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Factors Affecting the Permeability of Isolated Fat-Cells from the Rat to [^{42}K]Potassium and [^{36}Cl]Chloride Ions

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1. The effluxes of $^{42}K^+$ and $^{36}Cl^-$ from isolated fat-cells from the rat were studied under a variety of conditions known to affect the metabolism of the cells. 2. $^{42}K^+$ efflux from isolated fat cells was increased in a Na^+-free–high-K^+ medium and decreased in a K^+-free medium. The existence of K^+ exchange diffusion across the fat-cell membrane is suggested. 3. $^{36}Cl^-$ efflux from isolated fat-cells was decreased when the Cl^- component of the wash medium was replaced by acetate. The basal $^{36}Cl^-$ efflux is suggested to be partly by Cl^- exchange diffusion and partly in company with a univalent cation. 4. A variety of lipolytic stimuli, adrenaline, adrenocorticotrophic hormone, *N*-6,*O*-2′-dibutyryladenosine cyclic 3′:5′-monophosphate and theophylline, increased $^{42}K^+$ efflux from isolated fat-cells. The adrenaline stimulation was biphasic; an initial, rapid and transient increase in $^{42}K^+$ loss from the fat-cells was followed by a slower, more prolonged, increase in $^{42}K^+$ efflux. The initial phase was inhibited by phentolamine but not by propranolol. 5. Insulin increased $^{42}K^+$ efflux only after preincubation with the cells.

In an earlier paper (Perry & Hales, 1969) we described a technique for determining the rate of loss of radioisotopes from isolated fat-cells from the rat, and its use in the measurement of the intracellular concentrations and effluxes of the radioisotopes $^{42}K^+$, $^{22}Na^+$ and $^{36}Cl^-$. This technique has also been used to investigate the effects of hormones and other factors known to affect the metabolism of the isolated fat-cells on the effluxes of $^{42}K^+$ and $^{36}Cl^-$ from the cells. The results of these investigations are reported in this paper.

MATERIALS

Rats. Male albino Wistar rats (120–140g) were bred in the Department of Biochemistry, University of Cambridge, or purchased from Ralph Tuck Ltd., Rayleigh, Essex, U.K. The latter animals were allowed an acclimatization period of at least 3 days before use. All animals were fed *ad libitum*.

Radiochemicals. All radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Hormones. Bovine insulin (24i.u./mg) was given by Dr B. A. L. Hurn (Wellcome Research Laboratories, Beckenham, Kent, U.K.). A stock solution of 1mg/ml was prepared in 3mM-HCl and stored at −20°C.

Adrenaline (Hopkin and Williams, Ltd., Chadwell Heath, Essex, U.K.) was made into a stock solution of 1mg/ml in 0.1M-ascorbic acid and stored at 4°C.

Adrenocorticotrophic hormone was given by Dr H. B. F. Dixon (Department of Biochemistry, University of Cambridge). It was dissolved in Krebs–Ringer bicarbonate buffer immediately before use.

Chemicals. Ouabain and ascorbic acid were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Dibutyryl cyclic 3′:5′-AMP (*N*-6,*O*-2′-dibutyryladenosine cyclic 3′:5′-monophosphate) (sodium salt), glycerol kinase (EC 2.7.1.30), lactate dehydrogenase (EC 1.1.1.27), pyruvate kinase (EC 2.7.1.40), ATP (disodium salt), phosphoenolpyruvate (tricyclohexylammonium salt), NADH (disodium salt) and triethanolamine hydrochloride were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Glucose oxidase (EC 1.1.3.4), peroxidase (EC 1.11.1.7) and tris were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K., theophylline was from Halewood Chemicals Ltd., Manchester, U.K., phentolamine mesylate [2-(*N*′-*m*-hydroxy-*N*′-*p*-tolylphenylaminomethyl)-2-imidazoline mesylate; Rogitine] was from Ciba Laboratories Ltd., Horsham, Sussex, U.K., and propranolol hydrochloride [1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride; Inderal] was from Imperial Chemical Industries Ltd., Pharmaceuticals Division, Macclesfield, Cheshire, U.K. All other chemicals were A.R. grade or the purest grade available.

