but direct microscopic observation of bat wing arterial vessels following barbiturate injection showed platelet aggregates at arteriolar branch sites blocked flow into the capillary nets with the absence of arterial constriction.

Other studies relative to medical problems include observation of bat wing vessels in response to ethyl alcohol, both injected and ingested, to ascertain the vascular responses to various concentrations in the blood stream and an investigation to learn the mechanisms by which cold water acted to alleviate the pain and edema caused by 1st and 2nd degree burns of skin.

It is obvious from this brief review of the specific investigations in which the bat wing vessels have been used as the experimental site, that the bat, unanesthetized and with an unaltered cardiovascular system, lends itself to a wide variety of studies that have a direct application to the understanding and treatment of medical problems. The list presented here is not complete, but indicates the present and potential usefulness of the bat as an experimental animal.

## Interaction of Ca and K ions in governing spontaneous electrical and mechanical activity of bat wing veins

by J. G. Peristiany, H. Huggel and M.-L. Bisetti[1]

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The usefulness of blood vessels, that display rhythmically active vasomotion, in studying the mechanisms concerned with vascular smooth muscle activity, has now been adequately demonstrated. Bat wing vessels may present an additional advantage to several other models, in that they are easily accessible for comparative investigations in situ[2]. The aim of the present study was to determine the role of potassium and calcium ions in governing the electrical and mechanical events associated with spontaneous vasomotion in bat metacarpal veins. A sucrose-gap technique such as previously applied to the analysis of normal electromechanical coupling in the smooth muscle of such vessels was used for this purpose[3].

In the bat vein, this activity is essentially composed of single plateau-type action potentials (which occur at regular intervals), each of whom is accompanied by a contraction-relaxation cycle, measured in vitro as an isometric change in longitudinal force. During normal activity action potentials may be seen to arise either abruptly from a steady membrane potential (figure 1, A), or as the consequence of a slow wave of depolarization, resembling pacemaker activity, which rises to the threshold level of the following action potential (figure 1, B).

A segment of vein between 2 valves and approximately 2.5 cm in length was dissected from the neurovascular bundle, ligated at both ends, and allowed to rest for 1 h in normal C.S.3. physiological solution[4]. The vessel was then mounted in a sucrose-gap apparatus. One end was fixed and depolarized with high-$K^+$ solution, and the other end, which was connected to a force transducer (Grass FTO3), was bathed in normal C.S.3. solution at 35 °C. The section of muscle lying between the recording electrodes was superfused with isotonic sucrose solution. A passive tension of approximately 200 dyn/cm$^2$ was applied to the muscle before each experiment. Solutions with increased $K^+$ content were prepared by replacing NaCl with equimolar amounts of KCl, whereas $K^+$-low and $K^+$-free solutions were obtained by substituting sucrose for KCl on an equiosmolar basis. Variations in $Ca^{2+}$ were compensated by corresponding equimolar changes in sucrose content. All solutions were aerated with a gas mixture of 97% $O_2$ and 3% $CO_2$. The pH of the solutions was adjusted as necessary to a value of 7.4 by the addition of a small quantity of $H_2SO_4$.

The total removal of $K^+$ from the bathing medium was followed by a brief increase in the frequency of spike firing, and a reduction in the duration of the plateau phase and of the quiescent period (figure 2). During this time additional spike components could be seen in some veins to accompany each action potential. Portal vein preparations have similarly

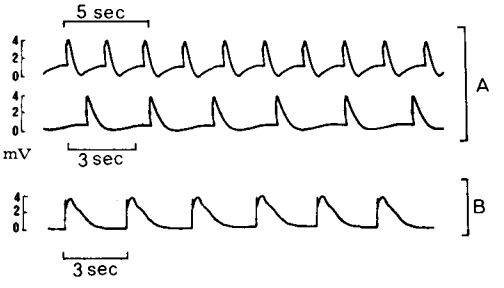


Fig. 1. 2 major types of electrical activity recorded in the bat metacarpal vein. *A* Repetitive-firing action potentials are each followed by a slow depolarization rising to the threshold level of the following action potentials. *B* Action potentials arise abruptly from a steady membrane potential without any preceding slow depolarization. The latter has been called a 'driven' as opposed to a 'pacemaker' activity.

