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CIRCULATION IN THE LEECH, *HIRUDO MEDICINALIS* L.

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SUMMARY

In the leech, the physiological significance of high-pressure phases (HIP) and low-pressure phases (LOP) of the lateral vessels was studied by intravascular pressure recordings and observation of blood flow in different parts of the circulatory system, and by measurements of the blood flow velocities in the dorsal vessel.

Different shapes of the pressure pulses were found in the anterior lateral vessel segments during HIP and LOP phases, according to the different modes of diastolic filling in both phases.

Pressure recordings in the lateral abdominal vessels showed the different action of the lateral abdominal sphincters in the HIP and LOP phases of the ipsilateral lateral vessel. The LOP contractions were responsible for the blood supply to the capillary networks of the organs and the body wall, with the possible exception of the intestine. The HIP contractions caused a forward bloodflow within the lateral vessel.

In the dorsal vessel, the blood pressure was about 0·9–1·9 kPa in different animals. The blood flow was discontinuous with velocities of 0·5–10 mm s^{-1}. Average blood flow in the dorsal vessel was 22·9 $\mu l\ min^{-1}$.

An improved model of the circulation in the leech is presented.

INTRODUCTION

The topography of the closed circulatory system of the leech, *Hirudo medicinalis* L., is well documented (Bourne, 1884; Gaskell, 1914; Boroffka & Hamp, 1969). The current model of circulation in the leech is based on vital microscopic observation and dye injection (Boroffka & Hamp, 1969). The dorsal and ventral vessels are non-contractile longitudinal vessels. The dorsal vessel collects blood from the capillary networks of the organs and the body wall and discharges into the intestinal capillaries and the caudal laterodorsal vessels. The ventral vessel encloses the ventral nerve cord. Two lateral vessels also run along the entire length of the animal. They are contractile and drive the circulation, and are therefore called heart tubes (Thompson & Stent, 1976; Calabrese & Peterson, 1983).

Blood flows anteriorly in the lateral vessels, and posteriorly in the dorsal and ventral vessels. The branching pattern of the lateral vessels and the localization of muscular sphincters within these vessels at the segment borders show the segmental

Key words: leech, *Hirudo medicinalis*, circulation, blood pressure, blood flow.

arrangement of the vascular system. Through the lateral abdominal vessels blood from the lateral vessel segments enters the capillary networks of the nephridia, the body wall and other organs. Each lateral abdominal vessel contains a muscular sphincter. The laterodorsal and laterolateral vessels collect blood from the body wall capillaries. At their outlets into the lateral vessels, there are valves which passively prevent blood from flowing back during contraction of the lateral vessel segment (Boroffka & Hamp, 1969; Hammersen, Staudte & Möhring, 1976).

Heartbeat in the leech is under nervous control (Thompson & Stent, 1976; Calabrese & Peterson, 1983). The lateral vessels contract with bilateral asymmetry, in antiphasic, peristaltic and non-peristaltic modes. In its anterior part (from the first to the ninth segment, segment numbers according to Kristan, Stent & Ort, 1974) one lateral vessel generates a peristaltic constriction wave travelling forwards, segment by segment. All segments behind contract nearly in concert as do those of the contralateral lateral vessel. Every 20–60 heartbeats the pattern switches sides.

Pressure recordings in the lateral vessels (Krahl & Zerbst-Boroffka, 1983) showed systolic pressures of about 50 mmHg (6·7 kPa) during the peristaltic mode, and 25 mmHg (3·3 kPa) during the non-peristaltic mode. Therefore, the period of peristalsis was called HIP (high-pressure phase), and the period of non-peristaltic constrictions was called LOP (low-pressure phase). The functional significance of these pressure phases remains unknown.

Additional information about the function of the lateral vessel segments and, especially, about the segmentally arranged distributing and collecting vessels is needed before a consistent model of circulation can be developed. The present study investigates the following problems: the mechanisms of diastolic filling of the lateral vessel segments, the distribution of blood by the lateral vessels, and the physiological significance of the high- and low-pressure phases.

