Effect of Insulin on Potassium Flux and Water and Electrolyte Content of Muscles from Normal and from Hypophysectomized Rats

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ABSTRACT It was reported previously that insulin hyperpolarized rat skeletal muscle and decreased K^+ flux in both directions. The observations on K^+ flux are now extended to take advantage of the greater sensitivity to insulin of hyperphysectomized rats. Insulin caused a shift of water from extracellular to intracellular space if glucose was present, but not in its absence. Insulin caused net gain of muscle fiber K^+, though not necessarily an increase in K^+ concentration in fiber water. It probably also decreased intrafiber Na^+ and Cl^-. Insulin decreased K^+ efflux. The effect was dose-dependent. Muscles from hypophysectomized rats were more sensitive to the action of insulin on K^+ flux than were those from normal rats. The effect was demonstrable within the time resolution of the system, suggesting that insulin's action is on cell surfaces. K^+ influx was also decreased by insulin. Bookkeeping suggests that some K^+ influx be called active. Insulin seemed to decrease active K^+ influx and passive K^+ efflux. It is not resolved whether insulin has a true dual effect or whether it acts only on passive fluxes in both directions (the apparent action on active K^+ influx being an artefact of incomplete definition of passive flux) or whether a single alteration in the membrane may affect both active and passive fluxes.

Insulin hyperpolarizes excised rat muscle and decreases K efflux and influx, whether or not glucose is present (1–3). At a concentration of 0.1 U/ml, insulin decreases efflux to a greater extent than influx, so that K accumulates in muscle. On the basis of these and related observations it was proposed that insulin acts by combining with the muscle membrane, the combination resulting in more positive (or less negative) fixed charges in the membrane than in the absence of insulin. Through such a membrane the flux of small cations would be decreased and the flux of small anions would be accelerated. The postulated decrease in Na permeability could lead to the observed hyper-

polarization, and the hyperpolarization was more than adequate to account for the net gain in intracellular K.

Among criticisms of the work cited above was the fact that the concentration of insulin was very large compared to that which is effective in vivo. Accordingly, to examine the biological relevance of these observations, similar experiments were performed with exhibition of smaller concentrations of insulin. As a further test of biological relevance, muscles from hypophysectomized rats were used as test material. Hypophysectomized rats have long been recognized as being more sensitive to insulin, at least with respect to the action of insulin on glucose uptake (4). If muscles from hypophysectomized rats proved to be more sensitive to insulin with respect to the changes in K flux, this would increase confidence in the likelihood that the effects observed in excised muscle were relevant to the hormonal action of insulin.

In addition to reexamining insulin's effect on K flux, the present paper also reports the distributions of water, K, Na, and Cl in muscles from normal and from hypophysectomized rats, and the effect of insulin on these distributions, data lacking in the earlier publications, and required for more rigorous analysis of the results.

As a result of analysis of the data to be presented, the hypothesis offered in earlier papers, that the action of insulin on K fluxes can be explained completely on the basis of a postulated increase in fixed positive charges in the muscle membrane, is no longer tenable. It is either incomplete or incorrect, or, if it is complete and correct, then notions discriminating active from passive transport require modification.

METHODS

Rats were from a strain different from those used in our previous experiments. Normal and hypophysectomized rats, Charles River CD strain, were obtained from the Charles River Breeding Laboratories, Inc., Brookline, Mass. Body weight was from about 50 to 80 g at the time of the experiment. Hypophysectomized rats received 5% glucose in their drinking water ad lib., and their muscles were studied 5 to 30 days posthypophysectomy.

Rats were anesthetized by intraperitoneal injection of Na pentobarbital. Both extensor digitorum longus muscles were removed by severing their tendons and freeing the muscle by sharp dissection. In those cases in which a muscle was to be analyzed as a zero time control, one member of each pair was weighed immediately on a torsion balance and placed at once in a deep freeze where it was held for subsequent analysis. In all other cases, ligatures were placed around the proximal and the distal tendon before the tendons were cut and the distance between the ligatures was measured and recorded as rest length. The muscle was then placed in one of two chambers at this rest length.

One chamber described previously (3), was used in studies of K flux and will be referred to hereafter as the flux chamber. The floor of this chamber formed the window

of a gas-flow Geiger counter. The chamber was washed continuously at a rate of about 10 ml/min by K^{42}-free solutions in a nonrecirculating, gravity-fed system described previously (3). The solutions were at approximately 25°C; temperature was measured but not controlled.

The second chamber, also described previously, was used in experiments, reported elsewhere, in which membrane potentials were measured and in certain experiments in which the distribution of water and electrolytes was to be assessed. Bathing solutions were recirculated by a pump through a volume of about 150 ml in the system, including the chamber, hereafter referred to as the recirculating chamber. Bathing solutions were also at approximately 25°C in this system.

All solutions were made with HCO_3 buffer at pH 7.4, gassed continuously with 95% O_2, 5% CO_2. Final concentrations (mM/liter) were: Na, 145; K, 4.5; Ca, 2.5; Mg, 1.2; Cl, 123; HCO_3, 28; HPO_4, 1.2; SO_4, 1.2; glucose, 11. In some experiments described under Results glucose was omitted. In some experiments gelatin, Eastman Kodak No. 1099, was added to a final concentration of 4 g/liter to prevent loss of insulin by adsorption on glass (5). This was abandoned when we showed that gelatin was unnecessary and because the surface tension of the gelation solution had a disruptive effect to be described under Results.

Distribution of muscle water was measured as follows: An extensor digitorum longus muscle was tied at rest length to a bent Lucite rod, placed in a 15 ml Warburg flask containing 3 ml of previously gassed buffer solution, contents as described above, and incubated, with continuous gassing, for 1 hr at 25°C. At the end of 1 hr inulin-C^{14}, 0.5 μc and 0.2 mg, was added to the flask, and simultaneously, insulin was added to flasks containing one muscle of each pair from a single rat. The final concentration of insulin was 0.01 U/ml. Muscles were incubated for an additional hour. (The inulin space measured was therefore comparable to that to be expected in muscles incubated for 2 hr in the other chambers, and the effect of insulin on inulin space was intended to mimic that in other experiments in which insulin was added for approximately 1 hr following approximately 1 hour of previous incubation.) At the end of the 2nd, or loading, hour the ligatures were removed from the muscle, the muscle was rinsed briefly in an inulin-C^{14}-free solution, blotted, and weighed. The muscle was then placed in a second flask containing 2 ml of fresh buffered solution, and shaken continuously for 2 hr. This was the unloading period. The muscle was then removed and dried at 95°C for at least 6 hr to constant weight. C^{14} content of loading and unloading solutions was measured and inulin space calculated. Total water was the difference between wet and dry weights. Fiber water was the difference between total water and inulin space.

K and Na content of muscles, from both flux and recirculating chambers and from zero time controls, was measured by flame photometry, and Cl content was measured in the Cotlove chloridometer. In the earlier experiments, including all those done in the flux chamber, electrolytes were extracted from the muscle by boiling in water for 2 hr. When it was shown that 1 hr in boiling water yielded complete extraction, this briefer period was used in later experiments, including all those done in the recirculating chamber.

K efflux was measured as follows: $K^{42}Cl$, obtained from Oak Ridge National

Laboratory and adjusted to pH 7, was injected intraperitoneally at least 2 hr before muscles were excised. One extensor digitorum longus muscle was tied at rest length in the flux chamber and radioactivity remaining in the muscle was monitored by recording the output from the Geiger counter either continuously through a ratemeter with a time constant of 50 sec or less, or, in most experiments, intermittently through a scaler at intervals of 6 min from beginning of one count to beginning of the next. Counting time varied from 2 to 5 min. Total counts were initially greater than 10,000.