been reported to exhibit almost continuous firing shortly after the removal of $[K^+]_o$[5]. The mechanical events, following the complete removal of $[K^+]_o$ in the metacarpal vein, were at first characterized by the development of a musle contracture and an increase in the frequency of phasic contractions. After 5–10 min, contractions usually slowed down, then ceased altogether, though action potential firing continued and may possibly have been related to the prolonged contracture registered at this time (figure 2, D). When $[K^+]_o$ was reduced to 3 mM only, the mechanical events that ensued followed a similar pattern as when $K^+$ was totally absent from the medium. Restoration of normal $[K^+]_o$ in either of these cases led to muscle relaxation and inhibition of all further electrical discharge for approximately 10 min, after which the muscle gradually recuperated, though phasic contractions tended to remain submaximal over longer periods of time. A similar loss of excitability, following a period of K-removal and return to normal solution, has been observed in other smooth muscle preparations[5,6] and in heart muscle[7]. The latter authors suggested that this 'inhibition' may well be due to a sudden increase in the $K^+$-permeability of the pacemaker-region cell membrane when the $[K^+]_o$ is again returned to normal. The above results following the removal of potassium ions surrounding the metacarpal vein were essentially similar, when twice and 3 times normal $Ca^{2+}$ concentrations accompanied the shift to the solution containing no potassium. However, when increased $Ca^{2+}$ concentrations were added to the superfusate 10 min after the removal of $K^+$, contracture tension was found to increase substantially at all times, though rhythmic vasomotion returned only occasionally and for short periods of time.

The simultaneous removal of calcium and potassium ions from the superfusate (figure 3) led invariably within 2–3 min to depolarization, to an increased rate of firing of spikes with decreasing amplitude and to contractions of diminished force. After this period, increasing membrane instability was noted until all electrical and mechanical events related to spontaneous vasomotion finally ceased. Returning to normal solution produced hyperpolarization and complete quiescence for 8–10 min, after which membrane potential returned to its usual resting value and more

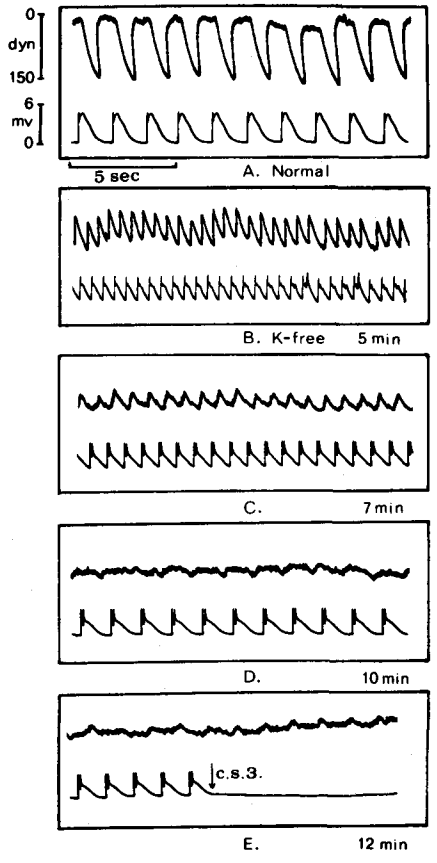


Fig. 2. Spontaneous electrical (lower tracings) and mechanical (upper-tracings) activity of the metacarpal vein and its response to $K^+$-free solution. A, Normal C.S.3. solution. B, 5th min in $K^+$-free solution, C, after 7 min, D, after 10 min, E, return to normal C.S.3. solution (arrow). Note the cessation of electrical activity and the muscle relaxation which follows return to C.S.3. solution. Electrical and mechanical tracings in B, C, D and E are in the same scale as in A.

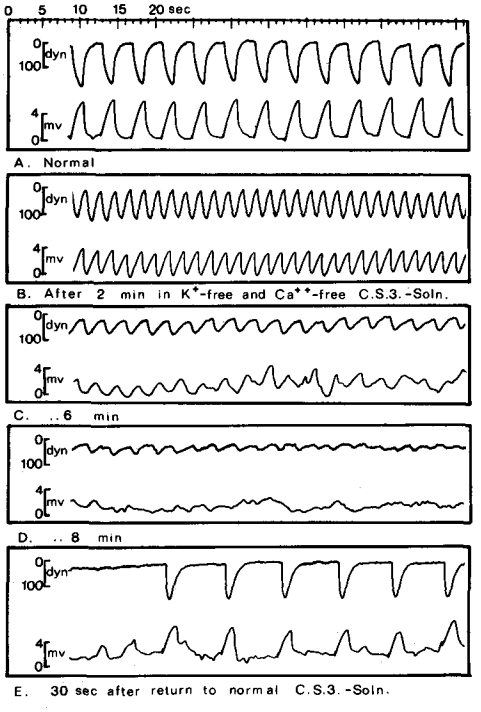


Fig. 3. The simultaneous removal of calcium and potassium ions from the superfusing solution of an isolated metacarpal vein. A, Normal activity; B, 2 min after shift to $K^+$-free and $Ca^{2+}$-free solution; C, 6 min later; D, 8 min later; E, 10 min after return to normal C.S.3. Note increasing membrane instability leading to cessation of all spontaneous activity.

