Inhibition of Active Sodium Transport in the Isolated Frog Skin

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The low concentration of sodium inside most cells appears to result from an active outward transport of sodium (1-5). Since it is technically difficult to study active sodium transport in muscle and nerve, a simpler system in which such transport occurs may be examined with profit.

It has long been known that an electrical potential difference (p.d.) may be measured across the isolated frog skin placed between two solutions of identical composition; usually the solution bathing the inside of the skin is positive relative to that bathing the outside by some 40 to 100 mv. The origin of this p.d. has been the subject of considerable experiment and speculation. On the basis of ion transport measurements with radioactive tracers, Ussing (6) advanced the hypothesis that the p.d. resulted from the active transport of Na\(^+\) inward across the skin. The problem has been greatly clarified by the recent work of Ussing and Zerahn (7) using a short-circuited isolated frog skin preparation in which the p.d. across the skin was maintained at zero. The total electric current generated by the skin then could be accounted for completely by the net inward transport of Na\(^+\). Their findings have been confirmed by Linderholm (8).

The short-circuited skin may be regarded as a type of battery in which the current results from movement of sodium ions (9). For an ion which is actively transported, such as sodium, the force acting upon the ion may be expressed in electrical terms as an emf. The essential components of the system are thus the transporting force acting upon the Na\(^+\) (E\(_{Na}\)) and the internal resistance to the movement of Na\(^+\) (R\(_{Na}\)). Substances influencing active sodium transport therefore may be characterized by their relative effects on these components of the sodium transporting system. In addition, however, certain substances may increase the electric current by initiating the active transport of a different ion species, e.g. chloride (10).

The effects of several substances on active sodium transport in the short-circuited frog skin have been determined previously. Posterior pituitary hormones produce a large increase in the p.d. of the non-short-circuited skin (11) and also increase the current in the short-circuited preparation. This effect seems to be primarily the result of a decrease in the sodium resistance (7). Since neurohypophysial hormones also increase the net flux of water across the skin (11), it was suggested that the effect of the hormones was to increase the pore size in some membrane, but presumably not the epithelial cell membrane where sodium transport probably occurs (9). Adrenaline (5 \(\times\) 10\(^{-6}\) M) added to the inside solution of the short-circuited skin increases the current, the influx and the outflux of sodium (10). The outflux is often increased more than 10-fold. However, adrenaline appears to initiate a new source of sodium transport (10).

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of current in the skin: active outward transport of Cl\textsuperscript{-}. Copper (3 \times 10^{-6} \text{M}) has little effect on sodium influx or outflux or current in the short-circuited skin, but increases the p.d. across the skin. Probably Cu\textsuperscript{++} decreases the permeability of the skin to Cl\textsuperscript{-}, which normally tends to short the potential (7). The use of 5 per cent CO\textsubscript{2} in the oxygenating mixture results in a fall of the current to zero in the short-circuited skin. The net sodium flux appears to be completely stopped by this treatment as the result of a great decrease in the sodium transporting potential (7, 8). Linderholm (8) has recently reported the effects of two diuretics on active sodium transport in the frog skin. Mersalyl decreased the current produced by the short-circuited skin by decreasing the sodium transporting potential. Aminophylline increased the conductance of the skin but had no effect on the sodium transport potential.

The experiments reported here describe the effects of a number of other substances on the electric current produced by the short-circuited frog skin. Several of these substances are shown to inhibit active sodium transport, determined by means of tracers, in a rather specific manner. Determinations of oxygen consumption of frog skin demonstrate that the effects of these substances on active sodium transport may occur without significant alteration in oxygen uptake.

**Methods**

Experiments were done during the winter months with the abdominal skin of *Rana temporaria*. The frogs were taken from a large indoor tank (ca. 5\textdegree C.) immediately before use. The skin was mounted in a lucite modification (10) of the apparatus described by Ussing and Zerahn. The Ringer solution on both sides of the skin contained NaCl 111 mM, KCl 2 mM, CaCl\textsubscript{2} 1 mM and NaHCO\textsubscript{3} 2.4 mM. The pH of the Ringer, after oxygenation with air, was about 8.2 (6). The paper of Ussing and Zerahn (7) may be consulted for details concerning the short-circuited frog skin preparation. Briefly, the p.d. across the skin is read from a potentiometer connected, through KCl-calomel electrodes, with agar bridges placed approximately 3 mm. on either side of the skin. An outer emf, applied through agar bridge electrodes placed approximately 4.5 cm. on either side of the skin, is adjusted so that the p.d. across the skin is maintained at zero. The current at zero potential is read from a microammeter. Measurement of current at two potential differences then permits calculation of the approximate d.c. resistance of the skin. Since the current-p.d. relationship is linear in this region only in some skins (8, 12, 13), no strict quantitative significance is attached to these resistance measurements.

