Energetics of Sodium Transport
in Frog Skin

I. Oxygen consumption in the short-circuited state

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ABSTRACT Sodium transport and oxygen consumption were studied simultaneously in the short-circuited frog skin. Sodium transport was evaluated from \( I_s/F \), where \( I_s \) is the short-circuit current measured with standard Ringer's solution bathing each surface and \( F \) is the Faraday constant. Oxygen tension was measured polarographically. Under a variety of circumstances the rate of oxygen consumption from the outer solution exceeded that from the inner solution, the ratio being constant (0.57 \( \pm \) 0.09 SD). Both \( I_s \) and the associated rate of oxygen consumption \( J_o \) declined nonlinearly with time, but the relationship between them was linear, suggesting that the basal oxygen consumption was constant. For each skin numerous experimental points were fitted by the best straight line. The intercept \( (J_o)_{t=0} \) then gave the basal oxygen consumption, and the slope \( dNa/dO_2 \) gave an apparent stoichiometric ratio for a given skin. The basal oxygen consumption was about one-half the total oxygen consumption in a representative untreated short-circuited skin. Values of \( dNa/dO_2 \) in 10 skins varied significantly, ranging from 7.1 to 30.9 (as compared with Zerahn's and Leaf and Renshaw's values of about 18). KCN abolished both \( I_s \) and \( J_o \). 2,4-dinitrophenol (DNP) depressed \( I_s \) while increasing \( J_o \) four- to fivefold. Anti-diuretic hormone stimulated and ouabain depressed both \( I_s \) and \( J_o \); in both cases apparent stoichiometric ratios were preserved.

INTRODUCTION

Much of the work on energetics of sodium transport places great emphasis on stoichiometry. A stoichiometric ratio is generally considered to relate the rate of active sodium transport to the rate of suprabasal oxygen consumption under all conditions of operation (e.g. the short-circuited and open-circuited...
states). It is also often tacitly assumed that the same ratio obtains in different preparations of a given kind.

In view of the marked differences between randomly selected tissues, the assumption of a unique ratio applicable to all tissues seems unwarranted. It is also by no means evident that a unique ratio would apply to any one tissue under all experimental conditions. This would be the case only if sodium transport and metabolism were completely coupled, so that any factor operating to change one must necessarily change the other in such a way as to keep their ratio constant. Partial decoupling could in fact come about in many ways, some of which have been considered elsewhere (Rottenberg et al., 1967; Essig and Caplan, 1968). The demonstration of linear dependence between transport and metabolism is often cited as evidence for a stoichiometric relationship. However, to say that there is a linear relationship between the rate of transport and the rate of metabolism is not equivalent to saying that the rate of transport is a constant multiple of the rate of suprabasal metabolism; linearity between two flows can exist whether coupling is tight or loose.

In this paper we shall consider the relationship between the rate of sodium transport and the rate of oxygen consumption in the short-circuited frog skin exposed to identical solutions at each surface. It will be shown that even in this well-defined state the relationship between sodium transport and suprabasal oxygen consumption varies from skin to skin.

METHODS

The studies were carried out in modified Ussing-Zerahn chambers (Ussing and Zerahn, 1951), permitting the simultaneous measurement of electrical current, transmembrane potential, and oxygen consumption (Fig. 1). Abdominal skins of *Rana pipiens* were mounted in a Lucite chamber, exposing an area of 7.1 cm$^2$, and equilibrated for at least 1 hr before study. Each half-chamber was connected to a microcentrifugal pump driven by a magnetic stirrer; the total volume on each side was 10.5 ml. Sodium Ringer agar "sensing" bridges were used for monitoring the electrical potential with saturated calomel half-cells, and an additional pair of bridges permitted the passage of current through silver-silver chloride electrodes. A voltage clamp was used to set the potential difference across the skin (a compensating circuit corrected automatically for the potential drop between the tips of the sensing bridges and the membrane). Electrical current was recorded continuously.

The partial pressure of oxygen in each compartment was continuously monitored by means of Clark oxygen electrodes with 0.001 inch Teflon membranes (Yellow Springs Instrument Company, Yellow Springs, Ohio), one of which was fitted to each micropump. Circulation at a rate of 2.3 ml/sec produced vigorous stirring at the surface of each electrode, providing stable pO$_2$ readings. Since the response of the oxygen electrodes is very sensitive to temperature the system was provided with water jackets supplied from a constant temperature (25°C) bath. Each oxygen electrode was connected to a polarographic circuit whose output voltage was directly
proportional to $pO_2$. When we were interested in the oxygen consumption from each solution the output voltages were recorded independently. Otherwise only the sum of the two voltages in series was recorded, thereby reducing the error in evaluating the slopes. During the experimental periods, clamping the inlet tubing isolated the solutions from air. Care was taken to avoid bubbles since these would lead to underestimation of oxygen consumption. At the beginning of each experiment the sensitivity of the circuit was adjusted so that, at the maximal pump rate, Ringer's solution equilibrated with air gave an output of 30 mv. The rate of oxygen consumption $J$, (in micromoles per second per square centimeter) was calculated from the relation:

