LETTERS TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in the Biophysical Journal *are invited. Receipt of such letters will not be acknowledged but those containing pertinent scientific comments and scientific criticism will be published.]*

Dear Sir:

A study of the spike potential of sartorius muscle fiber was recently published in this *Journal* (1). Propagated responses were recorded in a phase plane to facilitate extraction of several shapes and magnitude parameters which quantitatively describe the responses and which are related to the membrane currents and conductances (2). The 1963 publication was concerned with (*a*) the relationships between various spike parameters and V_s, the spike height, and (*b*) the effects of tris(hydroxymethyl)aminomethane buffer on these parameters. Figure 3 of reference 1, for example, indicated that the magnitudes of several parameters decreased at large V_s ($>$ 130 mv). Although this was unexpected, the experiments could not be ruled invalid. In addition, Table II of reference 1 showed a marked, depressive action of tris buffer on the spike parameters as compared to phosphate buffer. However, Dr. Allen Issacson has since reported to the author that this buffer substitution has *no* effect on the mechanical response of the sartorius muscle. Since it has not been demonstrated how the spike participates in the initiation of contraction, this report led us to a close reexamination of the tris effect. New experiments were performed with consistently *different* results from those already published (compare the previous Table II with the present Table I). An extensive search was then made through the old data for information which might resolve this disturbing result. In the process, a subsidiary event has appeared and is the main subject of this note.

TABLE I

AVERAGE AND STANDARD ERROR OF ACTION CURRENT PARAMETERS

V_s *mv*	V_n *mv*	V_r *mv*	V_{Na} *mv*	V_i *mv*	$\dot{V}+$ *V/sec.*	$\dot{V}-$ *V/sec.*	k_r *msec.*$^{-1}$	g_e *mmhos/cm*^{2}	g_i *mmhos/cm*^{2}	Buffer
124	19	89	44	77	485	116	7.2	18	140	Phosphate
(1.2)	(0.8)	(1.1)	(0.8)	(1.1)	(18)	(4)	(0.2)	(0.5)	(13)	51 fibers from 8 experiments
126	23	91	42	79	481	105	7.0	17	160	Tris
(0.6)	(0.7)	(1.5)	(1.2)	(1.4)	(11)	(8)	(0.2)	(0.4)	(16)	48 fibers from 8 experiments
Shift per cent*										
+0	+21	+3	−4	+3	0	−9	−4	−5	+12	

*Based on percentage changes occurring within each experiment. These may not agree with actual data averages because of rounding off errors.

Several uncontrolled variables (*e.g.* frog supply, laboratory temperatures, apparatus) which might conceivably affect the experimental results were first examined, but none appeared relevant. Eventually, a month-by-month plot of all spike parameters was constructed. To reduce variations associated with the resting potential, data were restricted to those experiments conducted in a "standard" Ringer's fluid (110 mM NaCl, 2.5 mM KCl, 1.0 or 2.0 mM $CaCl_2$, 1.5 mM phosphate at pH 7-7.5).

In Fig. 1 several variables are plotted on a 2 year chart. Each point represents an average determined for each muscle of the series from measurements on at least five separate fibers. The slight increase of V_r data in 1963, as compared to 1962, was traced to a minor change during August, 1962, in the $CaCl_2$ concentration of the Ringer's fluid,

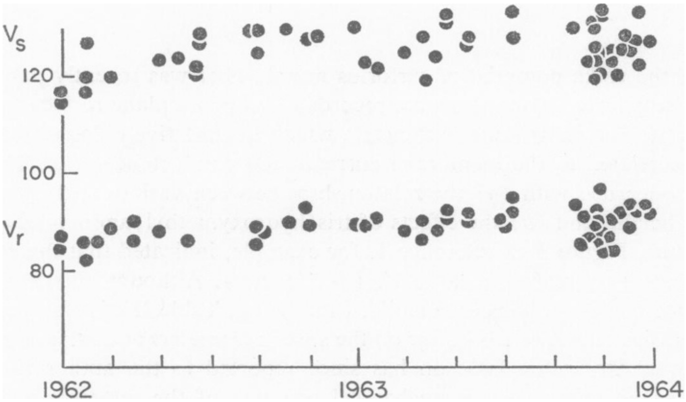

FIGURE 1*a* The resting potential V_r and spike potential V_s in millivolts plotted against experiment date. No seasonal cycle is apparent. The early rise in V_s was caused by apparatus improvements, while the upward jump of V_r in the Fall of 1962, occurred when $CaCl_2$ concentration was slightly changed (see text).

