10 attempts), after having been kept for 48 h at 7 °C in a physiological solution which was oxygenated twice each day. Below 4 °C and above 45 °C isolated vessels lost their ability to contract rhythmically. In the upper temperature range, at any rate, this is most probably due to an irreversible protein denaturation.

The difficulty of presenting, at this moment, a complete working model for the autoregulation of blood flow in the bat wing is relevant to nearly all other vascular territories. Any such endeavour must take into consideration not only the functional characteristics of the smooth muscle elements and other close lying structures, but also the superimposition of nervous (reflex and central), hormonal and metabolic influences, which are present at all times. Nevertheless, it is hoped that some of the problems raised in this paper may be considered as an initial contribution to this study as well as a starting point for further investigation. The bat wing lends itself as a particularly well suited model to the study of these parameters.

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Histology of the vascular wall and its innervation

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Mammalian veins have been categorized according to their fibre content. Accordingly, there are 3 large groups of veins: fibrous, fibroelastic and muscular. This latter group has been further subdivided into fibromuscular and musculoconnective.

The metacarpal vein of the bat is classified, along with veins such as the plantar, tibial, spermatic, internal iliac, hepato-portal, etc., with the musculoconnective type of muscular vein, because of the presence of loose collagenous bundles and a predominance of helicoidal muscle cells in the media[1].

Histology of the venous wall

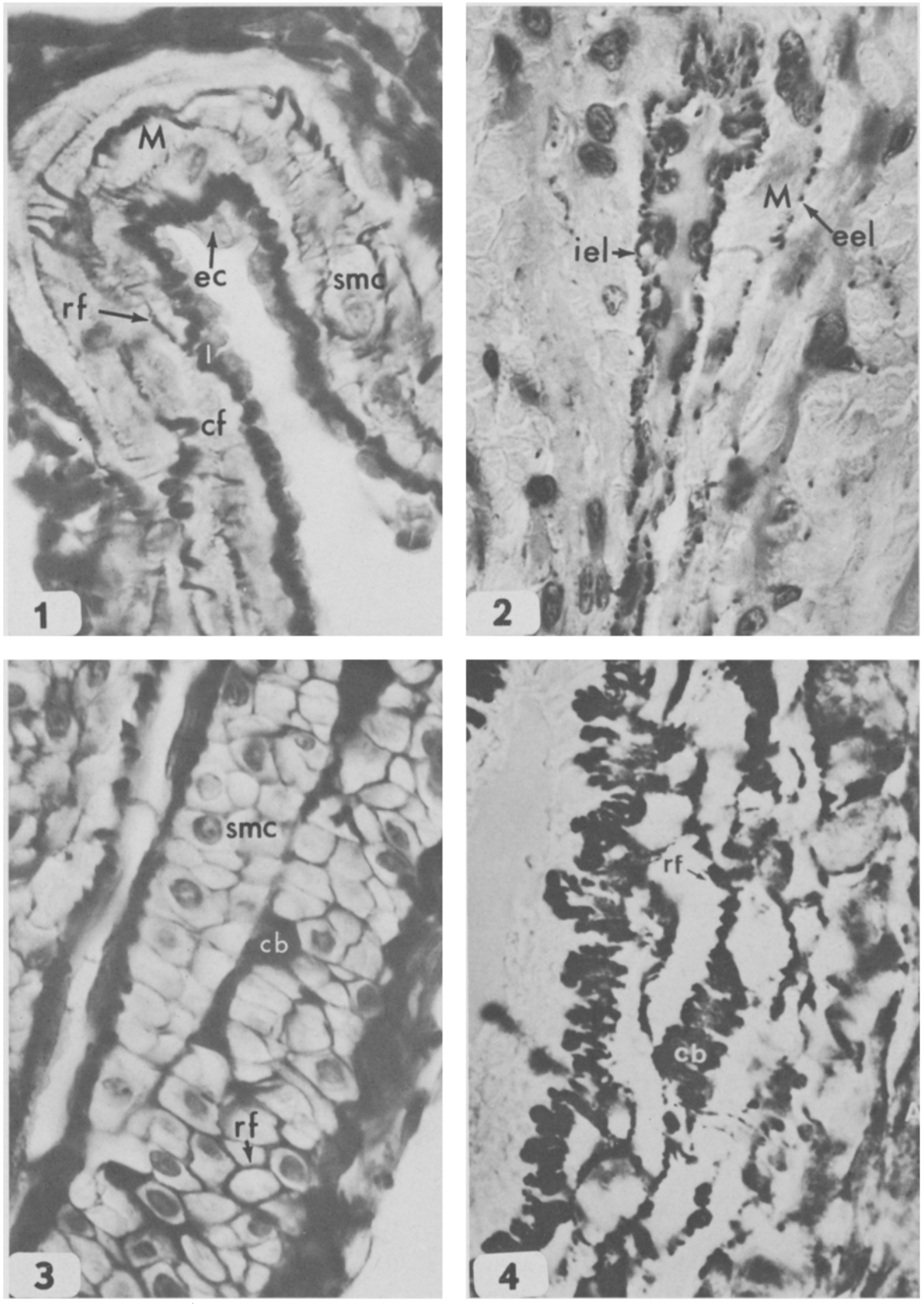
a) The *neurovascular bundle,* composed of a mixed nerve located between a muscular artery and vein[1,2], is ensheathed by connective tissue composed of both cellular and fibrous elements[1,3]. This sheath, which jackets the neurovascular structures, anchors the components to the metacarpal bone as well as the dermal elements of the upper and lower wing membranes. The *metacarpal artery* feeds small arteries and arterioles that are accompanied by adrenergic nerve fibres. The *metacarpal veins* receive blood from small muscular veins. Valves, which regulate the influx of venous blood, are found at the juncture of vein and small vein[3,4]. Valves, however, are not restricted to these junctures. They are also found strategically placed at nonjunctional segments. No report of a vasa vasorum in the arterial or venous wall has been noted.