$$J = \frac{aV}{E_{air}A} \left( P - P_{H_2O} \right) \frac{0.2095 \times 10^3}{760 \times 22.4} S,$$

where $a$, the solubility coefficient of oxygen at 25°C, is 0.0271 ml of $O_2$/ml Ringer solution (expressed at STP), $P$ is the barometric pressure (in millimeters of Hg), $P_{H_2O}$ is the water vapor pressure (in millimeters of Hg), $V$ is the volume of a half-chamber (in milliliters), $E_{air}$ is the output voltage of the polarographic circuit when each oxygen electrode is equilibrated with aerated sodium-Ringer's solution (in millivolts), $A$ is the membrane area (in square centimeters), and $S$ is the slope of the recorded plot of output voltage against time (in millivolts per second), measured visually. The volume fraction of oxygen in air has been taken as 0.2095.

\[1 \text{ Computed from tables in Linke (1965).}\]
The response of the electrodes in the above system was tested in Ringer's solution equilibrated with air, nitrogen, and nitrogen-oxygen mixtures of 10.33 and 15.47% oxygen. As shown in Fig. 2, the response was linear for pO2 values ranging from zero to that of air. This was true for each electrode, justifying the summation of the two voltages. Preliminary studies were also made of the importance of leakage and sorption of oxygen. For this purpose the system was the same as during experiments, but with a Parafilm membrane in place of the skin. After equilibration with air the chambers were rapidly filled with Ringer's solution equilibrated with a gas mixture of 15.47% oxygen in nitrogen. The system was then closed promptly and the pO2 was recorded for half an hour; it was found to be constant within experimental error. This shows that the solutions were adequately isolated from atmospheric air, and that desorption of oxygen from the walls of the Lucite chambers could be neglected. In the following experiments the pO2 was never allowed to fall below 85% of that of air. Periodically the depleted fluids were replaced by aerated solutions, or supplemented with small volumes of oxygenated solutions sufficient to bring the pO2 to that of air. Maximal O2 consumption by each electrode in air was $4.5 \times 10^{-2}$ μmole/sec (as calculated from a current of $1.75 \times 10^{-8}$ amp). This was negligible as compared with the rates of O2 consumption of the skins.

The standard glucose-Ringer's solution consisted of 110.0 mM NaCl, 2.5 mM KHCO3, 1.0 mM CaCl2, and 10.0 mM glucose, freshly prepared from concentrated stock solutions before use. The pH was 8.2 and the osmolarity 220 mosmol/liter. Streptomycin sulfate (Pfizer Labs., Div. Chas. Pfizer & Co., Inc., New York) was added at a concentration of 0.1 mg/ml to prevent bacterial growth. Other drugs and reagents used were antidiuretic hormone (Pitressin, Parke, Davis & Co., Detroit, Mich.), ouabain (Sigma Chemical Co., St. Louis, Mo.), 2,4-dinitrophenol (2,4-
DNP) (Eastman Organic Chemicals, Rochester, N.Y.), and oxygen-nitrogen mix-
tures (Medical-Technical Gases, Inc., Medford, Mass.). Frogs (Rana pipiens) pur-
chased from the Lemberger Company, Oshkosh, Wis. were kept in a moist environ-
ment at room temperature, without food, for a period of no longer than 2 wk.

Results are presented as the mean value ± the standard error (SE) if not otherwise
indicated. Straight lines were fitted by the method of least squares.

RESULTS

1. Oxygen Uptake from the Outer and Inner Surfaces

In these experiments the oxygen tension of each solution was monitored
continuously for 20-min periods. The rate of oxygen consumption from the
inner solution was always less than that from the outer solution; for short-
circuited skins the ratio had a mean value of 0.57 ± 0.09 SD, n = 77 (15
skins). Typical results for four skins are shown in Table I. The ratio was
independent of total oxygen consumption, electrical potential difference
across the skin, or the presence of glucose or 2,4-DNP. As a result of different
rates of oxygen consumption from the two baths an oxygen gradient developed
across the membrane. Despite the progressive increase of this gradient the
rates of oxygen uptake from the two baths were constant if the short-circuit
current was constant. This indicates that under the conditions of these
experiments the rate of diffusion of oxygen across the skin was insignificant.

2. Oxygen Consumption and Short-Circuit Current

In these experiments total oxygen consumption and short-circuit current
were measured simultaneously in skins exposed at each surface to identical
glucose-Ringer’s solutions. As is well known, when the frog skin is exposed to
standard sodium-Ringer’s solution the short-circuit current is equivalent to
the rate of net sodium transport (Ussing and Zerahn, 1951; Leaf and Renshaw,
1957a). We have assumed that the addition of 10 mM glucose to the solutions
does not alter this relationship.