In experiments with all substances except thiocyanate, small volumes of concentrated solutions or weighed amounts of dry material were added to the solutions bathing the skin. The pH of all solutions was adjusted so that addition to the Ringer solution produced changes of less than 0.1 pH unit. For experiments with thiocyanate a special 'CNS\textsuperscript{-} Ringer' was prepared from NaSCN, KSCN and Ca(NO\textsubscript{3})\textsubscript{2} such that the cation concentrations were unchanged from ordinary Ringer, but all of the Cl\textsuperscript{-} was replaced by CNS\textsuperscript{-}. The desired concentration of CNS\textsuperscript{-} was then obtained by mixing this solution with ordinary Ringer.

Chloride flux was determined with Cl\textsuperscript{36} (14) which was added to 20 ml. Ringer solution on one side of the skin. From 30 ml. of Ringer solution on the other side 10-ml. samples were taken at appropriate intervals and replaced with an equal volume of fresh solution. Simultaneously 25-ml. samples were taken from the high activity side and diluted to 10 ml. with fluid of the same composition as that on the low activity side. The chloride in each sample was precipitated with an excess of AgNO\textsubscript{3},
in acid solution, centrifuged, and the precipitate washed twice with distilled water. The precipitate was then transferred, with the aid of a few milliliters of glycerine and ethyl alcohol, collected with suction on filter paper discs in perforated aluminum counting dishes, smoothed with a nickel spatula and dried for a few minutes in an oven. The amount of Cl\textsuperscript{36} passing through the skin was expressed as percentage of the standard taken from the high activity side. Further calculations were essentially the same as those described by Ussing and Zerahn for sodium. In the short-circuited skin it was necessary to determine Cl\textsuperscript{−} flux in only one direction, since in these circumstances the influx and outflux are equal (16).

In other experiments the influx and outflux of sodium were determined simultaneously by means of a double-labeling technique (15). Outflux was determined with the short half life isotope Na\textsuperscript{24} (obtained from N. V. Philips-Roxane, Amsterdam) and the influx with the long half-life isotope Na\textsuperscript{22} (obtained from the Massachusetts Institute of Technology). For counting 0.5-ml. samples were taken in Carlberg pipettes from the low activity side. Standards were the same volume of 1:100 (for Na\textsuperscript{22}) or 1:1000 (for Na\textsuperscript{24}) dilutions from the high activity sides. Samples of Na\textsuperscript{24} were counted immediately using a 0.3 mm. aluminum filter to absorb the soft radiation of the Na\textsuperscript{22}. Samples of Na\textsuperscript{22} were counted after about 1 month when the Na\textsuperscript{24} had decayed. Because of a long half-life impurity in the Na\textsuperscript{24} the determinations of influx with Na\textsuperscript{22} were not possible in all experiments.

Thiocyanate concentration was determined by the method of Crandall and Anderson (16) with a Beckman spectrophotometer at 480 m\textmu completely 3 minutes after mixing (17).

Oxygen consumption of frog skin was determined in separate experiments by the direct method of Warburg at 25°C. with air as the gas phase. The skin was rinsed in Ringer solution, spread on filter paper, cut first along the mid line and then into transverse strips 2 to 4 mm. wide. The chemicals were added from the side arms after a 90-minute control period and the rate of oxygen consumption for the period 30 to 90 minutes after addition was expressed in relation to the rate for the same sample of skin for the period 15 to 75 minutes after setting. The oxygen consumption of about 100 mg. of skin from each of 10 frogs was measured for approximately 3 hours, during which the rate of oxygen consumption was linear after the first 15 minutes. The mean \( q_{02} \) of these control samples was 0.16 \( \mu \text{l/mg. wet weight per hour} \). If the water content is assumed to be 81 per cent (8) this is equal to 0.84 \( \mu \text{l/mg. dry weight} \).