b) *Non-nervous composition of the venous wall.* From the earlier articles to the present it has been agreed that the vein is composed of 3 distinct layers[1,5]. The intima is lined on the luminal side by tall endothelial cells (figure 1). These cells lie on an amorphous matrix that seems to lack muscular cells or even connective fibres. However, elastic fibres from the media do pierce this inner layer and accumulate at the base of the valves, some of which later may extend into the valve leaflets.

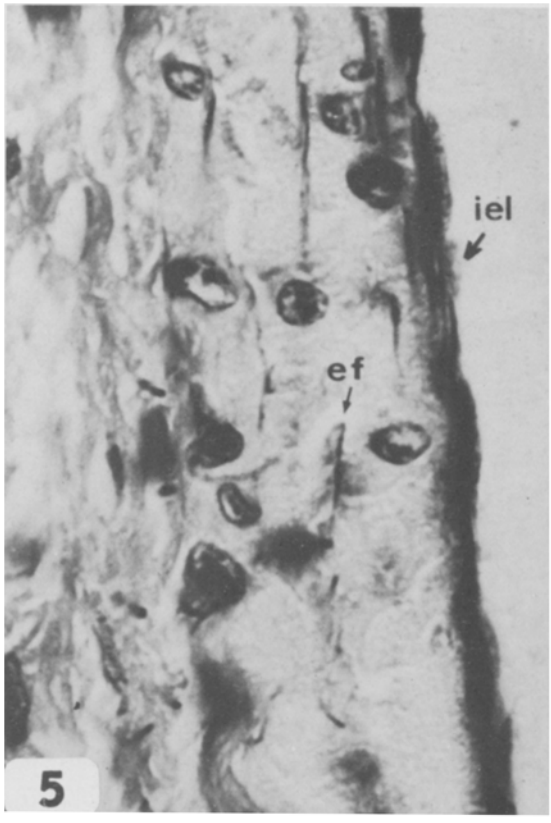
The venous valves are seen as a single flap[3] or composed of 2 connective leaflets[1,3]. These valves appear as connective outgrowths of the intima[1]. Their nonfibrous bodies are supplemented with few cells and rare elastic fibres at the base of the valve. Typically, they are lined with endothelial cells, thus presenting a continuous surface to the lumen. The overall thickness of the intima is between 3.5 and 5.7 µm[1].

