tical vibrations), and it sensitizes the A fibers or their premotor cells to wind-angle inputs. Neurons employed in other specific flight orientations may be similarly affected by the flight motor (*4, 18*). Therefore, this central oscillatory network is multifunctional.

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### References and Notes

1. T. Weis-Fogh, *Proc. Roy. Soc. Ser. B* **239**, 459 (1956).
2. J. M. Camhi, *J. Exp. Biol.* **50**, 335 (1969); *ibid.* **52**, 519 (1970); T. Weis-Fogh, *Nature* **164**, 873 (1949).
3. I. Waldron, *J. Exp. Biol.* **47**, 213 (1967).
4. J. M. Camhi, *ibid.* **52**, 519 (1970).
5. Ventral abdominal muscles and at least one fiber in the abdominal nerve cord are active in flight rhythm [I. Waldron, *ibid.* **47**, 201 (1967)].
6. Most of the yaw-correcting deflection of the abdomen occurs at this joint (*4*).
7. Also called the "tergal nerve of the first abdominal segment" [F. O. Albrecht, *The Anatomy of the Migratory Locust* (Athlone, London, 1953), p. 80].
8. E. H. Slifer and L. H. Finlayson, *Quart. J. Microsc. Sci.* **97**, 617 (1956).
9. These two fiber groups are more properly viewed as two extremes of fibers having a continuous range of properties (*10*).
10. M. Hinkle and J. H. Camhi, *Science* **175**, 553 (1972).
11. Relative sizes of different units were determined by comparing their spike heights in the main terminal trunk. That this measurement reflects relative sizes is indicated by two findings. (i) We could always record five large A-fiber spikes, and nerve cross sections show five large axons. (ii) There is a close correspondence between spike height and conduction velocity, a property generally strongly correlated with axon diameter.
12. Usually one or two axons show responses that are not consistent with this grouping into A and B units. A very common example is a small unit which is tonically active all during flight but is not active in the rhythmic firing pattern (Fig. 2). Embryos of this species have only seven or eight axons innervating the homologous muscle of the fourth abdominal segment. These may be B fibers, which are needed throughout life for respiration, while the flight-associated A fibers may develop closer to adulthood (M. Tyrer, personal communication.)
13. The unusually low frequency of wingbeats of 10 sec$^{-1}$ rather than the normal 17 sec$^{-1}$ (*1*) resulted from the necessity to remove the wings or wax them in place during recording periods. Stretch-receptor feedback, which normally doubles the wingbeat frequency [D. M. Wilson and E. Gettrup, *J. Exp. Biol.* **40**, 171 (1963)] was thereby largely lacking.
14. Though the effects of wind angle shown here are not overwhelming, the differences are larger if one considers only the first few bursts after a wind pivot. Also, since wind angle effects are bilaterally reciprocal, the actual magnitude of the effect is twice that shown here.
15. D. M. Wilson, *J. Exp. Biol.* **38**, 491 (1961).
16. E. Gettrup, *Acta Physiol. Scand.* **59** (Suppl. 213), 46 (1963).
17. A. C. Neville, *J. Exp. Biol.* **40**, 123 (1963).
18. A similar effect of central projections in determining the responsiveness level of sensory interneurons has been reported in crayfish. The origin of the central command is unknown but complex, and is correlated with the general level of limb motor activity [C. A. G. Wiersma and T. Yamaguchi, *ibid.* **47**, 409 (1967)].
19. Supported by NIH grant NS-09083 to J.M.C. We thank W. J. Davis and G. Wyse for their helpful comments and C. Monroe for technical assistance.