TABLE I

EFFECTS OF INSULIN AND OF HYPOPHYSECTOMY ON DISTRIBUTION OF MUSCLE WATER IN SOLUTIONS CONTAINING GLUCOSE

	Insulin	Total water	Inulin space	Cell water
	0.01 U/ml	*g/kg wet wt*	*g/kg wet wt*	*g/kg wet wt*
Normal rats	−	800±4.6	214±16	586±12.8
(N = 13)	+	802±6.0	189±8	612±5.8
Insulin effect		1.5	−25±13.5	26±13
			$0.1 > P > 0.05$	$0.1 > P > 0.05$
Hyp-x rats	−	785±2.6	191±5.2	594±5.3
(N = 10)	+	785±4.1	170±9.5	615±9.3
Insulin effect		0.8	−20±11.9	21±10.9
			$0.2 > P > 0.1$	$0.1 > P > 0.05$
Effect of hypophysectomy	−	−15.5±5.9	−23±19.4	8.1±15.8
	+	−16.2±8.0	−19±12.7	3.0±8.6
All rats	−		204±9.4	589±7.5
(N = 23)	+		181±6.4	613±1.6
Insulin effect			23±2.9	24±8.7
			$0.001 > P$	$0.02 > P > 0.01$

Insulin effect is calculated from paired difference. One muscle from a rat was incubated without insulin; the contralateral muscle was incubated with insulin. Data are means ± standard errors of mean. Data for all rats are pooled from normal and hypophysectomized rats.

RESULTS

Effect of Insulin on Distribution of Water

a. IN THE PRESENCE OF GLUCOSE The muscle from one hind limb of each rat was exposed to insulin during the 2nd hour of incubation in a bath containing glucose throughout the incubation period. Insulin had no effect on total water content but probably caused a shift of water from extracellular space to the inside of muscle fibers (Table I). Although the shift amounted to about 10% of extracellular space, variance about the analytical mean was so great that the significance of the insulin effect is in doubt if one examines separately only the data from normal rats or the data from hypophysectomized rats. When data from both normal and hypophysectomized rats are pooled,

confidence is gained that insulin did cause a shift of water, with an average increase in fiber water content of about 4%. It is interesting that the muscle fibers swelled at the expense of extracellular space, with no change in total water, indicating that the fibers were packed more tightly. This suggests that the sheath covering the entire muscle is poorly compliant.

There was about 2% less total water in muscles from hypophysectomized rats than in muscles from normal rats, a significant difference, probably due to a decrease in inulin space, although the significance of the latter decrease could not be demonstrated. The difference in water content was probably not due to differences in muscle weight. Muscles from normal rats ranged from 22 to 50 mg with a mean of 31.8. Muscles from hypophysectomized rats ranged from 22 to 32 mg with a mean of 28.8.

Inulin space and total water are greater in muscles from baby rats than in muscles from adults. There is a rather sharp decrease in inulin space as muscles from normal rats approach 25 mg fresh weight. Because the group of muscles included this critical weight, the variance of our results is greater than variances reported by others who examined the effect of insulin only in muscles from older rats. Creese and Northover (6) and Fritz and Knobil (7) have both found that insulin decreased inulin space in rat skeletal muscle by about 10%, as we confirm; indeed the mean values reported by them are very close to those we observed. However, both groups of investigators studied the effect of insulin on inulin space only when glucose was present in the bathing solution. Both used a glucose concentration of 200 mg per 100 ml.

b. IN THE ABSENCE OF GLUCOSE It occurred to us that the net shift of water from extracellular to intracellular space produced by insulin may have been a consequence of the translocation of glucose. It is an old observation, by Fenn (8) and others since, that deposition of glycogen is associated with increased water content of cells. Accordingly, we repeated the form of experiments described above, but omitted glucose from the bathing solution. We have found previously that for the excised extensor digitorum longus muscle of young rats incubated in the absence of glucose or a glucose substitute, the absolute value of the resting membrane potential falls, the muscle becomes increasingly leaky with respect to certain proteins, net K efflux becomes large, and muscle K content decreases. However, for reasons not understood, these changes are avoided when either cytidine or uridine, 4 mg per 100 ml, is added to the bath. The experiments to be reported were performed on muscles from normal rats incubated in a solution containing the same electrolyte concentrations as described above, with cytidine, 4 mg per 100 ml, and without glucose.

Under these conditions insulin caused no change in the distribution of water (Table II). It is concluded that the effect of insulin in decreasing inulin space and increasing cell water, observed in the previous experiments, was

secondary to the translocation of glucose, perhaps related to glycogen deposition. Since the membrane hyperpolarization and the decrease in K efflux produced by insulin are independent of the presence or absence of glucose in the medium, we conclude that the water shift is neither a cause nor a result of the action of insulin on membrane potential or K efflux.

Effect of Incubation on Potassium, Sodium, and Chloride Content of Muscle

In order to interpret studies on the action of insulin it is helpful to see first what spontaneous changes may occur as a result of incubation. Two groups of experiments were performed. In both groups one extensor digitorum longus muscle was removed, weighed, frozen immediately, and held for analysis. This is referred to as the unincubated muscle or zero time control. The contralateral extensor digitorum longus muscle was removed and placed at rest

TABLE II

LACK OF EFFECT OF INSULIN ON DISTRIBUTION OF MUSCLE WATER IN THE ABSENCE OF EXTERNAL GLUCOSE

No. of rats	Insulin	Total water	Inulin space	Cell water
	0.01 U/ml	*g/kg wet wt*	*g/kg wet wt*	*g/kg wet wt*
11	−	798±3	192±5	606±4
11	+	799±2	193±7	606±6

length in a chamber. In group A, the muscles were from rats previously injected intraperitoneally with K^{42}. These muscles were from either normal or hypophysectomized rats and were placed in the nonrecirculating chamber used for measurement of K flux. In group B, the muscles were from only normal rats and were placed for 1 hr at approximately 25° in the recirculating chamber. All muscles were incubated in the buffered glucose-electrolyte solution described under Methods. In addition to these two sets of experiments in which incubated and unincubated muscles were paired, we have a larger collection of data obtained by pooling our total experience with measurements of electrolyte content of the extensor digitorum longus muscle in rats of the size and strain used in these experiments (Table III).

Only K and Na measurements were made on muscles of group A. These data, with the per cent change in muscle weight, appear in Table IV. All the incubated muscles gained weight. The electrolyte contents were examined in two ways: in one case the total muscle was the unit of reference, in the other case the unit wet weight of the muscle was the reference base. Incubation produced no change in K content per muscle, but, because incubated muscles gained weight, K concentration per kilogram wet weight decreased in muscles from both normal and hypophysectomized rats. All incubated muscles gained considerable Na. The magnitude of the increase is not appreciated

TABLE III

POTASSIUM, SODIUM, AND CHLORIDE CONTENT OF UNINCUBATED MUSCLE, EXTENSOR DIGITORUM LONGUS MUSCLE, 15 TO 50 MG, C-R STRAIN

	Potassium	Sodium	Chloride
	mEq/kg wet wt	*mEq/kg wet wt*	*mEq/kg wet wt*
Normal	102±5.4 (49)	32.2±6.1 (49)	20.4±2.1 (15)
Hypophysectomized	103±5.3 (79)	30.6±6.2 (78)	18.7±1.8 (6)

Data are means ± standard deviation. Number of muscles given in parentheses.

if one looks only at Na concentration per kilogram wet weight. Indeed, in muscles from normal rats the gain in Na per kilogram wet weight was not statistically significant.

