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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2 J.G. Peristiany and H. Huggel, Proc. Int. Congr. comp. Physiol, p.591. Ed. L. Bolis, K. Schmidt-Nielsen and S.H.P. Maddrell, North Holland Publ. Co, Amsterdam 1973.
3 H. Huggel, B. Johansson and J. Peristiany, Experientia *29*, 977 (1973).
4 J.G. Peristiany, H.C. Lane and H. Huggel, Revue suisse Zool. *78*, 1202 (1971).
5 J. Axelsson, B. Wahlström, B. Johansson and O. Jonsson, Circulation Res. *21*, 609 (1967).
6 J. Axelsson, J. Physiol., Lond. *158*, 381 (1961).
7 B. Surawicz and L.S. Gettes, Circulation Res. *12*, 415 (1963).
8 G. Siegel, H. Roedel, J.N. Nolte, H.W. Hofer and O. Bertsche, in: Physiology of smooth muscle, p. 19. Ed. E. Bülbring and F. Shuba. Raven Press, New York 1976.
9 D.K. Anderson, Fedn Proc. *35*, 1294 (1976).

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.

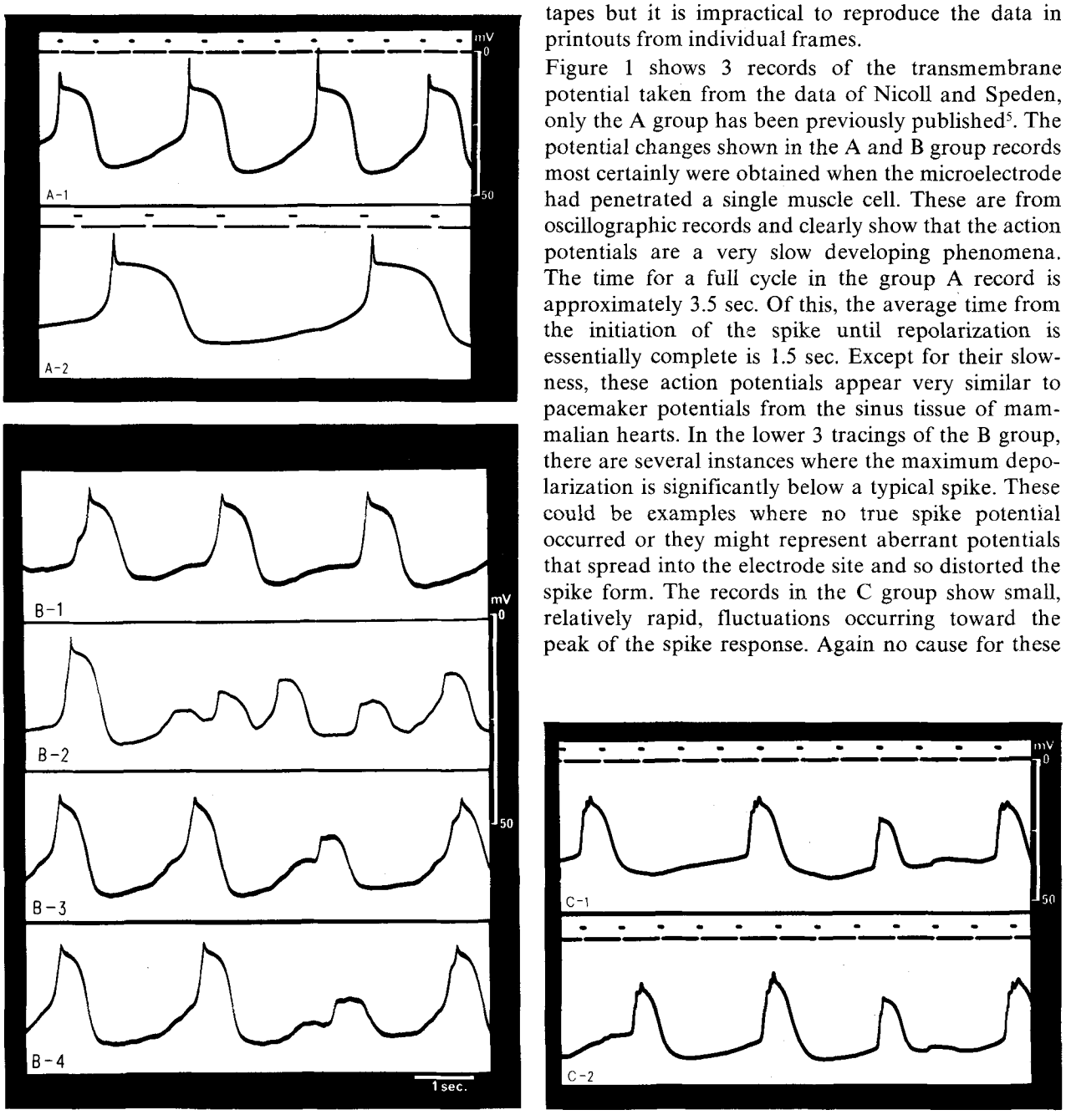
This action is usually described as a spontaneous activity and it continues unchanged in denervated wings[1,2]. However, unlike heart action, it is not always present even when the blood vessels are carrying an adequate flow. In preparations where the wing is held immobilized for direct microscopic observations, 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-

