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THE RELATIONSHIP BETWEEN THE TRANSPORT OF GLUCOSE AND CATIONS ACROSS CELL MEMBRANES IN ISOLATED TISSUES

III. EFFECT OF Na^+ AND HYPEROSMOLARITY ON GLUCOSE METABOLISM AND INSULIN RESPONSIVENESS IN ISOLATED RAT HEMIDIAPHRAGM

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SUMMARY

1. Total substitution of Na^+ in Krebs–Ringer bicarbonate buffer with choline or K^+ gave an irreversible, but not complete depression of glucose uptake, lactate release and glycogen deposition in isolated rat hemidiaphragm.

2. When Na^+ was replaced with Li^+, the glucose uptake and glycogen deposition were stimulated, whereas lactate release was diminished.

3. It is concluded that Na^+ is not essential for the uptake of glucose in diaphragm muscle.

4. Hyperosmolarity, whether induced by the addition of NaCl, LiCl, mannitol or choline chloride, gave an increase in glucose uptake. In the same hyperosmolar media, the effect of insulin was considerably, but reversibly, reduced, presumably as a result of the increased baseline level for glucose uptake.

5. Hyperosmolarity also accelerated glycogenolysis. This increase in energy production was also demonstrated in Na^+-free and in glucose-free buffer.

INTRODUCTION

The previous results of this study have indicated that the course of carbohydrate metabolism in rat diaphragm is influenced by alterations in active cation transport and the ionic milieu[1,2]. However, in this tissue, it has not been possible to demonstrate any significant change in glucose uptake under conditions which produce a decrease in the active coupled transport of Na^+ and K^+. On the other hand, experiments in which the extracellular Na^+ concentration was varied suggested that Na^+ might facilitate the entry of glucose into muscle cells[1]. This raised the question whether the glucose transport should have any specific or absolute requirement for Na^+. Therefore, this possibility was reinvestigated using media in which Na^+ was totally replaced by other cations.

It was recently reported that hyperosmolarity stimulates glucose uptake and CO_2 production in hemidiaphragm and epididymal fat pad from rats[3]. This pointed to the fact that the increased glucose uptake and lactate release found in media

containing 200 mM of Na^+ (ref. 1) were caused by the ensuing hyperosmolarity and not by any specific effect of the high Na^+ concentration. Therefore, the effects of media made hyperosmolar with Na^+ as well as with other substances were studied in more detail.

Hyperosmolarity imitates the action of insulin in facilitating glucose transport. If this were due to an effect on the same transport system, it was thought that hyperosmolarity would interfere with the action of insulin. Hence, the experiments with hyperosmolar media were combined with an evaluation of the effects of insulin under such conditions.

METHODS

Male albino rats of the Wistar strain, weighing from 100 to 150 g were fasted for 18 h. Hemidiaphragms were prepared and soaked in ice-cold glucose-free buffer.

Krebs–Ringer bicarbonate buffer containing 11.1 mM of D-glucose with or without addition of D-[$^{14}C_6$]glucose was used as the basic medium. D-Glucose was only omitted in the experiments presented in Table VII. When Na^+ was replaced with other cations, the completeness of substitution was controlled by flame photometry or electrometric chloride titration. The modifications used are given in the tables. In the experiments where Na^+ was totally replaced with choline, the $NaHCO_3$ in the control was also replaced with choline bicarbonate in order to exclude the possibility that the effects should be due to different buffer systems. The insulin was a pure crystalline preparation, dissolved in 0.004 M HCl. This solution, as well as all the buffers used for incubation, was freshly prepared on the day of experiment.

The methods for the estimation of glucose disappearance and lactate release, for the isolation and determination of glycogen, and for statistical analysis were the same as those described earlier[1,2].

For the determination of ^{14}C activity in total glycogen, the washed precipitates were dissolved in water and transferred to counting vials containing 5 or 10 ml of liquid scintillator[4], to which 5 % Cab-O-Sil (Packard) had been added. Without this gelling agent, the counting efficiency decreased with time. With Cab-O-Sil, the efficiency remained stable for weeks. The amount of glucose incorporated into glycogen was calculated and expressed in μmoles/g wet weight as previously indicated[2].

