Adipose Resting Membrane Potential: *In Vitro* Responses to Cl^- and K^+ (36835)

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There have been extensive investigations of adipose tissue resting membrane electrical potentials (RP) since they were first described in 1962 (1–4, 9, 12–14). Studies in this laboratory demonstrated depolarization of adipose tissue RP, *in vitro*, occurred in response to very high concentrations, 80 mEq/liter, of extracellular K^+ (3). Lower concentrations of K^+ were not tested. More detailed recent investigations of ionic effect on fat cell RP are presented in this communication. These studies and the isolated fat cell experiments of Perry and Hales (16) support firmly the hypothesis that Cl^- determines adipose tissue RP. However, the present data suggest that K^+ also plays a role in determining the RP of fat.

Materials and Methods. Fed male rats, weighing 210 g or less, were killed by decapitation. Fifty to 100 mg segments of epididdymal adipose tissue were quickly excised and placed in a plastic chamber containing Krebs–Ringer–bicarbonate (KRB) maintained at 30° by a constant temperature water bath. Each segment was equilibrated for 15 min following change of medium. All experiments were concluded within 2 hr of sacrificing the animal. Glass microelectrodes were prepared by utilizing an Industrial Scientific Associates' horizontal microelectrode puller. Each microelectrode was filled with 3 *M* KCl by gentle boiling in fresh 3 *M* KCl for 45–60 min. Ag–AgCl electrodes imbedded in agar bridges were utilized. A Dynagraph direct-writer, with electrometer coupling modified so that microelectrode tip resistance could be measured throughout the period of tissue penetration and tip potentials neutralized, was used. Microelectrodes with a resistance of 15–30 megohms with tip potentials less than 5 mV were utilized. Usually penetration was performed automatically using a motor which advanced the microelectrode at a rate of 10 μm/sec. Occasionally, penetrations were performed manually. Results were recorded on the basis of a dc potential configuration being maintained for at least 1 sec. The KRB solution was prepared according to the method of Umbreit, Burris and Stauffer (18), with slight modifications, and contained $K^+ = 5$ mEq/liter, $Na^+ = 142$ mEq/liter, $Cl^- = 128$ mEq/liter, $Ca^{2+} = 2.4$ mmoles/liter, $HCO_3^- = 24$ mmoles/liter, $HPO_4^- = 1.2$ mmoles/liter, and $SO_4^{2-} = 1.2$ mmoles/liter. This solution was modified according to the methods of Hodgkin and Horowicz (10) to obtain K^+ concentrations of 0, 10, 20, 40 and 80 mEq/liter, and Cl^- concentrations of 7 and 68 mEq/liter, without altering osmolality.

At least one control and one experimental series of measurements were made with the same segment of tissue from any single rat. Data in Tables I and III are calculated and presented according to the method of Williams (19). All of the dc potential values for the penetrations performed in any experiment are recorded and compared, employing appropriate statistical techniques with the equivalent control values obtained with tissue from the same animals. For example, in Table I, effect of 80 mEq/liter K^+ upon fat cell RP was determined with 157 microelectrode penetrations upon adipose tissue segments from 17 rats. These experiments are compared with 195 control values obtained with $(K^+)_c = 0$ mEq/liter upon the same segments of tissue from these 17 rats. The lowest concentrations of K^+ and Cl^-, 0 and 7 mEq/liter, respectively, were arbitrarily designated as controls in Tables I and III. There was some variation in control levels

TABLE I. Effects of K^+ on Adipose Tissue Resting Membrane Electrical Potentials (RP).

K^+ (mEq/liter)	No. rats	Control ($K^+ = 0$) No. determinations	Control ($K^+ = 0$) Mean ± SEM[a] (mV)	Experimental No. determinations	Experimental Mean ± SEM (mV)	p[b]	Calcd RP (mV)
0	23	259	27 ± 1				32
10	2	18	29 ± 1	12	29 ± 1	ns[c]	30
20	2	18	29 ± 1	13	27 ± 1	ns	27
80	17	195	28 ± 1	157	19 ± 1	<.001	16

$$V = 58 \log_{10} \frac{\text{pK } (K^+)_i + P_{Na} (Na^+)_i + P_{Cl} (Cl^-)_e}{\text{pK } (K^+)_e + P_{Na} (Na^+)_e + P_{Cl} (Cl^-)_i}$$

pK = 1.0
pNa = 0.2
pCl = 1.5

	(mEq/liter)
$(K^+)_i$	146
$(Na^+)_i$	19
$(Cl^-)_i$	43
$(Na^+)_e$	142
$(Cl^-)_e$	128

[a] Standard error of mean.

