

# Effects of Sodium Azide on Sodium Fluxes in Frog Striated Muscle

PAUL HOROWICZ and CARL J. GERBER

From the Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina, and the Department of Physiology, Washington University School of Medicine, St. Louis, Missouri

**ABSTRACT** Unidirectional Na fluxes from frog's striated muscle were measured in the presence of 0 to 5 mM sodium azide. With azide concentrations of 2 and 5 mM the Na efflux was markedly stimulated; the Na efflux with 5 mM azide was about 300 per cent greater than normal. A similar increase was present when all but the 5.0 mM sodium added with azide was replaced by choline.  $10^{-6}$  M strophanthidin abolished the azide effect on  $\text{Na}^{24}$  efflux. Concentrations of azide of 1.0 mM or less had no effect on Na efflux. The Na influx, on the other hand, was only increased by 41 per cent in the presence of 5 mM  $\text{NaN}_3$ . From these findings it is concluded that the active transport of Na is stimulated by the higher concentrations of azide. The hypothesis is advanced that the active transport of Na is controlled by the transmembrane potential and that the stimulation of Na efflux is produced as a consequence of the membrane depolarization caused by the azide.

## INTRODUCTION

Metabolic inhibitors and poisons provide useful experimental tools in the study of ionic regulation in tissues. Among the agents so employed, which have given useful results, is sodium azide. For example, Hurlbut (8) has demonstrated that frog sciatic nerves show net gains of Na when they are exposed to concentrations of azide between 0.2 and 5.0 mM. With 5.0 mM azide, he has also shown that the rate coefficient for Na efflux was reduced by about 50 per cent (9). In the giant axons from *Sepia* and *Loligo*, Hodgkin and Keynes (4) obtained approximately a 90 per cent reduction in the rate coefficient for Na efflux when the fibers were exposed to 3 mM  $\text{NaN}_3$ . In general, these effects are in accord with the known metabolic disturbances produced by azide.

With frog's sartorius muscle, on the other hand, Carey, Conway, and Kernan (1) found that 2 mM  $\text{NaN}_3$  had no effect on the net secretion of Na from muscles which had first been loaded with Na by an overnight immersion in

cold physiological salt solutions free of K ions. In the interpretation of their findings they state that the lack of an effect of azide on Na extrusion is in accord with Stannard's report in an abstract (14) that  $\text{NaN}_3$  has no effect on the  $\text{O}_2$  uptake of resting frog skeletal muscle for the range of concentrations between  $10^{-5}$  and  $10^{-1}$  M. When the data are examined, however, in the full length paper of Stannard (15), it is clear that with 2 mM azide there was a stimulation in the  $\text{O}_2$  consumption of resting muscle.

In the experiments presented in this paper, sodium azide at concentrations of 2 and 5 mM produced marked increases in the Na efflux in isolated striated muscle fibers from the frog. With 5 mM  $\text{NaN}_3$ , there was also an increase in the Na influx, but this effect is much smaller than the effect on Na efflux.

#### METHODS

Fiber bundles isolated from the semitendinosus muscle of the frog, *Rana pipiens*, were employed. The preceding paper (7) gives a description of the procedures used for the dissection and mounting of fibers. In preparing solutions containing  $\text{NaN}_3$ , this salt was simply added to the solutions as previously tabulated. For all solutions the pH was checked and found to be between 7.0 and 7.1. The apparatus used for the flux measurements and the methods of calibration were also the same as those described in the preceding paper.

#### RESULTS

##### *Stimulation of Sodium Efflux by $\text{NaN}_3$*

Fig. 1 illustrates an experiment typical of the effect of  $\text{NaN}_3$  on sodium efflux to be considered in this paper. The loss of  $\text{Na}^{24}$  into inactive Ringer's solution from a bundle of seven fibers, previously loaded with  $\text{Na}^{24}$  by stimulation, was first measured. At the time indicated by the arrow labeled A, the inactive bathing solution flowing past the fibers was changed to one containing 5 mM  $\text{NaN}_3$ . Soon thereafter, the loss of  $\text{Na}^{24}$  was considerably increased and this accelerated rate was maintained for the 30 minute period during which the azide was present. Upon removal of azide at time B, the loss of  $\text{Na}^{24}$  to inactive Ringer's solution returned to a rate which was slightly slower than its value prior to the application of the azide.

