Effects of Hormones upon Adipose Tissue Membrane Electrical Potentials.* (29149)

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Transmembrane or resting membrane electrical potentials (REP), demonstrated in rat epididymal adipose tissue, are increased by small (1-1000 micro-units/ml) concentrations of insulin(1,2). This effect diminishes in proportion to increase of total rat weight(1,2).

In this paper further observations are presented which demonstrate absence of nonspecific protein effects on adipose tissue REP. Gelatin, albumin, and insulin antibody have no significant effects. ACTH possesses no activity or has an equivocal effect. Data will be presented which suggest significant augmentation of REP by epinephrine and norepinephrine in physiological concentrations. In this report, unequivocal inhibition of insulin action on adipose tissue REP by insulin antibody is shown.

Methods. The apparatus, techniques for recording REP's and the design of the experiments have been previously 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. Previously detailed investigations demonstrate these to be cellular membrane DC potentials(1,2). It was possible during this study to perform a number of complete experiments using the same microelectrode.

Male Wistar rats, in the fed state, weighing 120-210 g were employed. The usual control medium was Krebs-Ringer bicarbonate buffer solution. In all experiments, 0.1% gelatin was added. As previously described, the experimental and control REP's were determined on the same tissue segment. Usually, 3-5-minute periods of recording REP's were alternated with 2-minute intervals of equilibration. In a few experiments, somewhat longer recording periods were utilized, but only the final 3-5 minutes were reported.

The insulin‡ and gelatin were prepared as previously described(1,2). Insulin antibody was obtained by immunizing guinea pigs by the method of Arquilla *et al*(3), employing alum-precipitated insulin. A 1/100 dilution of insulin antibody§ completely inhibited the effect of 1000 micro-units/ml of insulin upon rat epididymal adipose tissue glucose uptake. As little as one micro-unit/ml of crystalline insulin could be detected with this insulin antibody diluted 1/2000, employing the technique of Grodsky and Forsham(4).

Results. Presence of 0.1% gelatin, 0.1% serum albumin, or insulin antibody (1/1000-1/2400) did not significantly alter REP of rat epididymal adipose tissue (Tables I, II). Effect of 1 mU/ml ACTH‖ upon adipose tissue REP was ascertained, using tissue of 3 rats. Mean changes of REP, −6, −1, and +11 millivolts, reflected absence of significant change in 2 of the three. All 3 animals showed significant increase of adipose tissue REP in response to insulin, epinephrine, or norepinephrine.

Insulin antibody (1/1000-1/2400 dilution) had no significant effect upon adipose tissue

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‡ Supplies of re-crystallized insulin were generously provided by Dr. Lloyd Miller of U. S. Pharmacopia and Dr. Otto K. Behrens, Eli Lilly and Co.

§ Dr. Edward R. Arquilla, Dept. of Pathology, Univ. of California Med. School, Los Angeles, kindly donated a portion of the insulin antibody employed in this study.

‖ Corticotropin Injection, 25 units, Upjohn Co., Kalamazoo, Mich.

REP of 3 rats (Table II). All 3 animals demonstrated significant increase of adipose tissue REP in response to insulin. Insulin antibody significantly decreased the adipose tissue REP response to insulin. In Table III, the significant decreases of REP recorded for rats 1 and 2 were obtained by comparing response to insulin alone with that to insulin plus insulin antibody upon separate segments of adipose tissue. A single portion of adipose tissue was utilized in comparing insulin and insulin plus insulin antibody for rats 3-6

TABLE I. Effect of Protein on Electrical Potentials.

Rat No.	Wt (g)	Control*–no protein		.1% gelatin				.1% albumin			
		No.†	Millivolts‡	No.	Millivolts	Change§	P‖	No.	Millivolts	Change	P
1	175	11	32 ± 3	4	26 ± 3	−6	>.2	5	29 ± 3	−3	>.5
2	210	6	29 ± 2	7	23 ± 2	−6	>.05, <.1	13	26 ± 2	−3	>.3
3	180	8	32 ± 4	14	36 ± 3	+4	>.3	13	30 ± 3	−2	>.6
4	185	30	28 ± 1	5	28 ± 4	0	>.9				
5	180	11	29 ± 2	3	27 ± 1	−2	>.4				
6	120	7	42 ± 3	5	36 ± 7	−6	>.4				

* Krebs-Ringer bicarbonate buffer.
† No. of determinations.
‡ Mean value ± standard error of mean.
§ Mean change of electrical potential (millivolts) from mean insulin value.
‖ Probability of significance of change from control (from Fisher's tables, by student "t" test).

TABLE II. Insulin Antibody.