The seven muscles in group B were incubated for less than one-third the time for those in group A, yet the changes in weight were similar to those

TABLE IV

EFFECTS OF INCUBATION AND OF INSULIN ON WEIGHT AND ON Na AND K CONTENT OF MUSCLES USED IN STUDIES OF K EFFLUX

	Insulin	Total incubation time	Time insulin added	No. of pairs muscle	ΔK	ΔNa	ΔWt	ΔK	ΔNa
	U/ml	*min*	*min*		*% per muscle*	*% per muscle*	*% per muscle*	*mEq/kg wet wt*	*mEq/kg wet wt*
Normal	0	199 (192–210)		6	0.0 ±0.9	51* ±10	4.2* ±1.4	−4.0* ±1.1	13 ±8.4
Normal	0.001	186 (144–234)	60	6	4.7 ±3.4	20 ±14	1.5 ±2.1	2.8* ±2.3	4 ±5.0
Normal	0.01	144 (126–162)	60	6	4.0 ±2.6	26 ±10	5.3 ±2.4	−1.5 ±1.3	6 ±2.5
Hyp-x	0	206 (192–222)		6	−1.5 ±1.1	64* ±9	7.1* ±0.7*	−10.4* ±1.8	16* ±2.9
Hyp-x	0.001	182 (132–234)	85 (60–120)	15	3.5* ±1.5	54 ±11	1.7* ±0.4	−1.0* ±1.7	11 ±1.8
Hyp-x	0.01	149 (132–162)	62 (60–72)	6	6.3* ±2.5	42 +9	8.0 ±3.5	−2.1* ±1.9	9 ±1.8

Data are means ± standard errors of mean. Range is given in parentheses. All Δ's are values for incubated muscle minus those for unincubated muscle.

* By *t*-test, $P < 0.05$. For those muscles not exposed to insulin, *t*-tests compared means of data from incubated muscle to those from unincubated muscle. For those muscles exposed to insulin, *t*-tests compared means of data from muscles incubated with insulin to those incubated without insulin.

shown in Table IV, 4.6 ± 1.5 (SEM) %. These muscles did not lose K. There was an increase in K by 4.5 ± 2.3 (SEM) % per muscle, which was not significant, and because it nearly matched the gain in weight there was no change in K concentration per kilogram wet weight. The changes in Na were indistinguishable from those observed in muscles of group A. These muscles gained Na by 51 ± 13 (SEM) % per muscle and by 12 ± 3.3 (SEM) mEq per kg wet weight. They also gained Cl by 62 ± 3.9 (SEM) % per muscle and by 10.2 ± 3.0 mEq per kg wet weight. Thus with respect to the magnitude of the increase in weight and in Na, the changes observed after more than 3 hr of incubation (group A) were present at the end of 1 hr of incubation (group B). We have no explanation for the difference observed with respect to K between group A and group B, but it may be related to the fact that paired unincubated muscles in group A were richer in K, 104 ± 1.1 mEq per kg wet weight, than those in group B, 96 ± 1.0 mEq per kg wet weight. The two groups of unincubated muscles were similar with respect to weight and Na content.

Although we lack direct measurement of the distribution of water at zero time in unincubated muscle, we can draw certain plausible inferences from the change in weight and in Cl content of muscles in group B.

The wet weight of unincubated muscles averaged 33.7 mg, and of incubated muscles, 35.2 mg, an increase of 1.5 mg. Assume that gain in weight was entirely due to water. From Table I total water of incubated muscle was 800 g per kg wet weight, or the total water of incubated muscles in group B was 0.8 × 35.2 = 28.16 mg. The total water of unincubated muscles in group B was 26.66 (= 28.16 − 1.5) mg, from which it can be calculated that the total water content of these unincubated muscles was 791 g per kg wet weight. The average Cl content of unincubated muscles in this group was 19.45 mEq per kg wet weight. Assume that the Cl concentration of rat extracellular fluid is 110 mEq per kg. To calculate extracellular volume in unincubated muscle, we use the relation

$$\mathrm{Cl}_i = \frac{\mathrm{Cl}_w - \mathrm{Cl}_o V_o}{V_T - V_o},$$

where subscripts i, w, and o refer to Cl concentration in cell water, per kilogram wet weight, and in extracellular solution, respectively, and V_o and V_T are extracellular and total water, respectively in kilograms per kilogram wet weight.

An upper limit on V_o is obtained when Cl_i is set at zero, whence $V_o = 0.176$ and cell water, $V_i = 0.615$. This implies that incubated muscles, for which $V_o = 0.214$ and $V_i = 0.586$, gained at least 38 g of extracellular water per kg wet weight and lost at least 29 g of fiber water per kg wet weight. When

the unincubated muscle, weighing 33.7 mg on the average, gained 1.5 mg on incubation, we estimate from these calculations that extracellular water increased from 5.9 to 7.5 mg and fiber water decreased from 20.7 to 20.6 mg.

This estimate of V_o is not very different from that made on the assumption that the entire absolute increase in water was due to an absolute increase only in extracellular water. If the weight gain, 1.5 mg, were all extracellular, absolute extracellular water in unincubated muscle was 6.03 mg, or 0.179 kg per kg wet weight. Although this value is only slightly higher than that derived on the assumption that Cl_i was zero, it literally leads to a negative value for Cl_i, and implies that at least some of the increase in weight occurring with incubation may have been due to an absolute gain in fiber water.

Another estimate of V_o in unincubated muscle follows from the assumption that $Cl_o/Cl_i = K_i/K_o$. Unfortunately we cannot calculate K_i without knowledge of V_i. Since we know that 0.615 is a minimum estimate of V_i, an upper limit on K_i is obtained from the observed $K_w = 96$ for this group of muscles, from $K_o = 4.5$ and from $V_o = 0.176$ and $V_i = 0.615$. The ratio is 34.4 which leads to an estimate that $Cl_i = 3.14$, and from this it is calculated that $V_o = 0.164$ and $V_i = 0.627$. If these values for V_i and V_o had been used to calculate K_i, the ratio would have been 33.8 and Cl_i would be 3.07, which, in turn, leads to the estimate $V_o = 0.165$ and $V_i = 0.626$. If these latter figures are correct, then the 33.7 mg unincubated muscle gained 2 mg of extracellular water and lost 0.5 mg of fiber water on incubation. This is a decrease in fiber volume by about 2%.

Another estimate of V_o could be based on the assumption that the chloride equilibrium potential is the same as the resting membrane potential. In extensor digitorum longus muscles from rats of the age and strain used in these experiments we find that after about 1 hr of incubation the resting membrane potential is approximately −75 mv. Although this probably underestimates the membrane potential in vivo, it leads to an estimate of Cl_i of 6.18 mEq per kg of fiber water, whence it is calculated that V_o is 0.145 and V_i is 0.646 kg per kg wet weight. On this basis, there would have been 21.77 mg of cell water in a 33.7 mg unincubated muscle, and it would have decreased by 5% on incubation. This is likely to be an overestimate of the decrease in fiber volume.

