can remove substances from the tube (dotted lines), the remainder not (solid lines). With any such removal, the intratubular region with surface activity will have a relatively lower concentration. Thus, an axial concentration gradient will form, driving flow to the region with lower concentration. If the surface activity admits solutes instead of eliminating them, then the flow direction would be reversed.

Fig. 4. Potential driving mechanism of surface-induced flow. (A) In a tube with non-uniform diameter but uniform surface activity, an axial concentration gradient can form. (B) An axial concentration gradient can also form in a tube with uniform diameter but nonuniform surface activity.

To summarize the two conclusions above, the direction of the surface-induced flow should be determined by the direction of intratubular concentration gradient, which is in turn determined by the following two factors: (i) *type of surface activity* — whether the tubular surface introduces or removes substances; and (ii) *tube geometry* — how the surface-to-volume ratio changes along the tube's length.

Knowing those determining factors, we may then consider the expected directions of surface-induced flows in the full vascular system --- driven either by water-surface interaction, or, material exchange.

Flow Direction in the Full Vascular System

In considering blood-flow direction in the entire vascular system, we first note the results of previous research, which have shown that water-surface interaction commonly releases protons (30, 32, 46). If the vascular wall operates similarly, then, according to the model of the previous section, the issue of flow direction should rest principally with the architecture of the network.

The vascular network has a hierarchical branching structure (Fig. 5A), with each hierarchical level viewed as a set of vessels with similar diameter. The surface-to-volume ratio of these blood vessels is inversely proportional to their average diameter (for detailed analysis, see supplemental materials). This feature allows us to predict flow direction (assuming uniform behavior in all vessel walls). Based on the mechanism described in Figure 4A, blood should consistently flow from narrower to wider vessels.

The same is predicted in capillaries (Fig. 5A). Capillary flow should depend on the relative size of input and output vessels. Arterioles (red) have a narrower average diameter than venules (blue) – approximately one third of venule diameter (54). For a capillary bed lying in between arterioles and venules, then, the blood should flow from narrower arterioles to wider venules.

The predicted blood flow direction should therefore follow its natural direction in capillaries. It should also do so in veins, where the natural progression is from narrower to wider. But predicted flow in arteries should be opposite to the natural, because size progressively decreases from the heart to the periphery. As the model predicted, the flow in the large, near-heart arteries was indeed opposite to the natural direction just after the heart stopped beating (Movie 5S, 0:00:51 – 0:01:35). Hence, model predictions appear to match experimental observations for all vessel beds.

If the flow in arteries is against the flow in the capillaries and the veins, a natural question is: who plays the dominating role? The answer should be the capillaries and the veins: compared to the arterioles, the venules are higher in number (54) ; thus, more venules can generate flow. This conclusion is verified by the dynamics of the postmortem arterial blood flow. Postmortem flow in larger arteries was originally in the reversed direction, not the natural direction. Yet, the flow gradually resumed its natural direction from the peripheral region of the arterial network, indicating that the blood flowed into the capillaries and the veins. As the non-beating heart stopped replenishing blood to the arteries, ultimately, the arteries emptied. The emptied arteries indicate that the flow-driving capacity of capillaries and veins exceeds that of the arteries. Thus, all blood vessels drive the blood towards the natural direction.

Having considered water-surface interaction, we move to the other surface activity that could potentially drive blood flow: material exchange. That exchange occurs specifically in capillaries. There, materials may exchange mainly in two forms: (i) *trans-vascular flow* (“bulk flow”), which refers to the fluid movement across the capillary wall; and (ii) *diffusion*, which refers to gas and electrolyte movement across the capillary wall (53).

Both types of exchange can generate an axial material gradient between the arterioles and venules; and that gradient can drive flow through the capillaries. Regarding (i): Fluids enter and exit the capillary at different locations. At its arteriolar end, hydrostatic pressure drives fluid out through the capillary wall (Fig. 5B, left dashed rectangle), while at the venular end, osmotic pressure draws fluid into the capillaries (Fig. 5B, right dashed rectangle) (53). These activities alter the “water-concentration potential (or, water potential)” in the capillaries. Driven by this potential, water should flow from arteries to capillaries (as described in Fig. 4B), while a capillary-to-vein-directed flow should occur at the venular end (the reverse of that described in Fig.4B).