quence of the wall motion, the microelectrode was easily dislodged and it was not possible to follow the transmembrane potentials for any extended period. The longest any electrode remained in the cell was 15 min. Even then electrode contact with the cell was sufficiently large that leakage occurred and the resting membrane potential gradually fell to zero. Because of the brief contact, it has not been possible to change the chemical environment of the cells while making these potential measurements. Thus the role of any ion in determining the membrane charge or exciting contraction may only be deduced from indirect studies where the contraction is assumed to indicate excitation.

This assumption that contraction follows depolarization is based on some observations where the cell membrane potential was measured simultaneously with microscopic records of the wall contractions. The records were made on television tape and both phenomena were superimposed on the tape record. In every case the mechanical contraction followed immediately after the spike of the potential change and lasted throughout the depolarization phase. This data is available on 16-mm films made from the television tapes but it is impractical to reproduce the data in printouts from individual frames.

Figure 1 shows 3 records of the transmembrane potential taken from the data of Nicoll and Speden, only the A group has been previously published[5]. The potential changes shown in the A and B group records most certainly were obtained when the microelectrode had penetrated a single muscle cell. These are from oscillographic records and clearly show that the action potentials are a very slow developing phenomena. The time for a full cycle in the group A record is approximately 3.5 sec. Of this, the average time from the initiation of the spike until repolarization is essentially complete is 1.5 sec. Except for their slowness, these action potentials appear very similar to pacemaker potentials from the sinus tissue of mammalian hearts. In the lower 3 tracings of the B group, there are several instances where the maximum depolarization is significantly below a typical spike. These could be examples where no true spike potential occurred or they might represent aberrant potentials that spread into the electrode site and so distorted the spike form. The records in the C group show small, relatively rapid, fluctuations occurring toward the peak of the spike response. Again no cause for these



Transmembrane potentials recorded in vivo from spontaneously active venular muscle cells in the bat wing. Time marks=1-sec intervals. See text for discussion.

variations are known but they might represent multiple spikes closely grouped together from more than one cell. This could occur if the electrode happened to enter more than a single muscle cell. Although detailed histology of the relationship of muscle cells with adjacent cells is not known, early studies using methylene blue staining showed that the individual cells were sufficiently long to wrap more than 1 turn around these small venules and that adjacent cells appeared to overlap at their ends.

The problem mentioned above, of keeping a microelectrode in one of the muscle cells for any length of time, makes it impossible to directly detect the effect of any ion or other substance on either the resting or action potentials. The observation that contraction is always preceded by an action potential suggested that some indiregt information about the excitation might be gained by altering the environment of a small segment of a venule while observing the changes in contraction that occurred. It is, of course, also possible that any change in contraction is due to a modification of the contractile mechanism itself. Since these venular cells are similar to other vascular smooth muscle, comparison of results obtained in other vascular beds can help decide whether any contraction change represents an alteration of the excitation or the contraction mechanisms.