It is concluded that on incubation there was an increase in extracellular volume and probably a decrease in fiber water. The decrease in fiber water is not likely to have been as great as 5% and was probably close to 2%. This estimate is important in application of equations for calculation of K flux, in which simplification is gained if fiber volume is constant. The estimates given above justify the assumption that, for practical purposes, fiber volume was constant.

Effect of Insulin on Potassium, Sodium, and Chloride Content of Muscles

The effect of insulin on electrolyte content of muscle was studied in two groups of experiments. In group A are the muscles used in those experiments in which K flux was measured. This includes those muscles described as group A in the previous section in which the effects of incubation were examined. In group C muscles from four normal and from seven hypophysectomized rats were incubated in a pair of recirculating chambers. Both extensor digitorum longus muscles from one rat were incubated simultaneously for 3 hr. During the last 2 hr of the incubation period one of the pair of muscles was exposed to insulin, 0.01 U per ml. All bathing solutions were the buffered electrolyte-glucose solution described under Methods. Temperature was approximately 26°.

Results appear in Tables IV, V, and VI, and are considered with three different reference bases: per muscle, per kilogram wet weight, and per kilogram fiber water.

In muscles in group A, on the average there was a gain in K and loss in Na per muscle but the gain in K was statistically significant only for muscles from hypophysectomized rats, both in the presence of 0.001 U of insulin per ml and in the presence of 0.01 U per ml, and the loss of Na was not statistically significant in any of the subsets of experiments (Table IV). When the data from muscles from rats exposed to insulin were pooled, the decrease in Na was 28 $\pm$ 13% per muscle, which is significantly different from zero (P = 0.05).

In muscles in group C, in which muscles were paired for insulin effect, the average effects were similar to those in group A but, owing to the smaller variance accompanying paired experiments of this nature, the differences tended to be more significant.

Despite the average gain in K per muscle, muscles lost K per kilogram wet weight compared to unincubated muscle because water accumulated during incubation (Table IV). Excepted were those muscles from normal rats exposed to 0.001 U of insulin per ml. The concentration of K per kilogram wet weight was higher in muscles exposed to insulin than in those not exposed to insulin (Tables IV and V). There was, on the average, a decrease in Na per kilogram wet weight but this was significant only in muscles from hypophysectomized rats in group C. Chloride analysis was performed only on muscles in group C. Insulin decreased significantly the Cl concentration per kilogram wet weight in muscles from hypophysectomized rats.

Concentrations of electrolytes per kilogram fiber water were calculated with the aid of data on distribution of water given in Table I. Results appear in Table VI. In muscles of group A the average gain in K was matched by a proportional gain in fiber water so that K concentration per kilogram fiber water was unaffected by insulin. However, in muscles from hypophysec-

tomized rats of group C there was an increase in K concentration by 5%.

There tended to be a loss in Na in muscles from group A which was not significant. When the data from normal and hypophysectomized rats were pooled, there was a decrease in fiber Na by 7.2 ± 3.6 mEq per kg fiber water ($P = 0.06$). In muscles from hypophysectomized rats in group C there was a significant loss of both fiber Na and Cl (Table VI).

TABLE V

EFFECT OF INSULIN ON POTASSIUM, SODIUM, AND CHLORIDE CONTENT OF MUSCLE (PAIRED MUSCLES, GROUP C)

% change in quantity of electrolyte per muscle; quantity of electrolyte in muscle exposed to insulin minus quantity in paired muscle not exposed to insulin as % of the latter

	Potassium	Sodium	Chloride
Normal rats ($N = 4$)	3.8±0.80	−5.1±2.58	−5.0±2.09
	$P < 0.02$	N.S.	N.S.
Hypophysectomized rats	11.8±0.96	−7.4±2.53	−14.8±2.48
($N = 7$)	$P < 0.001$	$P < 0.05$	$P < 0.001$

Electrolyte content per kg wet weight

Average muscle wt	Insulin	Potassium	Sodium	Chloride
mg	*U/ml*	*mEq/kg wet wt*	*mEq/kg wet wt*	*mEq/kg wet wt*
	Normal rats, N = 4			
46.9	0	87.7±1.2	41.1±0.4	30.8±0.3
46.6	0.01	91.8±1.5	38.9±0.9	29.5±0.6
Insulin effect		4.1±0.68	−2.2±1.2	−1.3±0.9
		$P < 0.01$	N.S.	N.S.
	Hypophysectomized rats, N = 7			
26.8	0	94.0±1.7	47.2±1.7	33.9±1.6
27.5	0.01	102.2±2.1	42.5±2.2	28.1±1.7
Insulin effect		8.2±2.2	−4.7±1.1	−5.8±1.3
		$P < 0.01$	$P < 0.01$	$P < 0.01$

Muscles incubated in a pair of recirculating chambers for 3 hr. During last 2 hr insulin added to solution bathing one muscle of each pair from the same rat. Insulin effect is difference between pairs of muscles. Data are means ± standard errors of mean. N. S. is not significant.

In summary, in the concentrations and over the exposure time used in these experiments, insulin caused a gain in the amount of K in the muscles from hypophysectomized rats, and probably also in muscles from normal rats. However, in the presence of glucose in the bathing solution insulin also caused an increase in fiber water. Consequently, except for one group of muscles from hypophysectomized rats, there was no change in the concentration of K in fiber water. The total experience suggests that insulin may also have decreased the amount of Na and of Cl in muscle and may have decreased the concentration of Na and Cl in fiber water, although the effects were statistically significant only in the case of paired experiments on muscles

from hypophysectomized rats. These data are similar to those of Creese and Northover (6) who found that insulin, 0.01 U per ml, in 2 hr increased K and decreased Na per kilogram wet weight, and decreased Na per kilogram fiber water in rat diaphragms. Kernan (9) had previously shown that when frog sartorius muscles, previously made Na-rich by overnight immersion in K-free solution, were reimmersed in the same solutions to which K and in-

TABLE VI

EFFECT OF INSULIN ON POTASSIUM, SODIUM, AND CHLORIDE CONCENTRATION IN MUSCLE FIBER WATER IN NORMAL AND HYPOPHYSECTOMIZED RATS

Average muscle wt	Insulin	Potassium	Sodium	Chloride
mg	*U/ml*	*mEq/kg fiber water*	*mEq/kg fiber water*	*mEq/kg fiber water*
		Group A normal rats		
40.7 (6)	0	169±4.9	24.5±4.9	
40.3 (6)	0.01	167±3.8	16.2±5.6	
		Group A hypophysectomized rats		
30.7 (6)	0	159±4.3	30.1±5.6	
35.7 (6)	0.01	159±3.1	24.2±3.5	
		Group C normal rats		
46.9 (4)	0	148±3.8	17.2±4.1	8.7±0.5
46.6 (4)	0.01	149±2.9	18.8±2.4	11.1±1.1
		Group C hypophysectomized rats		
26.8 (7)	0	157±3.2	32.9±3.2	18.5±2.7
27.5 (7)	0.01	165±4.2	28.4±4.2	12.5±2.7
Insulin effect,		8.2±3.36	−4.5±1.33	−6.0±1.58
paired muscles		$P = 0.05$	$P < 0.02$	$P < 0.01$

Data are means ± standard errors of mean. Calculations based on distribution of muscle water given in Table I. Variance includes variance of water measurements as well as variance of electrolyte content. No insulin effects are significant except those in group C (hypophysectomized rats) for which all effects are significant when analyzed as differences between pairs of muscles from the same rat. Muscles in group A were not paired, and were those on which measurements were made of K efflux. Muscles in group C were incubated in a recirculating bath in paired experiments in which one muscle was not exposed to insulin while the other muscle from the same rat was exposed to insulin during the last 2 hr of a 3 hr incubation. Numbers of muscles are given in parentheses in the first column.

sulin were added, there was a decrease in Na content per unit wet weight of muscle. Moore (10) has confirmed Kernan's observation.