The origin of reagents and enzymes was given earlier[2]. The choline bicarbonate was obtained from Fluka, Buchs, Switzerland. The insulin was a gift from the Novo Laboratories, Copenhagen, Denmark.

RESULTS

The effects of total replacement of Na^+ with other monovalent cations are shown in Table I. It appears that the glucose disappearance decreased by about 40 %, when Na^+ was replaced with choline or K^+. On the other hand, when Li^+ was used as substituting ion, the glucose disappearance increased. The lactate release decreased to about the same level in all the three Na^+-free media. Whereas substitution with K^+ or choline caused a decrease in the ^{14}C activity of glycogen, Li^+ produced an increase.

Table II shows the effects of preincubation in Na^+-free media on glucose metabolism. The tissues were incubated for 120 min in buffers of the composition given

TABLE I

EFFECT OF Na^+-FREE KREBS–RINGER BICARBONATE BUFFER ON GLUCOSE DISAPPEARANCE, LACTATE RELEASE, AND ^{14}C ACTIVITY OF GLYCOGEN IN ISOLATED RAT HEMIDIAPHRAGM

The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses. In each experiment, the data of the control and the experimental group were obtained using "paired" hemidiaphragms, and all the differences between the pairs are significant ($P < 0.001$). Incubation time 120 min.

Incubation buffer	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g weight) wet*	*Change (%)*	*μmoles of glucose incorporated into glycogen per g wet weight*	*Change (%)*
Normal Krebs–Ringer	41.4 ± 2.2 (8)		36.6 ± 0.9 (8)		6.55 ± 0.51 (8)	
Krebs–Ringer: all Na^+ replaced with K^+	25.1 ± 1.3 (8)	−39	21.8 ± 0.8 (8)	−40	1.54 ± 0.23 (8)	−76
Krebs–Ringer: all $NaHCO_3$ replaced with choline bicarbonate	34.5 ± 1.1 (7)		34.3 ± 1.3 (7)		5.17 ± 0.36 (7)	
Krebs–Ringer: all Na^+ replaced with choline	19.4 ± 1.1 (7)	−44	22.1 ± 0.8 (7)	−36	1.03 ± 0.20 (7)	−80
Normal Krebs–Ringer	34.2 ± 1.6 (7)		41.5 ± 2.8 (7)		6.13 ± 0.51 (7)	
Krebs–Ringer: all Na^+ replaced with Li^+	43.0 ± 1.2 (7)	26	20.6 ± 0.9 (7)	−50	15.98 ± 1.04 (7)	151

TABLE II

EFFECT OF PREINCUBATION IN Na^+-FREE KREBS–RINGER BICARBONATE BUFFER ON GLUCOSE METABOLISM IN ISOLATED RAT HEMIDIAPHRAGM

For the second incubation, normal Krebs–Ringer bicarbonate buffer was used throughout. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses. Both the first and the second incubations lasted for 120 min.

Buffer used for the first incubation	*Glucose disappearance (μmoles/g wet weight)*		*Lactate release (μmoles/g wet weight)*		*μmoles of glucose incorporated into glycogen per g wet weight (second incubation)*
	First incubation	*Second incubation*	*First incubation*	*Second incubation*	
Normal Krebs–Ringer	35.4 ± 3.7 (6)	32.0 ± 2.7 (6)	34.6 ± 1.3 (6)	44.6 ± 2.8 (6)	1.75 ± 0.18 (6)
Krebs–Ringer: all Na^+ replaced with K^+	22.0 ± 1.9 (6)	3.1 ± 0.9 (6)	21.8 ± 1.1 (6)	11.0 ± 0.3 (6)	0.024 ± 0.002 (6)
Krebs–Ringer: all $NaHCO_3$ replaced with choline bicarbonate	31.8 ± 0.6 (6)	26.6 ± 1.6 (6)	33.4 ± 2.0 (6)	33.9 ± 2.6 (6)	1.52 ± 0.13 (6)
Krebs–Ringer: all Na^+ replaced with choline	19.3 ± 0.8 (6)	5.1 ± 0.6 (6)	23.2 ± 1.2 (6)	12.5 ± 0.6 (6)	0.043 ± 0.006 (6)
Normal Krebs–Ringer	38.6 ± 0.7 (4)	29.7 ± 1.7 (4)	37.5 ± 2.0 (4)	38.6 ± 2.3 (4)	1.32 ± 0.06 (4)
Krebs–Ringer: all Na^+ replaced with Li^+	44.1 ± 1.4 (4)	22.9 ± 3.2 (4)	20.2 ± 0.8 (4)	21.1 ± 1.9 (4)	8.29 ± 1.54 (4)

in the first column. They were then washed for 1 min in normal Krebs–Ringer bicarbonate buffer, transferred to this medium and incubated for another 120 min. D-[$^{14}C_6$]Glucose was only present during the second incubation period.