An acute change in the composition of the interstitial fluid around the venule is produced by insertion of a micropipette with sharpened tips ranging in their opening from 6 to 10 µm and loaded with the test solution. This is done with a pipette held in a sliding micromanipulator so the tip can be exactly positioned in relation to the venule under microscopic observation. Pressure in the micropipette is controlled by a micrometer drive and volumes as low as 0.5 µl can be accurately infused. The usual infusion volume ranges from 2.5 to 15 µl. Interstitial pressures are elevated but outward diffusion of the infusate allows conditions to return to normal within 10–30 sec as judged from the flow within the observed vessels. The technique uses unanesthetized bats[6].

As noted, the circulation is frequently rather poor in many of the arcuate microcircular beds and the infusion of even small volumes of most solutions initially produces relaxation of the arterioles and a marked increase in blood flow that will last from 2 to 30 min depending on the solution. This reaction is termed 'washout' and from the action of studies with Ca^{++} may represent a brief dilution of interstitial calcium ions. Specific responses to a particular ion or substance must be evaluated as a change beyond the 'washout' and one starting within 5 min of the infusion. It may last much longer, but since the vessels are carrying an adequate blood flow the venular behavior usually reaches a new steady state in 10–15 min. The modification is evaluated in terms of both the frequency and magnitude changes of vasomotion. Although semiquantitative data can be obtained from analysis of television records, the summaries below represent subjective observations.

Both lithium and choline replace sodium in the infusate without producing any significant modification in venular activity. Also changing the infusate osmotic value by dilution or addition of sucrose between approximately 225 and 350 mosmol have only minor effects on vasomotion. Probably related to the fact that the venules are carrying reasonable blood flow during the acute tests, changes within physiological ranges in P_{02}, P_{C02}, pH, and protein concentration may modify phasic durations of the vasomotion but do not initiate excitation.

It is generally accepted that calcium ions are necessary for contraction in smooth muscle. These venular structures conform to this calcium requirement. If calcium in the external solution is removed by either washing with Ca-free infusates or bound by added EDTA all activity is inhibited. Further, if external calcium ions are prevented from entering the cell by the use of verapamil, contraction is blocked even when vasoreactive agents are infused. Thus there must be little or no calcium ions available from an intracellular pool. Thus, calcium is necessary for contraction but it probably plays no role in excitation itself. Increase in potassium ion (20 mM/l) will enhance both frequency and magnitude of vasomotion provided adequate calcium is available but has no effect on the venules otherwise. This is probably related to a role of potassium ions in establishing the resting membrane charge.

Both epinephrine and norepinephrine in extreme dilutions (5.6×10^{-11} M), increase first the contraction frequency and with greater concentrations increase the degree of contraction. Isoproterenol on the contrary first inhibits contractions and then induces an extremely exaggerated response of both frequency and magnitude. The mechanism for this delayed intensification is unknown. Phentolamine (4.5×10^{-5} M), an alpha blocker, gradually depresses spontaneously active venules and finally inhibits them completely. The inhibition lasts about 30 min. Although additional study is essential, these results indicate that adrenergic excitants may be the stimulant of these spontaneous vasomotions even though central nervous activity is not involved.

1 P.A. Nicoll and R.L. Webb, Ann. N.Y. Acad. Sci. *46*, 697 (1946).
2 M.P. Wiedeman, Circulation Res. *5*, 641 (1957).
3 P.C. Johnson, Circulation Res., suppl. *14/15*, 1 (1964).
4 P.A. Nicoll and R. Speden, Physiologist *15*, 227 (1972).
5 P.A. Nicoll, Immun. Chem. *12*, 511 (1975).
6 P.A. Nicoll and R.L. Webb, Angiology *6*, 291 (1955).