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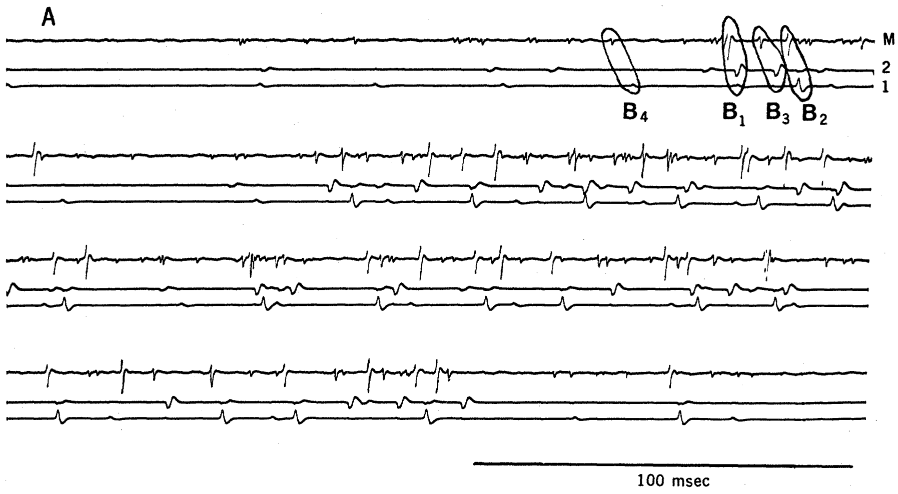
## Locust Motoneurons: Bursting Activity Correlated with Axon Diameter

*Abstract.* *A group of abdominal motoneurons in the locust can be driven by either or both of two central neuronal oscillators of different characteristic frequencies. Much of the motor cells' complex activity can be explained by the varying strengths of activation from these two rhythmic sources and by a cell-to-cell gradation in sensitivity to driving inputs, a property that is correlated with axon diameter.*

A current goal of behavioral neurophysiologists is to define the causal factors for specific patterns of output from an animal's nervous system. In most studies one or more natural or electrical stimuli are employed as inputs, and their interacting effects are studied as outputs at the motoneuron, muscle, or behavioral level. In many invertebrates, however, rhythmic motor activity can be produced independently of sensory stimuli by definable central networks, which comprise an important part of the neuronal machinery. We have studied the interaction of two such central neuronal oscillators on a specific group of locust motoneurons. We find that each of several output conditions is explained on the basis of summation of the two oscillatory signals, each with a range of possible amplitudes. The responsiveness of each motoneuron to this summed signal is correlated with its axon diameter.

The abdomen of the desert locust (*Schistocerca gregaria* ph. *gregaria*) engages in several stereotyped behaviors. During flight it (i) assumes a rigid horizontal posture about which it vibrates slightly in synchrony with the wingbeat, (ii) performs lateral yaw-correcting deflections, and (iii) performs vertical stall-avoidance deflections (*1*). At all times except approximately the first 10 seconds of flight, the abdomen also undergoes rhythmic ventilatory pumping motions (*2*). All of these behavior patterns result in part from contractions by the powerful dorsal longitudinal (DL) muscles in each



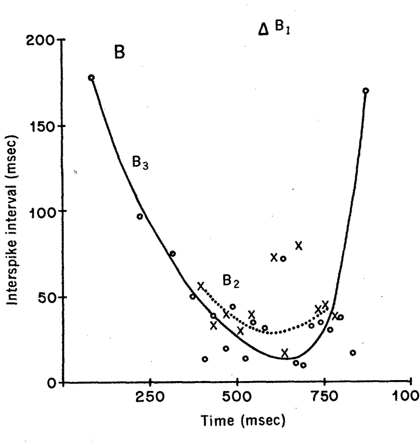


Fig. 1. Ventilatory bursting activity of abdominal B fibers. (A) A continuous record of a strong ventilatory burst was recorded from three points on the nerve: the main terminal trunk (*MT*), branch 1, and branch 2. One spike of each specifically identified B fiber is labeled. The burst included no A fibers. The recording shows that smaller spikes begin earlier and end later in the burst than larger spikes. (B) Graph of interspike interval as a function of time for units B$_1$ (△), B$_2$ (×), and B$_3$ (○) during a weak ventilatory burst. Unit B$_3$, first and last to fire, shows a smooth initial increase in frequency. Unit B$_2$, firing over the next longest interval, attains a slightly lower maximum frequency. Unit B$_1$ fires only twice and has a long interspike interval.

abdominal segment. The DL muscle of the first abdominal segment is innervated by approximately 14 motor axons. We have discussed the flight-associated activity of the five largest fibers, or A fibers (*3*). In this report we demonstrate that both the A fibers and a group of approximately seven motor axons of smaller diameter, which we call B fibers (*3*), receive synaptic input from both the central flight oscillator and a central ventilatory oscillator. The fibers can be driven by either oscillator or by both simultaneously (*4*).