MATERIALS AND METHODS

Animals and preparation

Leeches, *Hirudo medicinalis* L., were obtained from a commercial supplier and maintained in tap water at 20 ± 1 °C.

The animals were pinned in an extended position in a wax dish with needles through the anterior and posterior suckers, and covered with moist Kleenex tissue.

For pressure recordings and determination of blood flow velocities, the body wall was opened by a small incision exposing the relevant vessel. The connective tissue remained intact as far as possible. The preparation site was covered with physiological saline (Nicholls & Baylor, 1968).

All experiments were carried out on unanaesthetized animals because leeches seemed to suffer less from the preparation than from anaesthesia. Under ethanol, MS 222, or urethane anaesthesia the animals produced a large amount of mucus and tried to escape. After the experiments without anaesthetization, all animals showed normal behaviour.

Pressure recordings

The pressure recording system consisted of a glass capillary (tip diameter 30–50 μm) connected to a CP 01 pressure transducer (Century Technology Company) by a thick-walled polyethylene tube. The system was filled with de-aerated physiological saline. The pressure transducer was statically calibrated with a mercury calibration manometer (Gauer) at the experimental temperature, with the zero level set at the same level as the experimental animal. The dynamic properties of the system were determined following the method described by Jones (1970). The natural frequency of the transducer was 30 kHz. The frequency response of the whole system was tested up to 5 Hz.

Blood flow velocities

Blood flow velocities were recorded by means of blood cells which are present in the leech blood at $5\times10^4\,\mu l^{-1}$. Blood cells were labelled in microcaps which contained 100 μl of leech blood and 250 μl of buffer solution (90·85 mmol l^{-1} disodium hydrogen phosphate and 4·58 mmol l^{-1} citric acid) in which acridine orange dye (Chroma 1B307) had been dissolved at a concentration of 1 : 10 000. The incubation time was 20 min at 8 °C. The suspension was centrifuged at 350 ***g*** for 2 min. The supernatant was replaced by 250 μl of dye-free buffer solution in which the labelled blood cells were resuspended.

The suspension of labelled blood cells (10 μl) was injected into the exposed dorsal vessel in the fifth segment of a leech. The flow of labelled blood cells was microscopically detectable through the exposed walls of several vessels in the circulatory system in fluorescent light (Leitz Filterblock I2). Blood cells in the vascular system equilibrated within 15 min after injection. The circulation rate of labelled blood cells remained stable for several hours.

Blood cell movement was monitored in the dorsal vessel in the tenth segment of the leech with a Leitz microscope (oil-immersion objective, 10×) and a highly sensitive video camera (Bosch 9A, SIT 4804). Reflected fluorescence flashlight (xenon-lamp, Chadwick Helmuth) was applied. Recordings were made on a Sony VO-5800 PS video recorder.

Because of the flash frequency (50 Hz) each video still picture showed two positions of the same blood cell. The space between these marks was measured electronically on the video screen (video position analyser, FOR·A Comp.). Calibration with a graticule resulted in the absolute blood cell velocities.

The velocities of five blood cells were used to calculate the average blood flow velocity during an interval of 1 s. The velocity profile of the laminar flow in the dorsal vessel was rather flat. Only blood cells within the marginal cylinders of fluid showed significantly reduced velocities. For recording blood cell velocities, the objective was focused on half of the total depth of the dorsal vessel. The width of the dorsal vessel was divided into seven sections. Within each of the five central sections the velocity of one cell per interval was determined. The diameter of the dorsal vessel was measured

and, assuming a circular cross-section, the blood flow ($\mu l\,min^{-1}$) could then be calculated.

Direction of blood flow

After intravascular injection of fluorescence-labelled blood cells and exposure the wall of a vessel, the flow of labelled cells was directly visible under the microscope from the reflected fluorescent light. The main directions of blood flow within several vessels of the segmental circulation and within the branches of the dorsal vessel in the region of the intestine were determined.

