The Effect of Varying O_2 Tensions on Na Transport in the Toad Bladder[1] (35383)

BARBARA MCDOUGAL[2] AND LAWRENCE P. SULLIVAN[3]
(Introduced by E. B. Brown)
Department of Physiology, University of Kansas, Medical Center, Kansas City, Kansas 66103

Studies of the relationship between Na transport and oxygen consumption in epithelial tissue have largely been confined to the effect of the absence of Na from incubation media (1–3). The addition of Na and Na-free incubation media stimulates O_2 uptake in kidney slices (4), frog skin (5), and toad bladder tissue (1), and increases the arteriovenous difference in oxygen tension in intact perfused kidneys (6). However only a few studies have been concerned with the opposite problem, the effect of changes in oxygen tension on Na transport (5, 7). We present the results of such a study below.

Materials and Methods. The isolated urinary bladders of the toad, *Bufo marinus*, were used in all experiments. The means of keeping the animals prior to use, the design of the chambers and electrodes used for studying Na transport in the isolated bladder, and the instruments used have been described previously (8). The Ringer's solution consisted of 93 m*M* NaCl, 3 m*M* KCl, 0.9 m*M* $CaCl_2$, 10 m*M* glucose, 4 m*M* Na_2HPO_4, 1 m*M* NaH_2PO_4 and 20 m*M* glycylglycine, pH 7.2.

Short-circuit current (SCC) values were used for indicating changes in Na transport. Oxygen tension in the incubation medium was brought to a different level within 4 min by bubbling vigorously with gases of different oxygen concentrations, introduced directly into the chamber after passing through a gas washing bottle. An oxygen electrode was used to monitor the rate at which the solution became equilibrated with the new concentration.

The bladders were first incubated with 100% O_2 and the SCC was measured during a 30-min control period, then different O_2–N_2 mixtures were bubbled into the chambers, and the SCC was followed for 90 min. The SCC of the bladders was then depressed to the anaerobic level by 30-min incubation with 100% N_2. In some experiments, the original O_2–N_2 mixtures were reintroduced for 90 min. The changes in SCC that occurred when the different O_2 concentrations were used are expressed as the fraction of aerobic current remaining [$(SCC_t - SCC_{N_2})$/aerobic SCC]. The aerobic current is the difference between the SCC value obtained with 100% O_2 and that obtained with 100% N_2, and the $SCC_t - SCC_{N_2}$ is the SCC at time, *t*, minus the anaerobic SCC.

Results. Table I presents a summary of the effects of various oxygen tensions on the SCC in those experiments in which the cycle of exposure to O_2 then N_2 and then O_2 again were completed. Figure 1 presents the time course of the fall in SCC when the O_2 tension was reduced. These data are from the experiments represented in Table I and from additional experiments that were stopped after the exposure to N_2. Figure 2 presents the time course of the rise in SCC that occurred in those experiments in which oxygen was reintroduced into the chambers after the period of exposure to 100% N_2.

In Figure 3 the fraction of the aerobic SCC remaining after 90-min incubation with the various oxygen tensions is illustrated. The curve indicates that small increments in the O_2 tension above zero cause increases in the aerobic SCC and 50% of the aerobic SCC

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TABLE I. Summary of the Effect of Various Oxygen Concentrations on the SCC in the Toad Bladder.[a]

Series	n	%	Time (min)	SCC (μA/3.14 cm^2)	$\frac{SCC_t - SCC_{N_2}}{\text{aerobic SCC}}$[b]
I	6	100 O_2	0	235 ± 58	1.00
		100 N_2	90	23 ± 4	0.00
		100 O_2	180	187 ± 40	0.80 ± 0.08
II	9	100 O_2	0	170 ± 19	1.00
		1 O_2	90	53 ± 8	0.25 ± 0.04
		100 N_2	120	21 ± 4	0.00
		1 O_2	210	39 ± 6	0.13 ± 0.02
III	11	100 O_2	0	220 ± 22	1.00
		3 O_2	90	70 ± 10	0.24 ± 0.03
		100 N_2	120	17 ± 4	0.00
		3 O_2	210	66 ± 19	0.25 ± 0.09
IV	9	100 O_2	0	197 ± 34	1.00
		5 O_2	90	113 ± 13	0.56 ± 0.09
		100 N_2	120	34 ± 4	0.00
		5 O_2	210	89 ± 14	0.45 ± 0.14
V	9	100 O_2	0	218 ± 33	1.00
		10 O_2	90	169 ± 20	0.79 ± 0.05
		100 N_2	120	53 ± 4	0.00
		10 O_2	210	162 ± 21	0.83 ± 0.21

[a] Values are means ± SEM.

[b] Aerobic SCC is the difference between the SCC at 100% O_2 and 100% N_2, and $SCC_t - SCC_{N_2}$ is the SCC at time t minus the anaerobic SCC at 120 min.