Using the recording techniques described (*3*), we could identify individually all five A fibers and at least the largest four or five B fibers in most preparations. During quiescent, flightless periods, the B fibers were active in rhythmic bursts of about 20 per minute, while A fibers were silent (Fig. 1A). These bursts were synchronous with ventilatory pumping movements of the abdomen. This rhythmic bursting probably derives from a central ventilatory oscillator in the metathoracic ganglion (*5*), from which this nerve emerges, since we could record essentially identical bursts from the nerve of the totally isolated ganglion (*6*).

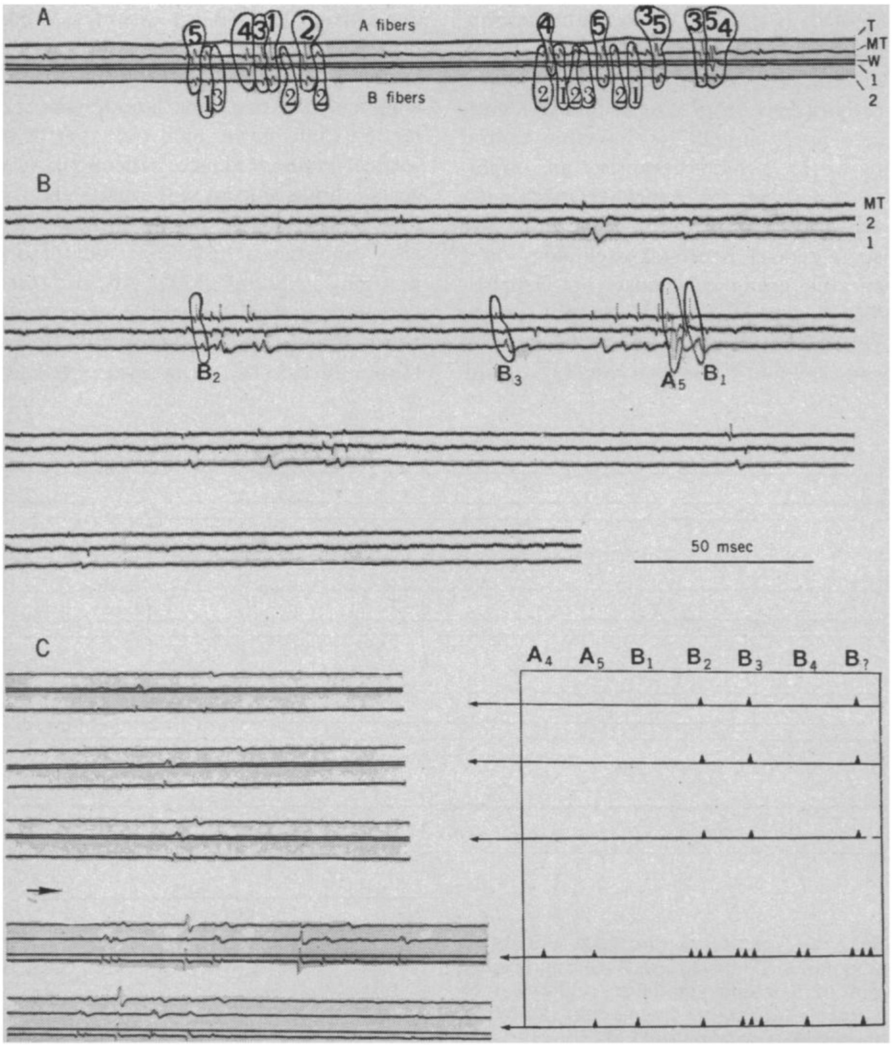


Fig. 2. Flight bursting activity of A and B fibers, recorded from the main terminal trunk (*MT*), branch 1, and branch 2. The time scale is the same for all sections. (A) Two consecutive bursts during flight are shown (*T*, thoracic flight movements; *W*, wind-angle monitor). Numbers identify spikes of specific A fibers (above trace) and B fibers (below trace). Smaller spikes are from unidentified B fibers. (B) A ventilatory burst is shown, with subbursts that presumably originate in the central flight oscillator. Consecutive subbursts show cells of increasing and then decreasing spike size, with the two largest units ($A_5$ and $B_1$) firing only in the middle subburst. (C) Consecutive flight bursts, three just before and two just after a wind pivot (arrow) from approximately 15° left to the center, were recorded from the nerve on the right side. The chart indicates spikes, within these bursts, of six identified axons and one unidentified axon, which are arranged left to right in order of decreasing fiber diameter. In general, the wind pivot evokes firing from larger fibers, which were silent beforehand, and increases the number of spikes per burst of previously active smaller units.

The ventilatory bursts of the resting locust show some characteristic features (Fig. 1A). Firing of the units of smallest diameter (*7*) usually begins and ends a burst. Occasionally the smallest one or two units fire right through the interburst interval. The larger B fibers begin later and fire most vigorously at about the burst's midpoint. In these respects, the burst behavior is identical to that shown by lobster swimmeret motoneurons (*8*). The largest B fiber, $B_1$, is often silent throughout especially weak bursts. Several of these properties are shown for three cells in Fig. 1B, which also shows another general feature: small axons often attain higher firing frequencies than do larger ones during the burst (*8, 9*). (We labeled fibers $A_1$ to $A_5$ and then $B_1$ to $B_7$ in order of decreasing size.) Finally, during periods of respiratory stress, such as after flight of several minutes' duration, the smallest of the A fibers, $A_5$ and $A_4$, often join in the ventilatory burst and give only a few spikes in the midpoint, even though all the B fibers are then firing vigorously.