Regarding (ii), diffusion-driven capillary flow, consider the exchange of gas as an example. In the systemic circulation, as the arterial blood enters the capillaries, the oxygen carried by blood diffuses into the surrounding tissues. Meanwhile, the CO₂ generated from those tissues diffuses into the capillaries. Thus, an axial O₂/CO₂ gradient forms across the capillary bed. In the pulmonary circulation, an O₂/CO₂ concentration gradient will form between the arterioles and venules in a similar manner. Theoretical analysis has suggested that such concentration gradients may drive capillary flow towards the veins, by so-called macromolecular chemotaxis (55). Thus, both trans-vascular flow and gas diffusion, two major forms of material exchange, may drive capillary flow from the arteries to the veins.

Fig. 5 Surface-induced flow in a capillary bed (A) Compared to venules (blue), the arterioles (red) are narrower; thus, the water-surface interaction drives capillary flow from arteries to veins. **(B)** As fluid (mainly water) leaves the capillaries through the capillary wall at the arteriolar end, a surface-induced flow is generated pointing from the arteries to the capillaries. Meanwhile, as fluid enters the capillaries at the venular end, a surface-induced flow is generated, pointing from the capillaries to the venules.

In sum, analysis of expected blood flow directions, driven by either water-surface interaction or material exchange, predict well-defined directionality. Both activities enable the blood vessels to drive the blood in the natural direction.

IR energy for facilitating venous return

In capillaries and veins, surface-induced flow drives blood towards the heart, facilitating venous return. A non-trivial amount of energy is needed: with capillary diameters sometimes narrower than a red blood cell, the resistance within each capillary is high.

With this high resistance, red blood cells may stall, or even flow in a reverse direction (56); yet when blood returns to the heart, it must flow at the same overall rate as the blood that leaves the heart.

To facilitate that return, IR energy can be used, via the surface-induced flow mechanism. Surface-induced flow converts chemical potential energy into kinetic energy. In this process, IR can operate two ways. It can: (i) accelerate proton production by enhancing water-surface interaction (48); and, (ii) accelerate the conversion of chemical potential energy to kinetic energy by elevating the temperature. In this context, each capillary may be viewed as a flow generator that runs on IR energy (18), sending the blood back to the heart.

IR energy is one of the most available energy sources. It can be both endogenous and exogenous: In terms of endogenous sources in the body, metabolic activities of living organisms generate heat, which according to Planck's law, can be emitted as IR energy (40, 57). In terms of exogenous sources, approximately 50% of solar energy received by earth is in the form of IR (58). Thus, capillaries have access to abundant IR energy for driving the flow, despite their high resistance.

Both the heart and the blood vessels drive the circulation

The importance of the vessels' contribution to driving blood flow is shown by the effect of IR deficiency. In the intact chick-embryo model, where neither any muscle pump nor respiratory pump is present to assist venous return, blood failed to flow out of the capillaries when IR energy was deficient (Fig. 3B). Thus, IR energy, which can be used by the blood vessels, apparently plays a critical role in maintaining the normal circulatory function. The results show that When IR is deficient, the heart alone cannot do the job.

Thus, as the two essential components of the circulatory system, the heart and the blood vessels, evidently work together to drive the blood flow. The heart pumps blood into the vessels; the vessels amplify the flow, returning the blood back to the heart.

Perspectives

The heart cannot be the sole driver of blood flow. If it were, then stopping the heart would stop all flow. Persisting flow after the heart stops beating has been reported in multiple studies (4-10). We found the same. In our case, various potential artifacts could be ruled out. Thus, some mechanism must supplement the cardiac driver.

Here, we demonstrated the existence of that second driving mechanism: surface-induced flow, originating in the blood vessels themselves. The circulation appears to be driven not only by the heart, but also by the vessels themselves. The fuel for this second driving mechanism lies in infrared energy, which emerges naturally from metabolic heat, as well as from external sources.

For any organism, the most fundamental requirement is metabolism. Metabolic activity generates heat. The heat is released in the form of IR energy, which can drive flow and hence nourish the tissues. Thus, metabolism facilitates the circulation, while the circulation facilitates metabolism. The two phenomena enable one another.