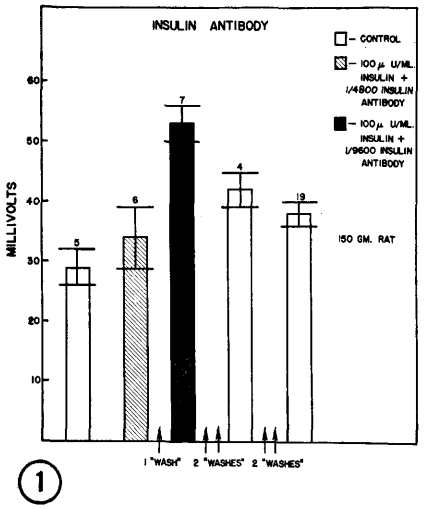
Rat No.	Wt (g)	Conc of insulin antibody	Electrical potential (millivolts)					
			Control*		Insulin antibody			
			No.†	Mean ± SEM‡	No.	Mean ± SEM	Change§	P‖
1	185	1/1000	15	50 ± 4	8	52 ± 2	+2	>.5
2	195	1/2000	13	22 ± 3	13	19 ± 1	−3	>.3, <.4
3	140	1/2400	5	56 ± 2	4	65 ± 6	+9	>.2, <.3

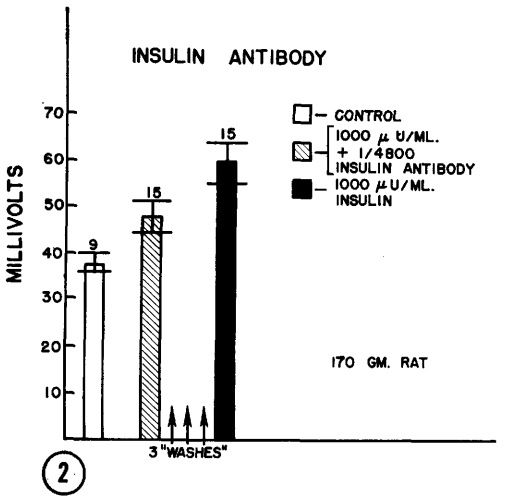
* Krebs-Ringer bicarbonate buffer.
† No. of determinations.
‡ Mean value of electrical potential (millivolts) ± standard error of mean.
§ Mean change of electrical potential (millivolts) from control.
‖ Probability of significance of change from control, by student "t" test.

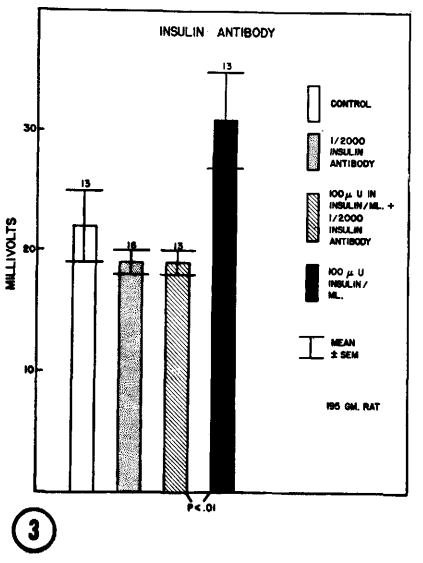
TABLE III. Effect of Insulin Compared with Insulin + Insulin Antibody on Electrical Potential.

Rat No.	Rat wt (g)	Insulin conc (μU/M)	Antibody conc	Control*		Insulin		Insulin + antibody			
				No.†	Millivolts‡	No.	Millivolts	No.	Millivolts	Change§	P‖
1	180	100	1/1000	3	27 ± 1	6	40 ± 4	5	31 ± 2	− 9	>.02, <.05
2	195	100	1/2000	13	22 ± 3	16	31 ± 4	13	19 ± 1	−12	<.01
3	140	100	1/2400	4	55 ± 3	5	63 ± 4	6	47 ± 2	−16	>.01, <.02
4	150	100	1/4800	5	29 ± 3	7	53 ± 3	6	34 ± 5	−19	>.02, <.05
5	170	1000	1/4800	9	38 ± 2	15	60 ± 5	15	48 ± 3	−12	>.02, <.05
6	195	100	1/9600	11	49 ± 4	5	61 ± 2	15	65 ± 4	+ 4	>.4

* Krebs-Ringer bicarbonate buffer.
† No. of determinations.
‡ Mean value ± standard error of mean.
§ Mean change of electrical potential (millivolts) from mean insulin value.
‖ Probability of significance of change from insulin (from Fisher's tables, by student "t" test).