The patterning of ventilatory bursts suggests that these motoneurons have as a common input the central ventilatory oscillator. The cell-to-cell differences in bursting behavior suggest that one factor determining the nature of the activity is the size principle; that is, for a given level of input common to several neurons, the sensitivity of any one neuron to that input is inversely related to the neuron's diameter (*10*).

In testing the generality of the size principle in determining firing patterns in these cells, we took advantage of the fact that the cells can also be driven by another definable oscillator, the central flight motor (*11*). When a tethered locust begins to fly in response to an airstream directed over its head, the ventilatory pumping movements cease, and most B fibers, as well as A fibers, begin bursting at wingbeat frequency (Fig. 2A). Like the A fibers (*3*), these units now appear to be driven by the central flight motor rather than by rhythmic sensory feedback, since recordings from the nerve of a locust whose thoracic and abdominal cord is totally isolated from the periphery show the same bursting when the flight motor is activated by wind over the head.

Also, the B fibers respond to wind-angle changes—just as do the A fibers—by a change in the number of spikes per burst but not in burst frequency or phase.

Not only can the A and B fibers follow either of two separate central oscillators, but they commonly follow both simultaneously and apparently summate the two rhythmic inputs (*4*). After about 10 seconds of flight, ventilatory pumping returns, and all fibers are clearly driven by two separate oscillators, the number of spikes per flight burst rising and falling with the ventilatory rhythm. The larger fibers fire only during the peak of the ventilatory bursts, although patterned into rapid flight bursts.

A particularly useful way of observing this interplay of the two oscillators upon fibers of different diameter is shown in Fig. 2B. This recording is from a locust mounted in the chamber with the cut forewing stumps waxed in an uplifted position. During quiescent, flightless periods, such animals usually give ventilatory bursts that have a subburst microstructure, as the recording shows. Since the subburst frequency was about 10 per second, in the range of wingbeat frequencies recorded under our conditions (*12*), the subbursts probably resulted from partial activation of the central flight motor. This motor receives excitatory inputs from tonic wing-stretch receptors, which are activated by wing elevation (*12*). We found that low-speed wind over the head hairs often produced similar effects, results that support our notion that the subbursts result from activity of the central flight motor.