The theoretical basis for experiments of this type has been reviewed recently by Ussing (9) so that only a brief summary need be given here. The amount of a substance that passes across unit area of membrane in unit time is designated flux (\( M \)). Net flux (\( \Delta \)) is then equal to \( M_{\text{in}} - M_{\text{out}} \). For a free ion diffusing through a membrane, neglecting transfer of water across the membrane, the following equation was shown to be valid:

\[
\frac{M_{\text{in}}}{M_{\text{out}}} = \frac{d_0 - C_o}{d_i - C_i} \cdot f_o \exp \left( -\frac{zF}{RT} (\psi_i - \psi_o) \right)
\]

where \( d \) = electrochemical activity, \( C \) = concentration, \( f \) = activity coefficient, \( z \) = charge of the ion, \( F \) = Faraday's number, \( R \) = gas constant, \( T \) = absolute temperature, \( \psi_i - \psi_o \) = potential difference across the membrane and the subscripts \( i \) and \( o \) refer to the inside and outside solutions, respectively. This equation does not hold for an actively transported ion; in this case it is necessary to introduce a term for the transporting force acting on the ion in question. In the present instance where
the p.d. across the membrane is kept at zero and the solutions on both sides of the skin are identical

\[
\frac{M_{in}}{M_{out}} = \exp \left( - \frac{zF}{RT} \cdot E_{Na} \right)
\]

(2)

where \( E_{Na} \) is the transporting force acting on the sodium ion. Then

\[
E_{Na} = \frac{RT}{zF} \ln \frac{M_{in}}{M_{out}}
\]

(3)

If \( E_{Na} \) is expressed in volts and \( M_{in} - M_{out} = \Delta \) in amperes, the partial sodium conductivity is given by

\[
k_{Na} = \frac{\Delta}{\frac{RT}{zF} \ln \frac{M_{in}}{M_{out}}}
\]

(4)

The partial sodium resistance, \( R_{Na} \), is the reciprocal of \( k_{Na} \).

RESULTS

After mounting the skin in the apparatus it was necessary to wait 60 to 90 minutes for the current to reach a more or less constant value. Readings were then begun and the substance to be tested was added 30 to 60 minutes later. If an effect was produced the reversibility was usually determined by replacing the solutions with fresh Ringer solution after another 30 to 90 minutes. Infrequently the same skin was used again with a higher concentration of the same substance or a new one. This second test usually served only for orientation and the results are rarely included in the data. In order to conserve space it has been necessary to omit many experiments from the tables. Usually concentrations less than the lowest given were tried and found to be ineffective.

The equations relating flux to electrochemical activities require a steady state between the isotope in the solution and that in the cells. For \( \text{Na}^+ \) and \( \text{Cl}^- \) the time required for equilibration is of the order of 30 minutes (9). In these experiments at least 45 minutes elapsed between initial addition of the isotope and the beginning of the first period of flux determination. However, when, in order to preserve osmotic equality, part of the Ringer solution was removed and replaced by isotonic solution of the inhibitor, additional radioactive isotope had to be added. Then more time was required for attainment of equilibrium. This interval has been reduced to 20 minutes, but even this delay prevented measurements during the period when current was transiently increased by some of the substances tested.

Thiocyanate. The addition of CNS\(^-\) in sufficient concentration to either or both sides of the skin produced a temporary increase in current (table 1). If the concentration was sufficiently high the current then fell below the initial level, but could be restored to approximately the initial value by adding fresh Ringer solution. Similar, but less marked, changes in p.d. were produced by CNS\(^-\) in the open-circuited skin. Skin resistance decreased in a more or less reciprocal manner during the period of increased current, but never fell to less than 50 per cent of the initial level. Thio-
cyanate added to only one side of the skin produced a greater increase in current and a more rapid decrease in current when added inside than when added outside. Event-
ually a fall in current occurred regardless of the side to which the CNS\(^-\) was applied.
Table 1. Effect of Various Chemicals on the Short-Circuit Electric Current and on Oxygen Consumption of Frog Skin