An important conclusion from our data, for subsequent use in application of equations for calculating K flux, is that insulin did not alter K concentration per kilogram fiber water in those muscles with which K flux was studied (group A, Table VI).

Transit Time of Insulin from Reservoir to Muscle Chamber and Concentration of Insulin Bathing Muscle

If insulin acts on cell membranes one might expect its action to be prompt. Measurement of the response time is obscured by the fact that there is a large

dead space in the apparatus used in experiments in which K flux was measured. In order to estimate the response time of the muscle to insulin it was therefore necessary to measure the transit time of insulin from the reservoir holding the bathing solution to the muscle chamber. This was accomplished in two ways. Evans' blue dye, T-1824, was added to the reservoir and a concentration-time curve determined by serial sampling from the muscle chamber. Bovine insulin-I^{131}, purchased from Abbott Laboratories, Oak Ridge, Tenn., was added to the reservoir so that the final concentration of insulin

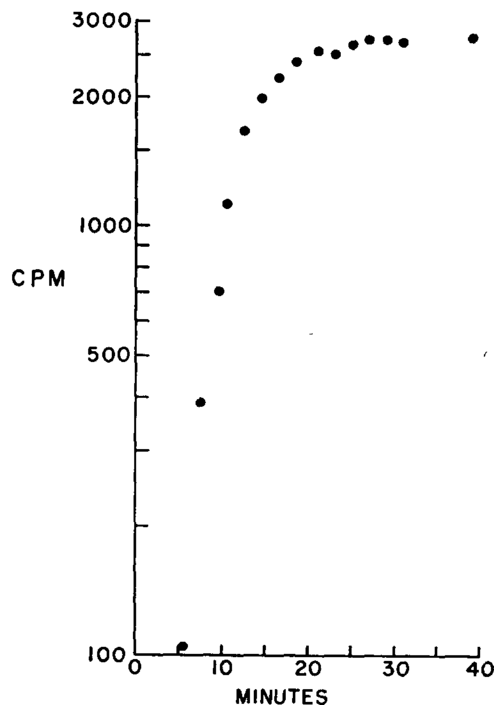

FIGURE 1. Time course of appearance of insulin-I^{131} from the reservoir to the muscle chamber. Insulin effect on K^+ efflux was delayed by a time commensurate with the delay in insulin delivery.

was either 0.01 or 0.001 U per ml. Radioactivity was measured in serial samples from the muscle chamber by means of a gamma spectrometer.

With either blue dye or insulin-I^{131}, half the plateau concentration in the chamber was reached in from 5 to 12 min. Plateau concentration occurred in 20 to 30 min (Fig. 1). Therefore, when large concentrations of insulin are added to the reservoir an effect should occur earlier than when small concentrations are added.

There were reasons to anticipate that the concentration of insulin bathing the muscle might be less than that in the reservoir. Two potential means of loss of insulin were: (*a*), adsorption to the extensive glass surface of the apparatus from the reservoir to the muscle chamber, and (*b*) denaturation or trapping of insulin in the bubble films formed during gassing of the bathing solution.

The first of these potential sources of loss of insulin was demonstrated to be insignificant by three experiments in which the concentration of radioactivity in the muscle chamber reached a plateau that was between 79 and 92% (average, 86%) of the concentration of radioactivity in the reservoir.

The second of these potential sources of loss of insulin was also demonstrated to be insignificant. Samples from the reservoir and from the muscle chamber were paper-chromatographed by the method of Berson *et al.* (11). Native insulin is said not to migrate from the initial spot in ascending paper chromatograms in barbital buffer at pH 8.4, whereas denatured insulin ascends. Chromatograms of solutions from the muscle chamber were identical with those of solutions from the reservoir. In both cases more than 95% of the radioactivity remained at the origin; that is, there was no evidence of substantial denaturation of insulin.

It is concluded that the muscles were indeed exposed, after a delay of 20 to 30 min, to approximately the concentration of native insulin added to the reservoir.

"*Rate Constants*" *for Potassium Efflux*

a. IN NORMAL RATS NOT EXPOSED TO INSULIN

Nineteen normal rats were injected intraperitoneally with $K^{42}Cl$ and, after at least 2 hr, the extensor digitorum longus muscles were removed. One was frozen immediately and held for analysis. The other muscle was tied at rest length in a chamber over the window of a gas-flow Geiger counter. The chamber was washed continuously with a K^{42}-free solution. The logarithm of the counts per minute remaining in the muscle was plotted against time. In thirteen of the nineteen muscles log CPM was linear with time until the end of the experiment, at least 2½ hr later, or until insulin was added to the bath. In six of the muscles there was a different initial curve, lasting no more than 30 min, followed for at least 1 hr by an apparently linear relation between log CPM and time. In those six cases the data reported here were calculated from the later, longer lasting linear portion of the curve. From each of these nineteen observations a value was calculated for k in the expression

$$\text{CPM}(t) = \text{CPM}(0)e^{-kt}.$$

This equation is discussed in a companion paper (12). For the time being k will be called the rate constant for K efflux. The results appear in Table VII, divided into three groups according to the subsequent nature of the experiment. In each group k was 0.183 or 0.184 hr^{-1}. This value for k is larger than that reported previously, 0.16 hr^{-1}, for the same muscle in a different strain of rats (3).

In six of the nineteen experiments no insulin was added. Incubation continued for at least 2½ hr to determine spontaneous changes in rate of decrease of remaining radioactivity. After 1½ to 2 hr there was a spontaneous decrease in rate constant in two of these six muscles. The smallest rate constant was 0.155. In the other four muscles the logarithm of CPM was linear with time from time zero to the end of the observation period, 192 to 210 min later. The mean and standard error of the mean of the rate constant for these six muscles, calculated from the 90th min of incubation to the termina-

TABLE VII

EFFECT OF INSULIN ON K EFFLUX, NORMAL AND HYPOPHYSECTOMIZED RAT MUSCLE

	Insulin concentration					
	None		0.001 U/ml		0.01 U/ml	
	k	k'	k	k'	k	k'
Unoperated	0.183 (6) ±0.012	0.175 ±0.011	0.184 (7) ±0.007	0.162 ±0.009	0.183 (6) ±0.021	0.145 ±0.014
Hypophysectomized	0.205 (6) ±0.014	0.194 ±0.012	0.206 (16) ±0.007	0.165 ±0.005	0.182 (6) ±0.006	0.121 ±0.010

k, rate constant, hr^{-1}, before addition of insulin or during first 90 min of experiment. k', rate constant reached after addition of insulin or spontaneously after 90 min of experiment. Numbers in parentheses are numbers of rats. Data are means ± standard errors of mean. Days postoperative, no insulin, average 15, range 9 to 30; 0.001 U/ml, average 9, range 5 to 21; 0.01 U/ml, average 15, range 7 to 22.

tion of the experiment, are given as the appropriate k' in Table VII. The spontaneous decrease in rate constant is not significant.

b. EFFECT OF INSULIN IN NORMAL RATS

After the logarithm of CPM had remained satisfactorily linear with time for at least 1 hr, insulin was added to the bath. In seven experiments the concentration of insulin was 0.001 U/ml, and in six experiments it was 0.01 U/ml. In the presence of 0.01 U/ml there was a decrease in the rate of loss of radioactivity from muscle which was evident within 15 to 30 min after insulin was added to the reservoir. This delay is approximately the time required for full concentration of insulin to reach the muscle chamber. In the presence of 0.001 U/ml there was approximately a 40 min delay preceding the decrease in rate of loss of radioactivity. The rate constant for K efflux following addition of insulin is recorded as k' in Table VII. In Fig. 2 these data are presented as the per cent change in rate constant for K efflux plotted against the concentration of insulin. In Fig. 2, data for the effect of the largest

concentration of insulin are taken from a previous report (3). The effect of insulin is concentration-dependent.