METHODS

Isolated fat-cells were prepared by the method of Rodbell (1964). The details of the methods for loading the cells with $^{42}K^+$ and $^{36}Cl^-$ and for determining the rate of loss of the radioisotopes from the cells have been

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published earlier (Perry & Hales, 1969). To investigate the effect of a given stimulus on the rate of radioisotope loss from cells containing $^{42}K^+$ or $^{36}Cl^-$, two halves of a fat-cell preparation were washed in parallel, one in the presence and the other in the absence of the stimulus. The two regression coefficients $b \pm$ S.E. of the semi-logarithmic radioisotope efflux plots were calculated as described earlier (Perry & Hales, 1969), and the significance of the difference between the two regression coefficients determined (Davies, 1957). The results are expressed as the regression coefficient $b \pm$ S.E. of the plot of log(intracellular c.p.m./100mg of cells) against the number of washes (i.e. against time).

Two experimental designs were used.

(1) *Acute experiments.* The cell preparation was halved after loading with the radioisotope and the hormone or test substance was added to one half of the cells after the cell sampling for the second wash (i.e. between washes 2 and 3) and was present throughout the rest of the experiment. In the experiments with theophylline and dibutyryl cyclic 3′:5′-AMP the test substance was added in the medium for the second wash.

(2) *Preincubation experiments.* Both halves of a cell preparation were incubated in separate equal volumes of radioisotope-containing medium. The hormone or test substance was preincubated with one-half of the cells for the final 30min of a 60 or 90min period of incubation with the radioisotope and was present throughout the wash period.

To determine the radioisotope efflux into an ionically modified medium the cells were washed with the modified medium from the first wash onwards, a control in normal medium being washed in parallel.

In each experiment to determine the radioisotope efflux from isolated fat-cells, a small fraction of the cells were checked for their sensitivity to insulin or adrenaline. The insulin stimulation of glucose uptake or the adrenaline stimulation of glycerol production by the cells was determined. Glucose was determined by the glucose oxidase method (Huggett & Nixon, 1957) and glycerol by the method of Garland & Randle (1962). This served as a control of the general integrity and hormone sensitivity of the cell preparation. On the very rare occasions when the cells did not respond to hormonal stimulation the experiment was discarded.

Modified media. All media were made with deionized water and contained 2% or 4% (w/v) bovine serum albumin. The normal incubation medium was Krebs–Ringer bicarbonate buffer containing half (1.3mM) the stated concentration of Ca^{2+} (Cohen, 1957). All media based on the normal medium were gassed with O_2+CO_2 (95:5) to pH7.4 and the pH was checked before use. The modified media used were: (1) K^+-free, normal medium with K^+ replaced by equimolar Na^+; (2) Na^+-free, normal medium with Na^+ replaced by equimolar K^+; (3) acetate, normal medium with Cl^- replaced by equimolar acetate; (4) sucrose–Ca^{2+}, 0.31M-sucrose+3mM-calcium acetate and brought to pH7.4 with crystalline tris.

RESULTS

$^{42}K^+$ *efflux*

Lipolytic stimuli. Various lipolytic stimuli were investigated for their effects on the rate of $^{42}K^+$ efflux from isolated fat-cells by using both the acute and preincubation techniques for the addition of the stimulus.

Adrenaline (0.1–1 μg/ml) increased the regression coefficient for $^{42}K^+$ efflux from 0.065 ± 0.007 (mean ±

Table 1. $^{42}K^+$ *efflux from isolated fat-cells: effect of lipolytic stimuli*

$^{42}K^+$ efflux rates from isolated fat-cells loaded with $^{42}K^+$ in Krebs–Ringer bicarbonate buffer containing 4% (w/v) of bovine serum albumin and 0.3–2 μCi of $^{42}K^+$/ml and washed in Krebs–Ringer bicarbonate buffer containing 2% (w/v) of bovine serum albumin. Results are expressed as the regression coefficient $b \pm$ S.E. of the plot of log(intracellular c.p.m./100mg of cells) against the wash number, with the significance (P) of the difference between the stimulated and control effluxes. All stimuli, whether added acutely or preincubated with the cells, were present throughout the wash period. N.S., Not significant.

