again in early October, whereas the Southern type spawns predominantly in June and July.

The evidence suggests that three distinct forms of *R. pipiens* occur in Arizona. All three bear consistently different hemoglobin phenotypes which are in turn correlated with morphological differences; in cases of geographic replacement of one form with another, the transition is discontinuous with respect to these features. Furthermore, the small number of hybrids detected in regions where two forms are sympatric indicate either that mating is preferential for the conspecific type or that, if mating is random, hybrid survival is very low.

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4. The Lowland type is widely distributed in Arizona at elevations between 300 and 1100 m, with one population at approximately 1600 m.
5. The hemoglobin band closest to the cathode (Fig. 1) is probably due to dimerization and is not seen in Lowland samples first treated with β-mercaptoethanol.
6. The Northern type has a white narrow stripe along the upper lip, from a point a short distance posterior to the angle of the jaw to the tip of the snout. In both the Lowland and Southern types any narrow pigmented lip stripe extends anteriorly no further than a point below the eye.
7. All three forms have been heard calling during fieldwork and can be readily differentiated by ear alone.

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Electrogenic Sodium Pump and High Specific Resistance in Nerve Cell Bodies of the Squid

Abstract. *An electrogenic sodium pump contributes to the membrane potential in squid nerve cell bodies, imparting a temperature dependence to the resting potential that is abolished by strophanthidin. The existence of a potential produced by the pump in the soma but not the axon is correlated with a higher membrane resistance in the soma. Thus, membranes from different parts of a neuron may have functionally significant differences in resistance.*

The giant stellar axon of the squid arises by a fusion of the processes of several hundred small nerve cell bodies (*1*). Although the squid axon has been a classic preparation for the study of the ionic events underlying nervous electrical activity there has been little interest in the cell bodies, since the somata are not electrically excitable and do not form synaptic contacts (*2*).

There are, however, some interesting questions concerning the relative electrical properties of somata and axons that can be examined in these cells. In several molluscan nerve cell bodies an electrogenic Na^+ pump is responsible for generation of a sizable fraction of the resting membrane potential and imparts a temperature dependence to the resting membrane potential that is much greater than would be predicted from the passive electrical properties of the membrane (*3*). In the squid axon the resting membrane potential is not determined by an electrogenic pump (*4*) and is not temperature dependent to any marked extent (*5*). It has been proposed that the difference between the molluscan nerve cell bodies and squid axon is not in the character or rate of the Na^+ pump but rather is due to a greater specific membrane resistance, R_m, in the somata (*6*). Whereas R_m for squid axon is about 1000 ohm cm^2 and the membrane time constant, τ, is about 1 msec (*7*), in both *Aplysia* (*6*) and *Anisodoris* (*8*) somata R_m is higher by a factor of about 100 and τ has a value that may be as great as several hundred milliseconds.

Intracellular recordings were made from the cell bodies of neurons in the stellate ganglion of the squid, *Loligo pealii*, with glass micropipettes filled with 3*M* KCl and having resistances of 15 to 30 megohms. Most recording techniques were as in (*6*). The reference electrode consisted of a heavily and freshly chlorided silver wire. Junction and tip potentials were kept as small as possible and the temperature dependence of the d-c level when the electrode was in seawater was always monitored. Ganglia were dissected under flowing seawater with entering and exiting nerve bundles tightly tied. The ganglion was pinned to a soft resin in a Lucite chamber and perfused with oxygenated seawater. Temperature was changed by flowing the perfusate through an ice bath and was monitored through a small thermistor near the ganglion.

A total of 34 cells were studied in some detail. Many other cells were penetrated but were injured so badly that useful information was not obtained. The cell bodies studied were between 50 to 150 μm in diameter; those of the giant fiber lobe tended to be considerably smaller than many cells found in other parts of the ganglion. Resting membrane potentials ranged from 30 to 60 mv (average 43 mv). In agreement with Miledi (*2*) most cells were electrically silent and on depolarizing current pulses would show only very small all-or-nothing action potentials (2 to 15 mv). Occasionally cells were found that showed small spikes after penetration, presumably as a result of the injury. These small spikes probably result from electrotonic propagation of potentials originating in an axonal process (*2*).