Three types of mechanisms can be considered in explaining the movement of labeled Na out of frog muscle cells. First, labeled sodium can leave the cell passively as a consequence of thermal agitation because the membrane is permeable to sodium ions. Second, labeled sodium can also leave the cell passively *via* a mechanism involving the exchange of a labeled sodium on the inside for an unlabeled Na outside; that is, it can leave by the so called "exchange diffusion" process (12, 16). Finally, labeled sodium can leave the cell *via* the Na "pump" mechanism which requires a source of free energy other than thermal agitation and electrical gradients.

The purpose of the experiments to be described further in this paper was to establish which of these three processes for Na efflux was being stimulated by azide and to characterize the mechanism responsible for this stimulation.

*Dependence of Na Efflux on External  $\text{NaN}_3$  Concentration*

Not all concentrations of azide below 5 mM produce an increase in the Na efflux. The behavior of the rate coefficient for loss of  $\text{Na}^{24}$  when the external azide concentration was varied in the range 0 to 5 mM in a number of different experiments is given in Table I.

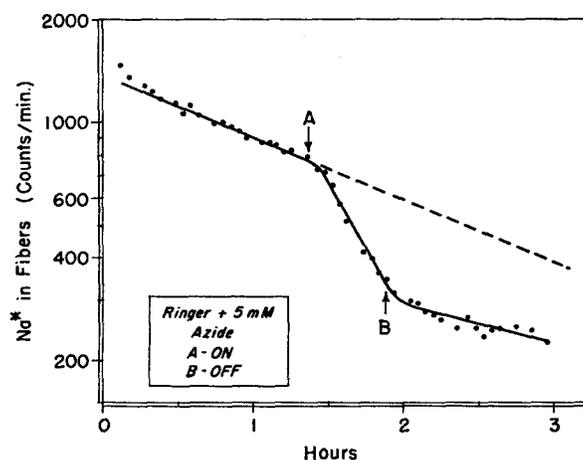


FIGURE 1. Effect of 5 mM sodium azide added to Ringer's solution on the efflux of sodium. The fibers were loaded with  $\text{Na}^{24}$  by exposing them to  $\text{Na}^{24}$  Ringer's solution for 4 minutes during which time they were stimulated 204 times at a frequency of about 1 shock/second. The end of the exposure to the active solution was at the time taken as zero for this figure. Inactive Ringer's solution flowed past the fibers except for the period between the arrows labeled A and B. The dashed line is drawn as a continuation of the curve initially obtained for the loss of  $\text{Na}^{24}$  in inactive Ringer's fluid. Experiment B75; a bundle of seven fibers. Temperature 20°C. 100 counts/minute in fibers are equivalent to 2.96 pmole of  $\text{Na}^*$ /cm of fiber.

As was the case with the K-stimulated  $\text{Na}^{24}$  efflux (7), after the first 5 to 10 minutes in the azide, the loss of  $\text{Na}^{24}$  from the fibers can be approximated by a constant rate coefficient for about 40 minutes; the ratio of this rate coefficient to the rate coefficient for loss of  $\text{Na}^{24}$  in normal Ringer's solution provides a measure of the stimulating effectiveness of azide. Using this ratio as an index, no detectable stimulation in the Na efflux was found for external azide concentrations less than 1 mM. On the other hand, with 2.0 and 5.0 mM azide the acceleration of the Na efflux was marked.

The initial control rates for Na loss in the experiments with 1 mM azide or less are somewhat higher than the control rates for the experiments in which

2.0 and 5.0 mM azide were used. However, there is in fact a large range for the values of the resting rate coefficients in normal fibers; since a factor of about 4 between the slowest and fastest rate coefficient is not atypical there is no reason for rejecting any of the experiments using the low concentrations of azide on the ground of unusually high resting rate coefficients. On the other hand, it cannot be supposed that fibers which have a high resting rate coefficient