Stimulus	Experimental design	Control efflux (b)	Stimulated efflux (b)	P
Adrenaline (1 μg/ml)	Acute Expt. 1	−0.058 ± 0.010	−0.130 ± 0.007	<0.001
	Acute Expt. 2	−0.086 ± 0.014	−0.196 ± 0.016	<0.001
	Acute Expt. 3	−0.050 ± 0.002	−0.105 ± 0.002	<0.001
	Acute Expt. 4	−0.052 ± 0.003	−0.089 ± 0.003	<0.001
Adrenaline (1 μg/ml)	Preincubation Expt. 1	−0.095 ± 0.005	−0.128 ± 0.007	<0.001
	Preincubation Expt. 2	−0.054 ± 0.007	−0.089 ± 0.006	<0.001
	Preincubation Expt. 3	−0.060 ± 0.003	−0.086 ± 0.003	<0.001
Adrenocorticotrophic hormone (1 μg/ml)	Acute Expt. 1	−0.058 ± 0.006	−0.106 ± 0.011	<0.01
	Acute Expt. 2	−0.053 ± 0.011	−0.085 ± 0.006	<0.05
Dibutyryl cyclic 3′:5′-AMP (1mM)	Acute Expt. 1	−0.051 ± 0.010	−0.045 ± 0.006	N.S.
	Acute Expt. 2	−0.059 ± 0.008	−0.073 ± 0.011	N.S.
	Preincubation Expt. 1	−0.095 ± 0.004	−0.147 ± 0.006	<0.001
	Preincubation Expt. 2	−0.056 ± 0.002	−0.098 ± 0.003	<0.001
Theophylline (10mM)	Acute Expt. 1	−0.064 ± 0.011	−0.112 ± 0.008	<0.01
	Acute Expt. 2	−0.058 ± 0.009	−0.096 ± 0.004	<0.01

S.E.M. of seven determinations) to 0.130 ± 0.024 (mean ± S.E.M. of four determinations) in acute experiments and to 0.091 ± 0.007 (mean ± S.E.M. of seven determinations) in preincubated experiments. Both increases were significant ($P < 0.001$ for acute and $P < 0.05$ for preincubated experiments by Student's t test). Table 1 shows the results of seven experiments in which the effect of adrenaline on $^{42}K^+$ efflux from one-half of an isolated fat-cell preparation was compared with the unstimulated efflux from the other half of the same cell preparation. There is a degree of variability in the regression coefficients for $^{42}K^+$ efflux from different preparations of isolated fat-cells, and this probably explains the lower degree of significance in the pooled preincubated experiments above. There is also variation in the size of the adrenaline effect on $^{42}K^+$ efflux from different isolated fat-cell preparations. However, in every experiment adrenaline produced a highly significant increase in $^{42}K^+$ efflux. In these terms therefore, the adrenaline effect was reproducible and consistent in experiments with different preparations of isolated fat-cells. Consequently it was decided to accept an effect when a significant change in the rate of radioisotope loss from isolated fat-cells was observed in duplicate experiments. The results of duplicate experiments with adrenocorticotrophic hormone, dibutyryl cyclic 3′:5′-AMP and theophylline are also shown in Table 1. In each case the agreement between the duplicate experiments was good, and it was concluded that $^{42}K^+$ efflux from isolated fat-cells was stimulated by the acute administration of adrenaline, adrenocorticotrophic hormone or theophylline. The effect of the stimulus was apparent within 5min (i.e. one wash) after the addition of the stimulus. Acute administration of dibutyryl cyclic 3′:5′-AMP did not change $^{42}K^+$ efflux, but on preincubation of the nucleotide with the cells for 30min before the determination of the radioisotope efflux the rate of loss of $^{42}K^+$ from the cells was increased. The adrenaline stimulation was still apparent after preincubation of the cells with the hormone. The cause of the delay in onset of the K^+-mobilizing action of dibutyryl cyclic 3′:5′-AMP is unknown, but may be a reflection of poor permeability of the fat-cell membrane to the nucleotide or of the need for the dibutyryl derivative to be hydrolysed to the *N*-6-monobutyryl derivative before being active (Sutherland, 1964).