C. IN HYPOPHYSECTOMIZED RATS NOT EXPOSED TO INSULIN

Twenty-eight hypophysectomized rats were injected intraperitoneally with $K^{42}Cl$ and, as in the case of normal rats described above, one extensor digitorum longus muscle was placed at rest length in the chamber over the Geiger counter. As was true for muscles from normal rats, in about two-thirds

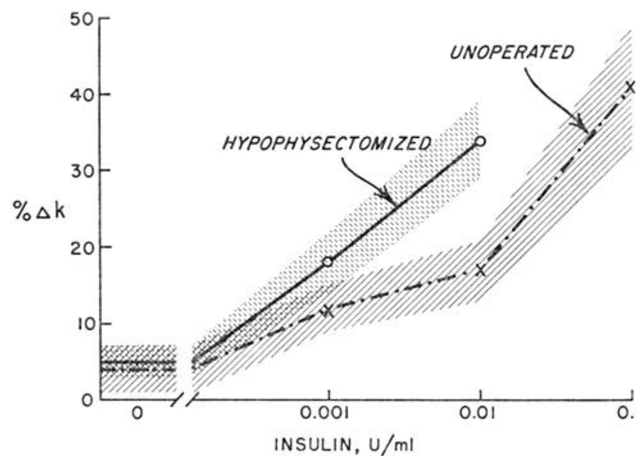

FIGURE 2. Concentration dependency of insulin response. % change in rate constant for K efflux plotted against insulin concentration. Points are mean values and shaded areas represent range of ±1 SEM. Changes at zero concentration indicate observed decrease in rate constant with incubation prolonged to times comparable to those during which muscles were exposed to insulin Reference rate constant is that calculated for each muscle during at least 1 hr before addition of insulin, or in the case of no added insulin, reference rate constant is calculated over a time comparable to that used in experiments in which insulin was added.

of these muscles the logarithm of CPM remaining in the muscle was linear with time from time zero until the experiment was terminated or until insulin was added and achieved its effect. In the remaining one-third of the muscles log CPM did not become linear with time until about 30 min after incubation started, and, again, the rate constant reported for these muscles is that observed during the linear period, lasting from the 30th to at least the 90th min of incubation. The results appear in Table VII divided into three group according to the subsequent nature of the experiment. In two of these groups, k was 0.205 or 0.206 hr^{-1}, and in the third group it was only 0.182 hr^{-1}. There was no obvious difference among the three groups of rats with regard to body weight or number of days posthypophysectomy, and we cannot explain why k was smaller in the third group. For the entire group of twenty-

eight muscles from hypophysectomized rats, k was 0.201 ± 0.005 hr^{-1}. For the entire group of nineteen muscles from normal rats k was 0.184 ± 0.008 hr^{-1}. The rate constant for K efflux was therefore significantly higher in muscles from hypophysectomized rats than in muscles from normal rats. In view of observations (13) that growth hormone decreases K efflux, it is likely that the larger rate constant in muscles from hypophysectomized rats reflects lack of growth hormone.

Six muscles from hypophysectomized rats were incubated for 192 to 222 min without addition of insulin to determine spontaneous changes. In four of the six there was no change in rate constant; in two there was a spontaneous decrease in rate constant. The rate constants for these six muscles during the period following the 90th minute of incubation are given as k' in Table VII. The mean decrease from $k = 0.205$ to $k' = 0.194$ is not significant.

d. EFFECT OF INSULIN IN HYPOPHYSECTOMIZED RATS

Insulin was added to the bath in a concentration of 0.01 U/ml in six experiments, and in a concentration of 0.001 U/ml in sixteen experiments. In both cases, after a delay of about 30 min the rate constant for K efflux decreased to the values reported as k' in Table VII.

Of the sixteen muscles exposed to insulin at a concentration of 0.001 U/ml, three were from rats hypophysectomized more than 2 wk previously. Data from these three experiments are included in Table VII and in Fig. 2. Without these three experiments, the value for k among the remaining thirteen muscles was 0.212 hr^{-1}, and the value for k' among these thirteen muscles during exhibition of insulin was 0.161 hr^{-1}.

When the effect of insulin is calculated as per cent change in rate constant; that is, as $(k - k')100/k$, muscles from hypophysectomized rats were more sensitive to insulin than were muscles from normal rats. The per cent decrease in rate constant was greater for muscles from hypophysectomized rats for a given concentration of insulin (Fig. 2).

Before it had been determined that gelatin was not necessary to prevent loss of insulin in these experiments, a series of observations were made in which gelatin, 4 g/liter, was added to the control solution and to solutions containing insulin 0.001 U/ml. Only muscles from hypophysectomized rats, 6 or 7 days postoperative, were used. Persistent bubbles formed when these gelatin solutions were gassed. These made the muscle rock irregularly, grossly altering its relation to the Geiger tube and yielding erratic counts. Despite the technically unsatisfactory nature of the experiment, rate constants could be calculated. Rate constants, k, before addition of insulin averaged 0.158 hr^{-1}, considerably smaller than in similar muscles bathed in gelatin-free solutions, and rate constants, k', during exposure to insulin averaged 0.116 hr^{-1}. The coefficients of variation were 15 and 17%, respectively, for the

two sets of data, much larger than observed in experiments with gelatin-free solutions. Nevertheless, they are consistent with the other observations that insulin reduces the rate constant for K efflux.

Potassium Efflux and Influx

a. METHOD OF CALCULATION

In a companion paper (12) it is shown that if there is a rate constant for potassium efflux in a muscle containing cylindrical fibers of different diameter, it is described by the equation

$$k = 2F_o/\gamma r C_i \qquad (1)$$

where k is the rate constant obtained as the slope of the semilogarithmic plot of radioactivity remaining in the muscle as a function of time, F_o is efflux of native potassium in units of mass · time^{-1} · length^{-2}, γ is the ratio of the volume of fiber water to the total volume of all fibers, r is the mean radius of single fibers in the muscle, and C_i is concentration of native potassium in fiber water.

The rate at which the quantity of native potassium changes in fiber water is the difference between influx and efflux, or

$$\frac{dN_i}{dt} = SF_i - SF_o \qquad (2)$$

where N_i is the quantity of native K^+ in fiber water, S is the sum of surface areas of all fibers, and F_i is influx per unit surface area.

If SF_o can be calculated from equation (1) and if dN_i/dt can be measured, SF_i can be calculated from equation (2).

The fluxes, F_i and F_o, can both be considered to include a passive component, M_i and M_o, respectively, and an active component, A_i and A_o, respectively.