[b] Probability of difference between means of control and experimental being statistically significant (from Fisher's Tables).

[c] Not significant.

from animal to animal, but the results from any single animal were quite constant, and the variation between animals was sufficiently small that calculations were made on the basis of absolute values rather than RP change. In Tables II and IV data from experiments restricted to 3 animals are presented in the usual manner. The sequence varied in which differing concentrations of K^+, Na^+, and Cl^- were tested.

Experiments also were conducted with lipid squeezed manually from epididymal adipose tissue. Large agglomerations of this lipid, while floating on the surface of physiologic solutions, were penetrated by microelectrodes under direct vision. There was no shift of base line as the microelectrode tip was advanced from air into lipid, electrical resistance remaining infinite. As the microelectrode was advanced from lipid into the electrolyte-containing physiologic solution, the change in base line was similar to that obtained when the microelectrode tip was advanced directly from air into solution. There was no resemblance to the electrical activity observed when adipose tissue is penetrated by microelectrodes. Microelectrode tip resistance, measured throughout penetration of rat epididymal adipose tissue, did not change significantly, and was not related to the magnitude of electrical potential. The dc potentials were not elicited from adipose tissue left standing at room temperature 2–3 days, or from dead adipose tissue.

Results. In Table I, effects of K^+ on adipose tissue RP are presented. Observed mean values are: Control = 27–29 mV, 10 mEq/liter = 29 ± 1 (mean ± SEM), 20 mEq/liter = 27 ± 1, 80 mEq/liter = 19 ± 1, with the statistically significant decrease in

TABLE II. Effects of K^+ on Adipose Tissue Resting Membrane Electrical Potentials (3 Rats).[c]

K^+ (mEq/liter)	No. determinations	Mean ± SEM[a] (mV)	p[b]	Calcd RP (mV)
0	23	25 ± 1		24
40	13	14 ± 1	<.001	17
80	13	11 ± 1	<.001	12

[c] Formula, ion concentration, constants, and footnotes same as in Table I, except that pNa = 0.5.

TABLE III. Effects of Cl^- on Adipose Tissue Resting Membrane Electrical Potentials.[c]

Cl^- (mEq/liter)	No. rats	Control (Cl^- = 7 mEq/liter) No. determinations	Control Mean ± SEM[a] (mV)	Experimental No. determinations	Experimental Mean ± SEM (mV)	p^b	Calcd RP (mV)
7	8	43	15 ± 1				13
68	5	21	13 ± 2	50	20 ± 1	<.01	24
128	6	37	16 ± 1	60	28 ± 1	<.001	31

[c] Formula, ion concentrations, constant, and footnotes same as in Table I.

response only at 80 mEq/liter. Assuming relative ionic permeabilities of approximately the same magnitude as indicated by Shanne and Coraboeuf (17) for liver, and adipose tissue ion concentrations described by Perry and Hales (16), adipose tissue RP values, calculated by the Goldman equation, appear to be in reasonable agreement with the observed values. Table II presents adipose tissue RP changes of 3 rats in response to 0, 40, and 80 mEq/liter K^+, the statistically significant decreases of RP being observed at 40 and 80 mEq/liter K^+. These observed values are also in quite good agreement with RP calculated as in Table I except that the assumption is made that pNa is elevated to 0.5. In Tables III and IV, the mean observed adipose RP tissue values at 7 mEq/liter $(Cl^-)_e$ are 13–16 mV, at 68 mEq/liter $(Cl^-)_e$ are 20–22 mV, and at 128 mEq/liter $(Cl^-)_e$ is 28 ± 1 mV. These observed values are in good agreement with RP calculated as in Table I.

Discussion. These studies have shown that osberved alterations of adipose tissue RP associated with extracellular Cl^- changes are consistent with calculated values. Perry and Hales (16) calculated by the Nernst equation a Cl^- potential in isolated fat cells of 28 mV, in remarkably close agreement with the base line adipose tissue RP observed in this laboratory. However, the data from this laboratory indicate that K^+ also plays a role in determining adipose tissue RP.