The effect of the double incubation *per se* is seen from the experiments with the control media (normal Krebs–Ringer or Krebs–Ringer where $NaHCO_3$ was replaced with choline bicarbonate). It appears that in the second period of incubation the glucose uptake was somewhat lower, the lactate release a little higher in one of the experiments, while glycogen deposition, when compared with the results illustrated in Table I, shows a considerable depression.

The experiments where K^+- or choline-substituted media had been used in the first period of incubation demonstrate that severe damage to the tissue had been produced, the glucose uptake and the lactate release appearing much more reduced upon change to one of the control media than in the experiments where the tissue was incubated in one of the control media twice, and particularly the incorporation of ^{14}C activity dropping to extremely low values.

On the other hand, when Na^+ had been replaced by Li^+, the glucose metabolism seemed to be less impaired during the subsequent period. The glucose disappearance was only a little lower than in the control. Lactate release was somewhat diminished, but, in contrast to the other substitution experiments, a surprisingly large ^{14}C activity was found in glycogen.

Table III shows the effect of insulin in normal Krebs–Ringer bicarbonate buffer. Insulin produced an increase in all 3 parameters measured. When expressed in %, these changes were very pronounced in the 30-min experiments. With longer incubations, much smaller effects were found. Both with and without insulin, the ^{14}C activity of glycogen seemed to reach a plateau after the first 60 min of incubation.

It appears from Table IV that in hyperosmolar media, the glucose uptake in the vessels without insulin was always found to exceed that obtained in normal Krebs–

TABLE III

EFFECT OF INSULIN ON THE GLUCOSE METABOLISM OF ISOLATED RAT HEMIDIAPHRAGM IN NORMAL KREBS–RINGER BICARBONATE BUFFER (143 mM OF Na^+)

The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses. In each experiment, the data of the control and the experimental group were obtained using "paired" hemidiaphragms, and all the differences between the pairs are significant ($P < 0.001$).

Duration of incubation (min)	*Insulin (0.1 I.U./ml)*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*
30	0	8.9 ± 0.8 (8)	179	13.9 ± 0.9 (8)	46	2.89 ± 0.18 (8)	324
	+	24.8 ± 0.7 (8)		20.3 ± 0.6 (8)		12.26 ± 0.42 (8)	
60	0	21.4 ± 0.9 (8)	85	20.4 ± 1.4 (8)	38	6.07 ± 0.51 (8)	197
	+	39.5 ± 1.5 (8)		28.1 ± 1.1 (8)		18.00 ± 1.12 (8)	
120	0	41.6 ± 1.7 (12)	53	40.6 ± 1.6 (11)	32	6.99 ± 0.45 (12)	151
	+	63.6 ± 1.6 (12)		53.6 ± 1.0 (11)		17.53 ± 0.88 (12)	