Thus,

$$F_i = M_i + A_i \qquad (3)$$

$$F_o = M_o + A_o. \qquad (4)$$

We define passive flux as that flux which is determined only by the electrochemical potential. We cannot, from measurement only of electrochemical potential differences across a muscle fiber membrane, calculate either M_o or M_i, but we can, in accordance with proposals by Teorell (14), Linderholm (15), and Ussing (16), calculate the ratio

$$\rho = \frac{M_o}{M_i} = e^{(E-\psi)F/RT}, \qquad (5)$$

where, for the steady state, $-E = \frac{RT}{F} \ln \frac{C_i}{C_o}$, assuming that we deal only with monovalent ions and that the activity coefficients inside and outside the fiber are identical. ψ is the observed resting membrane potential.

Equations (3), (4), and (5) contain four unknowns, M_i, M_o, A_i, and A_o, so the result is indeterminate. We cannot separate the elements of active and passive transport by this means alone.

Unfortunately, efforts to set up a system of simultaneous equations of the type of equations (3) to (5) by altering parameters to a new steady value or by using metabolic inhibitors to eliminate active transport, for example, are not conclusive because every such maneuver is attended by unknown effects on other parameters. If E is changed by changing C_i or C_o or both, the consequent change in electrical potential may be accompanied by (cause?) a change in permeability, as demonstrated by Hodgkin and Horowicz (17). Metabolic inhibitors not only block active transport, but also may alter passive flux by increasing membrane permeability (18).

A solution that has been used, sometimes only implicitly, is based on the assumption that, for potassium, $A_o = 0$. The three equations (3) to (5) are then adequate to yield the remaining unknowns, M_o, M_i, and A_i. Even though we shall make use of this assumption later, there is no firm warrant for it.

b. EFFECTS OF INSULIN ON FLUXES

When, in the experiments reported in this paper, insulin was added and k changed to, say, k', the per cent change in k, $(k - k')100/k$, was approximately the same as the change in F_o, since r and C_i were virtually constant. We write the set of equations (3) to (5) to describe fluxes obtained in response to insulin:

$$F'_i = M'_i + A'_i \tag{3 a}$$

$$F'_o = M'_o + A'_o \tag{4 a}$$

$$\rho' = \frac{M'_o}{M'_i} = e^{(E'-\psi')F/RT}. \tag{5 a}$$

The total K^+ efflux from a muscle of total surface area S is

$$SF_o = kN_i .$$

The total K^+ influx into a muscle is

$$SF_i = SF_o + dN_i/dt.$$

Table VIII gives values for SF_o, SF_i, and for $S'F_o'$, the fluxes during exposure to insulin, calculated from the data presented in Tables III, IV, and VII.

S and S' were estimated as follows: From examination of cross-sections of rat extensor digitorum longus muscles fixed at rest length, the average radius was about 15 μ in normal muscle not exposed to insulin. The total number of fibers per muscle was about 3800. The total volume of muscle fibers is known by subtracting the inulin space (Table I) from the wet weight. These values then fix fiber length at 1.10 cm, which is not greatly out of line with estimates from longitudinal sections of these muscles (about 1 cm). S was calculated on the assumption that there were 3800 cylinders of the given radius and length, and end areas were neglected. Owing to fiber swelling with insulin, and to the fact that the initial wet weight of muscles exposed to insulin was slightly greater than that of muscles incubated without insulin, radius and length were different for muscles exposed to insulin. Values used for S and S' for muscles from normal and from hypophysectomized rats are given in Table VIII

TABLE VIII
TOTAL POTASSIUM FLUXES

Normal or Hyp-x	Insulin	Total efflux	Total influx
	0.01 U/ml	*μEq/hr*	*μEq/hr*
N	–	0.68	0.67
N	+	0.56	0.64
H	–	0.56	0.54
H	+	0.42	0.55

Calculated from data from experiments cited in Tables IV, VI, and VII. Total efflux is SF_o; total influx is SF_i. S was calculated for each muscle on the basis of individual muscle weight, assuming 3800 fibers and the values for r given in Table IX. Values are the means of individual SF_o and SF_i, not the products of mean S and mean F_o or mean F_i.

These calculations show that insulin decreased both the influx and the efflux of K^+ in muscles from both normal and hypophysectomized rats.

The ratio of passive fluxes, ρ, was decreased by insulin because there was an increase in resting membrane potential with essentially no change in the K^+ equilibrium potential. (Data on effects of insulin, 0.1 U/ml, have been reported (1, 2), but are probably an underestimate of hyperpolarization due to neglect of a decrease in membrane potential observed in control muscles with repeated impalement and prolonged incubation. Data on effects of insulin, 0.01 U/ml, have been reported in abstract (19) and will be reported *in extenso* later.) The decrease in ρ means that insulin changed either passive influx or passive efflux or both. Since insulin decreased F_o and since most of K^+ efflux is probably passive, it is reasonable to suppose that at least one reason for the decrease in ρ was a decrease in M_o.

If insulin decreased passive efflux by somehow making the membrane less permeable to K^+, as we have proposed previously (3), then the decreased

permeability should be bidirectional; passive influx should also be decreased unless the hyperpolarization is sufficiently large to compensate for the decreased permeability.

If insulin decreased only passive flux, then the six equations (3) to (5) and (3 *a*) to (5 *a*) can be solved to yield absolute values for active and passive flux by setting $A_i' = A_i$ and $A_o' = A_o$. When the set of six equations was solved, using data from normal and from hypophysectomized rats, with and without insulin, 0.01 U/ml, A_o was 76% of F_o for muscles from normal rats and 57% of F_o for muscles from hypophysectomized rats. It is unlikely that active efflux is so high a fraction of total efflux, if one is willing to extrapolate from observations on amphibian muscle by Keynes and Maisel (20) and on cephalopod axons by Hodgkin and Keynes (21), in which there was no evidence of an effect of metabolic inhibitors on K^+ efflux.

We therefore reject the hypothesis that the effect of insulin was limited to reduction of passive K^+ fluxes.

If one assumes that there is no active K^+ efflux from these muscles, that is, that $F_o = M_o$ and $F_o' = M_o'$, then the set of six equations can be solved for M_i, A_i, M_i', and A_i'. Results appear in Table IX. With this assumption, it follows that insulin not only affected permeability of the membrane to K^+, but also reduced active K^+ influx. In muscles from normal rats, insulin-induced hyperpolarization more than compensated for decreased permeability, and there was a small increase in passive influx. In muscles from hypophysectomized rats, insulin-induced hyperpolarization was not adequate to compensate for the decreased permeability, and there was a small decrease in passive influx. Active influx was decreased by about the same proportion in muscles from hypophysectomized rats as in muscles from normal rats.

These calculations are sensitive to the values given for ρ and ρ', which in turn depend on the observed resting membrane potentials. Because the resting membrane potentials in these muscles are some 20 to 25 mv less than the K^+ equilibrium potential, it is conceivable that, due to leaks around the electrode probe, they may be falsely low. If we assume that the highest observed potentials, occurring in response to insulin, were really equal to the K^+ equilibrium potential, then we set $\rho' = 1$, and on the assumption that insulin affected only passive fluxes, we solve for ρ. The solution yields a value of 90 mv for the resting membrane potential in the absence of added insulin. Insulin would then have increased the resting potential by only 3 mv, a value significantly less than the observed effects. Even if the absolute values of the observed resting potentials are in error, it is unlikely that the insulin effect on membrane potential would be overestimated (indeed, the leak is more likely to lead to an underestimate of an increased membrane potential). It does not seem possible, therefore, to escape the conclusion that insulin decreased the passive permeability of the membrane to K^+ and also decreased active K^+ influx.