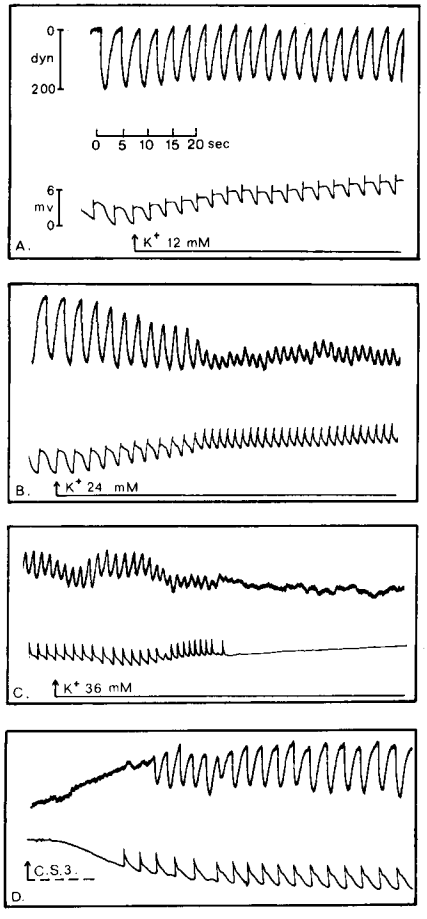


Fig. 4. Responses of the metacarpal vein to increased extra-cellular $K^+$ concentrations. A, Normal tracing, followed by shift (arrow) to solution containing 12 mM $K^+$; B, change (arrow) to 24 mM $K^+$; C, further change (arrow) to 36 mM $K^+$; D, return (arrow) to normal C.S.3. solution. Note progressive depolarization, shortening of the relaxing phase of the mechanogramme and the ultimate development of a maintained contracture. Scales are identical in A, B, C and D.

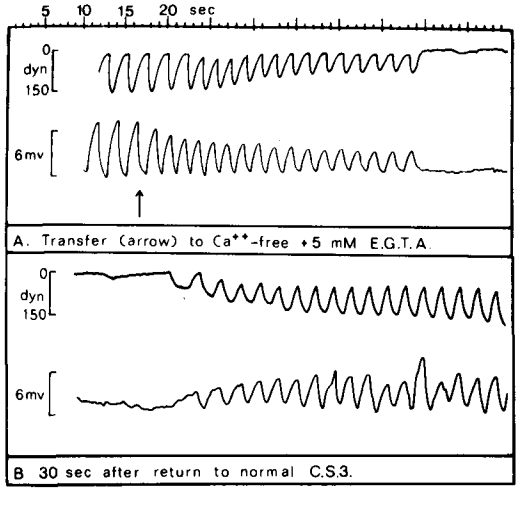


Fig. 5. The effects of a $Ca^{2+}$-free + EGTA medium. A, Normal recording, followed (arrow) by shift to $Ca^{2+}$-free + 5 mM EGTA solution; B, 30 sec after return to normal C.S.3., following 5 min of superfusion with $Ca^{2+}$-free + EGTA solution.

or less normal electro-mechanical activity usually returned.

Figure 4, A, shows the immediate effects of passing from a normal bathing solution to one containing 12 mM $K^+$. A distinct depolarization quickly followed as well as a slight increase in the frequency of contractions. A 4fold increase to 24 mM $K^+$ lowered the resting potential difference still further and gave rise to repetitive high speed electrical firing (figure 4, B). During this time tension in the muscle increased and the amplitude of phasic contractions diminished to about 20% of their previous value. A 6fold increase (36 mM $K^+$) led to further depolarization and to a sustained contracture in the absence of any further spike activity or phasic contractions (figure 4, C). A return to normal solution was at all times followed by a rapid recovery of normal resting potential value and electro-mechanical coupling.