Several inferences can be drawn from the data in Fig. 2B. (i) The central flight oscillator can produce rhythmic outputs at a proper frequency (*12*) but with a wide range of output levels. (ii) As the size principle predicts, the B fibers, smaller than A fibers and wing motoneurons (*13*), are most sensitive to low-strength activation by the flight motor, since all but one spike in the burst were from B fibers. (iii) There is a progressive increase and decrease during the ventilatory burst in the size of the fibers activated in each flight subburst. The largest B fiber, $B_1$, fires only once, in the middle subburst; while the next largest fiber, $B_2$, is present in all but the first subburst; and $B_3$, in all but the last. The number of $B_2$ and $B_3$ spikes per subburst rises and falls smoothly throughout the burst. As is not uncommon, the smallest A fiber, $A_5$, also fires in this burst, and its one spike occurs in the middle subburst.

These results lend further support to the importance of cell dimensions in determining the activity of both the B and A fibers. A final observation which lends force to this argument is that during the first 10 seconds of flight, when only the flight motor is active, some locusts show weak flight-correlated bursts that are characterized by a smaller than normal number of spikes per burst. In such cases the largest A fibers ($A_1$ to $A_3$ or $A_4$) are almost always silent, and the smaller fibers ($A_5$ and B), while active, give fewer than normal spikes per burst.

Figure 3 shows a model that suggests how the interplay of the two oscillatory inputs upon cells of different sizes (*7, 14*) produces the observed firing patterns. The model's underlying assumptions are that (i) each central oscillator independently produces a fluctuating synaptic drive, which at any moment is of similar level in all follower cells (*15*) and (ii) axon diameter correlates positively with some cell feature that determines the cell's sensitivity to this synaptic input (*10*). Sections A to D of the model are generally well supported by our findings. One prediction from this model is that since the level of flight oscillator output, which directly or indirectly drives these units, rises and falls with slopes approaching infinity (Fig. 3, B to D), there should be little or no tendency for flight bursts or subbursts to begin and end with their smallest fibers as the ventilatory bursts do. Smaller cells should, however, give more spikes per burst. Our results (Fig. 2, A and B) support these predictions (*16*), but they do not exclude other, more complicated models, such as ones involving different levels of input to different follower cells.

The apparently general role of the size principle in this integrating system suggests that it may be involved also in the strengthening and weakening of flight bursts evoked by wind-angle changes during flight (*3*). According to Fig. 3E, there should be a particular combination of wind angle and of activation level of the flight motor which would cause B fibers to be active but A fibers to be silent during