In most skins, despite an equilibration period of 1–1.5 hr, both the short-
circuit current $I_s$ and the associated rate of oxygen consumption $J_{so}$ declined
with time, although often with superimposed fluctuations. A representative
experiment is shown in Fig. 3 (such behavior was seen both in the presence
and in the absence of glucose). Despite the decline and fluctuation of both
$I_s$ and $J_{so}$, on relating the two values for each period a linear relationship
was observed. Table II gives the results for 10 skins, untreated with drugs,
in which there were sufficient spontaneous changes of $I_s$ and $J_{so}$ to permit the
demonstration of their relationship.

The existence of a linear relationship between $I_s$ and $J_{so}$ permits the evaluation
by extrapolation of the rate of oxygen consumption which would have
obtained in the absence of short-circuit current: $(J_{so})_{I_s=0}$. This quantity,
which we consider to represent the basal rate of oxygen consumption, was
about one-half the total rate of oxygen consumption in a representative freshly mounted skin. (The validity of the use of \(J_{\text{r}o}\) as a measure of oxygen consumption unrelated to transepithelial sodium transport will be discussed below.)

A subject which has received a great deal of attention is the quantitative relationship between suprabasal oxygen consumption and active sodium transport (Ussing, 1960). Several workers have evaluated a mean ratio,

\[
\frac{J_r}{J_o} = \text{mean ratio}
\]

**Table 1**

**Typical Rates of Oxygen Consumption in Four Skins**

<table>
<thead>
<tr>
<th>(\Delta \phi)</th>
<th>(J^r_{\text{r}})</th>
<th>(J^o_{\text{r}})</th>
<th>(J^r_{\text{t}})</th>
<th>(\frac{J^r_{\text{r}}}{J^o_{\text{r}}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{mm}Hg)</td>
<td>(\mu \text{mole/sec/cm}^2)</td>
<td>(\mu \text{mole/sec/cm}^2)</td>
<td>(\mu \text{mole/sec/cm}^2)</td>
<td>(\mu \text{mole/sec/cm}^2)</td>
</tr>
<tr>
<td>0</td>
<td>27.0</td>
<td>51.2</td>
<td>78.2</td>
<td>0.53</td>
</tr>
<tr>
<td>0</td>
<td>30.1</td>
<td>50.0</td>
<td>80.1</td>
<td>0.60</td>
</tr>
<tr>
<td>0</td>
<td>27.0</td>
<td>44.6</td>
<td>71.6</td>
<td>0.61</td>
</tr>
<tr>
<td>0</td>
<td>25.4</td>
<td>43.0</td>
<td>68.4</td>
<td>0.59</td>
</tr>
<tr>
<td>0</td>
<td>23.5</td>
<td>41.4</td>
<td>64.9</td>
<td>0.56</td>
</tr>
<tr>
<td>0</td>
<td>24.2</td>
<td>43.0</td>
<td>67.2</td>
<td>0.56</td>
</tr>
<tr>
<td>0</td>
<td>22.7</td>
<td>38.3</td>
<td>61.0</td>
<td>0.59</td>
</tr>
<tr>
<td>0</td>
<td>24.2</td>
<td>38.3</td>
<td>62.5</td>
<td>0.63</td>
</tr>
<tr>
<td>0</td>
<td>19.9</td>
<td>41.8</td>
<td>61.7</td>
<td>0.48</td>
</tr>
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<td>41.1</td>
<td>60.3</td>
<td>0.46</td>
</tr>
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<td>39.1</td>
<td>58.3</td>
<td>0.49</td>
</tr>
<tr>
<td>0</td>
<td>16.8</td>
<td>37.1</td>
<td>53.9</td>
<td>0.46</td>
</tr>
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<td>-100</td>
<td>22.7</td>
<td>48.1</td>
<td>70.8</td>
<td>0.47</td>
</tr>
<tr>
<td>-100</td>
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<td>40.7</td>
<td>60.6</td>
<td>0.49</td>
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<td>18.4</td>
<td>38.3</td>
<td>56.7</td>
<td>0.48</td>
</tr>
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<td>14.1</td>
<td>27.4</td>
<td>41.5</td>
<td>0.51</td>
</tr>
<tr>
<td>0</td>
<td>13.7</td>
<td>28.2</td>
<td>41.9</td>
<td>0.49</td>
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<td>15.2</td>
<td>29.3</td>
<td>44.5</td>
<td>0.52</td>
</tr>
<tr>
<td>0</td>
<td>15.6</td>
<td>31.3</td>
<td>46.9</td>
<td>0.50</td>
</tr>
<tr>
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<td>13.7</td>
<td>28.5</td>
<td>42.2</td>
<td>0.48</td>
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<td>36.8</td>
<td>54.4</td>
<td>0.48</td>
</tr>
<tr>
<td>-100</td>
<td>16.8</td>
<td>34.4</td>
<td>51.2</td>
<td>0.49</td>
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<tr>
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<td>37.1</td>
<td>53.9</td>
<td>0.45</td>
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<td>25.4</td>
<td>39.1</td>
<td>0.54</td>
</tr>
<tr>
<td>0</td>
<td>37.5</td>
<td>62.9</td>
<td>100.4</td>
<td>0.60</td>
</tr>
<tr>
<td>0</td>
<td>33.2</td>
<td>62.9</td>
<td>96.1</td>
<td>0.53</td>
</tr>
<tr>
<td>+100</td>
<td>24.2</td>
<td>35.6</td>
<td>59.8</td>
<td>0.68</td>
</tr>
<tr>
<td>+100</td>
<td>25.0</td>
<td>37.1</td>
<td>62.1</td>
<td>0.67</td>
</tr>
<tr>
<td>+100</td>
<td>23.5</td>
<td>35.2</td>
<td>58.7</td>
<td>0.67</td>
</tr>
<tr>
<td>0</td>
<td>37.5</td>
<td>56.7</td>
<td>94.2</td>
<td>0.66</td>
</tr>
<tr>
<td>0</td>
<td>89.9</td>
<td>153.3</td>
<td>243.2</td>
<td>0.59</td>
</tr>
<tr>
<td>0</td>
<td>82.5</td>
<td>133.3</td>
<td>215.8</td>
<td>0.62</td>
</tr>
</tbody>
</table>