<table>
<thead>
<tr>
<th>Substance Added</th>
<th>Conc. (mM)</th>
<th>Current</th>
<th>O2 Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15-20 min</td>
<td>30-60 min</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>121</td>
<td>103</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>182</td>
<td>99</td>
<td>3</td>
</tr>
<tr>
<td>58</td>
<td>171</td>
<td>79</td>
<td>4</td>
</tr>
<tr>
<td>100</td>
<td>186</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>110**</td>
<td>132</td>
<td>107</td>
<td>3</td>
</tr>
<tr>
<td>110*</td>
<td>195</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3*</td>
<td>73</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8*</td>
<td>107</td>
<td>79</td>
<td>1</td>
</tr>
<tr>
<td>23*</td>
<td>20</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>23**</td>
<td>114</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl sulfanilamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.7*</td>
<td>68</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>p-Toluene sulfonamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8*</td>
<td>111</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>3.8*</td>
<td>139</td>
<td>94</td>
<td>4</td>
</tr>
<tr>
<td>5*</td>
<td>133</td>
<td>87</td>
<td>1</td>
</tr>
<tr>
<td>18*</td>
<td>62</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Prontosil Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>133</td>
<td>96</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>p-Aminobenzoate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.7*</td>
<td>123</td>
<td>105</td>
<td>1</td>
</tr>
<tr>
<td>5.8</td>
<td>82</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>105</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>81</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0.008</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>0.05</td>
<td>47</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>0.5</td>
<td>62</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>p-Nitrophenol</td>
<td>0.05</td>
<td>107</td>
<td>1</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2,4,6-Trinitrophenol</td>
<td>0.05</td>
<td>112</td>
<td>1</td>
</tr>
<tr>
<td>0.2</td>
<td>106</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.01</td>
<td>114</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>22</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>p-Benzquinone</td>
<td>0.01</td>
<td>83</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>27</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>100*</td>
<td>110</td>
<td>2</td>
</tr>
</tbody>
</table>

The current and oxygen consumption are expressed in relation to control values for the same experiment which are taken as 100. The current at the time given is expressed in relation to the average of that during the 30-minute period immediately before addition of the chemical. When only one value is given for current no increase was observed. In experiments marked * the chemical was added to the inside of the skin only; in those marked ** it was added to the outside only. In all other experiments the chemicals were added to both sides simultaneously.
Table 2. Effect of various chemicals on sodium and chloride flux

<table>
<thead>
<tr>
<th>SUBSTANCE ADDED</th>
<th>CONC.</th>
<th>LENGTH OF PERIOD</th>
<th>MEAN CURRENT</th>
<th>$\mu A/cm^2$</th>
<th>$\mu A/cm^2/hr.$</th>
<th>(\Delta M_{\text{in}}/M_{\text{out}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiocyanate no. 48</td>
<td>0</td>
<td>60 min.</td>
<td>21.6</td>
<td>1.6</td>
<td>25.5</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>23.3</td>
<td>1.7</td>
<td>24.8</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>60 min.</td>
<td>27.4</td>
<td>2.1</td>
<td>16.3</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>60 min.</td>
<td>14.4</td>
<td>4.5</td>
<td>20.5</td>
<td>20.1</td>
</tr>
<tr>
<td>Thiocyanate no. 51</td>
<td>0</td>
<td>60 min.</td>
<td>18.9</td>
<td>1.6</td>
<td>20.5</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>23.0</td>
<td>1.6</td>
<td>24.9</td>
<td>24.9</td>
</tr>
<tr>
<td></td>
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<td>30 min.</td>
<td>23.8</td>
<td>2.1</td>
<td>13.9</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>60 min.</td>
<td>8.3</td>
<td>5.6</td>
<td>5.6</td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>20.1</td>
<td>2.3</td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
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<td>75 min.</td>
<td>24.4</td>
<td>2.6</td>
<td>20.2</td>
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</tr>
<tr>
<td></td>
<td>18</td>
<td>60 min.</td>
<td>25.0</td>
<td>2.9</td>
<td>11.6</td>
<td>11.6</td>
</tr>
<tr>
<td>p-Toluene sulfonamide no. 24</td>
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<td>60 min.</td>
<td>29.7</td>
<td>4.4</td>
<td>41.0</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>27.3</td>
<td>3.7</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>40 min.</td>
<td>20.4</td>
<td>3.7</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
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<td>60 min.</td>
<td>18.6</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
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<td>15.0 min.</td>
<td>12.4</td>
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<tr>
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<td>28.4</td>
<td>0.5</td>
<td>28.0</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>27.3</td>
<td>0.5</td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>40 min.</td>
<td>19.7</td>
<td>0.5</td>
<td>20.2</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
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<td>60 min.</td>
<td>10.9</td>
<td>0.7</td>
<td>11.6</td>
<td>11.6</td>
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<tr>
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<td>41.0</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>39.2</td>
<td>2.8</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
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<td>60 min.</td>
<td>23.9</td>
<td>3.2</td>
<td>21.6</td>
<td>27.1</td>
</tr>
<tr>
<td>Dinitrophenol no. 31</td>
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<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60 min.</td>
<td>20.7</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>60 min.</td>
<td>20.6</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>65 min.</td>
<td>6.4</td>
<td>1.4</td>
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<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>55 min.</td>
<td>3.0</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Dinitrophenol no. 35</td>
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<tr>
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<td>25.1</td>
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<tr>
<td></td>
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<td>60 min.</td>
<td>13.8</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>60 min.</td>
<td>4.1</td>
<td>3.1</td>
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<td>3.1</td>
</tr>
<tr>
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<tr>
<td>Dinitrophenol no. 47</td>
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<td>1.2</td>
<td>54.3</td>
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<tr>
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<td>60 min.</td>
<td>42.4</td>
<td>1.4</td>
<td>40.3</td>
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<td>23.7</td>
<td>1.5</td>
<td>25.2</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>45 min.</td>
<td>8.4</td>
<td>1.1</td>
<td>12.8</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>45 min.</td>
<td>4.0</td>
<td>3.7</td>
<td>12.7</td>
<td>7.7</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>0.05</td>
<td>35 min.</td>
<td>8.6</td>
<td>1.4</td>
<td>10.0</td>
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<tr>
<td></td>
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<td>3.9</td>
<td>3.0</td>
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</table>
Flux values were measured in the short-circuited skin with radioactive tracers as explained in text. Sulfonamides and alcohol were added to the inside only. All other substances were added to both sides. $M_{out} =$ outflux. $M_{in} =$ influx. $A =$ electric current. The last column gives sodium influx calculated from the mean current plus the sodium outflux.

