compared the rates of $O_2$ consumption of stimulated and control homogenates and found them to be not significantly different. Therefore, we have not, as yet, determined what initiates this rise in catalase activity.

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## Effects of inhibition and stimulation of $Na^+$-$K^+$ active transport on the resting membrane input conductance of the guinea-pig ventricle[1]

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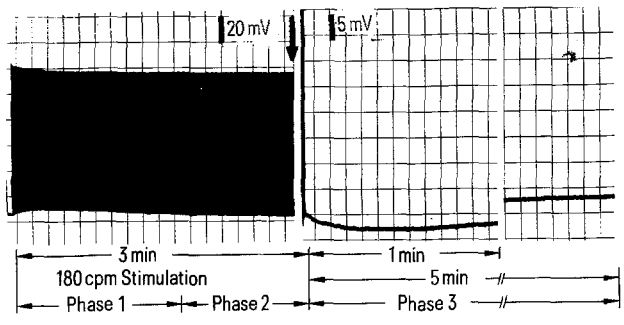
*Summary.* The effects of inhibition by ouabain and stimulation by high frequency drive of the sarcolemmal $Na^+$-$K^+$ active transport system on the resting input conductance ($g_i$) of guinea-pig ventricular muscles were determined. Although both pump inhibition and stimulation were associated with changes in electrophysiological properties of the muscles, neither had a significant effect on $g_i$.

The resting membrane potential of excitable cells is determined by the distribution of ions across the sarcolemma, the conductance of the membrane to these ions, and the net current produced by the $Na^+$-$K^+$ active transport system. It is generally accepted that $Na^+$ and $K^+$ active transport via the ouabain sensitive sarcolemmal $Na^+$-$K^+$ ATPase maintains the transmembrane concentration gradients for $Na^+$ and $K^+$ and that the $Na^+/K^+$ active transport coupling ratio and level of $Na^+$-$K^+$ ATPase activity determine the magnitude of the net current produced by the pump. Shanes[3] proposed that the $Na^+$-$K^+$ active transport system can also influence the bioelectric properties of a cell by preventing ions from penetrating the membrane and thereby influencing membrane conductance. This hypothesis has been supported by the results of experiments on frog skeletal muscle[4], cardiac Purkinje fibres[5], and crayfish giant axons[6]; inhibition of the pump by ouabain significantly increases the resting membrane conductance of these preparations while stimulation of $Na^+$-$K^+$ ATPase in the crayfish axon by papaverine produces a significant decrease in membrane conductance[7]. The purpose of this study was to determine the effects of inhibition and stimulation of the $Na^+$-$K^+$ active transport system on the resting input conductance of the ventricular myocardium.




Response of the transmembrane potential to 3 min of suprathreshold old stimulation at 180 cpm (Exp. No.121675). Note the changes in vertical scale and time base at the arrow.

Male guinea-pigs, weighing 200–400 gm, were stunned by a blow to the head and their hearts rapidly removed. A muscle strip was cut from the right ventricle and mounted in a bathing chamber through which a solution of the following composition flowed: 128 mM NaCl, 5.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Tris HCl, 2.2 mM Tris base, and 10 mM dextrose (pH = 7.41). Either gluconate or isethionate, 2 relatively impermeant anions, replaced 100% of the $Cl^-$ in the $Cl^-$-free media. The $Ca^{++}$ activity of these solutions was titrated to control levels to compensate for $Ca^{++}$ binding to the impermeant anions[8]. All media were saturated with 100% $O_2$ and maintained at 30 °C for the pump inhibition experiments and 37 °C for the pump stimulation experiments. One end of the muscle was attached to a fixed post while the other end was attached to an isometric strain gauge for recording mechanical activity. An isolated electronic stimulator excited the muscle strips through 2 platinum wires placed next to the preparation. The transmembrane potential was recorded from cells on the endocardial surface of the muscles by an electrometer through a glass microelectrode (15–20 M $\Omega$ resistance) filled with 2.5 M KCl. The electrical and mechanical signals were displayed on an oscilloscope and strip chart recorder for graphical analysis.