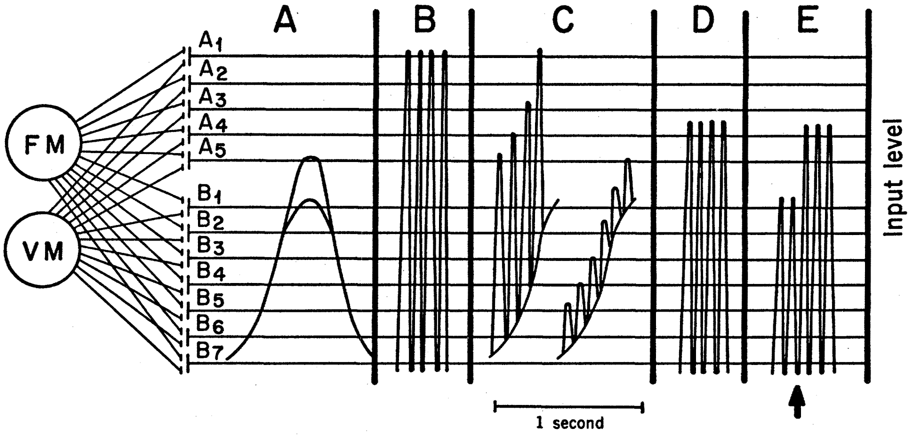


Fig. 3. A model of the interplay of two oscillators on motoneurons of different sizes. For simplicity, all motor cells are presumed to receive an approximately equivalent input from the flight motor (*FM*) and also from the ventilatory motor (*VM*), which are both in the metathoracic ganglion from which the axons emerge. The specific axons, represented as horizontal lines, are ordered according to fiber diameter, with the largest at the top. This order also represents the sensitivity to inputs impressed simultaneously upon all the cells from one or both oscillators. Virtually all of the behavior expected from the model is observed (*15*). (A) Two levels of *VM* input are shown. Each evokes a burst in which larger fibers fire later and more briefly, and attain lower maximum frequencies than smaller ones. The burst represented by the higher curve includes fiber $A_5$, which is absent from the burst shown by the lower curve. (B) A strong *FM* input evokes high-frequency bursts in which all units fire. (C) Simultaneous *VM* and *FM* activation is shown in locusts during prolonged flights (left) and in flightless locusts with forewings elevated (right). In both cases each succeeding flight burst or subburst includes firing from larger units and more spikes from each active unit. (D) Weak *FM* activation evokes bursts that have no spikes from cells $A_1$ to $A_3$ and fewer spikes from other units than are seen in B. (E) Bursts increase in response to ipsilaterally directed wind pivot about the head. The A fibers, silent before the pivot (arrow) are reactivated afterward, and firing frequency of B fibers increases.

flight. A wind-angle change in the appropriate direction would then restore activity of A fibers and increase that of B fibers. Figure 2C shows an example of just such behavior. More precise testing of this scheme has not proved fruitful due to the apparent closeness of the threshold levels of units of like size and to neuronal noise in the system. These findings argue, however, that wind-angle change has a common effect on all the follower cells in this population. It seems reasonable that wind-angle inputs may produce their effects at some common premotor point rather than at each of the 12 separate motoneurons.

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### References and Notes

1. J. M. Camhi, *J. Exp. Biol.* **52**, 519 (1970); *ibid.* p. 533.
2. P. L. Miller, *ibid.* **37**, 264 (1960).
3. J. M. Cambi and M. Hinkle, *Science* **175**, 550 (1972).
4. Simultaneous drive of a motoneuron by two different oscillators has been observed in crickets [D. Bentley, *J. Insect Physiol.* **15**, 677 (1969)].
5. P. L. Miller, *J. Exp. Biol.* **37**, 224 (1960).
6. It was sometimes necessary to stimulate bursting in the isolated ganglion by perfusion of the saline with $CO_2$. The $CO_2$ receptors in this ganglion have indirectly been shown to stimulate a local ventilatory oscillator (*5*).
7. The justification for scaling axon diameters by spike height (*3*), relies upon histological examination and measurements of spike-conduction velocity.
8. W. J. Davis, *J. Neurophysiol.* **34**, 274 (1971).
9. This generalization holds primarily for the larger B fibers and even then is not absolute.
10. Motoneuron size is an important and sometimes major determinant of the firing properties of vertebrate motoneurons [see E. Henneman, G. Somjen, D. O. Carpenter, *J. Neurophys.* **28**, 560 (1965)] and some crayfish motoneurons (*8*). The size principle is generally interpreted in terms of an inverse relation between postsynaptic cell size and the cell's input resistance [B. Katz and S. Thesleff, *J. Physiol. London* **137**, 267 (1957); see also (*8*). Since in our experiments axon diameter rather than dendrite diameter is correlated with cell physiology, this suggests a correlation, within each cell, of the diameter of the axon with that of the dendrites, the presumed synaptic loci. We are unable to rule out alternative correlations between axon diameter and presynaptic properties or some dendrite property other than diameter, although dendrite diameter seems the simplest possibility.
11. D. M. Wilson, *J. Exp. Biol.* **38**, 491 (1961).
12. The frequency of the flight oscillator in the absence of input from stretch receptors, one located on each wing, is about half the normal wingbeat frequency of the intact locust (17 per second). Each of the four stretch receptors elevates this frequency in about equal increments [D. M. Wilson and E. Gettrup, *J. Exp. Biol.* **40**, 171 (1963)]. Thus input from only the forewing stretch receptors, in a greatly adapted condition [E. Gettrup, *ibid.*, p. 323; H. Pabst and S. Schwartzkopff, *Z. Vergl. Physiol.* **45**, 369 (1962)] could be expected to produce a flight motor frequency of about the observed 10 per second.
13. A. C. Neville, *J. Exp. Biol.* **40**, 123 (1963).
14. This is an elaboration of model presented to explain bursting in lobster swimmeret motoneurons (*8*).
15. By level of synaptic drive we mean the magnitude of some synaptic event just prior to the step influenced by individual motoneuron properties. Specifically, if dendrite size is the factor causing individual motoneuron differences, then level could be taken to mean strength of synaptic current.
16. One deviation from perfect compliance with the model is that during prolonged flights, the smallest two or three B fibers are often silent. Another factor is that during simultaneous flight and ventilatory oscilator activity, the flight interburst intervals often show no activity (Fig. 2B) rather than the low activity predicted by Fig. 3C. This silence, which may reflect inhibition from antagonistic elements of the flight motor, will be discussed (M. Hinkle and J. M. Camhi, in preparation).
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## Sieving Behavior of a Series Membrane System