Complete replacement of Ringer solution on both sides of the skin with 'CNS- Ringer' resulted in decrease in both current and p.d. to 10 to 15 per cent of the initial value in 1 hour; this effect was irreversible.

Data showing the effect of two concentrations of CNS- on the rate of oxygen consumption of the skin are given in table 1. It is clear that the current may be decreased by a concentration (58 mM) which produced little decrease in oxygen consumption. Higher concentrations decreased both current and oxygen consumption.

The results of experiments in which sodium influx and outflux were determined by the double-labeling technique before and after addition of CNS- are given in table 2. The last column gives the influx calculated as current + outflux, since previous experiments have demonstrated that this is valid in most circumstances (7). These experiments demonstrate that CNS- produces both increase in Na+ outflux and decrease in Na+ influx. The net flux, represented by the current, decreases, particularly during the last periods of measurement. The implications of these results are discussed below.

**Sulfonamides.** Data showing the effect of four sulfonamides on the current of the short-circuited skin and on oxygen consumption are given in table 1. In most experiments these compounds were added only to the inside compartment in order to obtain the greatest possible increase in current. High concentrations of sulfonamides were necessary to decrease the current below the initial level; these concentrations of $p$-toluene sulfonamide produced marked inhibition of oxygen consumption. Changes in skin resistance after addition of the sulfonamides were relatively minor.

Prontosil Red (4-sulfonamido-2':4'-diaminoazobenzene) and acetylsulfanilamide ($p$ acetamidobenzenesulfonamide) were difficult to work with because of their low solubilities. In high concentrations (obtained by addition of a warm solution) acetylsulfanilamide crystallized and obstructed the circulation of fluid in the apparatus. Measurements of Cl$^-$ and Na$^+$ flux were therefore made with $p$-toluene sulfonamide and sulfanilamide only (table 2). The decrease in current is clearly the result of decreased sodium influx. The outflux of Na$^+$ and of Cl$^-$ appears to be unaffected even by high concentrations of the compounds tested.

**Nitrophenols.** Three nitrophenols were tested on the current in the short-circuited skin preparation and on oxygen consumption of the skin (table 1). 2,4-Dinitrophenol (DNP) in concentrations of from 0.008 to 0.5 mM decreased the current (reversibly) and greatly increased the oxygen consumption. There was no indication