\(J^r_{\text{r}}\) and \(J^o_{\text{r}}\) represent uptake from the inner and outer solutions, respectively. \(\Delta \phi\) is the electrical potential in the inner solution minus that in the outer solution. The duration of each period was 20 min; at the end of each period the solutions on each side were replaced with fresh aerated Ringer's solution.

*2,4-DNP was added to the inner solution to give a concentration of 0.5 mM.
FIGURE 3. Variation of short-circuit current $I_o$ and the associated rate of oxygen consumption $J_{ro}$ with time. The inset shows the relationship between $I_o$ and $J_{ro}$.

TABLE II

ANALYSIS OF THE RATE OF OXYGEN CONSUMPTION AT SHORT-CIRCUIT $J_{ro}$ AS A FUNCTION OF THE SHORT-CIRCUIT CURRENT $I_o$ DURING THEIR SPONTANEOUS DECLINE WITH TIME (NO DRUGS ADDED)

<table>
<thead>
<tr>
<th>Skin</th>
<th>$J_{ro}$</th>
<th>$dJ_{ro}/dI_o$</th>
<th>$n$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu M/\sec \ per \ cm^2$</td>
<td>$\mu M/coul$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>$39.6 \pm 5.3$</td>
<td>$0.774 \pm 0.132$</td>
<td>8</td>
<td>0.923</td>
</tr>
<tr>
<td>2</td>
<td>$23.1 \pm 9.2$</td>
<td>$1.082 \pm 0.218$</td>
<td>7</td>
<td>0.912</td>
</tr>
<tr>
<td>3</td>
<td>$66.0 \pm 2.1$</td>
<td>$0.955 \pm 0.132$</td>
<td>21</td>
<td>0.857</td>
</tr>
<tr>
<td>4</td>
<td>$39.9 \pm 9.5$</td>
<td>$0.827 \pm 0.160$</td>
<td>12</td>
<td>0.853</td>
</tr>
<tr>
<td>5</td>
<td>$51.2 \pm 3.4$</td>
<td>$0.488 \pm 0.139$</td>
<td>7</td>
<td>0.843</td>
</tr>
<tr>
<td>6</td>
<td>$58.6 \pm 4.1$</td>
<td>$0.360 \pm 0.083$</td>
<td>7</td>
<td>0.889</td>
</tr>
<tr>
<td>7</td>
<td>$56.2 \pm 4.0$</td>
<td>$0.456 \pm 0.080$</td>
<td>13</td>
<td>0.864</td>
</tr>
<tr>
<td>8</td>
<td>$53.6 \pm 8.1$</td>
<td>$1.031 \pm 0.295$</td>
<td>7</td>
<td>0.842</td>
</tr>
<tr>
<td>9</td>
<td>$37.1 \pm 11.4$</td>
<td>$1.114 \pm 0.178$</td>
<td>7</td>
<td>0.542</td>
</tr>
<tr>
<td>10</td>
<td>$56.3 \pm 7.2$</td>
<td>$1.492 \pm 0.212$</td>
<td>8</td>
<td>0.942</td>
</tr>
<tr>
<td>Mean</td>
<td>$48.2 \pm 12.9$</td>
<td>$0.854 \pm 0.344$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data were fitted to a straight line by least squares analysis. $(J_{ro})_{I_o=0}$ is the extrapolated rate of oxygen consumption at zero short-circuit current ±s.e.; $dJ_{ro}/dI_o$ is the slope ±s.e.; $n$ is the number of observations; and $r$ is the correlation coefficient. For $n \geq 7$ a value of $r \geq 0.798$ is significant at the 0.01 level.
Figure 4. Effect of antidiuretic hormone (0.25 units/ml), ouabain (1 mM), and potassium cyanide (1 mM) on short-circuit current $I_o$ and the associated rate of oxygen consumption $J_{ro}$. All agents were added to the inner solution. Bottom, plots of $I_o$ and $J_{ro}$ against time. Top, plot of $J_{ro}$ against $I_o$. These data are from a single experiment.
based on a single experimental determination for each skin. In contrast, Table II is based on numerous experimental points for each skin; hence statistical analysis of the slopes permits the evaluation of an appropriate ratio for each. The mean slope $dJ_o/dI_o$ was $0.854 \pm 0.344$ mole/coul, corresponding to an average value of 14.6 sodium ions transported per molecule of oxygen consumed. However, the various skins show significantly different values.