RESULTS

Pressure recordings

Pressure recordings in the sixth segment of a lateral vessel (Fig. 1) showed the characteristic HIP and LOP phases (Fig. 2, upper trace). Mean systolic pressure in the HIP phases was $8{\cdot}9 \pm 4{\cdot}7$ kPa (mean ± S.D., 22 animals, 4–6 pressure phases in each animal evaluated). In the LOP phases the systolic pressure was $3{\cdot}3 \pm 2{\cdot}0$ kPa (22 animals). In both phases, the diastolic pressure was 0·1–0·5 kPa. The heart rate stabilized within 20–30 min after preparation at $5{\cdot}5 \pm 1{\cdot}3$ pulses min^{-1} (22 animals). These results are in agreement with those of Krahl & Zerbst-Boroffka (1983). In both phases, either systolic pressures or the shapes of the pressure waves differed considerably in different animals. However, the form of the pressure wave in a HIP pulse in an anterior lateral vessel segment was always significantly different from that in a LOP pulse. The HIP pulse showed two different modes of pressure generation. When the diastolic filling of the lateral vessel segment became maximal, the pressure increased significantly from the diastolic level (Fig. 2, arrows in the lower trace). It was therefore called presystolic pressure. The following peak was caused by the contraction of the lateral vessel segment. It was observed that the presystolic pressure in the HIP pulse appeared simultaneously with the contraction of the next posterior lateral vessel segment. This indicated a difference in the mechanism of diastolic filling of the anterior lateral vessel segments in the HIP and LOP phases. In the diastole of a heart cycle in the HIP phase, blood entered the lateral vessel segment through the laterolateral and the laterodorsal vessels first. When the next posterior segment contracted, blood was forced forward to the next lateral vessel segment, which was dilated to its maximum by the additional volume. This caused the presystolic pressure. In the diastole of a heart cycle in the LOP phase, blood entered the lateral vessel segments only through the laterolateral and laterodorsal vessels.

Pressure recordings in the laterodorsal and laterolateral vessels (see Fig. 1) were only possible for 3–10 min because their muscular walls were thin and easily ruptured. Although these vessels are contractile, the recorded pressures were irregular and varied from zero to 0·8 kPa.

Pressure recordings in the lateral abdominal vessel (see Fig. 1) during the HIP phase of the ipsilateral lateral vessel showed two pressure waves which occurred alternately. Pulses up to 1·3 kPa (Fig. 3, circles) appeared simultaneously with the

