ity of the skeletal muscle. Matkovics and Nowak[14] also found that the activity of the peroxidase assayed (EC 1.11.1.7) was higher in the H_2O_2-supplied group in kidney and skeletal muscle whereas our results from the assay for Se-dependent GSHPx (EC 1.11.1.9) showed a decrease in the enzyme activity of kidney, liver and skeletal muscle. The experimental design of Matkovics and Novák[14] was similar to ours but they used the CFY-strain of rats and they had no water-deprived group similar to ours.

The GSHPx inhibits lipid peroxidation in vitro[15]. Selenium is necessary for the H_2O_2 decomposing activity of Se-dependent GSHPx and various organs contain different amounts of Se and Se-dependent GSHPx activities. For example, rat kidney contains Se 1.45 $mg \cdot kg^{-1}$, liver 1.29 $mg \cdot kg^{-1}$, heart 0.37 $mg \cdot kg^{-1}$ and muscle 0.16 $mg \cdot kg^{-1}$ [16]. The activity of Se-dependent GSHPx changes logarithmically in several tissues of rat with respect to the Se concentration in the diet[17]. The liver and kidneys are the organs most susceptible to degeneration induced by Se deficiency, whilst abnormalities in heart are rare[18]. H_2O_2 as an oxidizing agent could have an effect on the oxidization of the biologically effective organic selenium compounds of the food to inorganic derivatives which are not so bio-available[18]. This reduces the Se intake and causes a decrease in the activity of Se-dependent GSHPx. Smith et al.[19] found a reduced activity of GSHPx (cumene hydroperoxide as substrate) in kidney, red cells and liver in Se deficiency. The effects of Se deficiency, as well as those of oral H_2O_2-supply, are highly selective in various tissues, which suggests tissue differences in antioxidant regulation.

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Altered K^+ movement in liver mitochondria from alloxan diabetic rats

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Summary. Potassium movements were monitored in liver mitochondria from control and alloxan diabetic rats with a cationic electrode. There was net accumulation of K^+ after Ca^{2+} addition to the mitochondria with the diabetic but not with the control.
Key words. Diabetes; K^+; mitochondrial permeability; energy metabolism.

Liver mitochondria from alloxan diabetic rats were used to assess cellular energy production in the diabetic state[1,2]. Stimulation of respiration by addition of adenosine diphosphate (ADP) and Ca^{2+} to the mitochondria and the production of ATP or accumulation and retention of Ca^{2+} by mitochondria are standard methods for evaluating mitochondrial energy production[3]. The diabetic mitochondria are less efficient in energy production than mitochondria from untreated rats[1] and the accumulation and retention of Ca^{2+} in the diabetic mitochondria is lower than in normal mitochondria[2]. Changes in the ionic composition of the medium helped to elucidate these differences and indicated that a description of the interaction of K^+ with the mitochondria could help explain the altered pattern in the diabetic mitochondria[2].

In an earlier study the ionic content of the mitochondria was measured after a set incubation time using a flame photometer[2]. In that study changes in respiration were followed continuously and indicated that a continuous monitoring of changes in mitochondrial K^+ would be informative. Consequently the studies reported here were performed with continuous monitoring of net K^+ flux in mitochondria with a K^+ electrode. The patterns of K^+ flux in normal and diabetic mitochondria before and after Ca^{2+} stimulation are reported here. The purpose of these studies was to determine the pattern of K^+ movement in the diabetic state compared to control animals and to correlate these, if different from normal, to changes reported in the diabetic animal.