3. Effects of Antidiuretic Hormone, Ouabain, Potassium Cyanide, and 2,4-Dinitrophenol on the Relationship Between Short-Circuit Current and Oxygen Consumption

Since there was a linear relationship between $I_o$ and $J_o$, as these parameters varied spontaneously, it was of interest to examine the relationship between them when they were altered by specific agents. The addition of antidiuretic hormone (ADH) to the solution bathing the inner surface of the frog skin has been reported to result in an increase of both short-circuit current and oxygen consumption (Ussing and Zerahn, 1951; Zerahn, 1956; Leaf and Renshaw, 1957a). Ouabain may be expected to cause a decrease of these parameters (Koefoed-Johnsen, 1957; Levy and Richards, 1965). Both effects can be seen in Fig. 4, which refers to the skin in which ADH produced the greatest effect. Again the relationship between $I_o$ and $J_o$ appears to be linear. (The two points below the line following ouabain were obtained when the short-circuit current was still changing rapidly [see Fig. 4, bottom]. Such observations were made in only 2 of 10 skins, and are therefore omitted from statistical calculations.)

**Table III**

<table>
<thead>
<tr>
<th>Skin</th>
<th>$(J_o)_{short}$</th>
<th>$dJ_o/dI_o$</th>
<th>$n$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.7±3.9</td>
<td>0.783±0.088</td>
<td>14</td>
<td>0.933</td>
</tr>
<tr>
<td>2</td>
<td>34.4±4.8</td>
<td>0.800±0.098</td>
<td>12</td>
<td>0.933</td>
</tr>
<tr>
<td>3</td>
<td>66.1±1.7</td>
<td>0.940±0.091</td>
<td>27</td>
<td>0.900</td>
</tr>
<tr>
<td>4</td>
<td>43.4±5.8</td>
<td>0.764±0.091</td>
<td>16</td>
<td>0.913</td>
</tr>
<tr>
<td>5</td>
<td>51.1±2.2</td>
<td>0.490±0.068</td>
<td>11</td>
<td>0.923</td>
</tr>
<tr>
<td>6</td>
<td>38.7±3.8</td>
<td>0.395±0.060</td>
<td>12</td>
<td>0.800</td>
</tr>
<tr>
<td>7</td>
<td>55.4±2.9</td>
<td>0.478±0.044</td>
<td>18</td>
<td>0.928</td>
</tr>
<tr>
<td>8</td>
<td>54.7±5.4</td>
<td>0.375±0.131</td>
<td>12</td>
<td>0.874</td>
</tr>
<tr>
<td>9</td>
<td>41.1±6.9</td>
<td>1.042±0.099</td>
<td>11</td>
<td>0.962</td>
</tr>
<tr>
<td>10</td>
<td>60.8±4.9</td>
<td>1.283±0.117</td>
<td>12</td>
<td>0.961</td>
</tr>
<tr>
<td>Mean</td>
<td>50.5±10.4</td>
<td>0.795±0.280</td>
<td></td>
<td></td>
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</tbody>
</table>

Symbols are the same as in Table II. For $n \geq 11$ a value of $r \geq 0.684$ is significant at the 0.01 level.
The results of a least squares analysis of the 10 skins is shown in Table III, representing combined data obtained before and after treatment with ADH. Similar results were found on combining these data with those obtained after the addition of ouabain; the results are shown in Table IV. An examination of Tables II–IV suggests that all data for a given skin lie on a single straight line. A stringent statistical analysis supported this view (see Appendix).

Fig. 5 shows the apparent stoichiometric ratios for the 10 skins. Again we see that although there appears to be a characteristic ratio for each, independent of the presence of ADH or ouabain, this ratio differs from skin to skin. This is most evident in considering the pooled data.