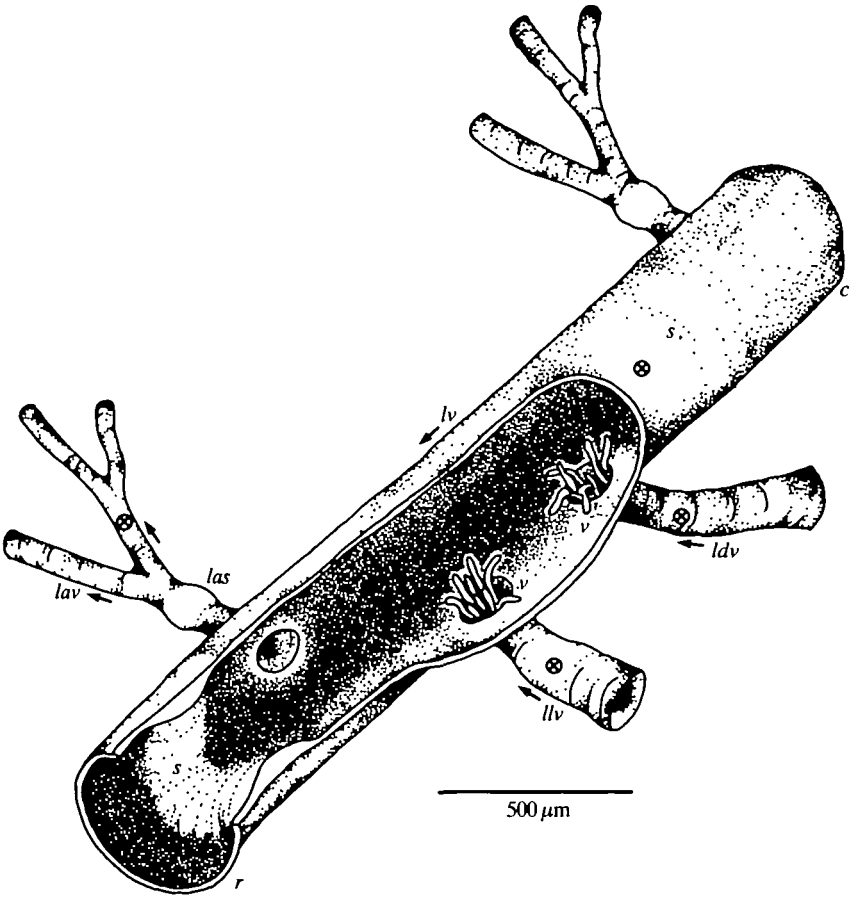

Fig. 1. One midbody segment of the lateral vessel, with the anterior part opened dorsally. *c*, caudal; *las*, lateral abdominal sphincter; *lav*, lateral abdominal vessel; *ldv*, laterodorsal vessel; *llv*, laterolateral vessel; *lv*, lateral vessel; *r*, rostral; *s*, main sphincter; *v*, valve, ⊗; site of pressure recordings. Arrows indicate the directions of blood flow.

maximal diastolic filling of the corresponding lateral vessel segment. Pulses up to 0·9 kPa (arrowheads) appeared simultaneously with the contractions of the lateral vessel segment. The lateral abdominal sphincter, which is located between the lateral and the lateral abdominal vessels (see Fig. 1), allowed an equilibration of pressure only up to the point when the filling of the lateral vessel segment was maximal. Just before the contraction of this lateral vessel segment occurred, the lateral abdominal sphincter was closed.

During the LOP phase of the ipsilateral lateral vessel, the lateral abdominal sphincter acted in a different way. In this case, the sphincter was open during the lateral vessel contraction. The pressure which was generated in the lateral vessel was detected in the lateral abdominal vessel (Fig. 3, arrowheads).

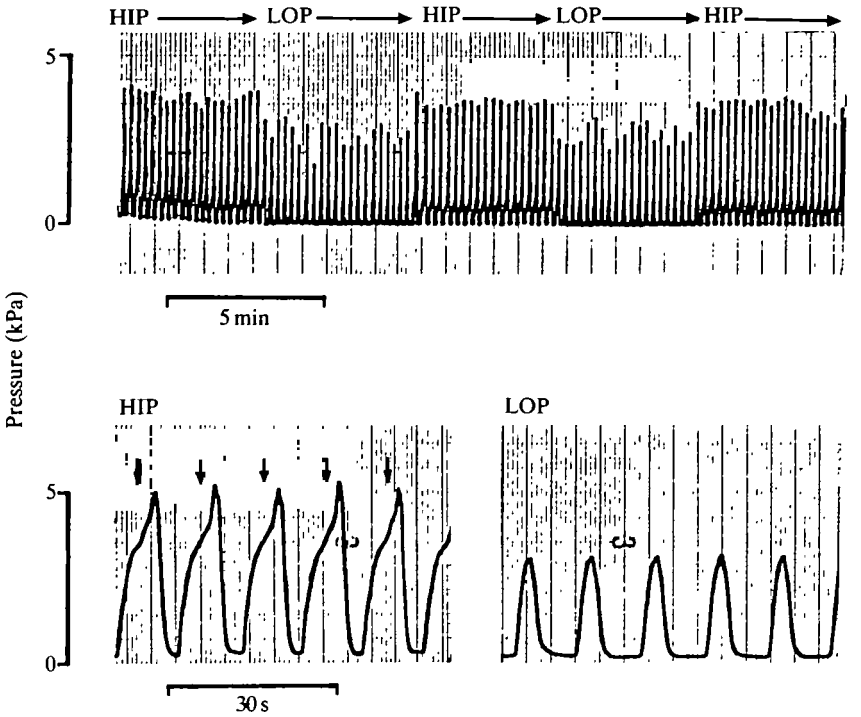

Fig. 2. Pressure recordings in the sixth segment of a lateral vessel. Upper trace: pressure pulses in HIP and LOP phases. Lower trace: HIP pulses and LOP pulses. Arrows mark the presystolic pressure in each HIP pulse.