Figure 1 shows results of one experiment with a recording from a cell not in the giant fiber lobe. In normal seawater the resting membrane potential reversibly increased by 10 mv when the temperature was increased from 5° to 20°C. This increase in the resting membrane potential was abolished and even reversed after exposure to strophanthidin, where now there is a 5-mv depolarization with a similar temperature change. These results suggest that strophanthidin abolishes a current which generates a potential that is the sum of the hyperpolarization seen in the control and the depolarization seen in presence of strophanthidin. In this case the total potential generated is 15 mv for a 15°C temperature change. We conclude that an electrogenic Na^+ pump contributes to the resting membrane potential in squid somata as in a variety of other molluscan nerve cell bodies (*3*). The depolarization on warming in the presence of the glycoside is also similar to what is seen in other preparations and probably reflects a greater temperature dependence of the passive Na^+ than K^+ conductance (*6*, *9*).

The greater role of the Na^+ pump in generation of the resting membrane potential in squid somata than in axons might reflect a greater pump rate, a different pump mechanism such as a larger coupling ratio of Na^+ to K^+ transported, or simply a greater mem-

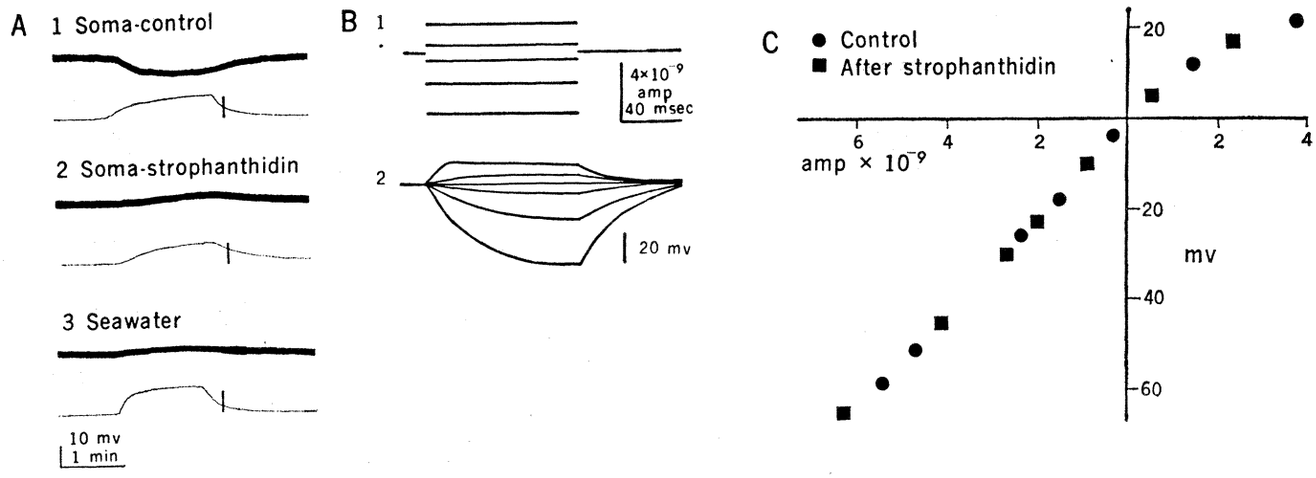

Fig. 1. Electrical properties of the squid nerve cell body. (A1) Intracellular recording from a soma shows that the membrane potential (upper trace) increases reversibly by 10 mv when temperature (lower trace) is raised. (A2) The same temperature change results in a 5-mv depolarization 6 minutes after exposure to $10^{-7}M$ strophanthidin. (A3) The temperature variation of d-c level when the electrode is in seawater but not a neuron. Temperature calibration marks indicate 5° and 20°C at the lower and upper ends of the vertical line, respectively. (B and C) The voltage changes occurring on application of constant current pulses of varying amplitudes and the current-voltage (*I-V*) relation thus obtained. Data are from the cell illustrated in A1 and were obtained at 10°C. The membrane time constant, τ, is equal to the time required for the voltage trace to reach 0.63 of its maximum value, and is equal to 27 msec. The *I-V* relation is linear over the most of the voltage excursion except for the delayed rectification seen on depolarizing pulses and is not changed in presence of strophanthidin.

brane resistance. Although differences in pump kinetics are difficult to assess the membrane resistance is easily measured. Figure 1, B and C, shows voltage changes associated with constant current pulses and the current-voltage (*I-V*) relation, respectively (*10*). The input resistance of this neuron is 10 megohms. The τ can be measured from the constant current pulses and is equal to the time required for the voltage trace to reach 0.63 of the steady-state value. In this cell τ has a value of 27 msec. Of 11 neurons in which complete *I-V* relations were obtained the average input resistance was 9 megohms (range 3 to 15) and the average τ was 19 msec (range 13 to 27). In general there was a direct correlation between the magnitude of the input resistance and the strophanthidin-sensitive potential change observed on warming. In these 11 neurons the average hyperpolarization on warming from 5° to 20°C was 7 mv (range 0 to 10 mv); in four of these cells exposed to strophanthidin, the average total change in membrane potential at 20°C was 13 mv (range 12 to 15 mv).