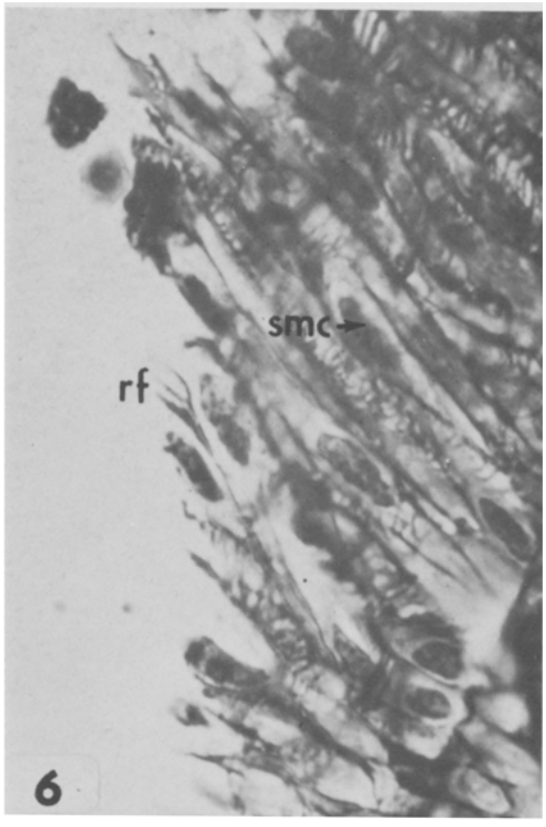
Underlying the intima, and delimiting it from the media, are evenly spaced elastic fibres running the length of the vessel (figures 2 and 3). Here, quite atypically, the elastic fibres are not fused into an internal elastic lamina, but are closely aligned to form an elastic boundary[1].

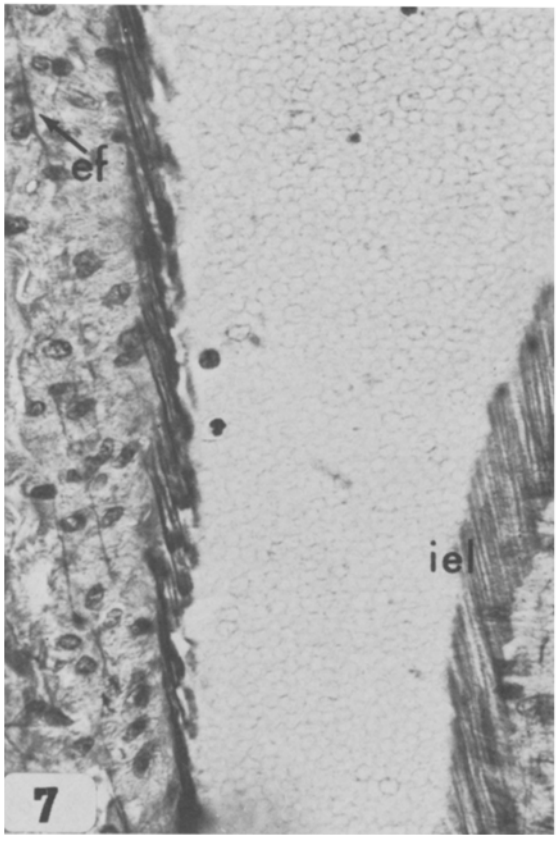
The media is the thickest coat, being 20–30 µm thick. Here, the muscle cells form 3–4 layers of helicoidal or annular elements (figure 3) running in a circular orientation[1,5,6]. Running between the muscle layers are bundles of collagen fibres (figure 4). These crisscross in a general longitudinal direction and form a loose meshwork of variable thickness. Elastic fibres, lying in the longitudinal plane are seen in the media (figures 5 and 6). They are not numerous here and

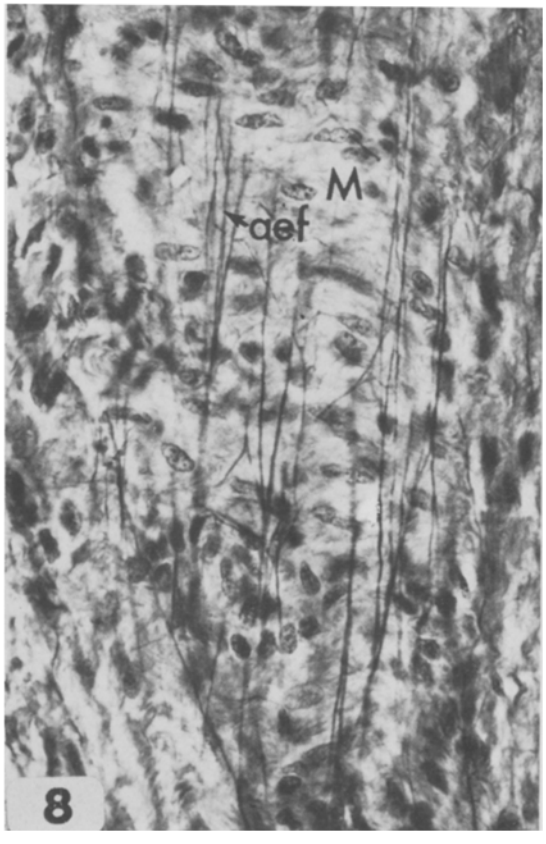


Figures 1-4. Cross section of metacarpal vein. ×570. Fig. 1. Humason-Lushbaugh differential stain. Fig. 2. Tanzer-Unna elastic stain. Fig. 3. Humason-Lushbaugh differential stain. Fig. 4. Gomori's collagen stain. M, Media; ec. endothelial cell; cf, collagen fibre; i, intima; smc, smooth muscle cell; rf, reticular fibre; iel, internal elastic layer; eel, external elastic layer; cb, collagen bundle.