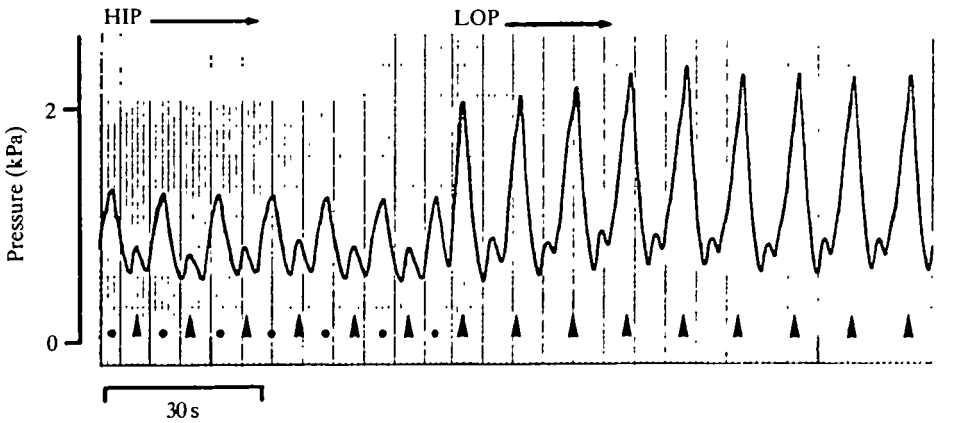

Fig. 3. Pressure recording in the lateral abdominal vessel in the eighth segment of a leech. Pulses marked with arrowheads were observed simultaneously with the contraction of the corresponding segment of the ipsilateral lateral vessel in HIP and LOP phases. The circles mark those pulses which appeared simultaneously with the maximal diastolic filling of the lateral vessel segment in the HIP phase.