The actions of α- and β-adrenergic blocking agents on the adrenaline stimulation of glycerol production by, and $^{42}K^+$ efflux from, isolated fat-cells were investigated. At $10\mu g$/ml the β-blocking agent propranolol abolished and the α-blocking agent phentolamine slightly but significantly ($P < 0.001$ by Student's t test) potentiated the lipolytic response of isolated fat-cells to adrenaline measured in terms of glycerol production (Table 2).

When adrenaline was added acutely to $^{42}K^+$-containing fat-cells in the presence of phentolamine or propranolol ($10\mu g$/ml) evidence was obtained for two phases in the adrenaline stimulation of $^{42}K^+$ efflux. In these experiments isolated fat-cells were loaded with $^{42}K^+$ by incubation for 60–90min in normal medium containing 4% (w/v) of bovine serum albumin and 0.3–2μCi of $^{42}K^+$/ml. Both the control fraction of the cells and the fraction to which adrenaline was added were washed with normal medium containing 2% (w/v) of bovine serum albumin and $10\mu g$ of phentolamine or propranolol/ml. The adrenaline was added acutely between the second and third washes and the regression coefficients for radioisotope efflux in the presence and absence of adrenaline were calculated. The results were expressed as a percentage:

$$\frac{(\text{regression coefficient for } ^{42}K^+ \text{ efflux} + \text{adrenaline})}{(\text{regression coefficient for } ^{42}K^+ \text{ efflux} - \text{adrenaline})} \times 100$$

Table 2. *Effects of phentolamine and propranolol on adrenaline stimulation of glycerol production by isolated fat-cells*

Glycerol production by isolated fat-cells incubated in Krebs–Ringer bicarbonate buffer containing 4% (w/v) of bovine serum albumin. Results are the mean ± S.E.M. of four observations. Significance (P) of the difference from the basal glycerol production was estimated by Student's t test. N.S., Not significant.

	Glycerol production (μmol/h per 100mg of cells)	P
Basal	0.17 ± 0.04	—
Adrenaline ($1\mu g$/ml)	2.16 ± 0.08	<0.001
Adrenaline ($1\mu g$/ml)+ phentolamine ($10\mu g$/ml)	2.67 ± 0.04	<0.001
Adrenaline ($1\mu g$/ml)+ propranolol ($10\mu g$/ml)	0.13 ± 0.01	N.S.
Phentolamine ($10\mu g$/ml)	0.18 ± 0.02	N.S.
Propranolol ($10\mu g$/ml)	0.23 ± 0.04	N.S.

Table 3. *Effect of adrenaline on $^{42}K^+$ efflux from isolated fat-cells in the presence of phentolamine or propranolol*

Isolated fat-cells were loaded with $^{42}K^+$ by incubation in Krebs–Ringer bicarbonate buffer containing 4% (w/v) of bovine serum albumin and 0.3–2 μCi of $^{42}K^+$/ml, and washed with Krebs–Ringer bicarbonate buffer containing 2% (w/v) of bovine serum albumin. Blocking agents added in the first wash medium were present throughout the wash period. Adrenaline was added acutely after the second wash and was present throughout the rest of the wash period. Results with phentolamine and propranolol present are the mean percentage ± S.E.M. of three experiments, with the significance (P) of the difference between the percentage increases with adrenaline over the first 5 min and the last 20 min after adrenaline addition calculated by Student's t test. N.S., Not significant.

$$\frac{\text{(regression coefficient for } ^{42}K^+ \text{ efflux+adrenaline)}}{\text{(regression coefficient for } ^{42}K^+ \text{ efflux−adrenaline)}} \times 100$$