TABLE I  
EFFECT OF VARIOUS SODIUM AZIDE  
CONCENTRATIONS ON SODIUM EFFLUX

Experiment reference	No. of fibers	Temperature	[NaN <sub>3</sub> ]	Rate coefficient for loss of Na <sup>24</sup> min. <sup>-1</sup>			$\frac{k_{OE}}{k_{OR}\S}$
				In Ringer's before NaN <sub>3</sub> $k_{OR}$	In NaN <sub>3</sub> ‡ $k_{OE}$	In Ringer's after NaN <sub>3</sub> $k_{OR}$	
		°C	mM				
B138	14	21	0.5	0.0242	0.0200 (50)	0.0164	0.99
B142	20	21	1.0	0.0143	0.0121 (50)	0.0121	0.92
C1	4	21	1.0	0.0121	0.0082 (45)	0.0042	1.01
Mean							0.97
B14	9	18	2.0	0.0078	0.0255 (40)	—	3.27
B32	ca. 60	19	2.0	0.0071	0.0131 (40)	0.0107	1.47
B71	7	20	2.0	0.0069	0.0085 (40)	0.0057	1.35
Mean							2.04
A140	8	18-21	5.0	0.0073	0.0219 (50)	0.0114	2.34
B75	11	20	5.0	0.0067	0.0287 (30)	0.0057	4.63
Mean							3.52

‡ The time interval (in minutes) for which the rate coefficient for Na<sup>24</sup> loss was estimated is given in parentheses.

§ When more than one determination of the efflux rate coefficient in normal Ringer's solution was made, the average was used for this calculation.

cient for loss of Na can't be further stimulated. In Table VI of the preceding paper, experiment B4 on a single fiber which had an initial resting rate coefficient comparable to that of experiment B138 of this paper (Table I) shows that 10 mM [K]<sub>o</sub> can increase the Na efflux in such fibers by a factor of 2.5. Consequently, it can't be argued that fibers which start with rate coefficients of about 0.02 min.<sup>-1</sup> can't be stimulated to values of at least as high as 0.05 min.<sup>-1</sup>. On pooling all the resting data in Table I for the experiments with 1.0 mM azide or less, the resulting mean resting rate coefficient is 0.014 min.<sup>-1</sup>. Even if 2.0 or 5.0 mM azide could reduce the maximum rate coefficient from 0.05 min.<sup>-1</sup> or greater to about 0.03 min.<sup>-1</sup>, then there still could have been size-

able increases in Na efflux with 1.0 mM azide. The data, clearly, do not show this. Consequently, it is evident that with the lower azide concentrations there was no detectable stimulation of Na efflux while with higher concentrations of azide there was a pronounced stimulation of Na efflux.

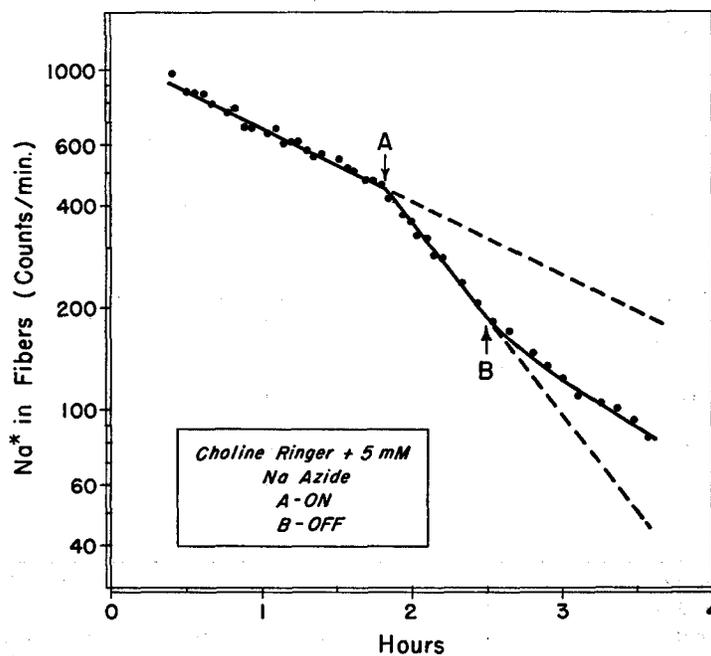


FIGURE 2. Effect of 5 mM sodium azide added to choline Ringer's solution on the efflux of sodium. Fibers stimulated 201 times at a frequency of about 1 shock/second while in  $\text{Na}^{24}$  Ringer's solution for 4 minutes. The exposure to the active solution was started 8 minutes after the time taken as zero for this figure. Inactive Ringer's solution flowed past the fibers except for the period between the arrows labeled A and B. The dashed lines are drawn as continuations of the fitted curves (1) prior to time A and (2) for the period between times A and B. Experiment B83; a bundle of fifteen fibers. Temperature 19°C. 100 counts/minute in fibers are equivalent to 2.65 pmole of  $\text{Na}^*$ /cm of fiber.