---

**Table 2. Continued**

<table>
<thead>
<tr>
<th>Substance Added</th>
<th>Conc.</th>
<th>Length of Period</th>
<th>Mean Current</th>
<th>$\text{Cl}^- M_{out}$</th>
<th>$\text{Na}^+ M_{out}$</th>
<th>$\text{Na}^+ M_{in}$</th>
<th>$\text{Na}^+ (\Delta M_{out})$</th>
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<tr>
<td>Quinone no. 50</td>
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<td>60 min</td>
<td>29.3 $\mu$A cm$^{-2}$ hr$^{-1}$</td>
<td>1.2</td>
<td>1.4</td>
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<td>16.0</td>
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<tr>
<td>Alcohol no. 50</td>
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<td>26.3 $\mu$A cm$^{-2}$</td>
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<td></td>
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<td>9.2</td>
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<td></td>
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<tr>
<td></td>
<td>400</td>
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<td>23.5 $\mu$A cm$^{-2}$</td>
<td>15.5</td>
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</table>
of transient increase in current with DNP. p-Nitrophenol produced effects on current and oxygen consumption similar to those produced by DNP but the effective concentration was about 10 times that of DNP (18). Slight transient increase in current was produced by p-nitrophenol. These nitrophenols had little effect on resistance. In contrast to these two compounds 2,4,6-trinitrophenol did not decrease current or increase oxygen consumption in concentrations as high as 0.2 mM.

Determinations of Na\(^+\) and Cl\(^-\) flux were made only with 2,4-dinitrophenol (table 2). Large decreases in current were found to occur without change in Cl\(^-\) flux; however when the current was maintained at a low level for some time Cl\(^-\) flux increased in one experiment. Although moderate increase in Na\(^+\) outflux occurred during the latter part of the experiments, the most significant effect is the great reduction in sodium influx.

\(p\)-Benzoquinone and Hydroquinone. Both these substances reduced the current and had no effect on oxygen consumption in concentrations of 0.01 mM (table 1). While the current decreased within 10 minutes after addition of quinone, it first increased and then decreased 40 minutes after addition of the same concentration of hydroquinone. Higher concentration (0.1 mM) of both acted much faster and the delayed action of hydroquinone was not evident. Solutions of both substances were prepared within 3 minutes of the time of addition to the skin. Older solutions of hydroquinone had a more immediate effect than did fresh ones. These differences seem to indicate that hydroquinone is not, in itself, effective in decreasing the skin current.

Table 2 shows that the decrease in current produced by quinone occurs without change in Na\(^+\) outflux. As with the other substances so far discussed the effect is a decrease in sodium influx.

Miscellaneous. Several other substances are included in table 1. \(p\)-Aminobenzoate was tested because of its structural relationship to the sulfonamides. It was found to decrease current in a concentration which had no consistent effect on oxygen consumption. Sodium benzoate, on the other hand, had practically no effect on either current or oxygen consumption.

Ethyl alcohol is included to emphasize the rather specific effects of the other substances. Alcohol (0.4 M) produced very large decreases in skin resistance (as much as 90 per cent in one experiment) but had little effect on the current in the short-circuited skin (table 1). The p.d. of the open-circuited skin was markedly decreased. The maintenance of a rather constant current suggests that alcohol does not act specifically on the active sodium transport. Chloride flux was determined in the short-circuited skin before and after the addition of alcohol to the inside solution (table 2). In this experiment the current did not vary by more than \(\pm \)10 per cent throughout the experiment. The p.d. before short-circuiting the skin was 70 mv. and at the end of the experiment was 13 mv. It may be seen that the Cl\(^-\) outflux was tremendously increased by this concentration of alcohol. Replacement of the alcoholic solution on the inside of the skin with fresh Ringer solution resulted in restoration of both p.d. and resistance to approximately the initial values.

**DISCUSSION**

**Effects of Inhibitors on the Sodium Transporting Mechanism.** It is clear from these and other experiments that various substances may alter either sodium influx, sodium outflux or both. Alterations in influx and outflux may be produced by change in the partial sodium resistance of the skin. The net sodium flux, which in the short-
circuited skin is the source of the electric current, may be reduced by either increased outflux or decreased influx. Complete inhibition of the ‘sodium pump’ would result in $M_{in} = M_{out}$ (net flux equal to zero). If the short-circuited skin is regarded as a battery then the electric current depends upon the emf acting upon the sodium ions ($E_{Na}$) and the partial sodium conductivity ($k_{Na}$) or its reciprocal ($R_{Na}$). These characteristics, calculated from equations 3 and 4 are given in table 3. The sodium influx used in these calculations is that derived from current plus outflux (table 2, column 8).