TABLE IV

EFFECT OF HYPEROSMOLARITY ON GLUCOSE METABOLISM AND INSULIN RESPONSIVENESS IN ISOLATED RAT HEMIDIAPHRAGM

Krebs–Ringer bicarbonate buffer was used as the basic medium; osmolarity was increased by the additions given in the first column. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Additions (mM)	*Duration of incubation (min)*	*Insulin (0.1 I.U./ml)*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*
NaCl (57)	120	o	55.1 ± 2.4 (8)	23 ($P < 0.001$)	69.8 ± 3.3 (8)	7 ($P > 0.10$)	3.94 ± 0.34 (8)	83 ($P < 0.001$)
		+	67.7 ± 2.3 (8)		75.0 ± 3.6 (8)		7.22 ± 0.40 (8)	
	30	o	16.1 ± 1.7 (4)	30 ($P < 0.05$)	30.1 ± 3.3 (4)	21 ($P > 0.10$)	1.98 ± 0.17 (4)	103 ($P < 0.001$)
		+	21.0 ± 1.5 (4)		36.5 ± 1.6 (4)		4.01 ± 0.15 (4)	
NaCl (107)	60	o	33.1 ± 1.9 (4)	19 ($P < 0.01$)	38.5 ± 2.1 (4)	15 ($P > 0.05$)	2.94 ± 0.39 (4)	36 ($P > 0.10$)
		+	39.5 ± 2.1 (4)		44.4 ± 1.4 (4)		3.99 ± 0.81 (4)	
	120	o	61.7 ± 2.5 (12)	17 ($P < 0.001$)	76.2 ± 4.1 (12)	14 ($P < 0.01$)	2.89 ± 0.21 (12)	30 ($P < 0.001$)
		+	72.3 ± 3.6 (12)		87.1 ± 3.8 (12)		3.76 ± 0.24 (12)	
LiCl (107)	30	o	17.9 ± 1.2 (4)	32 ($P < 0.02$)	29.5 ± 2.2 (4)	13 ($P > 0.10$)	2.48 ± 0.20 (4)	71 ($P < 0.02$)
		+	23.6 ± 1.2 (4)		33.2 ± 2.7 (4)		4.25 ± 0.52 (4)	
	60	o	38.1 ± 1.1 (3)	6 ($P > 0.05$)	48.0 ± 1.7 (3)	0 ($P > 0.10$)	7.35 ± 0.43 (3)	5 ($P > 0.10$)
		+	40.2 ± 1.5 (3)		48.0 ± 1.6 (3)		7.71 ± 0.89 (3)	
Choline chloride (107)	120	o	47.1 ± 1.4 (8)	17 ($P < 0.005$)	63.3 ± 1.9 (8)	18 ($P < 0.005$)	0.55 ± 0.02 (8)	13 ($P > 0.10$)
		+	55.3 ± 1.6 (8)		74.4 ± 2.1 (8)		0.62 ± 0.05 (8)	
Mannitol (214)	30	o	17.5 ± 0.8 (4)	33 ($P < 0.001$)	36.0 ± 0.9 (4)	7 ($P > 0.05$)	1.89 ± 0.17 (4)	62 ($P < 0.005$)
		+	23.3 ± 0.6 (4)		38.4 ± 0.6 (4)		3.07 ± 0.16 (4)	
	60	o	32.9 ± 1.4 (4)	19 ($P < 0.001$)	58.3 ± 1.8 (4)	11 ($P < 0.01$)	5.22 ± 0.56 (4)	28 ($P > 0.10$)
		+	39.2 ± 1.4 (4)		64.6 ± 1.9 (4)		6.67 ± 0.64 (4)	

Ringer bicarbonate buffer (Tables I, II, and III). Hyperosmolarity induced by LiCl, NaCl and mannitol gave approximately the same increase, whereas choline chloride caused a comparatively small change. A considerable stimulation of lactate release was found in all the experiments. When the medium was made hyperosmolar with NaCl, the ^{14}C activity of glycogen was low. After 60 min of incubation, no further increase could be detected in the controls, while in the presence of insulin, the same activity was found irrespective of the duration of the experiments.

Addition of choline chloride reduced the ^{14}C activity of glycogen to about one-tenth of the value found in normal buffer.

In the experiments with LiCl and mannitol, the incorporation of ^{14}C activity seemed to continue. In the vessels without insulin the 60-min values were even more than double the 30-min values.

In all the hyperosmolar media, insulin (in the same concentration as in the experiments illustrated in Table III) produced changes which were only a fraction of those seen in normal Krebs–Ringer bicarbonate buffer. Sometimes, the effects were even insignificant.

The incubation led to some reduction in wet weight of the hemidiaphragms. This change was significantly more pronounced in the hyperosmolar media (Table V).