Appreciating the existence of this second circulatory driver opens the door to fresh understanding of cardiovascular disease, as well as to unforeseen therapies for combatting that disease. Thus, we may anticipate novel therapies appearing in the future.

Supplementary Materials

Materials and Methods

On-stage incubator

An on-stage incubator was used to maintain the temperature of the egg during the experiment. The incubator was made by wrapping a flexible strip-heating element (TEMPCO, SHS80389) around an aluminum tube (inner diameter: 4.5 cm; outer diameter: 5.1 cm; height: 6.5 cm). The heating element heated the aluminum tube, while the tube's thermal radiation heated the egg. The power of the heating element, which determined the heating rate, was controlled by a power controller (Payne, 18TBP-1-15). The temperature of the heating element was controlled by a temperature controller (AGPtek, STC-1000). A temperature sensor read the stage temperature and provided feedback to the temperature controller. The temperature of the incubator was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the experiment.

Embryo model preparation

Pathogen-free white leghorn chicken eggs (Charles River, specific pathogen-free, research grade, fertile, 55-59 grams) were incubated for 72 to 75 hours (corresponding to Hamburger–Hamilton stage 18-20 (59)) in a commercial egg incubator (Brinsea Mini Advance Hatching Egg Incubator). The temperature of the incubator was set at 37.5°C and the humidity of the incubator was maintained by continuously evaporating water in a built-in water reservoir. An integrated motor of the incubator rotated the assembly of eggs 15 degrees every 45 minutes.

After incubation, the embryo was ready for experiments. The egg was transferred to the on-stage incubator, in an upright position with the blunt end up. Extra padding was added at the bottom of the egg to ensure stability, and to adjust the height of the egg. A portion of the eggshell and the eggshell membrane were carefully removed by using a pair of tweezers (Dumont tweezer, Style 55, Domostar, 72707-01) to expose the embryo.

For post-mortem experiments, the embryonic heart was arrested by directly injecting 10-30 μl 3 M potassium chloride (J.T. Baker, 3040-01) solution into the heart with a syringe outfitted with a $27\text{G} \times \frac{1}{2}$ inch needle (BD, 309623)(7, 19). Occasionally, the intrusive nature of this procedure caused cardiac hemorrhaging; those specimens with hemorrhage were excluded from the study.

For the qualitative studies of postmortem arterial and microcirculatory flow, the entire contents of the egg were poured into a 50-ml beaker (Fisher, fb-100-50), which was kept warm in the on-stage incubator. This configuration allowed visualization of the boundary portion of the embryo's blood vessel network, which was ordinarily blocked by the eggshell.

Flow data acquisition and quantification

The embryo was illuminated with white light from an LED flashlight (CREE, XLAMP XP-E LED). In quantitative studies, blood flow was recorded using a video camera (Edmund Optics, EO-3112C) outfitted with a 32x objective lens (Leitz Wetzlar, 32x, N.A. = 0.40). The lens was mounted onto the camera by a C-Mount to a DIN adapter (Edmund Optics, #03-627). The camera was connected to a computer via a USB interface. By decreasing the camera ROI through camera-software control, the frame rate could reach 80 frames per second.

With red blood cells as tracers, the flow video could be analyzed by Particle Image Velocimetry (PIV) to obtain red blood cell velocity(25-28). Due to changes of blood-

stream width, the flow velocities were routinely measured at the middle section of the vessels, where red blood cells were always available for tracking.

In the qualitative studies of postmortem arterial flow and postmortem microcirculation, videos were recorded with the same camera but using a 6x or a 10x objective lens (Ernst Leitz Weltzar, 6x, N.A. = 0.18; Bausch & Lomb Opt. CO., 10x, N.A. = 0.25) instead of the 32x lens. The relatively larger field of view permitted easy tracking of circulatory dynamics in different vessels simultaneously.

External IR application

To apply infrared radiation to the chick embryo, a 150-watt ceramic heat emitter (Zoo Med, CE150) was used. This lamp emitted IR at wavelengths ranging from 1 μm to 14 μm , with a peak at 2.9 μm .