The input conductance ($g_i$) of the muscle was measured by the 2-electrode technique which has previously been described in detail[9]. A current-passing microelectrode (<3 M $\Omega$ resistance), positioned in the muscle within 30 µm of the voltage-recording electrode, injected a 30 msec duration square current pulse from an isolated stimulator. The magnitude of the current (averaging 8 µA) was chosen to produce a subthreshold steady-state voltage response of less than 8 mV at the recording electrode. $g_i$ was calculated as the ratio of the injected current to the steady-state voltage change. Because cell geometry and the relative placement of the current and recording electrodes influence the value of $g_i$, data was accepted only from experiments in which the placement of the electrodes was maintained constant during the test and pre-test periods.

Partial inhibition of $Na^+$-$K^+$ active transport was produced by the addition of $1.2 \times 10^{-6}$ M ouabain to the bathing media. This was the highest dose that could be consistently used without the induction of toxic side effects charac-

Effect of stimulation on the diastolic membrane potential

| | Maximum response during phase A (mV) | B (mV) | C (mV) |
|---|---|---|---|
| Control (n=8) | −3.3±0.4* | +1.8±0.7 * | +3.8±0.2* |
| $1\times10^{-6}$ M ouabain (n=3) | −2.8±0.7* | −2.3±0.8** | +1.3±0.4*** |

Depolarizations from the pre-stimulation resting membrane potential are expressed as negative values while hyperpolarizations are expressed as positive values. The data are reported as the mean±SE. * Signifies a significant ($p<0.05$) change in the diastolic membrane potential as determined by a paired Student's t-test. ** Signifies a significant ($p<0.05$) difference between the response of control muscles and muscles pre-treated with ouabain to stimulation as determined by Student's t-test.

terized by membrane depolarization, spontaneous activity, and contracture.

Because of the dependence of the oubain effect on the frequency of stimulation, the muscle was stimulated at 60 cycles per min. $g_i$ was measured during the resting phase between action potentials. The relative placement of the recording and current electrodes remained constant for 30–65 min (42±7 min average) after the introduction of ouabain in 7 experiments. At this time there were no significant changes in either the resting potential or the resting tension of the muscle. There was, however, the characteristic shortening of the action potential (−15±4% at 90% repolarization) and increase in developed tension (+224±47%) associated with pump inhibition by cardiac glycosides. These electromechanical changes were not associated with a significant change in the resting input conductance; a 2±3% increase was observed.

Because the level of $Na^+$ and $K^+$ active transport is directly related to the intracellular $Na^+$ concentration ($[Na]_i$)[10] the pump can be stimulated by a high frequency train of suprathreshold stimuli which raises $[Na]_i$ by allowing extra $Na^+$ to enter the cell during the upstroke of the action potential. Driving ventricular fibres at a rate of 180 cpm was used to stimulate $Na^+$-$K^+$ active transport above resting levels in the 2nd series of experiments. The response of the muscles to a 3-min stimulation period is illustrated in the figure. Following a quiescent period, stimulation transiently depolarized (phase A) and then slightly hyperpolarized (phase B) the ventricular fibres. Cessation of stimulation led to a transient hyperpolarization (phase C) lasting 4–5 min. The table quantifies this response which is similar to that described for cardiac Purkinje fibres by Vassale[11]. That phases B and C of the response were ouabain sensitive suggests that the electrogenic (hyperpolarizing) pump is stimulated at these times. There was no significant difference between the input conductance measured during the resting period immediately before stimulation and at the peak of the phase C response to drive; $g_i$ during phase C was 1±3% (n=8) less than at rest.