	First 5 min after adrenaline	Last 20 min after adrenaline	P
Adrenaline (1 μg/ml)+ phentolamine (10 μg/ml)	208 ± 12	173 ± 25	N.S.
Adrenaline (1 μg/ml)+ propranolol (10 μg/ml)	279 ± 28	125 ± 4	<0.01
Adrenaline (1 μg/ml) Expt. 1	290	207	—
Adrenaline (1 μg/ml) Expt. 2	300	159	—

The two phases in the adrenaline stimulation of $^{42}K^+$ efflux were apparent when the percentage increase in the presence of adrenaline was compared over two time-intervals, the first 5 min (i.e. one wash) and the last 20 min (i.e. the final five washes), after the addition of adrenaline. The results (Table 3) are the mean percentage increases from three experiments over the two time-intervals after the addition of adrenaline. In the presence of propranolol adrenaline produced a significantly greater increase in $^{42}K^+$ efflux over the first 5 min than over the next 20 min after its administration. When phentolamine was present the increase in $^{42}K^+$ efflux was not significantly different between the two time-intervals. The initial rapid decrease in intracellular $^{42}K^+$ seen in the presence of propranolol was not apparent. Propranolol decreased the adrenaline stimulation of $^{42}K^+$ efflux during the last 20 min to slightly above the control efflux in the presence of propranolol alone. Also included in Table 3 are the results of two experiments in which adrenaline was added acutely to isolated fat-cells loaded with $^{42}K^+$ and washed with normal medium containing 2% (w/v) of bovine serum albumin in the absence of the blocking agents. In both experiments the stimulation of $^{42}K^+$ efflux by adrenaline was greater over the first 5 min than over the next 20 min after the hormone administration.

These results have been interpreted in terms of two phases in the adrenaline stimulation of $^{42}K^+$ efflux.

(1) An initial, rapid, response to adrenaline that was inhibited by phentolamine and not by propranolol. This effect was transient, being complete within 5 min, and small, representing a loss of 13.4 ± 3.2% (mean ± S.E.M. of three experiments) of the intracellular radioisotope present at the point of addition of adrenaline.

(2) A slower, more prolonged, increase in $^{42}K^+$ efflux, which was not completely inhibited by either propranolol or phentolamine and may contain both α- and β-adrenergic components, with the β-adrenergic component predominating since this phase of the efflux increase was inhibited to a greater extent by propranolol than by phentolamine. It is possible that this phase is related in some manner to the increased lipolytic activity caused by adrenaline, since both were inhibited by propranolol.

Modified media. Isolated fat-cell suspensions, loaded with $^{42}K^+$ by incubation in normal medium containing 4% (w/v) of bovine serum albumin and 0.3–2 μCi of $^{42}K^+$/ml, were halved. One half was washed with the modified medium from the first wash onwards and the other half was washed with the normal medium. The results obtained with K^+-free and Na^+-free media are shown in Table 4. Also included are two single experiments in which adrenaline was added acutely to $^{42}K^+$-loaded isolated fat-cells washed with either the K^+-free or Na^+-free medium.

$^{42}K^+$ efflux from fat cells was decreased in the K^+-free medium and increased in the Na^+-free–high-K^+ medium. In single experiments adrenaline produced a significant increase in $^{42}K^+$ efflux from isolated fat-cells in the K^+-free and the Na^+-free media.

Insulin. In a limited number of experiments the effect of insulin on $^{42}K^+$ efflux from isolated fat-cells was tested (Table 5). The only significant effect

Table 4. *Effects of* K^+*-free and* Na^+*-free wash media on* $^{42}K^+$ *efflux from isolated fat-cells*

For details of basic procedure and expression of results see Table 1. Normal medium was Krebs–Ringer bicarbonate buffer. The compositions of the modified media are given in the Methods section. Adrenaline (1 μg/ml) was added acutely after the second wash.