The purpose of this technical comment is to clarify a recent report by Bresler *et al.* (*1*). The illustrative example that Bresler *et al.* presented is carefully qualified, but, as it stands, it could be misleading. We shall show here that it is correct to require a removal mechanism in the steady state, but that it is erroneous to conclude that only the first membrane in an array generally determines the array's sieving characteristics. We shall also show that the arguments in (*1*) do not call into serious question present-day concepts of the interactions involved in membrane separations.

In the steady state, all concentrations and all fluxes in the system are defined to be constant in time. The steady-state concentrations within any compartment and the steady-state fluxes between any pair of compartments are determined by the properties of the separating membranes and the boundary conditions of the problem. For series membrane systems, the steady-state fluxes through each membrane are the same and, like the compositions in the internal compartments, have values determined by the properties of all the membranes in the system, the applied hydrostatic pressure difference, and the composition of the ambient baths.

No conceptual difficulty is encountered in trying to understand the constancy of the composition of the solutions in the internal compartments, since the fluxes of solute and solvent entering the compartment are the same as those of the solute and solvent leaving the compartment. In a sieving experiment, there is also no difficulty in understanding the constancy of the concentration of filtrate, $C_s^B$; it is made up of what has passed through the membrane assembly. Thus

$$C_s^B = J_s/J_v$$

where $J_s$ and $J_v$ are the solute and volume fluxes, respectively, each constant in time.

If the volume of the filtrand compartment, $V_A$, is infinite, its composition can remain at the steady-state value, $C_s^A$, indefinitely; otherwise, it will become richer in solute with the passage of time. Hence, the use of steady-state concentrations in describing flow through a series of membranes implies either that $V_A$ is so large that $C_s^A$ is substantially unchanged during the period of time in which the steady-state description is proposed to apply, or that the accumulating solute is removed from the filtrand in some unspecified fashion. This is the "removal" of which Bresler *et al.* write. Since the solute is, in this sense, removed from the filtrand, and inasmuch as all material passing through the first membrane in the series passes through to the filtrate ($J_s$ and $J_v$ are the same for each membrane), the statement that "all effective sieving still occurs at the first membrane" is merely a rephrasing of the assumptions implicit in the definition of the steady state.

It is incorrect to deduce, however, that the sieving behavior of a series membrane system is determined by the structure of the first membrane alone. It is as reasonable to try to pour water through a funnel faster than it can discharge fluid and then to deduce from the site of the overflow that the principal resistance to flow through a funnel is at the wide end. The site of a cause is not a priori the site of its effect. Consider, for example, the case of a membrane M with a nonzero rejection coefficient, located downstream of another membrane N. There is no accumulation of solute between the two; rejection is always from the filtrand. Yet the overall sieving characteristics of the system, including the concentration of the filtrate