It was shown in Table I that hemidiaphragms took up glucose in a Na^+-free medium. However, some Na^+ may have been lost from the tissue during the incubation. In other experiments, hemidiaphragms were preincubated for 60 min at 37° in a Krebs–Ringer bicarbonate buffer in which all Na^+ was replaced by Li^+. The tissues were then blotted briefly on dry filter paper and transferred to vessels containing either the same medium or another Na^+-free Krebs–Ringer bicarbonate buffer with a Li^+ concentration of 250 mM. D-[$^{14}C_6$]Glucose was only added to the second series of vessels. After removal of the tissue, the preincubation medium contained 1.68 ± 0.06 mM of Na^+ ($n = 16$). In the next series of vessels, the final Na^+ concentration was 0.084 + 0.004 mM ($n = 8$). It appears from Table VI, that even at this extremely low extracellular concentration of Na^+, glucose was taken up and metabolized. In this essentially Na^+-free milieu, hyperosmolarity did not produce any significant increase

TABLE V

EFFECT OF INCUBATION (30 min) ON WET WEIGHT OF ISOLATED RAT HEMIDIAPHRAGM

Results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Incubation buffer	*Insulin (0.1 I.U./ml)*	*Wet weight at the end of incubation in % of initial wet weight*	*Significance of difference between tissues incubated in normotonic and hypertonic media*
Krebs–Ringer bicarbonate (143 mM of Na^+)	0	97.5 ± 0.9 (8)	
	+	96.3 ± 1.0 (8)	
Krebs–Ringer + 107 mM NaCl	0	89.0 ± 2.0 (4)	$P < 0.001$
	+	90.4 ± 1.9 (4)	$P < 0.005$
Krebs–Ringer + 107 mM LiCl	0	86.1 ± 1.1 (4)	$P < 0.001$
	+	87.2 ± 0.7 (4)	$P < 0.001$
Krebs–Ringer + 214 mM mannitol	0	84.4 ± 0.4 (4)	$P < 0.001$
	+	84.5 ± 0.4 (4)	$P < 0.001$

TABLE VI

EFFECT OF HYPEROSMOLARITY ON THE GLUCOSE METABOLISM OF ISOLATED RAT HEMIDIAPHRAGM IN A Na^+-FREE KREBS–RINGER BICARBONATE BUFFER

All Na^+ was replaced with Li^+. After preincubation in Na^+-free buffer, the tissues were incubated for 60 min. For details, see the text. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Li^+ concn. (mM)	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*
143	21.0 ± 1.0 (8)	4 ($P > 0.10$)	11.7 ± 0.2 (8)	69 ($P < 0.001$)	10.94 ± 0.68 (8)	−20 ($P < 0.02$)
250	21.8 ± 0.5 (8)		19.8 ± 0.9 (8)		8.78 ± 0.33 (8)	

in glucose disappearance. However, there was an increase in lactate release and a decrease in the ^{14}C labelling of glycogen.

Hyperosmolarity also stimulated the release of lactate in a glucose-free medium, whereas the glycogen content was considerably lowered (Table VII). At the end of incubation, the buffer was shown to contain no glucose.

Table VIII shows an experiment performed to see if hyperosmolar incubation produced an irreversible change of insulin responsiveness. The hemidiaphragms were preincubated for 30 min at 37° in hyperosmolar Krebs–Ringer bicarbonate buffer containing 250 mM of Na^+. All the tissues were washed for 15 min in normal Krebs–Ringer bicarbonate buffer, transferred to other vessels, and incubated for another 60 min. The last incubation medium was either normal (143 mM of Na^+) or hyperosmolar (250 mM of Na^+) Krebs–Ringer bicarbonate buffer. Paired hemidiaphragms

TABLE VII

EFFECT OF HYPEROSMOLARITY ON LACTATE RELEASE AND TOTAL GLYCOGEN CONTENT IN ISOLATED RAT HEMIDIAPHRAGM

Tissues were incubated for 60 min in glucose-free Krebs–Ringer bicarbonate buffer. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses. All differences between data obtained with normal and hyperosmolar buffer are significant ($P < 0.001$).

