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Effects of Electrolytes Upon Adipose Tissue Membrane Electrical Potentials.* (28816)

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Transmembrane or resting membrane electrical potentials (REP) have been demonstrated in rat epididymal adipose tissue(1,2). A dose-response relationship has been established between increase of adipose tissue REP and insulin concentrations of 1-1000 micro-units/ml. The REP response to insulin diminishes in proportion to increase of rat weight(2). More recent studies have shown that insulin antibody blocks the insulin effects.

The investigation reported here reveals significant diminution of adipose tissue REP associated with increase of K^+ in the medium. This effect was not demonstrated when adipose tissue from older, larger animals was employed.

Methods. The apparatus and techniques for recording REP's and the design of the experiments have been described(1,2). Segments of male Wistar rat epididymal adipose tissue were penetrated with glass microelectrodes and the DC potentials recorded on motion picture film. Evidence that these were true electrical potentials has been presented (1,2). In many experiments, it was possible to employ a single glass micro-electrode for a single complete experiment or for a number of experiments, in some cases extending over several days' duration.

Normal male Wistar rats, in the fed state, weighing 160-390 g were employed. The usual control medium was Krebs-Ringer bicarbonate solution containing (mM) $Na^+ = 140$, $K^+ = 5$, $Ca^{++} = 2.5$, $Mg^{++} = 1$, $Cl^- = 125$, $H_2PO_4^- = 1$, $HCO^- = 25$ and $SO_4^= = 1$. Variations of potassium and sodium ion were obtained with media described by Hodgkin and Horowisz(3), the composition being (mM), $Ca^{++} = 8$, $HPO_4^= = 1.08$, $H_2PO_4^- = 0.43$, $SO_4^= = 48$, Sucrose $= 113$, $Cl^- = 0$. One solution, designated for convenience "high K^+", contained 80 mM of K^+ and 3 mM of Na^+. A second solution, designated "high Na^+", contained 83 mM of Na^+, and no K^+. Relative tonicity and ionic strength were approximately equivalent to the Krebs-Ringer control buffer, so that successive determinations of REP with control, "high K^+", and "high Na^+" solutions were usually characterized by a stable base-line. If major base-line alterations occurred, REP values were discarded. As previously described, the experimental and control REP's were determined on the same tissue segment. Usually, 3- to 5-minute periods of recording REP's were

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TABLE I. Effect of K^+ and Na^+ upon Electrical Potentials of Adipose Tissue.

Rat No.	Rat wt (g)	Control* No.§	Control* Millivolts‖	High K^+† No.	High K^+† Millivolts	High Na^+‡ No.	High Na^+‡ Millivolts	Change¶	p**
1	160	16	23 ± 1	8	15 ± 1	16	26 ± 1	+11	<.001
2	175	14	30 ± 4	31	31 ± 3	30	38 ± 3	+ 7	>.05, <.1
3	180			7	22 ± 2	8	33 ± 1	+11	<.001
4	190	15	27 ± 2	13	17 ± 1	11	23 ± 1	+ 6	<.001
5	190	12	25 ± 2	6	15 ± 2	13	31 ± 3	+16	<.001
6	195	37	22 ± 2	26	14 ± 1	26	30 ± 1	+16	<.001
7	210	20	19 ± 2	12	14 ± 2	29	22 ± 1	+ 8	<.001
	Total mean††		26		18		29	+11	
	P								<.001
8	235	15	24 ± 1	17	36 ± 5	15	35 ± 3	- 1	
9	290	33	24 ± 2	15	19 ± 1	24	21 ± 1	+ 2	>.1, <.2
10	300	15	27 ± 2	12	27 ± 3	11	35 ± 4	+ 8	>.1, <.2
11	370	8	38 ± 3	12	24 ± 1	8	27 ± 2	+ 3	>.1, <.2
	Total mean		28		26		29	+ 3	
	P								>.2, <.3

* Krebs-Ringer bicarbonate buffer.
† $K^+ = 80$ and $Na^+ = 3$ mM.
‡ $Na^+ = 83$ mM and $K^+ = 0$.
§ No. of determinations.
‖ Mean value of electrical potential ± standard error of mean (SEM).
¶ Mean change of electrical potential, "High Na^+" compared with "High K^+."
** "P"—Probability of difference being significant. Calculated by Student "t" test.
†† Calculated on basis of single mean value for each rat.

alternated with 2-minute intervals of equilibration.