The conductances measured in these experiments are input conductances ($g_i$), changes in which reflect changes in the specific membrane conductance ($g_m$) of ventricular muscle within the limits of the measurement technique[12]. Because no significant changes in $g_i$ were observed upon either stimulation or inhibition of the pump, it is necessary to estimate the minimal change in $g_m$ that could be detected by measuring $g_i$. It is well accepted that $Cl^-$ makes a significant contribution to the resting membrane conductance of cardiac cells[13]. This contribution has been estimated to be somewhere between 15 and 43% of $g_m$[8,12,14]. To estimate the lower limit of the sensitivity of $g_i$ to changes in $g_m$, let us assume the upper value of 43% for ventricular muscle. The standard errors of the $g_i$ measurements made here are 3–4%. Therefore, a change in $g_i$ of at least 8% would have been shown to be statistically significant. The theoretical relationship between $g_i$ and specific membrane resistance depends on the model one visualizes. It has been argued that in a 3-dimensional network changes of membrane conductance should have little influence on $g_i$[12]. However, the relationship to be expected critically depends on details of the model such as the distance between branching points[13]. To avoid having to rely on theoretical considerations, the sensitivity of $g_i$ was experimentally tested by omitting from the bathing solution one of the main carriers of charge, $Cl^-$. In $Cl^-$-free solution a 44±4% (n=12) decrease in $g_i$ was observed, attesting to a good sensitivity of changes of $g_i$ to changes of $g_m$ in the preparation used.

Considering the physical dimensions of a typical ventricular cell (10–20 µm in diameter and 40–100 µm in length), it is most likely that the recording- and current-electrodes were in adjacent cells. The experimental interventions used here elevate intracellular $Ca^{++}$ and, therefore, could decrease the conductance of the nexal junctions between cells and affect $g_i$[16]. However, a decrease in nexal conductance is preceded by an increase in resting tension[17]. No change in this parameter was observed under any test conditions indicating that the $g_i$ values measured were not secondary to changes in intercellular conductances.

The non-toxic dose of ouabain used in these experiments inhibited $Na^+$-$K^+$ ATPase activity by approximately 37%[18] and had no significant effect on $g_i$. This is in contrast to the results of Kassebaum[5] and Lieberman & Lane[6] who reported decreases in $g_i$ in response to ouabain in sheep Purkinje fibers and crayfish giant axons, respectively. However, Kassebaum used a dose of ouabain which decreased $Na^+$-$K^+$ ATPase activity to a greater extent (55%) than that used here and also produced a depolarization of the membrane – a sign of ouabain toxicity. Lieberman and Lane inhibited $Na^+$-$K^+$ ATPase to an extent (95–100%) that produced toxic effects in the ventricle.

The membrane potential response to a high frequency drive reported here is similar to that previously described in neurons[19], sheep Purkinje fibres[11], and dog ventricular muscle[20]. It is well accepted that the hyperpolarization which follows driving the preparation is the result of stimulating the active transport system. $g_i$ was not changed from its pre-drive resting value during the period of pump stimulation. This result is identical to that reported by Vassalle[11] in Purkinje fibres. Collectively the experiments with ouabain and high frequency stimulation suggest that moderate levels of inhibition and stimulation of the $Na^+$-$K^+$ active transport system, although associated with physiological effects, do not significantly alter the membrane conductance of resting ventricular muscle.

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## Dietary utilization of aliphatic alcohols by *Drosophila*

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*Summary.* Metabolic utilization was measured by the increase in life duration of adults receiving a low concentration of alcohol as the sole energy source. Flies were able to use primary alcohols as food in the following order: butanol > ethanol > propanol. Methanol and secondary alcohols were not metabolized. These results cannot be explained by considering only the specific activity of alcohol dehydrogenase upon these substrates.

Adaptation to resources containing alcohol is a well established property of *Drosophila* species living in wine cellars[2-4]. This adaptation corresponds to a capacity to tolerate high amounts of ethanol[4,5] and also to use small amounts as a food for energy production[6-8]. For these 2 different physiological traits, alcohol dehydrogenase (ADH) plays a key role since ADH negative mutants are both sensitive to ethanol and unable to metabolize it[9,10].