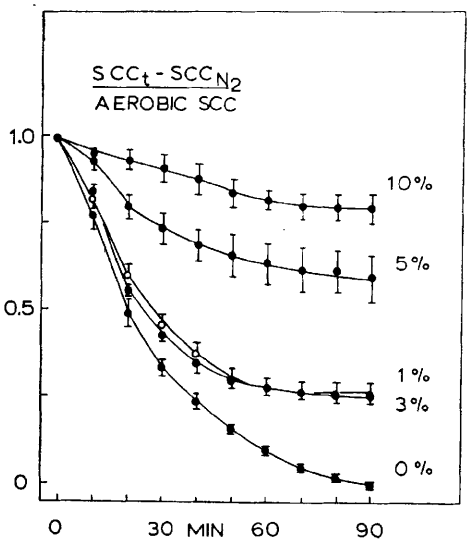

FIG. 1. Reducing the O_2 tension depressed the aerobic SCC to 0.27 ± 0.03 for 1% (n = 20), 0.26 ± 0.02 for 3% (n = 24), 0.60 ± 0.07 for 5% (n = 20), and 0.80 ± 0.04 for 10% (n = 18) in 90 min. The effects of 1 and 3% O_2 were not significantly different.

can be sustained by less than 5% O_2. However, 10% O_2 will not support maximal transport. It is doubtful that increases in O_2 tensions above that in air cause much additional increase in SCC.

Discussion. Implicit in the analysis of the present data is the assumption that the amount of the SCC supported by anaerobic metabolism does not change as the oxygen tension in the incubation media was changed. It is possible that the Pasteur effect could cause an increase in the support of Na transport provided by glycolysis. The data obtained in these experiments do not support or deny this possibility.

The data indicate that this amphibian tissue can sustain periods of anoxia with little loss of the ability to transport Na. In series I of Table I, the aerobic SCC returned to within 80% of control levels after 90-minutes exposure to oxygen-free solutions.

Francis reported in 1934 (7) that the potential difference across the frog skin fell

when the solutions bathing the skins were saturated with a gas containing less than 20% oxygen. He also showed that the rate of respiration fell and the curve relating the rate of respiration to the oxygen content of the gas used closely resembles the curve in Fig. 3. In 1956, Zerahn (5) reported that he obtained low short-circuit currents in three of four experiments on frog skin with oxygen concentrations less than 10%. He also reported that the oxygen consumed in transporting Na did not change in these experiments. These data apparently do not agree with the results obtained by Francis (7). However Zerahn's experiments are few in number and were performed without adequate controls.

The polarographic oxygen electrode is increasingly being used for measurement of oxygen consumption in epithelial tissue. The electrode measures oxygen tension as it decreases in a vessel closed to the atmosphere. The data reported here indicate that, in using this electrode in studies concerned with Na transport and oxygen consumption, the oxygen tension should not be allowed to fall to a point much below that in room air.

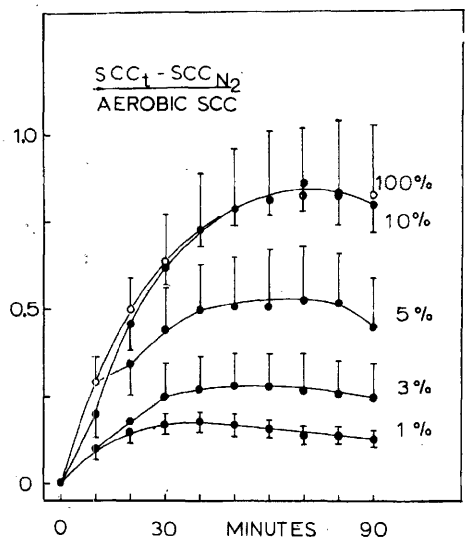

FIG. 2. Toad bladders were incubated with 100% N_2 prior to addition of various O_2 concentrations at 0 time. Recovery from anaerobiosis amounted to 0.83 ± 0.21 of the aerobic SCC for 10% O_2 (n = 9), 0.80 ± 0.08 for 100% (n = 6), 0.45 ± 0.14 for 5% (n = 9), 0.25 ± 0.09 for 3% (n = 11), and 0.13 ± 0.02 for 1% (n = 9).

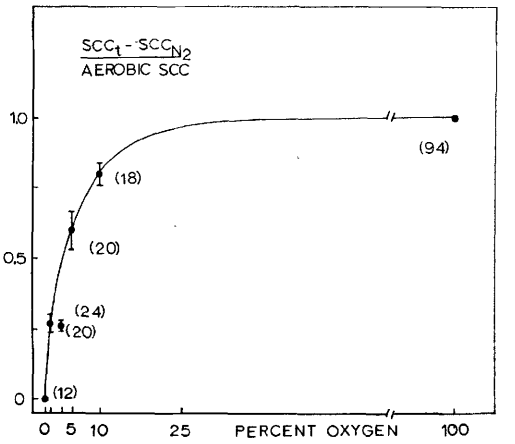

FIG. 3. Fractional changes in the aerobic SCC after 90-min incubation of the bladders with various O_2 tensions. The plot indicates that maximal Na transport would be supported by an O_2 concentration of 20–25%.

Summary. We have studied the effect of changes in the oxygen tension of incubating media on Na transport in the toad bladder. Solutions containing 10% oxygen will support approximately 80% of the aerobic short-circuit current obtained with 100% O_2. When the oxygen tension is reduced below 10%, the SCC falls sharply. The toad bladder will survive exposure to 100% N_2 for periods of at least 90 minutes with little loss of its ability to transport Na.

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