(Table III). Fig. 1 demonstrates significant increase of adipose tissue REP, in response to 100 micro-units/ml of insulin, as the insulin antibody concentration is decreased from 1/4800 to 1/9600. The same piece of tissue was used throughout this experiment. Fig. 2 demonstrates no significant change of REP in a segment of adipose tissue treated with 1000 micro-units/ml insulin plus 1/2400 insulin antibody. The same piece of tissue subsequently demonstrated significant increase of REP in response to insulin without antibody. In Fig. 3, one segment of adipose tissue showed no change of REP as insulin antibody (1/2000) and, later, 100 micro-units/ml of insulin plus insulin antibody were substituted for the control buffer. Subsequently, this portion of adipose tissue showed a significant increase of REP in response to 100 micro-units/ml of insulin. This tissue demonstrated a normal range of REP's, 12-22 millivolts, when tested a few times in the presence of control buffer immediately preceding and fol-

FIG. 1. *Effect of varying concentrations of insulin antibody upon response of adipose tissue resting electrical potential (REP) to 100 micro-units/ml insulin.* REP mean values, in millivolts, given by height of bar. Brackets define standard error of mean (SEM). Open bars indicate controls, diagonally striped bar—100 micro-units/ml insulin /ml + 1/4800 insulin antibody, and closed bar represents 100 micro-units insulin/ml + 1/9600 insulin antibody. Bars placed in time sequence that experiments were performed. Numbers above bars represent number of determinations.

FIG. 2. Effect of 1/2400 insulin antibody upon response of adipose tissue resting electrical potential (REP) to 1000 micro-units/ml insulin. REP mean values, in millivolts, given by height of bar. Brackets define standard error of mean (SEM). Open bars indicate controls, diagonally striped bar —1000 micro-units/ml insulin + 1/2400 insulin antibody; closed bar represents 1000 micro-units/ml insulin. Bars placed in time sequence that experiments were performed. Numbers above bars represent number of determinations.

FIG. 3. Effect of 1/2000 insulin antibody upon response of adipose tissue resting electrical potential (REP) to 100 micro-units/ml insulin. REP mean values, in millivolts, given by height of bar. Brackets define standard error of mean (SEM). Open bars indicate controls, speckled bar—1/2000 insulin antibody, diagonally striped bar—1/2000 insulin antibody + 100 micro-units/ml insulin, and closed bar indicates 100 micro-units/ml insulin. Bars placed in time sequence that experiments were performed. Numbers above bars represent number of determinations. "P" indicates probability of significance of difference between indicated means (from Fisher's Tables, by Student "t" test).

TABLE IV. Effect of Epinephrine and Norepinephrine Upon Electrical Potentials.

Rat No.	Wt (g)	Control* No.†	Control* Millivolts‡	Epinephrine Conc§	Epinephrine No.	Epinephrine Millivolts	Epinephrine Change‖	P¶
1	145	14	51 ± 5	.1	21	54 ± 7	+ 3	>.6
2	170	18	49 ± 3	.1	38	59 ± 2	+10	<.01
2	170	6	56 ± 4	10.0	15	63 ± 6	+ 6	>.3 , <.4
3	195	10	32 ± 7	10.0	35	51 ± 3	+19	>.01, <.02
						Norepinephrine		
1	145	10	52 ± 6	.1	7	81 ± 1	+29	<.001
2	170	25	53 ± 4	.1	20	57 ± 4	+ 4	>.3
2	170	7	42 ± 2	10.0	18	61 ± 4	+19	<.001
3	195	6	40 ± 4	10.0	18	54 ± 3	+14	<.01

* Krebs-Ringer bicarbonate buffer.
† No. of determinations.
‡ Mean value ± standard error of mean.
§ Concentration in gammas per liter.
‖ Mean change of electrical potential (millivolts) from control.
¶ Probability of significant increase over control, by student "t" test.

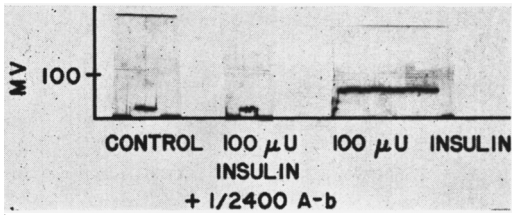


FIG. 4. Motion picture records of 3 different single cell membrane potentials from adipose tissue. Media designated, from left to right, in time sequence experiments performed upon single segment of adipose tissue are Krebs-Ringer control, 100 micro-units/ml insulin + 1/2400 insulin antibody, and 100 micro-units/ml insulin. Potential calibration indicated on vertical scale, time on horizontal scale.

lowing the addition of insulin without antibody. Inhibition by insulin antibody of adipose tissue REP response to insulin, as recorded on motion picture film, is depicted in Fig. 4. The time sequence of the experiments, using a single segment of tissue, is given as control, 100 micro-units/ml insulin + 1/2400 insulin antibody, and 100 micro-units insulin/ml, respectively, from left to right.