The experiments to be considered further in this paper were performed with azide concentrations of 2.0 and 5.0 mM since these concentrations consistently stimulated the Na efflux.

#### *Azide Stimulation of Na Efflux in Choline Ringer's*

The effect on Na efflux of an inactive solution which had its normal Na replaced by choline but to which 5 mM  $\text{NaN}_3$  had been added is shown in Fig. 2. After an initial period of washing into inactive Ringer's solution, the fibers were bathed, starting at the time indicated by the arrow labeled A, in the

azide- and choline-containing fluid. The loss of  $\text{Na}^{24}$  from the fibers was clearly accelerated. At the time indicated by B, 30 minutes later, the fibers were returned to normal inactive Ringer's solution and the rate of  $\text{Na}^{24}$  loss decelerated, gradually approaching its initial value.

In this experiment the rate coefficient for loss of  $\text{Na}^{24}$  increased by a factor of 2.6, a value which was within the range found for stimulation of Na loss in normal Na-containing Ringer's solution using this concentration of  $\text{NaN}_3$  (see Table I).

Keynes and Swan (11) have shown that the presumptive exchange diffusion

TABLE I  
EFFECT OF SODIUM AZIDE ON SODIUM INFLUX

Experiment reference	No. of fibers	Temperature °C	[ $\text{NaN}_3$ ] mM	Influx before $\text{NaN}_3$ exposure	Influx in solu- tion with $\text{NaN}_3$ ‡	Influx after $\text{NaN}_3$ exposure	Na influx ratio =
							$\frac{\text{Influx in } [\text{NaN}_3]}{\text{Influx in Ringer's fluid}}$
B71	11	20	2.0	—	0.0224	0.0229	0.98
B87	9	20	5.0	—	0.1033	0.0700	1.48
B113	2	20	5.0	§	§	—	1.48
B117	3	20	5.0	§	§	—	1.26
							1.41

‡ In all experiments, fibers were exposed to  $\text{NaN}_3$ -containing Ringer's solution for 9 minutes prior to the  $\text{Na}^{24}$  influx measurement.

§ In experiments B113 and B117 the calibrations were incomplete. The ratio was calculated directly from the measured uptake of  $\text{Na}^{24}$  in units of (counts/minute)/second of influx.

mechanism in frog's sartorius muscle is half-saturated at an external Na concentration of 30 mM. In the experiment described above, the external concentration of Na was only 5 mM and thus should have largely eliminated the stimulation of the Na efflux if the stimulation by azide was caused by an acceleration of the exchange diffusion mechanism.

#### *Effect of $\text{NaN}_3$ on the Na Influx*

An increased membrane permeability to Na ions caused by external azide could explain the stimulation of the Na efflux by azide. Since the electrochemical gradient across the membrane for Na ions is directed for net inward movement of Na ions, it is evident that an increase in the Na permeability of the membrane must be accompanied by an increase in the  $\text{Na}^{24}$  influx. An increase in the  $\text{Na}^{24}$  influx would also be the predicted result if the azide stimulated an exchange diffusion mechanism. For these reasons the effect of  $\text{NaN}_3$  on  $\text{Na}^{24}$  influx was measured.

A summary of the influx measurements on several preparations is given in Table II. A 9 minute soak in inactive test solution preceded each influx meas-

urement. This preliminary exposure to azide was adopted in order that the influx could be measured at a time when the azide-stimulated Na efflux was well developed (see Fig. 1). Considering only the data using 5 mM  $\text{NaN}_3$ , it is clear that there was a small increase in the  $\text{Na}^{24}$  influx; an average increase of 41 per cent over the resting influx was observed.