Incubation buffer	*Lactate release (μmoles/g wet weight)*	*Difference (μmoles)*	*Change (%)*	*Total glycogen content (μmoles of glucose/g wet weight)*	*Difference (μmoles)*	*Change (%)*
Normal Krebs–Ringer bicarbonate (143 mM of Na^+)	12.4 ± 1.3 (4)	14.5	117	15.54 ± 1.22 (4)	12.88	−83
Krebs–Ringer bicarbonate (250 mM of Na^+)	26.9 ± 0.7 (4)			2.66 ± 0.34 (4)		
Normal Krebs-Ringer bicarbonate (143 mM of Na^+)	13.5 ± 0.7 (5)	17.4	129	16.32 ± 1.04 (5)	13.27	−81
Krebs–Ringer bicarbonate (143 mM of Na^+ + 214 mM of mannitol)	30.9 ± 1.6 (5)			3.05 ± 0.31 (5)		

TABLE VIII

EFFECT OF INSULIN ON GLUCOSE DISAPPEARANCE AND GLYCOGEN DEPOSITION IN ISOLATED RAT HEMIDIAPHRAGM

All tissues were preincubated for 30 min in Krebs–Ringer bicarbonate buffer containing 250 mM of Na^+. Their insulin responsiveness was tested during reincubation for 60 min in media containing either 143 or 250 mM of Na^+. For details, see the text. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Incubation buffer	*Insulin (0.1 I.U./ml)*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen per g wet weight*	*Change (%)*
Normal Krebs–Ringer bicarbonate (143 mM of Na^+)	o	15.2 ± 0.8 (4)	80 ($P < 0.001$)	5.18 ± 0.46 (4)	122 ($P < 0.001$)
	+	27.3 ± 1.3 (4)		11.48 ± 2.72 (4)	
Krebs–Ringer bicarbonate (250 mM of Na^+)	o	26.4 ± 2.5 (4)	16 ($P > 0.10$)	1.48 ± 0.21 (4)	53 ($P < 0.005$)
	+	30.5 ± 0.9 (4)		2.26 ± 0.21 (4)	

were incubated with or without insulin (0.1 I.U./ml). D-[$^{14}C_6$]Glucose was only added to the last series of vessels. It appears that after preincubation in hyperosmolar medium, an approximately normal insulin responsiveness is retained as judged by the increase in glucose uptake and ^{14}C activity of glycogen (compare with Table III). On the other hand, when the hemidiaphragms were reincubated in the hyperosmolar medium instead, the effect of insulin was reduced to about the same level as found in the other experiment (Table IV).

DISCUSSION

It was found that rat hemidiaphragm takes up glucose from a Na^+-free medium. Substitution with choline or K^+ gave a depression, but the data in Table II makes it likely that this was a result of irreversible cell damage. When the tissue after the incubation was transferred to normal Krebs–Ringer bicarbonate buffer, both the uptake and the metabolism of glucose was found to be severely impaired. It was recently reported that in the same tissue, incubation in such media for 120 min leads to a considerable decrease in ATP content and the incorporation of amino acids into proteins[5]. Substitution with Li^+ seemed to be tolerated better; glucose uptake and glycogen deposition were even increased. Hence, it seems reasonable to assume that the presence of Na^+ in the extracellular milieu is not essential for glucose transport in isolated rat hemidiaphragm, a conclusion which is further supported by the data in Table VI.

However, since the muscle preparation used in the present study has cut edges, a change in the transport of sugar across the membrane proper might be concealed by a larger leak of rather non-specific nature directly into exposed cytoplasm. On the other hand, PARRISH AND KIPNIS[6] have demonstrated that in the intact diaphragm the transport of both galactose and 2-deoxyglucose occurs equally well in the presence and the absence of Na^+.

If the osmolarity is increased by the addition of Li^+ or mannitol without any change in Na^+ concentration, the glucose uptake is increased by about the same amount as when the medium is made hyperosmolar with Na^+ (Table IV). This confirms

the findings of KUZUYA, SAMOLS AND WILLIAMS[3]. Hyperosmolarity has also been shown to increase the glucose uptake in isolated fat cells (ref. 7 and J. LETARTE, personal communication). These results also suggest that the glucose uptake is not determined by the extracellular Na^+ concentration as such, but rather by the tonicity of the medium. However, the fact that lithium hyperosmolarity does not increase glucose uptake in an essentially Na^+-free medium (Table VI) gives an indication that Na^+ might be of some importance for the effect of hyperosmolarity on glucose permeation. This statement must be made with the reservation that Li^+, which is in itself a stimulator of glucose uptake[8], may possibly be able to increase this process to a level where further augmentation by hyperosmolarity is not possible.