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<tr>
<th>EXPER. NO.</th>
<th>SUBSTANCE ADDED</th>
<th>CONC.</th>
<th>$E_{Na}$</th>
<th>$R_{Na}$</th>
<th>$k_{Na}$</th>
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<td>mV</td>
<td>$\Omega$ cm$^{-2}$</td>
<td>$\Omega$ cm$^{-2}$ m$^{-1}$</td>
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<td>mV</td>
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<td>$\Omega$ cm$^{-2}$ m$^{-1}$</td>
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<td>$\Omega$ cm$^{-2}$ m$^{-1}$</td>
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2,4-Dinitrophenol, sulfonamides, thiocyanate and quinone all reduce $E_{Na}$. Their effect on the short-circuited current is therefore, at least in part, due to a reduction of the sodium transport potential. The effects of these compounds on sodium resistance are not consistent for the group. Thiocyanate appears to decrease sodium resistance slightly, but the effect is small compared to that produced by adrenaline or neurohypophysial hormones (7). For the other compounds studied the trend is to increase sodium resistance particularly if the initial resistance of the skin is low (e.g. experiment 47).

**Mechanism of Inhibition of Active Sodium Transport.** Thiocyanate and sulfonamides were used to test the hypothesis that carbonic anhydrase may be involved in
active sodium transport in the frog skin. Both of these substances have been reported
to inhibit carbonic anhydrase and active ion transport in the kidney (19) and gastric
mucosa (20). The results reported here show that both thiocyanate and sulfonamides
inhibit active sodium transport in frog skin. These data, however, should not be
interpreted as demonstrating that carbonic anhydrase is concerned in active trans-
port in this tissue. Although it is difficult to compare effective inhibitor concen-
trations in different systems, the concentrations of sulfonamides necessary to decrease
active sodium transport in the frog skin are exceedingly high (21). It should be em-
phasized that neither thiocyanate nor sulfonamides are specific inhibitors of carbonic
anhydrase. Both are goitrogenic, and it is of particular interest that the action of
thiocyanate on the thyroid is to inhibit the ability of the gland to concentrate iodine
(22, 23). Thiocyanate has recently been found to decrease the rate of oxidation of
certain amino acids by liver (24). Sulfonamides have been reported to inhibit lumi-
nescence (25), the activity of glucose-6-phosphate dehydrogenase (26) and carboxylase
(27), as well as oxygen consumption in certain organisms (28, 29). No determinations
of the carbonic anhydrase activity of frog skin were found in the literature. Experi-
ments carried out in this laboratory by Dr. L. B. Kirschner failed to demonstrate
the presence of carbonic anhydrase in this tissue. Therefore it may be concluded
tentatively that thiocyanate and sulfonamides inhibit active sodium transport in
the frog skin through inhibition of some processes other than those controlled by
carbonic anhydrase.

The current of the short-circuited skin preparation was also decreased by para-
aminobenzoic acid, which was tested because of its structural relationship to the
sulfonamides. When it was added after the current was already reduced by sulfon-
amides it produced a further decrease in current; there was no indication of an-
tagonsm (30).

Benzoic acid, reported by Klein and Kamin (31) to inhibit d-amino acid oxidase,
did not reduce either current or oxygen consumption in the frog skin.

Hydroquinone was tested on the current of the frog skin because it, as well as
thiocyanate, sulfonamides and para-aminobenzoic acid, has been reported to be
goitrogenic (32) and to inhibit synthesis of thyroxine in thyroid slices (33). Both
hydroquinone and quinone were extremely effective in decreasing the current in the
trog skin, and the latter substance was shown to decrease ENa. In contrast to the
other substances tested the inhibition produced by hydroquinone and quinone was
irreversible. Sufficient data are not available to warrant speculation as to the mech-
anism of action of these two inhibitors. However, various quinones have been re-
ported to act in low concentrations as enzyme inhibitors (34). Certain nitrophenols have long been known to inhibit various synthetic cellular
processes in concentrations which stimulate oxygen consumption (35, 36). The
mechanism by which the inhibition of synthesis is brought about now appears to be
through ‘uncoupling’ of phosphorylation from oxidation (37-40). Taggart and For-
ster (41) found that certain nitrophenols inhibited renal tubular transport of phenol
red and suggested that energy-rich phosphate compounds play a role in this transport
process. This work has been amply confirmed (42) and has been extended by Mudge
(43, 44) to ‘potassium accumulation’ in kidney slices. Such kidney slices lost potas-
sium and gained sodium in cold Ringer solution. When transferred to a warm nutrient
medium the potassium reaccumulation and sodium extrusion could be measured.
Mudge refers to these changes as ‘potassium accumulation,’ although he points out
that it is not known whether the potassium accumulation is primary or whether it is
secondary to active sodium extrusion. Regardless of which process is the primary one, the movement of the ions against concentration gradients could be prevented by certain nitrophenols. In view of the lack of evidence for active potassium transport, and the fact that active sodium transport can be demonstrated in other tissues, it may be suggested that sodium transport is the primary active process in Mudge's experiments. Cation transport in red blood cells was not inhibited by dinitrophenol (45).