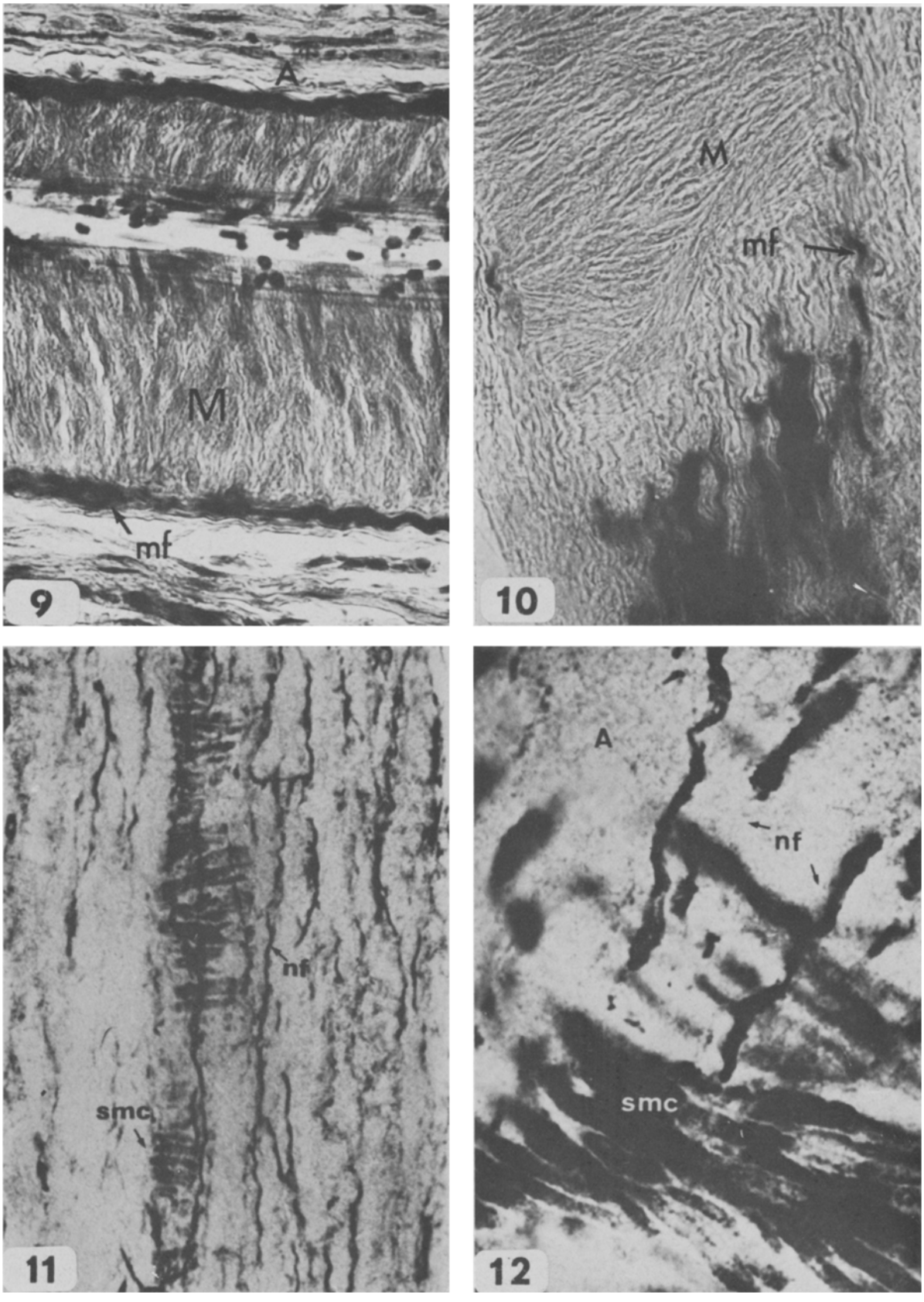




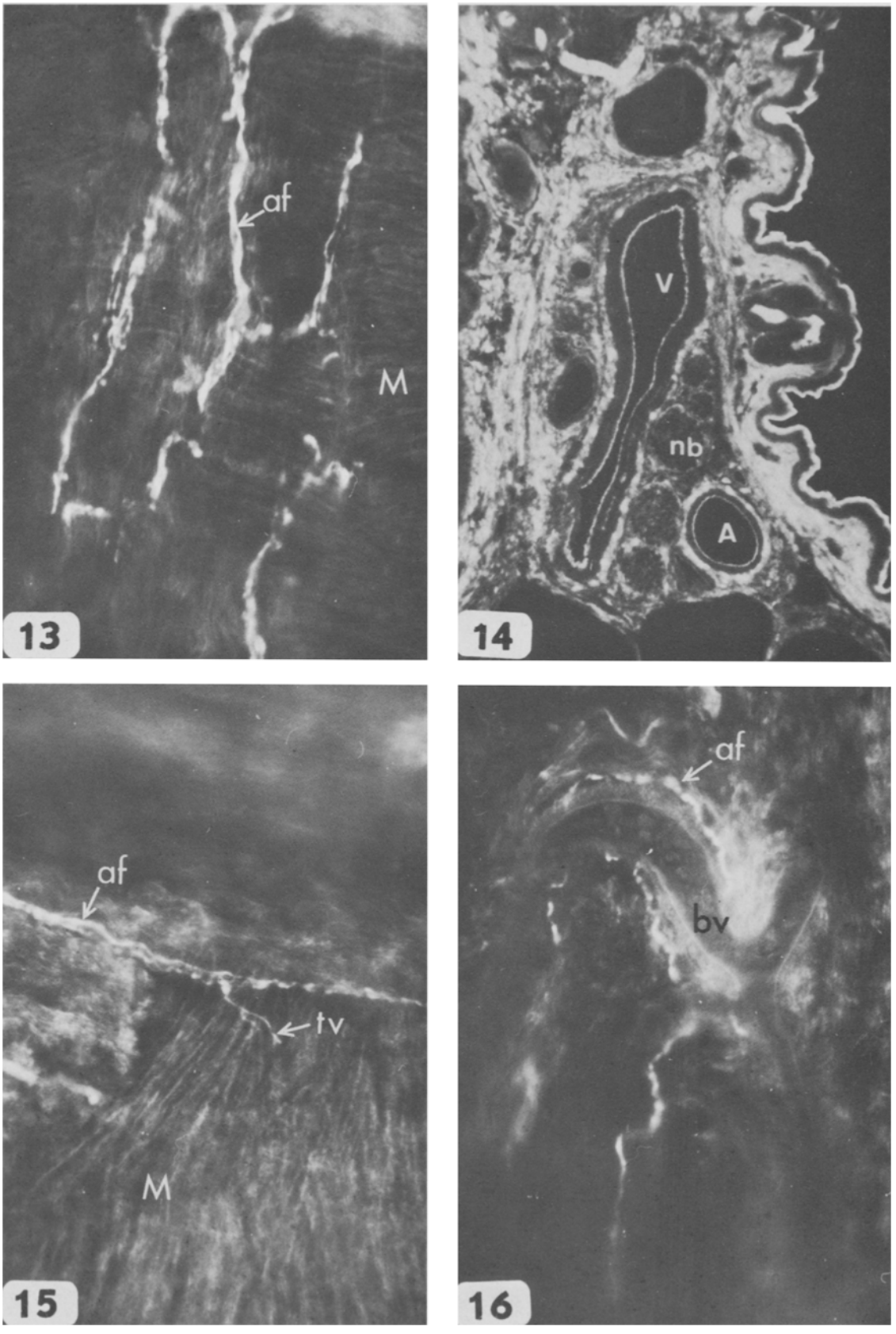




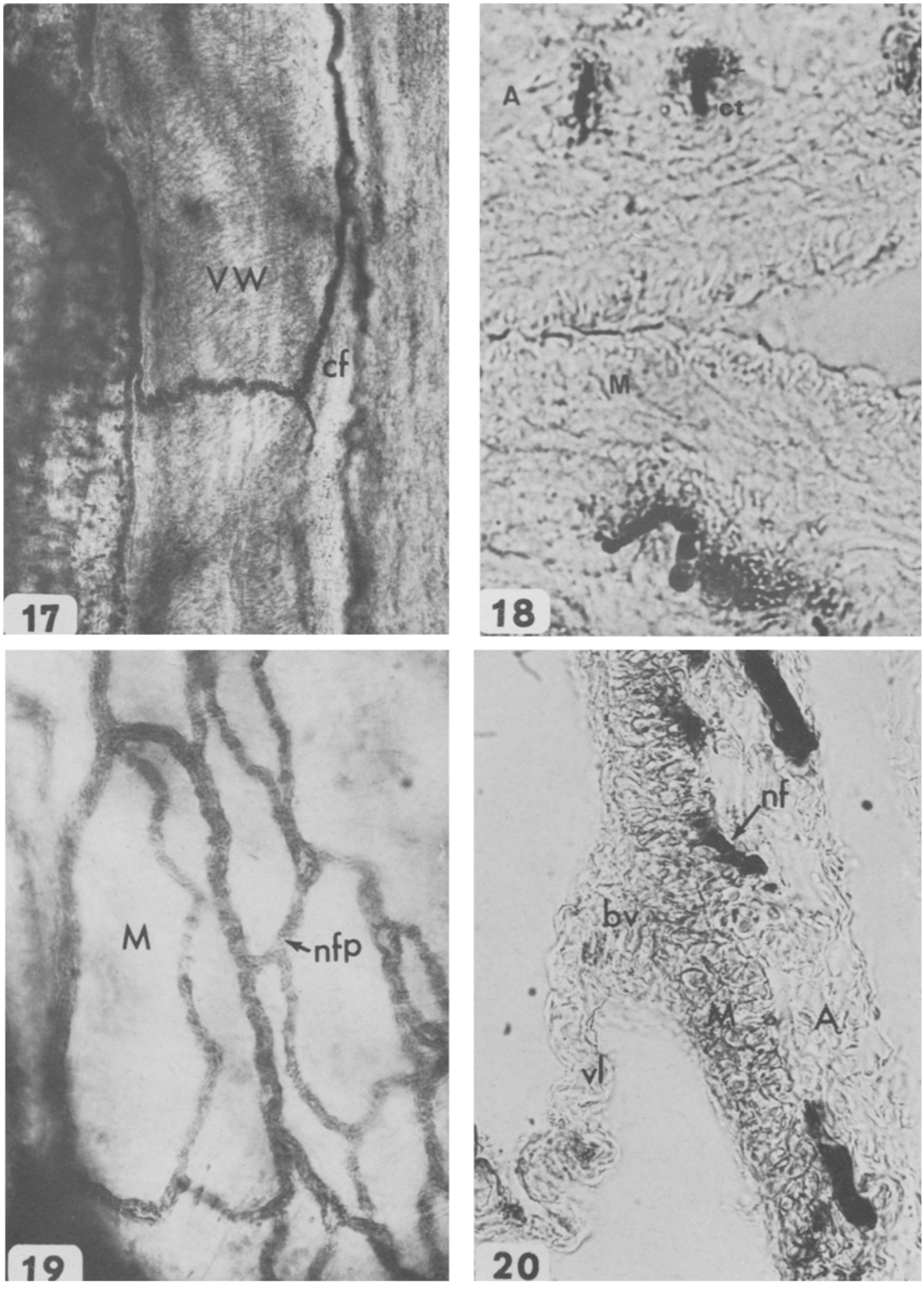
Figures 5-8. Fig. 5, 7 and 8. Longitudinal section of metacarpal vein. × 570. Tanzer-Unna elastic stain. Fig. 6. Tangential section of metacarpal vein. × 570. Humason-Lushbaugh differential stain. M, Media; iel, internal elastic layer; ef, elastic fibre; smc, smooth muscle cell; rf, reticular fibre; aef, adventitial elastic fibre.



Figures 9–12. Fig. 9 and 10. Longitudinal section of metacarpal vein. × 570. Loyez's myelin stain. Fig. 11. Longitudinal section of metacarpal vein. × 570. Gladden's silver nitrate stain. Fig. 12. Tangential section of metacarpal vessel. × 1471. Gladden's silver nitrate stain. M, Media; a, adventitia; mf, myelinated fibre; smc, smooth muscle cell; nf, nerve fibre.



Figures 13-16. Fig. 13. Longitudinal section of metacarpal vein. ×570. Fig. 14. Cross section of neurovascular bundle. ×150. Fig. 15. Longitudinal section of metacarpal vein. ×570. Fig. 16. Longitudinal section of a vascular juncture. ×570. Falck-Owmans fluorescent reaction. M, Media; ap, adrenergic plexus; V, vein; A, artery; nb, nerve bundles; af, adrenergic fibre; tv, terminal varicosity; bv, blood vessel.