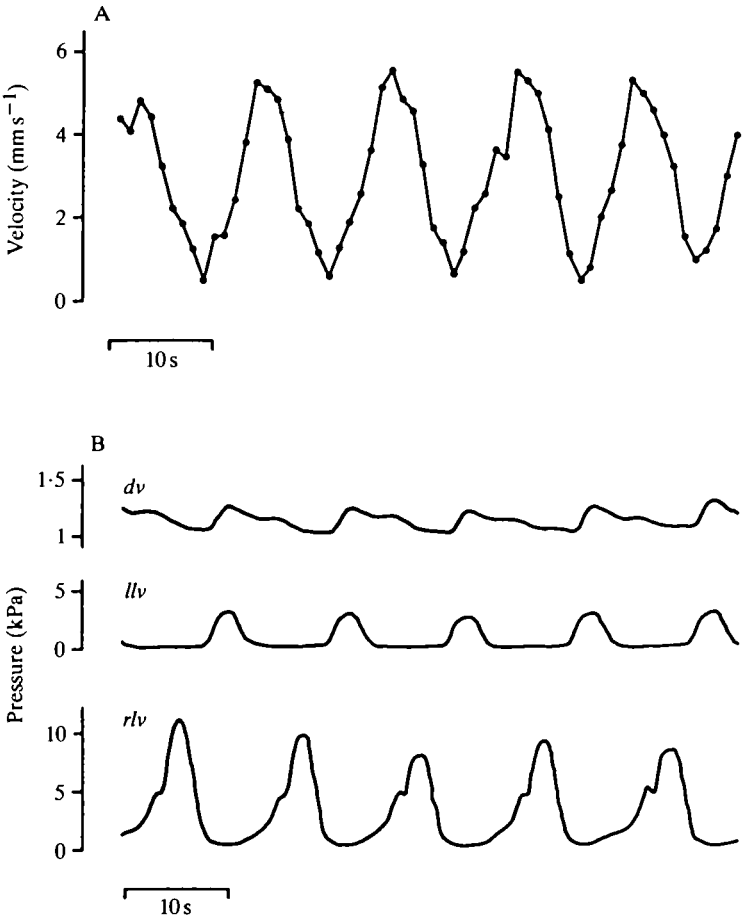

Fig. 4. (A) Periodically changing blood cell velocities in the dorsal vessel. (B) Simultaneous pressure recordings in the dorsal vessel (*dv*, tenth segment), and the left (*llv*) and right (*rlv*) lateral vessels (sixth segment). The left lateral vessel is contracting in LOP, the right one in HIP. Pulses in the dorsal vessel are generated by the LOP contractions of the lateral vessel. The periods of presystolic pressure in the anterior lateral vessel segments in the HIP phase cause the high pressure level in the dorsal vessel. The rate of blood pulses in the dorsal vessel is similar to the heart rate.

These results were complemented by simultaneous pressure recordings in the lateral vessels and the dorsal vessel (Fig. 4B). The pressure pulses generated by the LOP contractions of the lateral vessel were directly transmitted to the dorsal vessel damped by the capillary systems between both vessels. The pressure pulses of the HIP contractions of the contralateral lateral vessel were not detectable in the dorsal vessel. The pressure in the dorsal vessel, however, did not decrease at the end of the LOP systole. This was due to the transmission of pressure from the lateral vessel segments in HIP during their presystolic periods through the still-opened lateral abdominal sphincters. Because of the peristaltic action of the anterior lateral vessel

segments in the HIP phase, the pressure in the dorsal vessel remained high until the next LOP contraction of the contralateral lateral vessel occurred. The average pressures in the dorsal vessel ranged from 0·9 to 1·9 kPa in 17 animals. The pressure level in each animal remained constant for several hours.

These pressure recordings showed that the outflow of blood to the capillary networks of the nephridia, the body wall and all other organs through the lateral abdominal vessel is only possible during the LOP contractions of the lateral vessels and, less significantly, during the period of presystolic pressure in the lateral vessel segment in HIP. The HIP contractions caused primarily a forward blood flow within the lateral vessels and, secondarily, a blood pulse to the segmental circulation through the lateral abdominal sphincter of the next anterior lateral vessel segment.

Blood flow

Blood from the capillary networks of the organs and the body wall was collected by the dorsal vessel. The backward blood flow in it was discontinuous. In some animals, the blood flow stopped transiently. The maximal blood flow velocities were about $10\,mm\,s^{-1}$. Because periodic changes of blood flow velocities (Fig. 4A) showed the same rate as the contractions of one lateral vessel ($5–6\,min^{-1}$), it was concluded that they were caused mainly by the LOP contractions. Additionally, the pressure gradient between lateral and dorsal vessels during the periods of presystolic pressure in the lateral vessel segments supported blood flow in the dorsal vessel. The periods of minimal blood flow velocity could be shorter than the periods of diastolic pressure level in the lateral vessel in the LOP phase (Fig. 4).

The average blood flow velocity analysed for 80 s was $3·0 \pm 1·4\,mm\,s^{-1}$ (27 animals) in the dorsal vessel. The average diameter of the dorsal vessel was $0·403 \pm 0·014$ mm (27 animals) so that the mean value of blood flow was $22·9 \pm 3·1\,\mu l\,min^{-1}$.

Observations of the flow directions in the posterior part of the leech showed that blood from the dorsal vessel entered either the dorsal or the ventral intestinal vessels. There were numerous anastomoses between the dorsal and the lateral intestinal vessel (Boroffka & Hamp, 1969) so that only a portion of blood was observed to pass through the capillaries of the intestine (Fig. 5, *ci*). The other portion directly entered the connections between the lateral intestinal vessel and the laterodorsal vessels, which have already been described by Boroffka & Hamp (1969). Through the contractile laterodorsal vessels, blood re-entered the lateral vessels.

