the Brookhaven reactor. The temperature was 23° ± 2°C, and the ionic strength was 0.1*M*.

Besides determination of the equilibrium quotient κ for the reaction

$$Ag^+ + H^+R^- \rightleftarrows Ag^+R^- + H^+ \qquad (1)$$

measurements were made of the volumes of the beads by means of a microscope with a calibrated ocular micrometer.

Figure 1 shows the "equivalental" volumes and equilibrium quotients for three different beads plotted against the mole fraction of silver in the resin. Although the data for a given bead may show a spread as large as 20 percent, the variation from bead to bead is much larger —in fact almost 170 percent in "equivalental" volume and 260 percent in equilibrium quotient. There is also a correlation between the swelling and the equilibrium quotient: the larger the "equivalental" volume, the smaller the equilibrium quotient. This is what is to be expected if the degree of cross-linking is different for different beads, and it is very likely that, to a first approximation, bead-to-bead variations are due to differences in cross-linking.

Thirty-six different beads were studied. Values for the experimentally determined equilibrium quotient centered around 2.5 and 4.7, with a spread from 1.8 to 6.5. This large spread may be an extreme case; however, it should be emphasized that resins may contain beads with widely different cross-linkings. This is not unexpected in view of the variations in swelling properties noted by several workers in the field (*2*).

This heterogeneity effect may be of little practical importance. It is important, however, for an understanding of the ion-exchange process, that it may sometimes be so large that it contributes significantly to deviations from ideal behavior. When different models such as ion-pair formation, specific and nonspecific interactions, and so on, are discussed, the heterogeneity effect must be eliminated. For basic research with ion exchangers it would thus be very helpful if very homogeneous resins could be made available in the future (*3*).

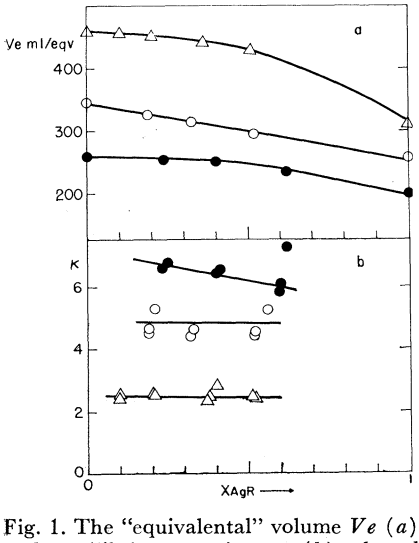

Fig. 1. The "equivalental" volume *Ve* (*a*) and equilibrium quotient κ (*b*) plotted against the mole fraction of silver in resin, X_{AgR}, for three different beads of a batch from a DVB 4 resin. Open circles, bead 1; solid circles, bead 2; triangles, bead 3.

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3. A detailed report on this work is in preparation. The research described in this study was performed under the auspices of the U.S. Atomic Energy Commission and was presented at the meeting of the American Chemical Society held in San Francisco in April 1958. I am grateful to Dr. William Rubinson for help with the English text and to Dr. W. C. Bauman and Dr. R. E. Anderson of the Dow Chemical Company for the resin used in the study. Through the courtesy of the Dow Chemical Company a special batch of DVB 4, as homogeneous as possible, is being synthesized, and it will be interesting to see how homogeneous a resin batch can be.

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23 June 1958

Activation Energy of Ventricular Contraction in Anionically Modified Solutions

Abstract. The activation energy of ventricular contraction and relaxation in chloride and nitrate-Ringer's solution was studied on the driven rat ventricle strip by measurement of the specific contraction and relaxation velocities over a 17°C range of temperature. Nitrate ion produced a small but significant reduction in the activation energy of contraction.