**Table IV**

Analysis of the Rate of Oxygen Consumption at Short-Circuit \( J_{sc} \) as a Function of the Short-Circuit Current \( I_o \) during Their Spontaneous Decline with Time and After the Administration of ADH and Ouabain (Combined Data)

<table>
<thead>
<tr>
<th>Skin</th>
<th>((J_{sc})_{I_o=0})</th>
<th>(dJ_{sc}/dI_o)</th>
<th>(n)</th>
<th>(r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.2±1.0</td>
<td>0.774±0.026</td>
<td>19</td>
<td>0.991</td>
</tr>
<tr>
<td>2</td>
<td>43.4±1.6</td>
<td>0.618±0.037</td>
<td>16</td>
<td>0.976</td>
</tr>
<tr>
<td>3</td>
<td>66.6±1.0</td>
<td>0.919±0.060</td>
<td>33</td>
<td>0.940</td>
</tr>
<tr>
<td>4</td>
<td>49.5±1.5</td>
<td>0.670±0.027</td>
<td>20</td>
<td>0.986</td>
</tr>
<tr>
<td>5</td>
<td>50.6±1.2</td>
<td>0.504±0.041</td>
<td>14</td>
<td>0.982</td>
</tr>
<tr>
<td>6</td>
<td>59.7±1.7</td>
<td>0.336±0.032</td>
<td>16</td>
<td>0.943</td>
</tr>
<tr>
<td>7</td>
<td>52.1±1.7</td>
<td>0.528±0.031</td>
<td>23</td>
<td>0.966</td>
</tr>
<tr>
<td>8</td>
<td>58.9±2.3</td>
<td>0.843±0.083</td>
<td>16</td>
<td>0.938</td>
</tr>
<tr>
<td>9</td>
<td>32.7±1.7</td>
<td>1.161±0.028</td>
<td>15</td>
<td>0.996</td>
</tr>
<tr>
<td>10</td>
<td>60.8±2.4</td>
<td>1.284±0.065</td>
<td>16</td>
<td>0.983</td>
</tr>
<tr>
<td>Mean</td>
<td>51.4±10.5</td>
<td>0.764±0.297</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Symbols are the same as in Table II. For \( n \geq 14 \) a value of \( r \geq 0.623 \) is significant at the 0.01 level.

**Figure 5.** Apparent stoichiometric ratios of short-circuited skins, calculated from \( 1/F(dJ_{sc}/dI_o) \). The bars represent 95% confidence limits.
The addition of KCN not only reduced active sodium transport and the associated oxygen consumption to very low levels, but also depressed basal oxygen consumption. The effect of KCN on basal oxygen consumption is shown in Fig. 4. The addition of 2,4-DNP caused a prompt decrease in $I_o$ and a transient increase in $J_o$ (Fig. 6). Following blockage of active sodium transport by ouabain, only an increase in $J_o$ was observed.

![Figure 6. Effect of ouabain (1 mM) and 2,4-dinitrophenol (0.5 mM) on the relation between short-circuit current $I_o$ and the associated rate of oxygen consumption $J_o$.](image)

**DISCUSSION**

It is of interest that the rate of consumption of oxygen from the outer solution is appreciably greater than that from the inner solution, the ratio being essentially constant (Table I). This was the case in all the conditions studied. This is not in agreement with Zerahn's findings. The reason for the difference in uptake at the two surfaces is uncertain. However, it seems probable that

2 In an attempt to minimize the contribution of bacterial contamination, all solutions were freshly prepared and contained streptomycin. However, Sharp et al. (personal communication) have recently noted continuing bacterial nucleotide turnover in toad bladders for as long as 4 hr after administration of streptomycin. We do not feel that the possibility of bacterial metabolism invalidates our main conclusions, for the following reasons: (a) bacterial contamination, which should be confined predominantly to the outer layers, would be expected to vary with time. In fact no change in the ratio $J_o/J_s$ was observed (Table I). (b) perturbation of $\Delta\Psi$ would be expected to change oxygen consumption related to sodium transport, but not that due to bacterial metabolism. No change in $J_o/J_s$ was noted with large changes in $\Delta\Psi$ (Table I). (c) If bacterial oxygen consumption were significant we would have expected to observe changes in the ratio $dNa/dO_2$ both with the passage of time (Fig. 3) and with alteration of the rate of sodium transport by specific agents (Fig. 5). No such changes were noted.
the mitochondria-rich epithelial cells will consume oxygen more rapidly than
the poorly cellular, underlying connective tissue. Also, as suggested by Martin
and Diamond (1966) for the rabbit gall bladder, the thick layer of connective
tissue may constitute a more effective permeability barrier to O₂ diffusion
than the thinner epithelial cell layer. Similar results have recently been
reported by Nellans and Finn (1970) in the toad bladder.

As mentioned above, a progressively increasing gradient for oxygen de-
velops, but constancy of $J'_o$ and $J'_e$ in those skins with constant $I_o$
indicates that the rate of diffusion of oxygen across the skin is insignificant compared
with the rate of oxygen consumption. In order to test this further we calculated
the rate of diffusion of oxygen across an unstirred layer of water of the approxi-
mate thickness of the frog skin, 300 $\mu$m, induced by a mean difference of pO₂ of
4.6 mm Hg, the largest value permitted here. The result was 5.6 $\mu$umole/sec
per cm², as compared with a basal oxygen consumption of 58.3 $\mu$umole/sec
per cm². Since the mean diffusion coefficient in the frog skin is presumably less
than in water, and since we have ignored the effects of unstirred layers, it
appears that the diffusion of oxygen across the skin is insignificant in these
experiments.