Figures 17–20. Fig. 17. In toto view of metacarpal vein. ×220. Gerebtzoff's AChE stain, incubation 4 h. Fig. 18. Cross section of metacarpal vein. ×877. Gerebtzoff's AChE stain, incubation 4 h. Fig. 19. Longitudinal section of metacarpal vein. ×570. Karnovsky-Roots AChE reaction, incubation 20 h. Fig. 20. Cross section of valve region of metacarpal vein. ×570. Karnovsky-Roots AChE stain, incubation 20 h. VW, vessel wall; cf, cholinergic fibre; M, media; A, adventitia; ct, cholinergic terminal; nfp, nerve fibre plexus; vl, valve leaflet; bv, base of valve; nf, nerve fibre.

seem to be contained in the intermuscular connective tissue.

Reticular fibres are also found in the media (figure 3). These seem more closely associated with the muscle cells, and in some images seem to envelope the cells completely. The fibrillar nature of these reticular elements can best be seen in figure 6, a tangential section, in which the venous wall was torn. Some images show the reticular fibres to be lightly tortuous on the surface of the muscle cell (figure 1).

Unlike most mammalian veins, which lack an external elastic lamina, the metacarpal vein has an external elastic layer[1]. This is not composed of fused fibres, but of widely spaced elastic fibres running mainly in the longitudinal axis (figure 2). These fibres represent, then, the medio-adventitial border. Interestingly, some rare elastic fibres are oriented in the circular plane in the dorsal and ventral regions whilst they are longitudinally arranged in the lateral walls[1].

The adventitia, measuring 15–17 µm in thickness, seems not to contain muscle cells. The adventitia is composed of layered collagen bundles running in both longitudinal and circular planes of the vessel. Elastic fibres are present (figure 8). These lie mainly in the longitudinal axis, but some are circularly and obliquely oriented. The orientation of these elastic fibres could play an important role in vasomotor activity and may behave much like elastic fibres do in elastic vessels, aiding in the pulsatile nature of venous action. Finally, toward the periphery of the adventitia pericytes are seen. These are few in number.

c) *Innervation of the venous wall.* Direct correlation between vasomotor activity and nervous influence has only recently been shown[1,7,8]. Since the automatism of the vein has been known for many years[6,9–12], nerve fibres from the neurovascular bundles were considered, at best, to use the vascular wall as a pathway to the periphery.

The fact that the vein is responsive to topically applied and perfused neurotransmitters[1,8,12–14] indicate a nervous involvement. Furthermore, sectioning of brachial and metacarpal nerve trunks has a profound effect on the perfused vein[1,7,8]. Although nerve fibres were seen associated with the vein[14] it was with the more recent, and more specific, staining methods, that the nature of the innervation was determined. The vein possesses a dual innervation[1,15]. This innervation is dual not only in terms of the neurotransmitters but also in terms of the fibre types. Both myelinated (figures 9 and 10) and unmyelinated (figures 11 and 12) fibres have been associated with the vessel wall.

The *adrenergic* innervation, as demonstrated by monoamine fluorescence, patterns itself on the unmyelinated structures seen with a more specific argyrophilic stain (figures 11 and 12). Adrenergic fibres run as a plexus in the longitudinal plane in the adventitia. They crisscross fairly often though dichotomise seldom (figure 13). The general appearance is that of a loose network running longitudinally in the wall. From these fibres will arise the terminal varicosities that seem to end at the media-adventitial border in Rousettus[1] (figures 14 and 15). In Pteropus, adrenergic elements have been observed between the muscle layers of the media and seem to extend to the internal elastic lamina. Here, then, the pattern is 3-dimensional[8,16].

The origin of these adrenergic plexi may be in the nerve trunk[1] as well as in elements entering the wing as preterminal fibres that continue their course as a plexus in the venous wall[8] and on to the web and its vasculature (figure 16).

No adrenergic fibres or structures have been seen in the mixed nerve bundles of the nerve trunk with the fluorescent method. However fibres are seen in the connective tissue surrounding the nerve bundles[1]. Injection of a monoamine inhibitor (Niamide 200 g/kg) produces within 6 h an increase in intensity and apparent diameter of the fluorescent fibres. Denervation at the brachial at metacarpal levels have been shown to have 2 responses[1,8]. After 14 h fluorescent intensity is not reduced appreciably, but after 3 days there is a constant fluorescent reduction[8]. After 28 days no fluorescence is seen other than background fluorescence of elastic components of the wall[1].