Since in resting isolated preparations the influx is only about 20 per cent greater than the efflux (2), it is clear that any condition leading to a 252 per

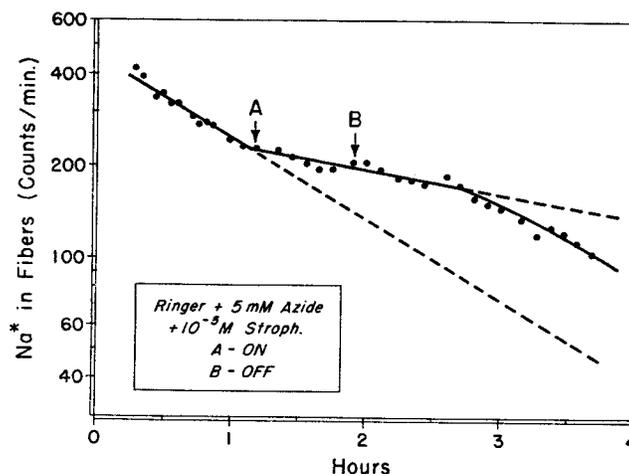


FIGURE 3. Effect of the simultaneous addition of 5 mM sodium azide and  $10^{-5}$  M strophanthidin to Ringer's solution on the efflux of sodium. Fibers stimulated 204 times at a frequency of about 1 shock/second while in  $\text{Na}^{24}$  Ringer's solution for 4 minutes. The exposure to the active solution was started 4 minutes after the time taken as zero. Inactive Ringer's solution flowed past the fibers except for the period between the arrows labeled A and B. Dashed lines are continuations of the fitted curves (1) prior to time A and (2) for the period between times A and B. Experiment B79; a bundle of five fibers. Temperature  $19^{\circ}\text{C}$ . 100 counts/minute in fibers are equivalent to 7.12 pmole of  $\text{Na}^*$ /cm of fiber.

cent increase in the Na efflux (see Table I for effect of 5 mM  $\text{NaN}_3$  on efflux) by the acceleration of any passive mechanism would require, as an absolute minimum, a similar increase in the influx. Although there is a small increase in the  $\text{Na}^{24}$  influx produced by azide, nevertheless, it is clear from the small magnitude of this effect that the stimulation of the Na efflux produced by  $\text{NaN}_3$  cannot be solely ascribed to passive mechanisms and must involve stimulation of an active transport system.

#### *Strophanthidin Inhibition of Azide Effect*

To further test the hypothesis that the azide-stimulated Na efflux involved the sodium pump, the effect of strophanthidin, an aglycone known to inhibit active transport (7, 10), on this stimulated Na efflux was studied. Fig. 3 illus-

trates an experiment in which the effect on Na efflux of the simultaneous addition of strophanthidin and  $\text{NaN}_3$  was measured. At the time indicated by A, which was preceded by a period measuring the loss of  $\text{Na}^{24}$  into inactive Ringer's solution, the solution flowing past the fibers was changed to one having 5 mM  $\text{NaN}_3$  and  $10^{-5}$  M strophanthidin added to it. It is clear that the stimulation usually produced by  $\text{NaN}_3$  was absent while the inhibition usually produced by strophanthidin (7) was the dominating effect. After normal Ringer's solution was readmitted, at the time indicated by the arrow B, there was a gradual recovery of  $\text{Na}^{24}$  loss from the fibers to the initial rate. The same result was obtained in another experiment of this type.

To the extent that strophanthidin preferentially inhibits the active transport component of the Na efflux, this observation is consistent with the interpretation that azide stimulated the Na pump.