Medium	Efflux in normal medium (b)	Efflux in modified medium (b)	Efflux in modified medium + adrenaline (b)	P
K^+-free Expt. 1	-0.054 ± 0.004	-0.037 ± 0.004	—	<0.001
Expt. 2	-0.046 ± 0.002	-0.038 ± 0.002	—	<0.01
Expt. 3	—	-0.041 ± 0.002	-0.082 ± 0.002	<0.001
Na^+-free Expt. 1	-0.052 ± 0.003	-0.101 ± 0.003	—	<0.001
Expt. 2	—	-0.112 ± 0.005	-0.145 ± 0.005	<0.001

Table 5. *Effect of insulin on* $^{42}K^+$ *efflux from isolated fat-cells*

For details of basic procedure and expression of results see Table 1. Insulin was added acutely, or preincubated with the cells for 30 min, or 10 min in the experiment marked*. N.S., Not significant.

Insulin concentration	Control efflux (b)	Stimulated efflux (b)	P
Acute experiments			
24 m-units/ml	-0.072 ± 0.005	-0.084 ± 0.004	N.S.
10 μ-units/ml	-0.079 ± 0.006	-0.085 ± 0.005	N.S.
Preincubation experiments			
1 m-unit/ml	-0.095 ± 0.003	-0.111 ± 0.003	<0.001
1 m-unit/ml	-0.064 ± 0.002	-0.101 ± 0.004	<0.001
10 μ-units/ml*	-0.042 ± 0.007	-0.056 ± 0.002	<0.001

observed was an increased $^{42}K^+$ efflux after preincubation of the hormone with the cells for at least 10 min.

$^{36}Cl^-$ *efflux*

It has been reported that lipolytic agents, adrenaline, theophylline and dibutyryl cyclic 3′:5′-AMP, increased $^{36}Cl^-$ efflux from isolated fat-cells (Hales, Chalmers, Perry & Wade, 1968). These observations were extended to investigate the effect of alterations in the anionic composition of the wash medium on the rate of loss of $^{36}Cl^-$ from the cells. Substitution of acetate for Cl^- in the normal medium produced a significant reduction in $^{36}Cl^-$ efflux (Table 6). The inhibition produced by the acetate medium was very close to that produced by washing the cells in the sucrose–Ca^{2+} medium, which, with the exception of 6 mM-acetate, was anion-free.

Table 6. $^{36}Cl^-$ *efflux from isolated fat-cells*

$^{36}Cl^-$ efflux was determined in isolated fat-cells loaded with $^{36}Cl^-$ in Krebs–Ringer bicarbonate buffer containing 4% (w/v) of bovine serum albumin and 1.2–2 μCi of $^{36}Cl^-$/ml and washed with the appropriate medium containing 2% (w/v) of bovine serum albumin. Results are expressed as regression coefficient b of the plot of log (intracellular c.p.m./100 mg of cells) against wash number (means ± S.E.M. of the numbers of determinations in parentheses). The significance (P) of the difference between the mean effluxes in standard medium and the modified medium was calculated by Student's t test. The compositions of the modified media are given in the Methods section.

Wash medium	Efflux (b)	P
Standard	-0.344 ± 0.007 (30)	—
Sucrose–Ca^{2+}	-0.242 ± 0.010 (9)	<0.001
Acetate	-0.237 ± 0.042 (5)	<0.001

DISCUSSION

K^+ efflux from cells is believed to occur by a passive process, energy being dissipated as K^+ moves down its concentration gradient. To maintain electrical balance, K^+ loss has to be balanced by Cl^- loss, Na^+ gain or K^+ gain. Exchange of intracellular K^+ for extracellular K^+ (K^+ exchange diffusion) has been demonstrated in strips of frog ventricle (Lorber, Walker, Greeve, Minarik & Pak, 1962), rat uterus (Daniel, 1963), frog muscle (Harris & Sjodin, 1961) and erythrocytes (Hoffman & Kregenow, 1966). The results in