In the bat vein, $[Ca^{2+}]_o$ is essential both for the emission of spike potentials and for the phasic and tonic components of the muscle contraction. When $[Ca^{2+}]_o$ is totally removed, the rate of spike discharge and of contractions at first gradually increase and then, after longer exposure to the medium, fall rapidly alongside with the force of the contractions, leading to a pronounced relaxation of the muscle and to the cessation of all further spontaneous activity. The addition of 5 mM EGTA to a solution containing no calcium (figure 5) led to complete quiescence within 20–30 sec, whereas superfusion with Ca-free solution alone required approximately 1 h before contractions came to a halt. The addition of 1.15 mM Ca to the solution, after 1 h of washout in Ca-free medium, gradually increased the tone of the muscle and led at such times to the return of vasomotion in which contractions remained of sub-maximal force throughout, though frequency returned to its initial value after approximately 30–40 min in this medium. A 2–3fold increase in normal $[Ca^{2+}]_o$ content increased moderately the amplitude and decreased similarly the frequency of contractions.

These results demonstrate that spontaneous phasic contractions in bat wing veins are directly related to single action potentials and that both contracture tension and the electro-mechanical process leading to rhythmical vasomotion are strongly influenced by the calcium and potassium content of the surrounding medium. Whereas variations in activity due to changes in $[K^+]_o$ are essentially similar in the bat vein to those reported by Axelsson et al.[5], (who worked with the rat portal vein, which also exhibits rhythmical vasomotion), they are inconsistent with the results of Siegel et al.[8] (who found membrane potential in the dog carotid artery to be relatively independent of $[K^+]_o$, as demonstrated by intracellular recordings following variations in external $K^+$ concentration) and, in contradiction with experimental data obtained

by Anderson[9] (in accordance with membrane potential values which he theoretically predicted for moderate changes in $[K^+]_o$, whilst assuming the presence of an electrogenic pump activated by extracellular potassium and intracellular sodium). Though it is not possible to establish a quantitative correlation between membrane potential and log $[K^+]_o$ by means of the sucrose-gap method, it did appear for the most part that membrane potential was affected by changes in extracellular potassium concentration (6–36 mM $K^+$) as would be expected, if the resting potential was at least partly determined by the gradient $[K^+]_i/[K^+]_o$. This would indicate that potassium ions are predominant in governing membrane potential at rest in this tissue. It is possible, therefore, that different ionic mechanisms may be involved in regulating membrane potential in vascular smooth muscles of different types.

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## Phasic activities in venular muscles of the bat wing

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The cyclic contraction-relaxation activity, usually termed vasomotion, observed in the venules and veins in the wing membrane of bats is associated in part with the organization of these vessels into intervalvular segments. Each segment starts as an enlarged region peripherally, into which protrude the valve or valves of the segments behind, and terminates centrally in a typically bicuspid valve that projects into the sinus of the segment ahead. In the smaller venules this segmental organization is not well developed but their walls exhibit contractions. These actions, especially the segmental ones which serve as pumps to aid in venous return are probably necessary during flight. Venous vasomotion has been observed in all species where it has been looked for and is more vigorous if the species is a strong flyer with well developed muscles in the wing membrane. A small species observed in Australia, *Nyctophilus timoriensis*, with a fluttering type flight, has almost no muscle in the wing membrane and exhibits very limited venous vasomotion.

This action is usually described as a spontaneous activity and it continues unchanged in denervated wings[1,2]. However, unlike heart action, it is not always present even when the blood vessels are carrying an adequate flow. In preparations where the wing is held immobilized for direct microscopic observations, one frequently finds little or no vasomotion even in the larger segmentally organized veins. Therefore, the action must either require certain conditions or is a response to some stimulant. As the responding element in the wall is vascular smooth muscle, their activation may be similar to the excitation of other vascular muscle. Such activation has been ascribed to either a change in the cell's membrane permeability that is reflected as a change in the resting membrane potential, or the entrance of some excitant into the cell such as may occur when catecholamines activate a specific receptor in the membrane. Activations of the excitation-contraction mechanism may arise from various modifications of the smooth muscle cell or its equilibrium with the immediate environment. Thus, neural actions may excite or mechanical deformations such as stretching the cell, can induce contraction. Also a wide variety of chemical agents can alter the excitability either in a positive or negative direction[3].

Nicoll and Speden[4] studied the transmembrane potentials of a muscle cell, or cells, in 30–40-µm venule exhibiting spontaneous contractions. This was done by inserting, without anesthesia, a microelectrode directly into a cell through a minute opening in the epidermis. A vigorous blood flow continued during the measurement and the entire venule was exhibiting significant wall movements. As a conse-