#### DISCUSSION

It is evident from the presentation of the experimental results that when 2 to 5 mM sodium azide is added to the normal Ringer's solution, an increase in the active transport of Na in the muscle fibers of frogs is produced. In looking for an explanation of the azide stimulation of active sodium transport, the fact that sodium azide is a rapid and potent depolarizing agent (6, 13) is of considerable relevance. A more detailed description and analysis of this depolarizing action of sodium azide will be given in later papers (5). For the purpose of this discussion what is important to note is that within a few seconds of the application of solutions containing azide in concentrations up to 5 mM the membranes of frog's striated muscle become depolarized to levels comparable to those achieved by increasing the external K concentration from its normal value of 2.5 mM to values as high as 15 mM. As was shown previously (7), this range of external potassium concentrations results in an increased active transport of sodium which, significantly, is comparable in magnitude to the increases in the Na pump produced by azide concentrations reported in this paper. Concentrations of either potassium or azide which produce depolarization less than 22 mv from the normal resting transmembrane potential of about -92 mv—that is, for  $[\text{K}]_o \leq 7.5$  mM (3) and  $[\text{NaN}_3] \leq 1.0$  mM at pH = 7.1 (5, 6)—have little effect on the active Na transport. On the other hand, concentrations of either potassium or azide which produce depolarizations greater than about 22 mv from the normal resting transmembrane potential—that is, for  $[\text{K}]_o > 7.5$  mM (3) and  $[\text{NaN}_3] \geq 2.0$  mM at pH = 7.1 (5, 6)—result in large increases in the active Na transport.

These statements are graphically illustrated in Fig. 4. The ratios of the rate coefficient for loss of  $\text{Na}^{24}$  determined in a given experimental solution to the rate coefficient for loss of  $\text{Na}^{24}$  in normal Ringer's fluid were averaged and plotted on the axis of ordinates. On the axis of abscissas is plotted the average

transmembrane potential found for the various experimental solutions which contained either increased external potassium concentrations or added sodium azide (3, 5, 6). It is clear that the relation between the sodium efflux and the transmembrane potential is the same for both the potassium and the azide experiments. The Na efflux was constant for internal potentials between  $-92$  and  $-70$  mv, while for internal potentials between  $-70$  and  $-60$  mv there

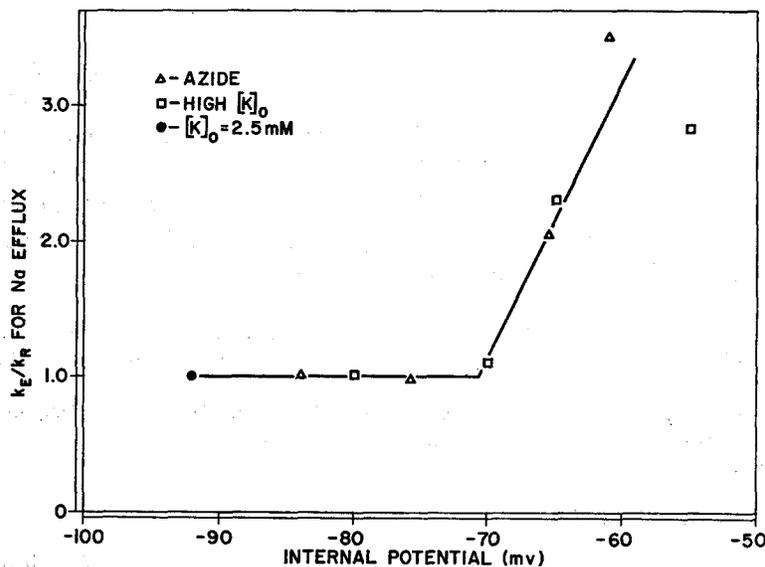


FIGURE 4. The relation between the transmembrane potential and the rate coefficient for loss of sodium. The ratio of the rate coefficient for loss of  $\text{Na}^{24}$  in experimental solution to the average rate coefficient for Na loss in Ringer's solution before and after exposure to the experimental solution is given as the ordinate; for the azide solutions, see Table I; for the high  $[\text{K}]_o$ , see Table VI of preceding paper (7). The membrane potentials for any given experimental solution are given as the abscissa; the values for the high  $[\text{K}]_o$  are computed from the equation  $V = 58 \log \frac{[\text{K}]_o + 0.01 [\text{Na}]_o}{140}$  given in reference (3); the values for the azide were measured by internal microelectrodes in fibers from the semitendinosus and are given in references (5 and 6).

was a marked dependence of sodium efflux on the membrane potential; within this range of potentials the dependence was roughly linear with the sodium efflux at  $-60$  mv being three times greater than it was at  $-70$  mv. These findings clearly suggest that the stimulated Na efflux produced by adding  $\text{NaN}_3$  and by increasing the external K concentration is referable to the depolarizations produced by these agents. The details of the mechanism by which the transmembrane potential regulates the rate of the Na pump are, for the present, obscure.