It is clear that in the frog skin 2,4-dinitrophenol and p-nitrophenol both inhibit active sodium transport although the effective concentrations are different (table 1). 2,4,6-Trinitrophenol was ineffective. Those nitrophenols which stimulated oxygen consumption also inhibited active sodium transport. These results are consistent with those of Clowes et al. on cell division and respiration in Arbacia eggs (46) and on cell-free phosphorylating extracts from these eggs (47). They differ from those of others (38, 41, 44) with respect to the action of p-nitrophenol which these authors report to be ineffective.

The results showing inhibition of active sodium transport in the frog skin by 2,4-dinitrophenol strongly suggest the participation of energy-rich phosphate compounds in this process.

Active Sodium Transport and Oxygen Consumption. Many workers have investigated the relationship between oxygen consumption of the frog skin and the potential difference across the skin (8, 11, 48-50). Since the p.d. is the result of active inward transport of sodium, a decrease in p.d. may be due to inhibition of active transport. However, p.d. may also be reduced by increased permeability of the skin to Cl\(^{-}\) (14) without decrease in the sodium transporting potential. For this reason the present discussion is confined to those instances in which active sodium transport has been determined or in which it can be ascertained from measurements of current. Poisoning of the skin with cyanide reduces both active sodium transport and oxygen consumption (6, 50). This is, of course, to be expected in view of the role of oxidative processes in the maintenance of the p.d. (51). Thus substances which reduce oxygen consumption may be expected to reduce active transport. But it is also now certain that inhibition of active sodium transport need not be accompanied by reduction in the rate of oxygen consumption of the frog skin (8). Of the substances studied here, thiocyanate, sulfanilamide, para-aminobenzoic acid, hydroquinone and quinone reduced active sodium transport without significant effect on oxygen consumption. Dinitrophenol and p-nitrophenol reduced active transport in concentrations which increased oxygen consumption. Prontosil Red, acetylsulfanilamide and p-toluene sulfonamide reduced both oxygen consumption and active sodium transport.

SUMMARY

Several groups of compounds were found to inhibit active sodium transport across the isolated short-circuited frog skin with Ringer solution on both sides. In most circumstances the electric current which can be drawn from the skin is equal to the net sodium influx. The influx and outflux of sodium and of chloride were determined by means of radioactive tracers. The effect of the same inhibitors on the skin oxygen consumption was determined.

Active sodium transport (net sodium influx) was inhibited by thiocyanate, p-benzoquinone, sulfanilamide, p-toluene sulfonamide and dinitrophenol. Sodium outflux was increased moderately in some experiments. The latter three compounds produced some increase in the permeability of the skin to chloride. Judging from the reduction

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of the electric current which could be drawn from the skin, active transport of sodium was also inhibited by acetylsulfanilamide, Prontosil Red, p-aminobenzoic acid, p-nitrophenol and hydroquinone. Benzoic acid and 2,4,6-trinitrophenol were ineffective.

Substances which reduced oxygen consumption reduced the active transport of sodium. However, other substances which had no effect on oxygen consumption, or which increased it, also reduced active sodium transport.

The experiments with dinitrophenol indicate that high-energy phosphate compounds are involved in active sodium transport in the frog skin. In spite of the inhibitory effect of sulfonamides on active transport, it is doubtful that carbonic anhydrase plays a role in this process.

I wish to express my gratitude to Professor H. H. Ussing for his interest in this work and for many helpful discussions, and to K. Zerahn for helping in many ways. I am grateful to Professor P. Brandt Rehberg and his staff for their hospitality during tenure of the fellowship. The sulfonamides were supplied through the courtesy of Professor K. A. Jensen. I especially wish to thank my wife, Geraldine J. Fuhrman, for her unfailing assistance throughout the course of the work.

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