DISCUSSION

Insulin has the following effects with respect to ions. It alters membrane permeability at least with respect to K^+ (3), confirmed in the present study. It hyperpolarizes the membrane (1, 2, 19), an observation confirmed in frog muscle l y Moore (10). It decreases the Na^+ content of muscle (6, 9, 10), confirmed by the present study, and it may also decrease Cl^- content. Creese (22) reported that insulin increased washout of radiosodium from rat diaphragm. We have confirmed this observation in the rat extensor digitorum longus muscle (unpublished data). Since nearly all Na^+ efflux is active, these observations on Na^+ efflux and Na^+ content of muscle suggest that insulin increases active Na^+ efflux from muscle.

TABLE IX

EFFECT OF INSULIN ON CALCULATED ACTIVE AND PASSIVE FLUXES OF POTASSIUM

	Normal rats		Hyp-x rats	
	insulin, 0.01 U/ml		*Insulin, 0.01 U/ml*	
	−	+	−	+
S, cm^2	42.6	42.9	33.1	39.1
F_o, $m\mu Eq \cdot hr^{-1} \cdot cm^{-2}$	16.6	13.8	17.0	10.8
dN_i/dt, $m\mu Eq \cdot hr^{-1}$	−6.0	79	−15.7	134
F_i, $m\mu Eq \cdot hr^{-1} \cdot cm^{-2}$	16.5	15.6	16.5	14.2
M_o/M_i	2.9	2.1	2.5	1.7
M_i, $m\mu Eq \cdot hr^{-1} \cdot cm^{-2}$	5.8	6.6	6.7	6.2
A_i, $m\mu Eq \cdot hr^{-1} \cdot cm^{-2}$	10.7	9.1	9.8	8.0

S, surface ; rea of a muscle with 3800 fibers; F_o and F_i, efflux and influx, respectively; dN_i/dt, change in p tassium content of muscle fibers per unit time; M_o and M_i, passive efflux and influx, respectively; A_i, active influx. Calculations are based on the assumption that there is no active efflux of K^+, so that $F_o = M_o$.

For calculation of M_o/M_i, the following values (mv) for ψ were used: in normal rats, without insulin 66, with insulin 74; in hyp-x rats, without insulin 68, with insulin, 78.

Probably not relevant to these effects of insulin is the fact that it also increases water content of rat muscle fibers, demonstrated by Creese and Northover (6) and by Fritz and Knobil (7) and by us. Since we have shown that this occurs only in the presence of glucose and that insulin hyperpolarizes the membrane and decreases K^+ flux whether glucose is present or absent, we need not consider further the effect on fiber water.

It is difficult to construct a single hypothesis to accommodate these observations. Kernan (9) suggested that Na^+ was pumped out of muscle by insulin-induced acceleration of metabolism, specifically by increased oxidation of lactic acid. But Kernan's observations were on the frog. Insulin does not increase oxygen consumption by mammalian muscle and causes only a trivial

increase in lactic acid production, and again, we have observed insulin effects in the absence of glucose.

If the primary effect of insulin were on a sodium pump, then one would have no obvious explanation for the effects on K^+ flux. If there is a one-to-one link between active Na^+ efflux and active K^+ influx, as is thought to be the case, one would expect increased Na^+ efflux to be associated with increased active K^+ influx, contrary to the conclusions reached from our data. Nor is it evident how increased active Na^+ efflux might alter membrane permeability to Na^+.

It is conceivable that what appears to be a multiple effect may ultimately prove to rest on a common basis. What we have arbitrarily called active K^+ influx is a matter of bookkeeping, and we may lack adequate information on all possible sources of potential energy driving passive K^+ flux. Furthermore our understanding of the mechanisms underlying active Na^+ efflux is still incomplete.

The conclusion reached here differs from that given in our earlier reports of the action of a larger concentration of insulin (1–3) entirely due to our earlier underestimate of the magnitude of hyperpolarization produced by insulin.

The data remain consistent with our earlier proposal that a small number of molecules of insulin combine with a few critical sites on the surface of the cell. That the combination occurs at the cell surface is likely because the change in K^+ flux occurs rapidly. It is proposed that there are a number of consequences of this combination between insulin and membrane. One consequence is a deformed membrane with decreased permeability to uncharged molecules (23). A second consequence is a hypothetical net increase in positive fixed charges (or decrease in negative fixed charges) within the membrane. The result of this is decreased permeability to cations and increased permeability to anions. The consequent changes in permeability to Na^+ and Cl^- consistent with the net changes in internal Na^+ and Cl^- concentration reported here, but not yet measured directly, are responsible for the hyperpolarization. If the processes concerned with active inward movement of K^+ are located in the membrane at the sites at which insulin keys, active transport of K^+ may also be impaired. Whether or not the effect of insulin on Na^+ movement can be fitted into this hypothesis remains to be seen.

These studies were aided by United States Public Health Service Research Grant AM-05524, the National Institute of Arthritis and Metabolic Diseases, and by a grant-in-aid from the Muscular Dystrophy Associations of America, Inc.

Received for publication 21 May 1965.

REFERENCES

1. ZIERLER, K. L., *Am. J. Physiol.*, 1959, **197**, 515.
2. ZIERLER, K. L., *Am. J. Physiol.*, 1959, **197**, 524.

3. ZIERLER, K. L., *Am. J. Physiol.*, 1960, **198**, 1066.
4. DE BODO, R. C., and ALTSZULER, N., *Physiol. Rev.*, 1958, **38**, 389.
5. BALL, E. G., MARTIN, D. B., AND COOPER, O., *J. Biol. Chem.*, 1959, **234**, 774.
6. CREESE, R., and NORTHOVER, J., *J. Physiol.*, 1961, **155**, 343.
7. FRITZ, G. R., and KNOBIL, E., *Biochim. et Biophysica Acta*, 1963, **78**, 773.
8. FENN, W. O., *J. Biol. Chem.*, 1939, **128**, 297.
9. KERNAN, R. P., *J. Physiol.*, 1962, **162**, 129.
10. MOORE, R. D., *Biophysical Soc. Abstracts*, 1965, p. 122.
11. BERSON, S. A., YALOW, R. S., BAUMAN, A., ROTHSCHILD, M. A., and NEWERLY, K., *J. Clin. Inv.*, 1956, **35**, 170.
12. ZIERLER, K. L., *J. Gen. Physiol.*, 1966, **49**, 423.
13. RABINOWITZ, D., ZIERLER, K. L., and ROGUS, E., *Clin. Research*, 1964, **12**, 202.
14. TEORELL, T., *Arch. sci. physiol.*, 1949, **3**, 205.
15. LINDERHOLM, H., *Acta Physiol. Scand.*, 1952, **27**, suppl. 97.
16. USSING, H. H., *Acta Physiol. Scand.*, 1949, **19**, 43.
17. HODGKIN, A. L., and HOROWICZ, P., *J. Physiol.*, 1959, **148**, 127.
18. ZIERLER, K. L., *Am. J. Physiol.*, 1958, **193**, 534.
19. HAZLEWOOD, C. F., and ZIERLER, K. L., *Fed. Proc.*, 1964, **23**, 410.
20. KEYNES, R. D., and MAISEL, G. W., *Proc. Roy. Soc. London, Series B*, 1954, **142**, 383.
21. HODGKIN, A. L., and KEYNES, R. D., *J. Physiol.*, 1955, **128**, 61.
22. CREESE, R., *Nature*, 1964, **201**, 505.
23. ZIERLER, K. L., *Bull. Johns Hopkins Hosp.*, 1961, **109**, 35.