Although there is a close similarity between  $\text{NaN}_3$  and increased  $[\text{K}]_o$  in

their effects on the Na efflux, nevertheless, there is a difference between these two agents in that azide produced an increase in the Na influx which did not occur with potassium. The simplest explanation is that this increase in Na influx is due to a small increase in the membrane permeability to Na. By using the flux-ratio equation of Ussing, it can readily be shown that the measured increment in influx due to a permeability change could only lead to a very small increase in Na efflux (7). Since Na influx was determined only for the period from the 9th to the 15th minute after the start of the exposure to azide, there is, at present, no information on either how rapidly this effect develops or how constant this effect remains. Nevertheless, it is clear that azide produces, at least for this period, an increase in the active transport of Na. Since strophanthidin abolished the effect for the entire 30 to 40 minute exposure period to 5 mM azide, it is plausible to suppose that the measured increment in Na efflux was due primarily to an acceleration of active transport processes through the entire exposure to 5 mM azide.

During the period of this investigation, one of the authors (C. J. G.) held a Postdoctoral Fellowship from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.

This study was supported by National Science Foundation Grants G-8131, G-16189, and G-24388 and by Grant RG08803 from the National Institutes of Health.

Received for publication, August 24, 1964.

#### REFERENCES

- CAREY, M. J., CONWAY, E. J., and KERNAN, R. P., Secretion of sodium ions by the frog's sartorius, *J. Physiol.*, 1959, **148**, 51.
- HODGKIN, A. L., and HOROWICZ, P., Movements of Na and K in single muscle fibres, *J. Physiol.*, 1959, **145**, 405.
- HODGKIN, A. L., and HOROWICZ, P., The influence of potassium and chloride ions on the membrane potential of single muscle fibres, *J. Physiol.*, 1960, **148**, 127.
- HODGKIN, A. L., and KEYNES, R. D., Active transport of cations in giant axons from *Sepia* and *Loligo*, *J. Physiol.*, 1955, **128**, 28.
- HOROWICZ, P., and CAPUTO, C., data to be published.
- HOROWICZ, P., CAPUTO, C., and ROBESON, J. A., Rapid depolarization of frog muscle membranes by  $\text{NaN}_3$ , *Fed. Proc.*, 1962, **21**, 151.
- HOROWICZ, P., and GERBER, C. J., Effects of external potassium and strophanthidin on sodium fluxes in frog striated muscle, *J. Gen. Physiol.*, 1965, **48**, 489.
- HURLBUT, W. P., Effects of azide and chloretone on the sodium and potassium contents and the respiration of frog sciatic nerves, *J. Gen. Physiol.*, 1958, **41**, 959.
- HURLBUT, W. P., Sodium fluxes in desheathed frog sciatic nerve, *J. Gen. Physiol.*, 1963, **46**, 1191.
- JOHNSON, J. A., Influence of ouabain, strophanthidin and dihydrostrophanthidin

- on sodium and potassium transport in frog sartorii, *Am. J. Physiol.*, 1956, **187**, 328.
11. KEYNES, R. D., and SWAN, R. C., The effect of external sodium concentration on the sodium fluxes in frog skeletal muscle, *J. Physiol.*, 1959, **147**, 591.
  12. LEVI, H., and USSING, H. H., The exchange of sodium and chloride ions across the fibre membrane of the isolated frog sartorius, *Acta Physiol. Scand.*, 1948, **16**, 232.
  13. LING, G., and GERARD, R. W., The membrane potential and metabolism of muscle fibers, *J. Cell. and Comp. Physiol.*, 1949, **34**, 413.
  14. STANNARD, J. N., Separation of the resting and activity oxygen consumptions of frog muscle by means of sodium azide, *Am. J. Physiol.*, 1939, **126**, P. 634.
  15. STANNARD, J. N., Separation of the resting and activity oxygen consumptions of frog muscle by means of sodium azide, *Am. J. Physiol.*, 1939, **126**, 196.
  16. USSING, H. H., Transport of ions across cellular membranes, *Physiol. Rev.*, 1949, **29**, 127.