In all experiments the IR source was turned on for 30 minutes prior to experimentation to stabilize the output. The IR lamp was fixed to a claw holder, with adjustable height and angle. This source was directed down to the surface of the exposed chick embryo at an angle of 45° with respect to horizontal surface. The distance between the center of the IR source and the center of the embryo surface was 10 cm (Fig. S1). The height and angle of the IR lamp was adjusted beforehand; and the location of the claw holder was marked on the table with tape. Thus, the IR lamp could be quickly applied and removed as needed.

In the postmortem circulation experiments, the first three minutes of the blood-flow recording immediately after cardiac arrest was considered as the control, while in the live circulation experiments, the control was taken as the first three minutes of the blood flow recording after the embryo had been properly prepared. In both experiments, the second three minutes of recording tracked the blood flow during IR exposure, while the final three minutes recorded the blood flow after IR exposure. These time points were marked in the video by blocking the illumination light briefly.

Temperature measurement

The temperature increase arising from IR treatment was measured with a thermometer (Omega, HH306A) outfitted with a type-K thermocouple. The thermocouple was inserted immediately underneath the surface of the egg yolk. Due to the principle of humane use of animals, the temperature measurements were performed on non-experimental samples, e.g., on abnormally developed or non-developed eggs.

Supplemental Text

A calculation of surface-area-to-volume ratio of a single blood vessel and a vascular network:

Consider a section of a cylindrical blood vessel. The diameter is D ; the length is L ; the surface area is S ; and the volume is V .

The surface-area-to-volume ratio of this piece of blood vessel is:

$$\frac{S}{V} = \frac{\pi D \cdot L}{\frac{1}{4}\pi D^2 \cdot L} = \frac{4}{D} \quad (1)$$

Thus, for a single section of blood vessel, the surface-to-volume ratio is inversely proportional to the diameter of the vessel.

Next, consider a vascular network. Regardless of geometric complexity, a vascular network can be considered as the sum of N segments of non-branching blood vessels. Assume the blood vessels are cylindrical, the length of the i th segment is L_i ; the diameter of the i th segment is D_i ; the surface-area of the i th segment is S_i ; the volume of the i th segment is V_i . The surface-area of the vascular bed is S , and the volume of the vascular bed is V . The surface-area-to-volume ratio of this vascular bed is:

$$\frac{S}{V} = \frac{\sum_{i=1}^N S_i}{\sum_{i=1}^N V_i} = \frac{\sum_{i=1}^N \pi D_i \cdot L_i}{\sum_{i=1}^N \frac{1}{4} \pi D_i^2 \cdot L_i} = 4 \frac{\sum_{i=1}^N D_i L_i}{\sum_{i=1}^N D_i^2 L_i} \quad (2)$$

Blood vascular network has a hierarchical structure. Within the same hierarchical level, the blood vessels have similar diameters. Assume the average diameter of blood vessels is D . From equation (2), we can get the surface-area-to-volume ratio of the blood vessels in a hierarchical level:

$$\frac{S}{V} = 4 \frac{\sum_{i=1}^N D L_i}{\sum_{i=1}^N D^2 L_i} = \frac{4}{D} \cdot \frac{\sum_{i=1}^N L_i}{\sum_{i=1}^N L_i} = \frac{4}{D} \quad (3)$$

Thus, like a single section of blood vessel, the surface-to-volume ratio at each hierarchical level is inversely proportional to the average diameter of the vessels.

Fig. S1.

Experimental configuration for applying IR to the chick embryo.

Fig. S2.

Chick embryo surface temperature change resulting from IR radiation. N = 5. Error bars (grey) show standard deviations.

Movie Captions:

Movie S1: Postmortem venous blood flow at ROI.

Movie S2: Postmortem venous blood flow at ROI, with brief reversal.

Movie S3: Postmortem venous blood flow at the boundary of the vascular network.

Movie S4: Postmortem blood flow in capillaries.

Movie S5: Postmortem arterial blood flow dynamics. This footage was taken on an embryo with the vascular network deformed, so as to expose the vitelline artery.

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Role of Z.L.: Data curation, formal analysis, investigation, methodology, project administration, software, validation, visualization, writing-original draft, writing-review & editing. Role of G.H.P.: Conceptualization, funding acquisition, project administration, resources, supervision, writing-original draft, writing-review & editing.

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Authors declare no competing interests.

Data and materials availability:

All data are available in the main text or the supplementary materials.

Supplementary Materials:

Movies S1-S5

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