Acute perfused liver experiments by Claret, Coraboeuf and Favier (7) and *in vitro* liver studies from this laboratory (5) indicated Cl^- may, at least in part, determine hepatic tissue RP. Other data (unpublished) from this laboratory suggested that K^+ may also play a role in determining liver RP if the pNa is very high, an assumption which appears to be correct according to recent data of Claret and Mazet (8). Claret, Coraboeuf and Favier (7) noted previously that prolonged exposure of hepatic cells to high concentrations of K^+ may convert the liver cell RP from a Cl^- to a K^+ potential. On the basis of their most recent detailed studies, Claret and Mazet (8) concluded that there may be two major components determining hepatic cell RP, a passive distribution of Cl^- and active transport of K^+ and Na^+ by an electrogenic pump. Their data and interpretations may also effectively explain the observations of Williams, Withrow and Woodbury (20) that *in vivo* liver RP cannot be ascribed solely to K^+, and that no simple

TABLE IV. Effects of Cl^- of Adipose Tissue Resting Membrane Electrical Potentials (3 Rats).[c]

Cl^- (mEq/liter)	No. determinations	Mean ± SEM[a] (mV)	p^b	Calcd RP (mV)
7	21	14 ± 1		13
68	27	22 ± 1	>.01, <.02	24
128	15	28 ± 1	<.000	31

[c] Formula, ion concentrations, constants, and footnotes same as in Table I.

hypothesis implicating a single ion would appear to be tenable.

The present data suggest that the mechanism of liver and fat RP may be quite similar, involving both Cl^- and K^+. Preliminary studies indicate inhibition of *in vitro* fat cell RP by cyanide, further resembling the *in vitro* hepatic cell RP (6). Both liver and fat cell *in vitro* preparations seem viable, and would appear to be valuable tools for electrophysiological studies. Lambotte (15), emphasizing the value of RP measurements as an index of hepatic cell viability in perfused preparations, has indicated that *in vitro* RP values are abnormal. Not unexpectedly, major electrophysiologic differences may be observed between *in vitro,* perfused, and *in vivo* preparations, but it may be premature to conclude that one preparation is indubitably and invariably superior to another.

Summary. In vitro rat adipose tissue resting membrane potentials (RP) appear to be determined by Cl^- with K^+ also playing a role. There are important similarities between adipose and hepatic tissue RP, and *in vitro* preparations of both appear to be "electrically" viable.

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1. Beigelman, P. M., and Hollander, P. B., Proc. Soc. Exp. Biol. Med. **110**, 590 (1962).

2. Beigelman, P. M., and Hollander, P. B., Diabetes **12**, 262 (1963).

3. Beigelman, P. M., and Hollander, P. B., Proc. Soc. Exp. Biol. Med. **115**, 14 (1964).

4. Beigelman, P. M., and Hollander, P. B., Acta Endocrinol. **50**, 648 (1965).

5. Beigelman, P. M., and Schlosser, G. H., Biochem. Med. **3**, 73 (1969).

6. Beigelman, P. M., and Thomas, L. J., J. Membrane Biol. **8**, 181 (1972).

7. Claret, M., Coraboeuf, E., and Favier, M. P., Arch. Int. Physiol. **78**, 531 (1970).

8. Claret, M., and Mazet, J. L., J. Physiol. (London) **223**, 279 (1972).

9. Girardier, L., Seydoux, J., and Clausen, T., J. Gen. Physiol. **52**, 925 (1968).

10. Goldman, D. E., J. Gen. Physiol. **27**, 37 (1943).

11. Hodgkin, A. L., and Horowicz, P., J. Physiol. (London) **184**, 127 (1969).

12. Horowitz, J. M., Horwitz, B. A., and Smith, R. E., Experentia **27**, 1419 (1971).

13. Horwitz, B. A., Horowitz, J. M., and Smith, R. E., Proc. Nat. Acad. Sci. U.S.A. **64**, 113 (1969).

14. Krishna, G., McCallum, Z., Miskowitz, J., Dempsey, P., and Brodie, B. B., Life Sci. **9**, 1353 (1970).

15. Lambotte, L., Eur. Surg. Res. **2**, 241 (1970).

16. Perry, M. C., and Hales, C. N., Biochem. J. **115**, 3865 (1969).

17. Schanne, O., and Coraboeuf, E., Nature (London) **210**, 1390 (1966).

18. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques," 4th ed., p. 132. Burgess, Minneapolis (1964).

19. Williams, J. A., Amer. J. Physiol. **211**, 1171 (1966).

20. Williams, J. A., Withrow, D. C., and Woodbury, D. M., J. Physiol. (London) **212**, 117 (1971).

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