Previous communications from this laboratory (*1*, *2*) have shown that nitrate and thiocyanate ions potentiate the hypodynamic ventricle's response to glucose and *k*-strophanthoside by augmenting the velocity of the effect as well as by increasing the magnitude of the final tension attained. According to previous interpretations, the increase in twitch-tension observed in amphibian skeletal muscles surviving in the presence of "abnormal" anions results from the influence of these anions in prolonging the duration of the active state. On the basis of work appearing from his laboratory it was proposed by Sandow (*3*) that the site of action of these anions was the excitable membrane rather than the contractile elements. Hill and Macpherson (*4*) concurred in this explanation but suggested as one of the alternatives the possibility that NO_3^-, Br^-, and I^- might act on the muscle by reducing the activation energy of the contractile process. As far as we were aware this proposition had not been directly tested, and it was the object of the investigation described in this report (*5*) to evaluate this idea with respect to the contractile velocity of the isolated rat's ventricle.

In the succeeding portions of this report the influence of anions on the activation energies of contraction and relaxation is described. These energies were tested by studying the velocities of contraction and relaxation as a function of temperature. The use of shortening velocity as the mechanical counterpart of the rate of liberation of chemical energy can be justified formally by two successful models of muscular contraction: Hill's (*6*) and Polissar's (*7*). In Hill's equation for muscular shortening

$$v(P + a) = b(P_o - P) \qquad (1)$$

the constant *b*, having the dimensions of velocity (muscle-length × sec^{-1}) increases rapidly with temperature, the Q_{10} being about 2.05 for frog muscle in the range between 0° and 10°C (*6*). The value of *a* (g wt/cm^2 of muscle cross-section) car, according to Hill, be derived from force-velocity as well as from heat-length data. The velocity of contraction in Polissar's (*7*) kinetic model is the mechanical counterpart of the net reaction rate of $L \rightarrow S$ in which *L* and *S* are the long and the short configurations of the contractile elements, respectively. Similarly, the speed of relaxation is an expression of the kinetics of conversion of $S \rightarrow L$. The two reactions are assumed to proceed by different metabolic paths, the initial stages of each process being sufficiently characterized by first-order kinetics.

Strips from the right ventricles of young adult Slonaker-Wistar rats, unselected as to sex, were prepared as described in earlier publications (*8*, *9*). They were mounted on immersible electrodes and maintained in muscle baths having a capacity of 220 ml. The media used were the "chloride" and the "nitrate" reference solutions, the composition of which has been described (*2*); in the presence of 100 percent O_2 as the gas phase, the *p*H of the solutions was 8.3.

The ventricle strips were stimulated at 85 shocks per minute from a constant-current square wave generator (*10*), the contractions being transduced by a mechanoelectrical myograph consisting of a Statham No. 315 G7A (0.15 to 500 ± 0.15 oz) strain gauge. The passive legs of the bridge and the batteries providing the source of the d-c potential were contained in a Statham type CB7 control box which was connected to the gauge

Table 1. Contraction and relaxation velocity constants, Q_{10} values, and activation energies for nitrate and chloride reference solutions.

Anion	T (°K)	$\log_{10} K$*	$\log K_2 - \log K_1$	Q_{10}	E_a (cal)
		Contraction velocity			
NO_3	300.1	0.9656	0.2541	1.80	10,819
NO_3	310.1	1.2197			
Cl	300.1	1.0274	0.3125	2.05	13,305
Cl	310.1	1.3399			
		Relaxation velocity			
NO_3	300.1	0.4003	0.4045	2.54	17,222
NO_3	310.1	0.8048			
Cl	300.1	0.4435	0.4330	2.71	18,436
Cl	310.1	0.8765			

* From equation of the line.

with shielded cable. The ventricle strip was attached to the lever arm of the gauge with nylon thread. As the muscle contracted it changed the resistance of the gauge, and the resulting signal was conducted from the control unit to a Tektronix (model 512) oscilloscope through the d-c input of the instrument. The sweep was triggered by the stimulator, and the oscilloscope traces were recorded photographically on Eastman Tri-X film.