Table 4 suggest that K^+ exchange diffusion also occurs across the fat-cell membrane. $^{42}K^+$ efflux from isolated fat-cells was decreased in a K^+-free medium and increased in a Na^+-free–high-K^+, medium. This dependence of $^{42}K^+$ efflux on extracellular K^+ was not complete, for even in the absence of extracellular K^+ there was an appreciable efflux of radioisotope. However, elevation of the extracellular K^+ concentration increased cyclic 3′:5′-AMP concentrations in rat parotid-gland slices (Rasmussen & Tenenhouse, 1968) and rat diaphragm (Lundholm, Rall & Vamos, 1967), fatty acid release by rat epididymal adipose tissue (Bleicher, Farber, Lewis & Goldner, 1966) and glycerol production by isolated fat-cells (M. C. Perry & C. N. Hales, unpublished work). It is possible that the increased $^{42}K^+$ efflux in the Na^+-free–high-K^+ medium was a result of increased lipolytic activity in the cells, since a variety of other lipolytic agents increased the rate of $^{42}K^+$ loss from isolated fat-cells (Table 1).

The replacement of Cl^- in the normal medium by acetate decreased the $^{36}Cl^-$ efflux from the fat-cells (Table 6). Under these conditions it may be asked whether the acetate anion can be regarded as impermeant. In absolute terms acetate must be able to cross the cell membrane, since it has been shown to be metabolized by adipose tissue (Winegrad & Renold, 1958). This does not necessarily imply ready permeability of the fat-cell membrane to acetate in that it can freely substitute for Cl^-. The inhibitory post-synaptic membrane of cat spinal motoneurones is permeable to Cl^- but not to acetate (Coombs, Eccles & Fatt, 1955; Araki, Ito & Oscarsson, 1961), presumably because the pores in the membrane are too small to allow free passage to the acetate anions. Evidence that a similar situation occurs in isolated fat-cells comes from the demonstration (Table 6) that in the acetate medium and the anion-free (except for 6mM-acetate) sucrose–Ca^{2+} medium the $^{36}Cl^-$ efflux was decreased to a similar extent. The acetate anion under these conditions appears to inhibit Cl^- exchange diffusion across the fat cell membrane. Similarly in phosphatidylcholine liquid crystals (Bangham, Standish & Watkins, 1965) and frog muscle (Harris, 1958) $^{36}Cl^-$ efflux was decreased by replacement of the Cl^- on the trans side of the membrane by larger impermeant anions.

The nature of the residual $^{36}Cl^-$ efflux in the acetate medium, in which Cl^- exchange diffusion was inhibited, was investigated by comparison with univalent cation effluxes when active-transport and exchange-diffusion mechanisms had been excluded. The residual $^{42}K^+$ efflux in K^+-free medium was 0.039 in terms of b (mean of three determinations of $^{42}K^+$ efflux in K^+-free medium in Table 4), corresponding to a first-order rate constant of $0.018\,min^{-1}$ and a $^{42}K^+$ efflux of 10.3nmol/min per 100mg of cells, calculated from the intracellular K^+ concentration of isolated fat-cells determined previously (Perry & Hales, 1969). The first-order rate constant for $^{22}Na^+$ efflux into normal medium containing 0.1mM-ouabain was $0.140\,min^{-1}$ (Perry & Hales, 1969), corresponding to a $^{22}Na^+$ efflux of 10.2nmol/min per 100mg of cells. This was little changed when Na^+ in the medium was replaced by equimolar K^+ (M. C. Perry & C. N. Hales, unpublished work). Thus the total univalent cation flux was 20.5nmol/min per 100mg of cells. The $^{36}Cl^-$ efflux regression coefficient in the acetate medium was 0.237, corresponding to an efflux of 18.4nmol/min per 100mg of cells. The close agreement between the residual univalent cation and Cl^- effluxes when active-transport and exchange-diffusion mechanisms have been inhibited suggests that these fluxes, if not linked, balance each other electrically.