For squid axon, τ has a value of about 1 msec. Thus τ in the cell body is very much longer than that of the axon. The greater value for τ can be due to a greater R_m or membrane capacitance (C_m) or both, since $\tau = R_m C_m$. The C_m appears to be reasonably constant for most biological membranes and usually has a value of about 1 μfarad/cm^2, except in special tissues like muscle which have complicated membrane specializations (*8, 11*). If we assume C_m to be 1 μfarad/cm^2 for the squid somata the specific resistance of the membrane of the neuron illustrated in Fig. 1 can be calculated directly from τ, and is equal to 27,000 ohm cm^2. This is at least 27 times the R_m of the axon (*12*).

The major finding is that the specific resistance of the squid soma membrane is much greater than that of its axonal membrane. The greater efficacy of the Na^+ pump in generation of the resting membrane potential presumably is only a result of the differences in R_m. Since the cell bodies are small and easily injured by penetration the value of R_m in intact somata may in fact be even larger than calculated here. The differences between R_m values for axon and soma in the squid, taken together with the high R_m values obtained in *Aplysia* (*6*) and *Anisodoris* (*8*), suggest that these differences may be characteristic of somata and axons, respectively. There is as yet no clear information about R_m of dendrites of higher nerve cell bodies, but it would appear likely that membranes of dendrites would be more like those of somata than axons. In any case it is not justified to take the value of 1000 ohm cm^2, characteristic of squid axon, as a general value of R_m of nerve cells.

Because they function as a current source, electrogenic Na^+ pumps contribute to the resting membrane potential in proportion to the R_m. In *Aplysia* neurons, where R_m is about 100,000 ohm cm^2, the pump can generate up to 50 mv (*6*). *Anisodoris* neurons also have a large R_m, and the resting membrane potential can be adequately described by the passive membrane properties in these cells only at low temperatures where the pump is not active (*9*). Although electrogenic pumps are known to contribute to the resting membrane potential even in some axons (*13*), the high R_m and the presence of an electrogenic pump in squid somata suggests that pumps may in general be of greater importance in somata than in axons.

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10. Current was passed through the recording electrode by means of a standard bridge circuit. It was impossible to get two independent electrodes into the same cell through the connective tissue capsule, and the cells tended to be

more damaged if the capsule was removed. Although current passage through a recording electrode can introduce errors in the measurement of resistance, care was taken to keep the bridge in balance. This is not particularly difficult with electrodes having resistances in the range of 15 to 30 megohms and with maximum currents of the order of 10^{-8} amp. Another danger is that the electrode resistance changes during the current passage by depleting ions from the tip. Any change of electrode resistance is easily detected as a change in bridge balance between the onset and end of the current pulse (usually about 100-msec duration). Results were discarded when such changes in balance occurred.

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12. The R_m can also be calculated from a measurement of total surface area and input resistance. For a 150-μm cell, surface area, assuming no indentations of the surface membrane and assuming the cell to be a sphere, would be 6.9×10^{-4} cm^2. For a sphere with an input resistance of 10^7 ohms there would be a limiting R_m of 6900 ohm cm^2. Some molluscan and many other invertebrate neurons have membrane indentations that increase the total surface area by up to 7.5-fold [M. Mirolli and S. R. Talbott, *J. Physiol. London* **227**, 19 (1972); T. H. Bullock and G. A. Horridge, *Structure and Function in the Nervous Systems of Invertebrates* (Freeman, San Francisco, 1965)]. Information as to the extent of indentations of the squid soma membrane is not available. In order to have agreement between the two calculations there would have to be a four-fold increase in surface membrane area over that of a single sphere.
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14. These experiments were done at the Marine Biological Laboratory, Woods Hole, Massachusetts. I thank K. S. Cole and W. J. Adelman, Jr., for the use of their facilities and for discussions, and A. F. Bak for assistance.