The enzymatic specificity of ADH does not, however, correlate with its physiological role in the live fly. ADH is known to be inactive on methanol, slightly active on ethanol, more active on primary propanol and butanol and still more on secondary propanol and butanol[11,12]. In the living fly[9] ADH is unable to detoxify methanol, is very efficient in promoting ethanol tolerance, is poorly active in detoxifying n-propanol and n-butanol and is still less efficient with secondary alcohols.

Recently, analysis of strains selected for ethanol tolerance showed that the capacity for metabolizing small quantities of alcohol was not related to the detoxification ability[13]. This possible genetic independence of the 2 traits led us to study the relationship between the nutritive value of various alcohols and their toxicity: our results demonstrate an absence of direct correlation: for example n-butanol, which is highly toxic, is better used as food than ethanol when in low concentration.

Experiments were made on a French strain (Colmar) of *Drosophila melanogaster*, homozygous for the F (fast) allele of ADH. Groups of 10 newly emerged adults were placed in air tight vials containing a small concentration of alcohol in water. Dead flies were recorded twice a day and average longevity calculated. As in previous studies with ethanol[5,6] the metabolic utilization was estimated by increase in life duration compared to control flies receiving only water.

Results obtained with ethanol, primary and secondary propanol and butanol are given in figure 1. In the different experiments survival of control flies ranged between 60 and 70 h. With the secondary alcohols, no gain in life duration was observed and a decrease, due to a toxic effect, occurred with concentrations higher than 1%; *Drosophila* adults cannot use these alcohols as an energy source. By contrast, a significant increase in longevity was observed with the 3 primary alcohols. All the curves have the same general shape: first, life duration increases with concentration, then it decreases when the toxic effects of higher levels overcome the beneficial ones. The rapidity of the decrease is proportional to the toxicity, and we see that ethanol is the least toxic and butanol the most toxic alcohol, thus confirming previous results[9].

The nutritive values of the various alcohols can be compared by considering longevities obtained with a small, non-toxic concentration. For example, with 1% alcohol, life extension is greatest with butanol (the most toxic at higher concentrations) and shortest with propanol.

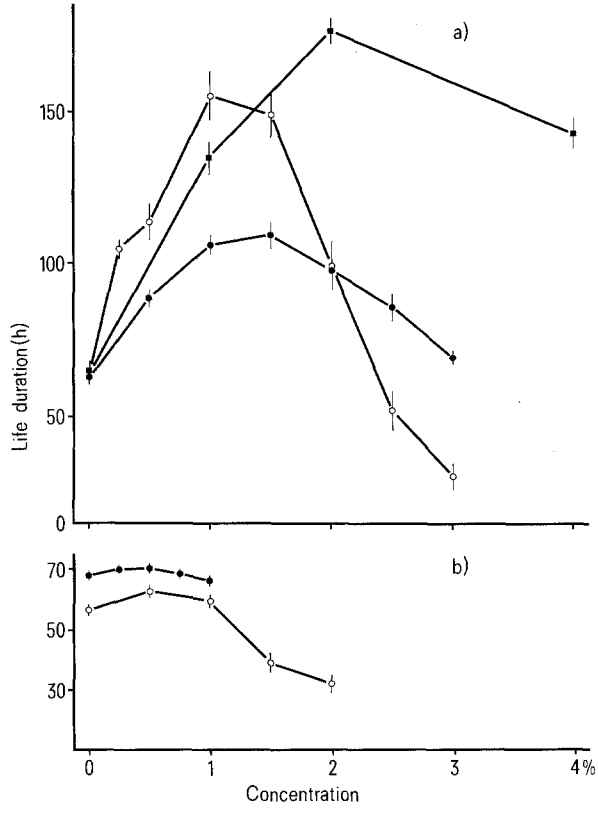


Fig. 1. Influence of alcohol concentration (% by volume) upon life duration of *Drosophila* adults. *A* Primary alcohols: ■, ethanol; ●, propanol; ○, butanol. *B* Secondary alcohols: ●, isopropanol; ○, isobutanol.