USSING has shown that hyperosmolar solutions of urea, creatinin or NaCl, when applied to the outside of isolated frog skin, produce a considerable (and reversible) increase in the permeability to Na^+, SO_4^{2-}, and even sucrose[9]. It was pointed out that hyperosmolarity might render the outside facing boundary of the epithelium more permeable to a variety of substances and lower this barrier's ability to discriminate among them. Earlier reports indicate that hyperosmolarity also increases the permeability of mammalian bladder to ^2H_2O and urea[10,11]. Furthermore, it was recently shown that hyperosmolarity induced by erythritol or dimethylsulfoxide increases the permeability of frog skin to mannitol, urea, thiourea and sucrose[12]. It is tempting to draw analogies between these observations and the present findings. Hyperosmolarity possibly alters the structure of the cell membranes in a rather non-specific way leading to an overall increased permeability. Glucose is presumably only one of the compounds accompanying an augmented flow of material across the membrane. It is interesting that the effect of hyperosmolarity on the glucose uptake and the insulin responsiveness of rat hemidiaphragms is reversible.

It has been reported that incubation in media with hyperosmolar levels of Na^+ stimulates energy production as estimated from the O_2 consumption in toad muscle[13] and rat hemidiaphragm[14,15]. NISSAN *et al.*[15], showed that when the osmolarity was increased by compounds other than Na^+, there was no increase in O_2 consumption. As the rise in respiration produced by high Na^+ could be abolished by ouabain, it was concluded that this change was a result of increased active transport of Na^+. These experiments were performed with a phosphate buffer. In a bicarbonate buffer KUZUYA, SAMOLS AND WILLIAMS[3] found that the $^{14}CO_2$ production from D-[1-^{14}C]glucose was stimulated equally well when the osmolarity was increased by sucrose, mannitol or NaCl. This discrepancy may be a result of the different buffer systems employed. In the present study it was found that hyperosmolarity increases the energy production as estimated from the release of lactate. This was increased in all the hyperosmolar media tested. The results in Table VI and VII make it unlikely that this is a consequence of increased glucose uptake and ATP consumption for glucose phosphorylation. The reduction in glycogen content induced by both mannitol and Na^+ in a glucose-free medium gives another indication of augmented catabolism (Table VII). Stoichiometric calculations show that only around 60 % of the difference in glycogen content could be accounted for by a rise in lactate release. This may be explained by a simultaneous stimulation of CO_2 production. It is interesting that an approximately similar degree of hyperosmolarity has been shown to increase this parameter by about 370 %, although it should be noted that these experiments were performed with a glucose-containing medium[3].

From the data in Table V it may be inferred that the cells undergo shrinking in hyperosmolar media. If this leads to an increase in the intracellular concentration of Na^+, the active extrusion of this ion may be stimulated. In frog sartorius muscle, KEYNES found that hyperosmolarity causes an immediate rise in the active transport of Na^+ (ref. 16). It seems reasonable to assume that this will act as a trigger to increase substrate catabolism. On the other hand it was shown in the present study (Table VI) that hemidiaphragms which were preincubated in a Na^+-free medium and exposed to hyperosmolarity, also in a Na^+-free medium, show an increase in the release of lactate and a decrease in glycogen deposition. However, these changes were smaller than those seen in Na^+-containing media.

Another interpretation of the increased catabolism induced by hyperosmolarity is offered by the possibility that a rise in the intracellular concentration of metabolites and enzymes leads to an acceleration of substrate breakdown.

Comparison of the data in Tables III, IV and VIII shows that in hyperosmolar media the stimulating effect of insulin on glucose uptake was considerably, but reversibly, reduced. KUZUYA, SAMOLS AND WILLIAMS[3] found that hyperosmolarity decreases the stimulating effect of insulin on the formation of $^{14}CO_2$ from D-[1-^{14}C]-glucose. A similar change was found in isolated adipose cells[7]. The present data show that the effect of insulin on lactate release and glycogen deposition was also reduced in hyperosmolar media.

Hyperosmolarity may increase the glucose uptake either by accelerating the operation of the normal transport system for glucose or by establishing other channels by which glucose can enter the cells. In either case it must be taken into consideration that the phosphorylative capacity of the hexokinase may approach its maximum. This will naturally prevent insulin from producing much further increase in the rate of glucose consumption.

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