The muscle chambers were immersed in a Precision (D8) serological water bath which was modified for more accurate temperature control by interposition of an electronic relay responding to mercury thermoswitches maintained in the bath. These switches were set to operate at 20°, 23°, 27°, 32°, and 37°C with a precision of ± 0.2°C.

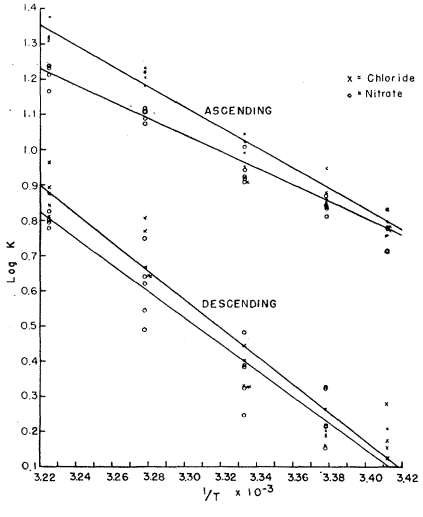

Fig. 1. Relationship between the logarithms of the velocity constants (log K) and the reciprocals of the absolute temperature. The regression equations corresponding to the several lines are: ascending Cl, $\bar{y} = 11.0214 - 3.002x$; ascending NO_3, $\bar{y} = 9.199 - 2.475x$; descending Cl, $\bar{y} = 13.877 - 4.033x$; descending NO_3, $\bar{y} = 12.755 - 3.709x$.

All experiments were made in "flowing" media: the solutions were dripped into the bottom of the muscle bath at a rate of 500 ml/hr, the level being maintained constant by aspiration from the upper surface. After a preliminary equilibration at 20°C for 90 minutes, the resting tension on the muscle strip was adjusted by means of a micrometer to the level giving a maximal contraction. All tests on a given muscle were made against the resting tension value determined at the 20°C point. After the appropriate records had been made at 20°C the temperature was raised and similar recordings were made successively at the higher temperatures, the muscle being allowed to equilibrate for 15 minutes at each temperature before the record was made. The dependence of contraction amplitude on temperature for both the chloride and the nitrate series is described by a parabola having a maximum at 27°C, confirming earlier studies made in "chloride" reference solutions (*9, 11*). In order to obtain values that were independent of amplitude, the velocity constants of contraction and relaxation were calculated by dividing the slopes of the linear portions of the ascending and descending limbs of the wave by the amplitude of the curve, and by correcting the values appropriately for the time scale and tension calibration. The common logarithms of these constants were plotted against the corresponding reciprocals of absolute temperature, and a line was fitted to each array of contraction or relaxation data by the method of least squares (Fig. 1). The regressions were tested for reliability by the method of Snedecor (*12*), and each line was found to represent a discrete array of data. The t values, calculated from the ratios of the slopes to their standard errors, were as follows: nitrate contraction, 28.33; relaxation, 11.77; chloride contraction, 24.18; relaxation, 17.05. The P values corresponding to these figures were less than .01 in each case.

The activation energy for each category was computed by means of the usual form of the Arrhenius equation in which E_a is the activation energy in calories, R is the gas constant, and T is the absolute temperature

$$\log \frac{K_2}{K_1} = \frac{E_a}{2.303R}\left(\frac{T_2 - T_1}{T_2 T_1}\right) \qquad (2)$$

using the values of K taken from the regression lines. Calculations of Q_{10} between 27° and 37°C are presented for reference, along with the activation energy data, in Table 1.

The data show a small but statistically significant reduction in the activation energy of contraction and relaxation when nitrate is substituted for chloride in the reference solution. Although the reduction in activation energy appears to be real, we feel that other factors such as the possible increased duration of excitability, produced by the slowing in the transmembrane Na transport, are of greater primary importance in explaining the potentiating effects of nitrate than its influence on the activation energy.

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