In the area of the anterior sucker, blood from the first lateral vessel segments in the HIP phase entered the dorsal vessel through small anastomoses between the lateral and dorsal vessels. This blood flow rate should not exceed $4\,\mu l\,min^{-1}$, calculated from changes in the diameter and the length of the lateral vessel segments during contraction.

Movements of the body wall caused considerable local unsteadiness of circulation, in the smaller vessels especially. Sometimes, this resulted in opposite flow directions

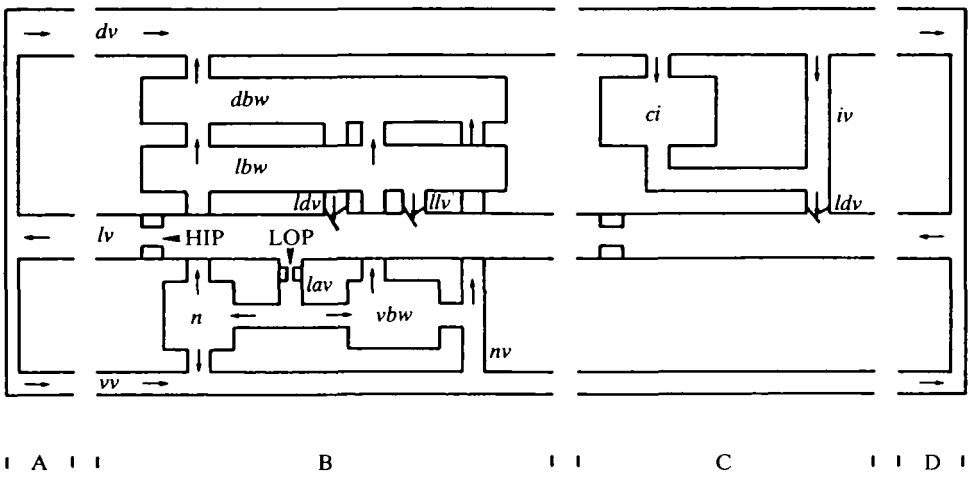

Fig. 5. Improved scheme of the circulatory system of *Hirudo medicinalis* basing on anatomical studies made by Boroffka & Hamp (1969). Arrows mark the directions of blood flow. (A) Six anterior segments fused; (B) scheme valid for the 21 somatic segments; (C) scheme valid for those five segments which contain the intestine; (D) seven posterior segments fused. *ci*, capillary network of the intestine; *dbw*, capillary network of the dorsal body wall; *dv*, dorsal vessel; HIP, direction of blood flow in the high-pressure phase; *iv*, lateral intestine vessel; *lav*, lateral abdominal vessel with sphincter; *lbw*, capillary network of the lateral body wall; *ldv*, laterodorsal vessel with valve; *llv*, laterolateral vessel with valve; LOP, direction of blood flow in the low-pressure phase; *lv*, lateral vessel with main sphincter; *n*, capillary network of the nephridium; *nv*, vessel enclosing the dorsal branch of the posterior segmental nerve; *vbw*, capillary network of the ventral body wall; *vv*, ventral vessel.

in the capillary networks. These effects, however, influenced the general course of circulation only to a small degree.

DISCUSSION

Pressure phases

It has been shown that the function of the two pressure phases in the anterior parts of the lateral vessels is to distribute blood along the entire length of the leech in the HIP phase, and to supply the segmental circulation mainly in the LOP phase. As Thompson & Stent (1976) have pointed out, true peristalsis occurs only in the anterior segments of the lateral vessel. Between the tenth and the seventeenth segments, all lateral vessel segments contract synchronously. Systolic pressures in the fourteenth segment, however, indicate HIP pulses, but the presystolic pressure, which characterizes the HIP pulse in an anterior lateral vessel segment, is absent (see fig. 3 in Krahl & Zerbst-Boroffka, 1983). Thus, it can be concluded that all segments contracting synchronously are filled with blood only through the laterodorsal and laterolateral vessels, whether the lateral vessel is in the HIP or in the LOP phase. In

contrast, the lateral vessel segments that contract in peristalsis, which occurs only in the HIP phase, are filled additionally by the contraction of the next posterior lateral vessel segment.