Zerahn raised the question as to whether the oxygen required for transport
is derived from the outside or inside solution. The observation of a constant
ratio of uptake from each solution even after the abolition of sodium transport
by ouabain, and the insignificance of oxygen transport across the skin, lead us
to believe that the oxygen is derived from both surfaces. In any event, it
cannot be assumed that the pattern observed in vitro applied also in vivo,
since in life the skin is vigorously perfused.

It is remarkable that in each skin there is a unique value of $dNa/dO_2$ which
is independent of treatment with either ADH or ouabain (Fig. 5; Tables II–
IV). This is of particular interest in view of the common belief that ADH acts
at an outer permeability barrier whereas ouabain acts at the pump. In terms
of a formalism presented previously (Essig and Caplan, 1968), the observed
results are consistent either with a change in the free energy of the metabolic
driving reaction, or with effects on the phenomenological coefficients of a
highly coupled system.

The above findings suggest also that there are two components of oxygen
consumption. One component is that associated with the process of net sodium
transport, linearly related to the short-circuit current. Extrapolating to the
state $I_o = 0$ then gives us the other component, oxygen consumption appar-
tently associated with all other cellular functions. We shall call this "basal"
oxygen consumption; we do not mean to imply, however, that this oxygen
consumption is necessarily unrelated to any cellular sodium transport, but
only that it is unrelated to net transepithelial sodium transport. The demon-
stration of two functional components of oxygen consumption is reminiscent
of the observations of Kidder et al. (1966) in their studies of hydrogen ion
secretion in bullfrog gastric mucosa. These workers interpreted relationships between oxidation-reduction states of cytochromes and active hydrogen ion secretion in terms of two parallel electron transport chains, one mitochondrial and the other extramitochondrial, the latter specifically involved in the transfer of hydrogen ions. Similarly, Siekevitz (1965) has adduced biochemical evidence for two discrete electron transport systems, one in mitochondria and the other in the endoplasmic reticulum.

It is of interest that there appears to be a basal oxygen consumption which remains essentially constant over an extended period. The alternative hypothesis is that both basal and nonbasal oxygen consumption vary linearly with short-circuit current, whether the variation in $I_o$ is a consequence only of the passage of time or also includes the effects of ADH or ouabain. This possibility seems to us a priori much less likely than a fairly constant rate of basal consumption. It is noteworthy that the basal rate calculated by extrapolation of $J_{ro}$ to $I_o = 0$ is equal to the rate of oxygen consumption observed in the presence of ouabain. This also supports the idea of near-constancy of the basal rate of oxygen consumption.

It may seem surprising that sodium transport and the associated oxygen consumption frequently decline with time while basal metabolism is well maintained. It might have been expected that they would decline in parallel owing to depletion of tissue substrates. Various possibilities could explain this behavior. For example, sodium transport may well depend on metabolic intermediates, cofactors, or hormonal agents, etc., readily depleted in the in vitro preparation, which are not important for basal metabolism. This would be consistent with the fact that the electron transport system of endoplasmic reticulum contains components lacking in mitochondria (Siekevitz, 1965).

Two metabolic inhibitors altered the regular pattern of behavior described above. The addition of 2,4-DNP uncouples sodium transport and oxygen consumption, as shown in Fig. 6 (see also Fuhrman, 1952, and Huf et al., 1957). The increase in $J_{ro}$ presumably reflects the characteristic effect of 2,4-DNP on mitochondrial oxidative phosphorylation. The addition of KCN abolishes not only active sodium transport and the associated oxygen consumption, but also basal oxygen consumption, presumably by the inhibition of cytochrome oxidase (Slater, 1966).

It is pertinent to ask to what extent $I_o$ may be supported by glycolytic metabolism. Leaf and Renshaw (1957b) noted that active ion transport occurs anaerobically in frog skin at 20–40% of the aerobic rate. However, in their

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3 We assume that the observed linearity is not due to the combination of two nonlinear rates of oxygen consumption. Previous data concerning the possibility of a small effect of ADH on basal oxygen consumption are conflicting (Leaf and Dempsey, 1960; Rasmussen et al., 1960; Swenson and Maffly, 1968); species differences may have contributed to this.
study glycolytic sodium transport in the presence of oxygen may have been small since lactate production increased markedly under anaerobic conditions. The linearity noted between sodium transport and oxygen consumption in the present study indicates that if glycolytic sodium transport is appreciable it must be constant. Therefore, if glycolytic sodium transport were in fact appreciable the basal rate of oxygen consumption calculated by extrapolation to \( I_s = 0 \) would be erroneously low and hence smaller than the value derived from the administration of ouabain; this was not the case. For these reasons we feel that glycolytic sodium transport was small in the present studies.