Preliminary experiments were performed in 3 rats, utilizing epinephrine and norepinephrine¶ in concentrations of 0.1-100 gamma/l (Table IV, Fig. 5). The data recorded in Table IV are the results of testing a segment of tissue with a single dose of epinephrine or norepinephrine. Rat No. 1 demonstrated a significant increase of REP in response to 0.1 gamma/l norepinephrine, but no such increment occurred with epinephrine. Adipose tissue REP of Rat No. 2 increased significantly in response to 0.1 gamma/l epinephrine and 10 gamma/l norepinephrine, but the response was not significant to either 0.1 gamma/l norepinephrine or, paradoxically, to 10 gamma/l epinephrine. In Rat No. 1, successive increases of epinephrine and norepinephrine concentration, utilizing a single seg-

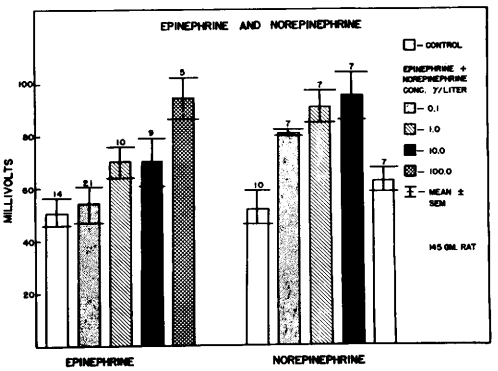


FIG. 5. Effect of epinephrine and norepinephrine upon adipose tissue resting electrical potential (REP). REP mean values, in millivolts, given by height of bar. Brackets define standard error of mean (SEM). Open bars indicate controls. Concentrations of epinephrine and norepinephrine in gamma/l: Open bar—0, speckled—0.1, lined—1.0, cross-hatched—100.0, closed—10.0. Epinephrine represented on left side of graph, norepinephrine on right. Bars placed in time sequence that experiments were performed. Numbers above bars represent number of determinations.

¶ We are indebted to Dr. Shannon Brunjes, Dept. of Medicine, Univ. of Southern California Med. School, for supplies of epinephrine and norepinephrine.

ment of adipose tissue for each hormone, were characterized by proportional increments of REP (Fig. 5).

Discussion. Previous reports have indicated that the increase of adipose tissue membrane resting electrical potential (REP) associated with insulin is a specific effect(1,2). The present data further confirm this conclusion. Other protein, including gelatin, human serum albumin, and insulin antibody exert no significant effect upon REP. Insulin antibody, in concentrations of 1/1000-1/4800, significantly blocks the effect of insulin, in concentrations of 100-1000 micro-units/ml, upon adipose tissue REP.

Epinephrine and norepinephrine, in at least one concentration between 0.1 and 10 gamma/l, significantly increased adipose tissue REP in each of 3 rats tested. There may be an important relationship between these hormones and intracellular substrate which, according to Maffly and Edelman(5), provide energy required to maintain membrane transport systems. It is possible that these hormones may operate in a similar manner to elevate REP. The fundamental interrelationships of electrical properties, transport mechanisms, and intracellular metabolism of the cell are emphasized by these studies.

Summary. Adipose tissue transmembrane or resting electrical potential (REP) is not altered by gelatin, serum albumin, or insulin antibody. Elevation of adipose tissue REP by insulin is blocked by insulin antibody. Epinephrine and norepinephrine significantly increase adipose tissue REP.

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Effect of Epinephrine on Metabolism of Thyroxine by Pituitary and Brain. (29150)

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After injection of I^{131}-labeled thyroxine (T4), triiodothyronine (T3) has been found in chromatograms of the pituitary gland (1-3) and of mouse pituitary tumors(4). Ford and Gross(5) reported the absence of T3 in the organs of the central nervous system (CNS) after injection of I^{131}-labeled T4. More recent publications have claimed, however, the presence of a compound with the same mobility as T3 in the brain, medulla, and spinal cord. The absence of T3 in the optic nerve was noted after intraperitoneal injection of I^{131}-labeled thyroxine(6).

The present study confirms the later findings(6), and provides additional information regarding the effects of epinephrine on the metabolism of I^{131}-labeled thyroxine in various organs. Radiothyroidectomized mice were injected with I^{131}-labeled T4 plus epinephrine, and chromatograms of the brains were made 30 minutes and 4 hours later. At 4 hours, a high percentage of a compound with the same mobility as T3 was found.

Materials and methods. Forty female Swiss white mice, body weight 20 $\pm$ 1 g, were kept at a constant room temperature of 22°C. For 10 days they were fed the Remington low-iodine diet and were given distilled water to drink. Then they were radiothyroidectomized by intraperitoneal in-