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Electrocardiogram Monitoring: Computerized Detection of Ventricular Changes Induced by Drugs

Abstract. *Cardiac arrhythmia was induced in mice by the injection of ouabain, lidocaine, and diphenylhydantoin. Temporal spreading of ventricular electrocardiographic complexes is shown consistently to follow injection of a cardioactive drug and to precede rhythm disturbances. A computerized monitoring technique based on temporal spreading and waveform distortion should find wide clinical and experimental application.*

Cardiac arrhythmias resulting from therapeutically administered digitalis, quinidine, procainamide, and lidocaine or from abnormal concentrations of blood electrolytes pose serious health problems (*1*). Associated premature ventricular contractions (PVC) and ventricular fibrillation which arise in these situations appear secondary to cardiac conduction impairments (*2*). Most advanced monitoring programs rely upon the detection and classification of rhythm disturbances obtained by a continuous, real time analysis of the electrocardiogram (ECG) (*3*). Such programs, however, are insensitive to gradual ECG changes which may precede critical rhythm disturbances. Detection should include indications of pending arrhythmias so treatments might be offered or modified to avoid patient exposure to critical cardiac conditions. Our objective was to determine whether such detection criteria can be physiologically and technically satisfied.

An initial assumption was that decreases in propagation velocity along the Purkinje network would cause delays in muscle activity proportional to distance. Thus, if a cardiac treatment operated homogeneously on Purkinje cells, simple spreading or time scaling of ventricular ECG complexes is expected. Selected effects might be expected to produce distortions in addition to time spreading since cardiac muscle masses would be activated in abnormal sequences. Accordingly, the computer was programmed to detect the time spread of QRS waveforms from samples of ECG's obtained both before and after drug treatments that produced cardiac arrhythmias. In addition, waveform correlation methods were designed to measure residual ventricular alterations not due to simple time spreading.

Table 1. Summary of results obtained with lower doses of ouabain (Ouab), lidocaine (Lido), and diphenylhydantoin (Dph). Latency shows elapsed time between treatment and first β alterations. Minimum β and qualitative alterations in the ECG are noted, and both occurred near the end of each recording session. The ρ_{max} was more than .95 during periods prior to arrhythmia.

Dose (mg/kg)	Latency (min)	Minimum β	Rhythm disturbances	Death
0.9 (Ouab)	45	0.82	None	No
3.4 (Ouab)	23	.75	PVC's	Died within 24 hours
4.0 (Ouab)	8	.62	PVC's and tachycardia	No
10.0 (Ouab)	4	.50	PVC's and fibrillation	Died within 2 hours
10.0 (Lido)	1	.87	None	No
10.0 (Dph)	60	.92	None	No
30.0 (Dph)	23	.87	PVC's	No

Twenty adult mice (weighing 28 to 32 g) were each anesthetized briefly with chloroform, and transthoracic electrodes, consisting of 9-mm stainless steel wound clips attached superficially to the epidermis, were implanted bilaterally. Flexible spectrastrip cable carried ECG signals from the mice to preamplifiers. During recording sessions, half the mice were restrained in a Plexiglas neck and leg harness and the other mice were lightly tranquilized with chlorpromazine (1 mg per kilogram of body weight). In either case a 30-minute period of adaptation was allowed immediately following implantation of electrodes and the ECG was continually monitored for several hours in all mice.

The ECG signals were amplified and recorded. Analysis began with filtering (1.6- to 250-hertz band pass, 60-hertz notch), synchronizing (narrow bandpass filter at 105 hertz followed by threshold detection), and digitizing (1000 samples per second) of the recorded signals. The synchronization pulse marked the QRS complex for digital sampling. Initially, 20 consecutive ECG beats were sampled and averaged to provide a reference waveform, $S_R(t)$.

Subsequent 20-beat samples were similarly averaged for continuous comparisons with the reference. Temporal position and time scale variables were searched iteratively to maximize the cross-correlation coefficient, ρ, between the reference waveform and each new averaged waveform. The search of the temporal position variable eliminated false discrepancies between reference and compared waveforms due to synchronization variations. The maximized ρ is thus described in the following way

$$\rho_{max} = \max_{\{\beta,\tau\}} \left\{ \frac{\int_0^T S_R(\beta(t-\tau))\, S_c(t)\, dt}{\left[\int_0^T S_R^2(\beta(t-\tau))\, dt \cdot \int_0^T S_c^2(t)\, dt \right]^{1/2}} \right\}$$