Results. A definite, significant diminution of REP occurred following marked increase of K^+ (and decrease of Na^+) in the buffer solution with adipose tissue from all, except one, small rats weighing less than 215 g (Table I). In these studies of smaller rats, subsequent replacement by buffer high in Na^+ and lower in K^+ was accompanied by restoration of REP to control or elevated levels of REP, although these changes were not generally significant compared with the control (Table I). Adipose tissue REP of larger rats, weighing more than 230 g, revealed no such consistent pattern when studied in an identical manner (Table I). In one experiment, substitution of control buffer by medium containing 40 mM K^+ was accompanied by significant diminution of adipose tissue REP. Subsequent replacement with medium containing 80 mM K^+ was associated with further decrease of REP. Compilation of the mean values and changes demonstrates that the only consistent significant change was the decrease of REP following immersion of adipose tissue from smaller, younger rats in high K^+ medium (Table I). Consistent with this conclusion is comparison of mean adipose tissue REP change associated with shifts from high K^+ to high Na^+ medium, demonstrating a highly significant difference for young, but not for older rats.

Discussion. The present studies of adipose tissue REP demonstrate that increase of K^+ in the medium is associated with a decrease of adipose tissue transmembrane or resting electrical potential (REP) in younger rats, as occurs with nerve and muscle. Reduction of K^+ and increase of Na^+ was accompanied by restoration of REP to the original level, or higher. It is difficult to compute, using the Nernst equation, predicted electrical potentials across the fat cell membrane since little is known concerning adipose tissue electrolytes. However, preliminary computations, employing data accumulated in studies of adipose tissue electrolyte and water content(4), suggest that the observed quantitative alterations in adipose tissue REP, in response to changes of extracellular K^+, are not in agreement with those expected on the basis of the Nernst equation. It should be

emphasized that recent work with muscle preparations indicate that the relationship between ion flux and REP may require a more complex explanation than provided by the Nernst equation(5).

Loss of K^+ from adipose tissue is significantly reduced by insulin, with no glucose in the medium(6). There is no effect of insulin on the net inward movement of Na^+ or water. The relative decrease of K^+ accumulating in the medium, in response to insulin, might be related directly to the increase of REP associated with insulin(1,2). The results given here, decrease of fat pad REP as K^+ in the medium is increased, are consistent with these findings. Alternatively, it may be proposed that as insulin causes transport of K^+ from an extracellular to intracellular locus an increase of REP would result. However, studies in muscle by Zierler(7) indicated that increase of intracellular K^+ was too small and too late to account for the rise in REP, in response to insulin. Zierler (7) proposed that the REP associated with insulin caused the K^+ shift. Similarly, it is possible that the primary effect of insulin or other hormones is upon fat cell REP which, in turn, alters transport rate of ions, such as K^+, and of substrate, such as glucose.

Summary. Adipose tissue transmembrane or resting electrical potential (REP) of young rats is decreased by elevation of K^+. Substitution of medium high in Na^+ for one high in K^+ restored the REP to its control level or slightly higher. Adipose tissue from aged rats did not demonstrate any significant change of REP in response to alteration of K^+ or Na^+.

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Purification of Thyroid Hormone-Transaminase from Rat Kidney Mitochondria.* (28817)

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We have previously shown that extracts of rat kidney mitochondria contain oxidase and transaminase systems which deaminate L-thyroxine(1). Recently we found that a partially purified preparation with transaminase activity obtained by stepwise elution of the crude transaminase on DEAE cellulose column catalyzes the deamination of thyroxine, triiodothyronine, and diiodotyrosine(2). The work herein reported was undertaken to determine whether the transamination of these iodinated amino acids is effected by a single transaminase.

Material and method. All preparative procedures were carried out at 0° to 4°C. The dialyzed ammonium sulfate fraction, labeled crude transaminase, prepared from extracts of rat kidney mitochondria(2) was purified by gradient elution from DEAE cellulose. The crude transaminase, which contained 60

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