Function of the lateral abdominal sphincter

The distribution of blood from the lateral vessel segments is controlled by the muscular lateral abdominal sphincters and the main sphincters within the lateral vessels. A different action of the lateral abdominal sphincters is postulated during contractions of the lateral vessels in the HIP and LOP phases. This might be under nervous control. Maranto & Calabrese (1984) looked for the terminals of two types of neurones innervating the heart tubes. These neurones did not show a special innervation of the sphincter regions. However, gold chloride staining (Zerbst-Boroffka, Bazin & Wenning, 1982) and cobalt hexamine chloride filling (A. Wenning, personal communication) of the nervous supply to the heart tubes showed dense innervation of the lateral abdominal sphincter. It is possible that the heartbeat rhythm and the distribution of blood flow are controlled by different neurones in the central nervous system.

Segmental blood flow

Pressure recordings in the lateral, lateral abdominal and dorsal vessels, as well as observations of the blood flow in the dorsal vessel, showed that the blood flow in the segmental capillary networks of the body wall and the organ systems in the leech is caused mainly by the LOP contractions of the lateral vessels. The segmental capillary systems are in series (Lankester, 1880; Boroffka & Hamp, 1969) and the peripheral resistance should be high. However, the pressure gradient between the lateral vessel in an LOP systole and the dorsal vessel (0·8–1·9 kPa) is capable of causing a slow blood flow in the capillaries of the epidermis. This provides efficient uptake of oxygen through the integument, even in a resting animal.

The high pressure level in the dorsal vessel (about 1 kPa) may feign a 'windkessel' effect (Frank, 1899) in the dorsal vessel. In contrast, the extreme variations of the blood flow velocities in the dorsal vessel (Fig. 4A) indicate the absence of a windkessel effect in the vascular system. Simultaneous pressure recordings in the lateral and dorsal vessels indicate the transmission of either systolic LOP pressure or presystolic HIP pressure to the dorsal vessel. The coordinated action of the lateral vessels causes the high pressure level in the dorsal vessel. The functional significance may be the maintenance of an almost constant pressure gradient for driving the blood flow in the capillary network of the intestine. Thus, the mechanism seems to be quite different from the windkessel effect but has similar consequences. How far the output from the dorsal vessel to the intestinal vessels is regulated is not clear. The lateral intestine vessels, the laterodorsal vessels, and the connections between both laterodorsal vessels in one segment (*Laterodorsalgefäß-Bögen*; Boroffka & Hamp, 1969) are contractile and could be involved in such a control mechanism.

Circulation model

In the 21 somatic segments of the leech (Fig. 5B) blood flows anteriorly along the lateral vessels in the HIP phases, or enters the segmental circulation through the lateral abdominal vessels mainly in the LOP phases of the lateral vessels. The capillary networks of the nephridia and the body wall discharge segmentally either into the ventral, the laterodorsal and the laterolateral vessels or into the dorsal vessel. In consequence, the total blood flow in the segmental circulation should be somewhat larger than has been measured in the dorsal vessel.

Through anastomoses in the area of the anterior sucker (Fig. 5A) blood enters the dorsal vessel. This is caused by the HIP contractions of the anterior lateral vessel segments. The amount of blood, however, is insignificant in comparison with the amount that is forced into the dorsal vessel by the LOP contractions of all segments of the contralateral vessel.

From the thirteenth to the seventeenth segment of the leech there is, additionally, another course of circulation (Fig. 5C). The dorsal vessel discharges partly into the capillary network of the intestine. Through numerous anastomoses, blood enters the lateral intestine vessels directly. These vessels are segmentally connected with the laterodorsal vessels (Boroffka & Hamp, 1969).

Blood from the dorsal vessel also enters the lateral vessels through anastomoses in the area of the posterior sucker (Fig. 5D).

The total blood volume in the ventral vessel should be insignificant because the ganglia and the connectives occupy most of the vessel lumen.

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