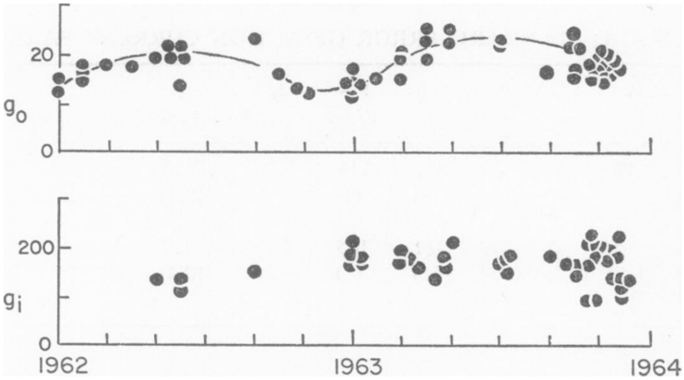

FIGURE 1*b* The outward conductance of g_o and inward conductance g_i in mmhos/cm^2. The strikingly consistent cycle for g_o extends over 2 years, whereas *none* is apparent for g_i.

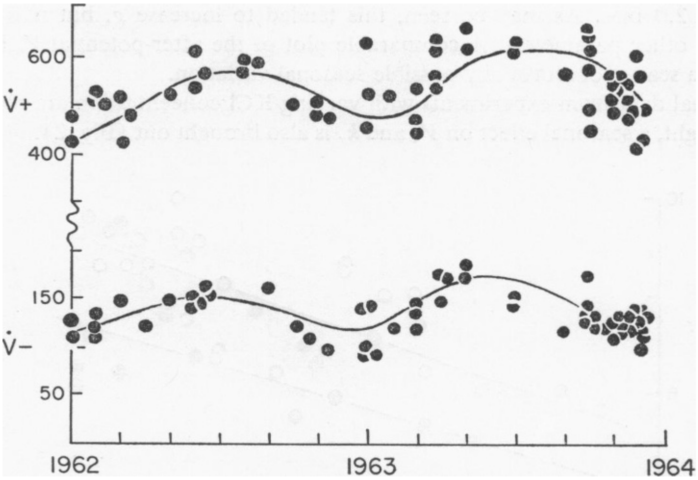

Figure 1*c* The maximum rate of rise $\dot{V}+$ and fall $\dot{V}-$ of the spike in V/sec. (note the scale change). These parameters are related to the maximum inward and outward currents, respectively. The $\dot{V}-$ cycle probably reflects seasonal changes in the associated conductance g_o. The $\dot{V}+$ cycle is probably linked with seasonal shifts in the ionic gradient, since g_i seems unaffected.

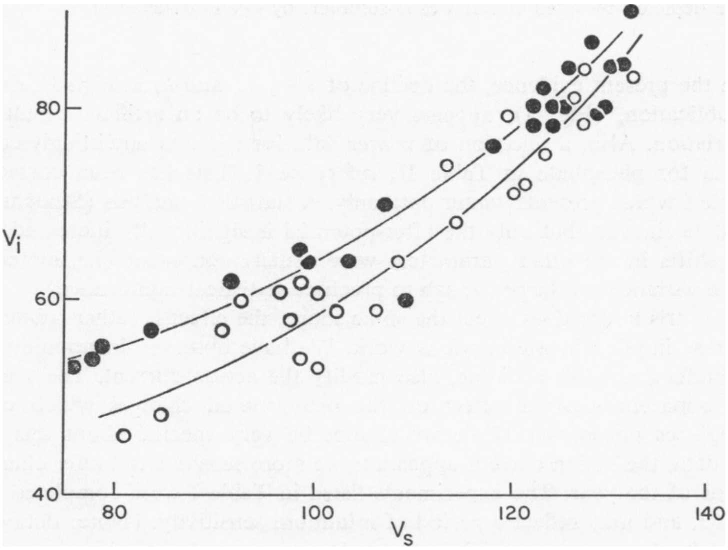

Figure 2*a* The potential of the first inflection point in millivolts plotted against the spike height V_s. V_i is closely related to the actual firing potential as measured from V_r. Note that the threshold for any given spike is larger during the winter season (●) than during the summer (○).

from 1.0 to 2.0 mM. As may be seen, this tended to increase g_t but had a negligible effect on the other parameters. A comparable plot of the after-potential V_n is not shown since the data scatter obscures any possible seasonal variation.