Adrenaline, added acutely to or preincubated with the cells, stimulated $^{42}K^+$ efflux (Table 1) and $^{36}Cl^-$ efflux (Hales *et al.* 1968). The effects of the two adrenergic blocking agents, phentolamine and propranolol, gave evidence for two phases in the adrenaline increase in $^{42}K^+$ efflux (Table 3). In rat epididymal adipose tissue phentolamine had no effect on the stimulation of cyclic 3′:5′-AMP production by adrenaline (Butcher & Sutherland, 1967), and did not inhibit the adrenaline stimulation of glycerol production by isolated fat-cells (Table 2). In guinea-pig smooth muscle, phentolamine inhibited the stimulation of $^{42}K^+$ efflux by noradrenaline (Jenkinson & Morton, 1967*b*), when a β-blocking agent pronethalol had no effect. Propranolol, a potent β-receptor antagonist (Black, Crowther, Shanks, Smith & Dornhorst, 1964), inhibited the adrenaline stimulation of lipolysis in isolated fat-cells (Fain, 1967; Table 2). Pronethalol abolished the elevation of cyclic 3′:5′-AMP concentrations in rat epididymal adipose tissue by adrenaline (Butcher & Sutherland, 1967). Acute addition of adrenaline to isolated fat-cells loaded with $^{42}K^+$ produced a rapid initial fall in intracellular $^{42}K^+$ followed by a slower more prolonged increase in $^{42}K^+$ efflux. The initial phase was inhibited by phentolamine but not by propranolol, and may thus be related to stimulation of α-receptors in the fat-cells by adrenaline. The second phase was partially inhibited by propranolol, which at the same concentration completely inhibited the lipolytic action of adrenaline, suggesting a possible connexion between the second phase in the $^{42}K^+$ efflux increase and increased lipolytic activity in the cells.

The diverse nature of the lipolytic agents, including dibutyryl cyclic 3′:5′-AMP, that increase $^{42}K^+$ efflux suggests that a factor distal to the

adenylate cyclase step in the activation of lipolysis is important in the stimulation of $^{42}K^+$ efflux from isolated fat-cells.

The ionic mechanisms underlying the adrenaline-stimulated $^{42}K^+$ efflux are uncertain. To determine whether the increases in $^{42}K^+$ and $^{36}Cl^-$ effluxes after adrenaline represented loss from the fat-cells of K^+ and Cl^- as an ion pair, the increments in the effluxes over the initial three washes after acute administration of the hormone were compared. In five experiments adrenaline produced increments in the $^{36}Cl^-$ regression coefficient *b* of 0.080, 0.074, 0.024, 0.076 and 0.032. The mean of these values, 0.057, corresponds to an increase in $^{36}Cl^-$ efflux of 4.4nmol/min per 100mg of cells. In four experiments the mean increase in $^{42}K^+$ efflux regression coefficient over the same time-interval after adrenaline administration was 0.071, giving an increase in ^{42}K efflux of 18.8nmol/min per 100mg of cells. This marked difference between the magnitude of the adrenaline effect on $^{42}K^+$ and $^{36}Cl^-$ effluxes suggests that loss of K^+ and Cl^- as an ion pair is not the underlying mechanism of the increased effluxes.

K^+ mobilization is a feature of catecholamine action in liver (Burton, Mondon & Ishida, 1967), intestinal smooth muscle (Jenkinson & Morton, 1967*a*) and brown adipose tissue (Girardier, Seydoux & Clausen, 1968). The transient nature of the initial K^+-mobilizing action of adrenaline in isolated fat-cells was also observed with catecholamine action on these tissues. In liver it has been suggested that the K^+ mobilization by glycogenolytic hormones was an integral part of their activation of adenylate cyclase (Craig & Honig, 1963; Finder, Boyme & Shoemaker, 1964). If K^+ mobilization were an important step in adenylate cyclase activation by lipolytic hormones in fat-cells, it should precede or parallel the rise of cyclic 3′:5′-AMP concentration in the cells. Butcher, Ho, Meng & Sutherland (1965) have shown that maximal concentrations of cyclic 3′:5′-AMP in rat epididymal fat-pads incubated with adrenaline occurred within 5min of the addition of the hormone. After 5min in the fat-cells, the initial rapid $^{42}K^+$ loss in response to adrenaline was complete. Within these limits, therefore, the time-responses of the two systems were similar. However, a direct involvement of the initial K^+ mobilization in the stimulation of adenylate cyclase activity by adrenaline seems unlikely, since phentolamine inhibited the initial K^+ loss in response to adrenaline, but at the same concentration actually potentiated the adrenaline stimulation of glycerol production (Table 2).

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