Both *cholinergic* and myelinated fibres seem to have the same pattern. In Rousettus, they originate in the bundles of the nerve trunk, located between the artery and vein. Here, long fibres that ramify rarely run longitudinally in the adventitia (figure 17). They can cross the wall perpendicular to the vascular axis to regain a longitudinal path on the opposite side[1,17]. In cross section the acetylcholinesterase (AChE)-stained fibres terminate as branching terminal varicosities at the medio-adventitial border (figure 18). 3–12 varicosities of the AChE-stained and fluorescent types thus ring the vessel (figures 14 and 18). Gross measurements show that the argyrophil and fluorescent fibres have the same dimensions and orientations. By the same token the cholinergic fibres, which are larger, seem to have the same dimensions and orientations as fibres stained for myelin[1]. Interestingly, after prolonged incubation periods of 16 h, in Pteropus[8,16] and of 20 h in Rousettus[18] the AChE staining reaction yields images resembling the adrenergic pattern (figures 19 und 20). After denervation, this pattern disappears[8,16]. Because of these results more work needs to be done to determine whether the preceding description of cholinergic patterns is due to staining artefact or whether there may be 2 cholinergic pathways. It could be that this latter data on Pteropus and Rousettus validates the postulate that AChE is associated in the membranes of adrenergic nerve fibres and that adrenergic and cholinergic plexi are very closely associated[19–22].

* Work conducted at: University of Geneva, Department of Animal Biology, Geneva, Switzerland.
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Morphological specializations: Ultrastructural

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In accordance with the special functional properties of the autonomously contractile veins in the wing membrane of Chiroptera, which have been demonstrated comprehensively in the works of Mislin[1-3], the electron microscopical studies of this organ in the flying fox, *Pteropus vampyrus* (Gray) and the bat *Myotis myotis* (Borkhausen) show some remarkable ultrastructural specializations of the vessel structure. These vascular specializations concern particularly the endothelium as well as the vascular smooth muscle and its innervation.

The general morphology of the 120-µm thick vessel wall is in accordance with that of other mammalian veins[4]. It is characterized by a tunica intima with an always closed endothelium layer, associated with collagenous fibres, few elastic structures but without smooth muscle cells. The following layer, the tunica media, is built up of only circular muscle cells which are surrounded by a fine network of collagenous fibres. The tunica adventitia contains few muscles and consists of a network of collagenous fibres, corresponding to those of the media in which in addition to the vasa vasorum may non-myelinated nerve fibres can be seen. These fibres have contact with the peripheral muscle cells of the tunica media.

The most prominent endothelial specializations of this contractile vessel are the many unipolar branched processes in the abluminal side of the cells. They have a length up to 10 µm and their terminal branches are fixed in the collagenous and elastic tissue marking the border between the tunica intima and media so that the endothelium has an arcade-like appearance (figure 1, a). Depending on the contractile state of the vessel, the distance of these arcades to the muscle tissue of the media changes. The degree of flexure of the central cell area expresses the high degree of adaptability of this layer to the considerable variations of the vessel lumen. According to the changeable tensile stress, the cytoplasm of the endothelial cells contain filamentous structures such as we see in other contractile vessels[4-7]. It is not yet clarified, whether these structures function as contractile myofilaments like the actin-myosin-system of muscle cells or solely as stabilizing elements. The functional importance of the intraplasmatic, lysosome-like dense bodies is also not yet known[8].

The spindle-like muscle cells of tunica media - building up 5-6 layers - show very close side-to-side interdigitations[4] (figure 1, b). As in other smooth muscle cells[9-11] we can distinguish 2 sets of myofilaments with diameters of 70 Å respectively 100 Å and dense patches or dense bodies in which the thin filaments insert (figures 1, c and 2, b). They appear to have a function similar to that of the Z-lines of striated muscle, with a sliding filament mechanism of contraction in which thick (myosin) and thin (actin) filaments slide relative to each other. In the peripheral sarcolemm the thin filaments are inserted by hemidesmosome-like structures (figure 2, e).

A very prominent feature of these vascular muscle cells is the significantly greater content of mitochondria than in non-autonomously contractile vessels (figure 1, c and 2, a). As in the muscle cells of other autonomously contractile blood and lymph vessels[4], we can see large accumulations of these organells (30-40) near the centrally situated nucleus and in streaks between the contractile apparatus reaching up to the cell surface. There they are associated with micropinocytotical vesicles, free ribosomes, glycogen particles, granular or lamellated dense bodies and a well developed sarcoplasmatic reticulum, generally of the smooth form (figure 2, d and e). More or less straight