If additional data from experiments with varying KCl concentration are plotted against the spike height, a seasonal effect on V_t and k_r is also brought out (Fig. 2).

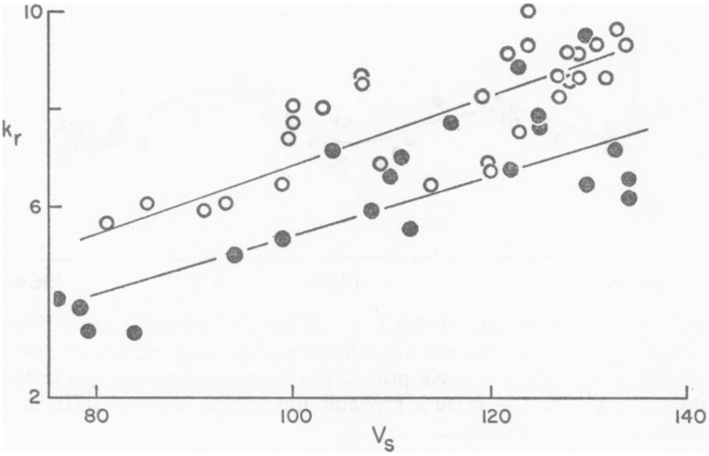

FIGURE 2*b* The foot of the action potential rises exponentially in time according to the expression $V \propto \exp(k_r t)$. Here k_r in msec.$^{-1}$ is plotted against the spike height V_s. The variable k_r is also equal to the factor $a/2RC\theta^2$, of the cable equation. Winter data are depicted by filled circles (●); summer, by open circles (○).

Based on the present evidence, the decline of $\dot{V}-$, k_r, and k_f at high V_s shown in the previous publication, (Fig. 3), appears very likely to be an artificial result caused by seasonal variation. Also, a selection of *winter* data for tris was unwittingly compared to *summer* data for phosphate in Table II, reference 1. This has been corrected in the present Table I which presents winter data only. A statistical analysis (Student's *t* test) of the recent data showed that only the after-potential is significantly increased by tris. Although the shifts in the other parameters were usually consistent (*i.e.* increased or decreased), the variance was large enough to preclude statistical significance.

Thus, while tris buffer does affect the spike shape, the effect is rather subtle and not as marked as was implied in the previous work. We have observed in unpublished studies that other buffers, notably histidine, also modify the action current. The relevant point here is an apparent seasonal effect on the proportional changes which occur when histidine replaces phosphate. While we cannot be very specific about this because of incomplete data, the action current appears to be more sensitive to buffer changes during some seasons of the year. The experiments listed in Table I were completed during December, 1963, and may reflect a period of minimum sensitivity. (Note: data on tris buffer obtained in the summer of 1957, fall below summer 1963, phosphate data, hence Table II of the previous paper may be correct in principle.)

The present observations cannot be attributed to the effects of hibernation alone, since all animals are kept indoors. Although water tank temperature fluctuates between 24°C (summer) and 17°C (winter), this is well above the temperature at which hibernation is initiated (V. Popovic, personal communication). Experiments are conducted after at least 1 hour of equilibration at room temperature, which usually ranges during the year between 22°C to 24°C. Although Q_{10} of some parameters is as high as 2 to 2.5 (unpublished data) this 2°C shift is not enough to account for the observed changes in the outward current and conductance.

REFERENCES

1. JENERICK, H., 1963, Phase plane trajectories of the muscle spike potential, *Biophysic. J.*, **3**, 363.
2. JENERICK, H., 1964, An analysis of the striated muscle action current, *Biophysic. J.*, **4**, 77.

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