Many people have considered the relationship between the rate of sodium transport and the rate of oxygen consumption in various tissues (Zerahn, 1956, 1958; Leaf and Renshaw, 1957a; Thurau, 1961; Kramer and Deetjen, 1964; Martin and Diamond, 1966). The earliest comprehensive studies involving simultaneous measurements in frog skin were carried out by Zerahn, who used the chemical method of Krogh to evaluate average rates of oxygen consumption. His experiments usually consisted of the determination of the average rate of oxygen consumption in three consecutive periods of unspecified length, two control periods bracketing an experimental period. In the experimental period the average rate of sodium transport was also evaluated. In the control periods the transepithelial active sodium transport was assumed to be eliminated by replacing the outer sodium-Ringer's solution with distilled water or magnesium-Ringer's solution. The difference between the rates of oxygen consumption in the experimental and control periods ("net oxygen consumption") was then expressed as a percentage of the rate of net sodium transport. In this way Zerahn calculated a mean value for the net percentage ("N.P.") of 22.5, i.e. 22.5 equivalents of oxygen consumed per 100 equivalents of sodium transported, and stated that "the N.P. was the same under all conditions." The factors which were varied included outer concentration of Na, electrical potential difference, \( pO_2 \), time of year, species of animal, temperature, presence or absence of posterior pituitary hormone, and methods for producing the blank. Zerahn's value corresponds to 17.8 equivalents of sodium transported per mole of oxygen consumed, as compared with the mean value of 14.6 for the untreated skins of the present study. We do not feel that there is any discrepancy between these values, since in each case the numbers are simply means of a large number of values which vary over a considerable range. For example, using short-circuited skins of \textit{Rana temporaria} with identical sodium-Ringer solutions at each surface, Zerahn found values of N.P. ranging from 14.3 to 27.8 (corresponding to \( dNa/dO_2 \) values of 14.4–28.0 eq/mole); for \textit{Rana esculenta} the values ranged from 14.9 to 34.0 (i.e. \( dNa/dO_2 \) values of 11.8–26.9 eq/mole). In these studies the blank averaged two-thirds of the total

\[dNa/dO_2\]
rate of oxygen consumption and Zerahn estimated that the standard deviation of the net oxygen consumption ranged from 3 to 20%. In view of the uncertainties in the chemical technique employed, and the fact that the value for each skin was derived from only a single experimental period, it was of course reasonable to emphasize only the mean value for N.P., rather than its variability.

Leaf and Renshaw (1957 a), utilizing a precise polarographic technique, studied the relationship between sodium transport and oxygen consumption in the frog skin in both the absence and in the presence of antidiuretic hormone. Relating increments in sodium transport and oxygen consumption induced by the administration of hormone, they calculated a mean value for $\Delta \text{Na}/\Delta \text{O}_2$ of 18.2. Again, however, the values were derived from a single experimental period, and again they varied considerably in individual skins. Excluding one grossly aberrant figure, the values ranged from 5.8 to 43.1. The present results, based on several experimental periods in each skin, show values of $d\text{Na}/d\text{O}_2$ ranging from 7.1 to 30.9 (Fig. 5).

We feel that our results demonstrate clearly that for short-circuited skins with glucose-Ringer's solution on both sides there is no unique value for $d\text{Na}/d\text{O}_2$; rather the value may differ significantly from one skin to another and possibly from one season to another. Although the reason for this variation is unknown, certain possibilities come to mind. One possibility is recirculation of actively transported sodium (Ussing, 1966). If the magnitude of such recirculation were to differ from one skin to another, the apparent stoichiometric ratio would vary. Another possibility is variation of skin thickness. Such variation might affect sodium transport and oxygen consumption differently. Still a third possibility is variation in the state of metabolism. More generally, there are several possible sources of nonstoichiometry of the process of oxidative phosphorylation which presumably supports active transport. These have been discussed elsewhere (Rottenberg et al., 1967).

**APPENDIX**

**Statistical Analysis**

Since the measured short-circuit current reflects sodium transport with high precision, we attribute all error to the measurements of oxygen consumption. Two hypotheses were tested.

**(A) The Data Obtained before and after Treatment with ADH Lie on the Same Straight Line**

This hypothesis was tested in two ways.

(a) For each skin a curve was constructed for the untreated and ADH points considered separately. A $t$ test on their slopes and intercepts gave the following results ($DF$ indicates degrees of freedom; $P$, the significance level, is the probability of
Taking all skins together the significance of the difference of the mean slopes was tested:

\[ \text{slope}_\text{untreated} - \text{slope}_\text{ADH} = -0.01 \pm 0.09 \text{ (SE)}. \]

Testing both the slopes and the angles of inclination gave the following results:

<table>
<thead>
<tr>
<th></th>
<th>( t )</th>
<th>( \text{DF} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slopes</td>
<td>-0.111</td>
<td>9</td>
</tr>
<tr>
<td>Angles, rad</td>
<td>-0.112</td>
<td>9</td>
</tr>
</tbody>
</table>

The above results show no significant difference between the untreated and ADH data.

(B) The Data Obtained after Treatment with Ouabain Lie on the Straight Line Obtained from the Untreated + ADH Data

For each skin the average distance of the ouabain points from the untreated + ADH line was computed. The mean distance for all skins was insignificantly different from zero (\( t = -0.151, \text{DF} = 9, P = 0.88 \)).

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