Methods. Care and preparation of the rats and preparation of the mitochondria (in 0.25 M sucrose) were described previously[1]. Potassium movements were monitored continuously with a Beckman cationic electrode (No. 39137) and an Orion 701 digital pH meter and recorded on a strip chart. The response of the electrode to various incubation medium components and to pH changes was evaluated to confirm that the changes monitored were those due to K^+ levels only. Mitochondria were incubated in 6 ml of medium containing: 100 mM choline chloride, 50 mM Tris HCl (tris(hydroxymethyl)aminomethane), 5 mM Tris succinate, 4 mM KCl, and 6-7 mg of mitochondrial protein/ml with a total osmolarity of 300 mosmoles. After equilibration, 0.5 mM $CaCl_2$ (80 nmoles/mg protein) was added and continuous recordings made for at least 5 min to monitor the changes in medium K^+ after Ca^{2+} addition. It should be noted that *net* K^+ movements are monitored in this system and increased influx and efflux of K^+ is measured only if there is a *net* change in medium content of K^+ due to these movements.

Volume changes in mitochondria as reflected by changes in absorption[4], which have been described as phase 1 or low amplitude changes, were monitored with a Perkin-Elmer Spectrophotometer at 520 nm and recorded on a strip chart. The incubation medium of 3 ml contained 225 mM sucrose, 20 mM Tris HCl, 5 mM succinate and 1 mg mitochondrial protein/ml. These volume changes occur as a normal part of the cycle seen in stimulated and resting mitochondira and are reversible[3]. After equilibration calcium or phosphate was added and the changes moni-

Net change in mitochondrial K^+ levels in medium with 4 mM KCl, pH 7.4

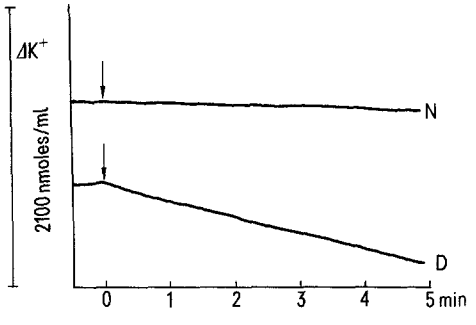
	nmoles K^+/mg mitochondrial protein net change	
	Normal	Diabetic
Add mitochondria + 0.5 mM Ca^{2+}	N.C.	N.C.
5 min later	(+) 16 ± 6 (7)	(+) 114 ± 23 (5)
10 min later	N.C.	(+) 207 ± 54 (5)

N.C. = no change; (+) = net influx. Freshly isolated mitochondria contain 82 nmoles K^+, after accumulation the normal contain 98 nmoles K^+/mg mitochondria protein and the diabetic contain 196 nmoles K^+/mg mitochondrial protein and 289 nmoles K^+/mg mitochondrial protein.

tored. The integrity of the mitochondria was monitored by measuring oxygen consumption with a Clark oxygen electrode by procedures described previously[1]. All studies were conducted at 21 °C.

Results and discussion. 1) Calcium stimulation of K^+ uptake at pH 7.4. Diabetic and normal mitochondria contained 82–85 nmoles K^+/mg protein as measured by a flame photometer[2]. For K^+ movement studies the mitochondria were incubated in the complete medium before calcium addition for electrode equilibration. There was no measurable net change in K^+ content during the equilibration (table and fig.). Addition of Ca^{2+} with no mitochondria present produced no electrode response. Under unstimulated conditions net movement of K^+ in the normal and diabetic mitochondria is the same. There can be considerable variation in the amount of K^+ movement in different normal mitochondrial preparations[5]. When normal mitochondria are added to an incubation medium and remain in state 4 respiration no significant *net* loss or gain of K^+ occurs at pH 7.4 unless the mitochondria are aged or specific ionophores are added[8,9]. Upon addition of Ca^{2+} and its uptake, with a change to state 3 respiration in the mitochondria, little or no net uptake of K^+ occurs at pH of 7.4[9]. In the control preparations in this study with Ca^{2+} addition there is a small uptake of 16 nmoles/mg. There is a considerably higher uptake of K^+ in the diabetic reaching 114 nmoles/mg after five minutes and 207 nmoles/mg after 8–10 min (table and fig.).

Earlier we reported that in 80 mM KCl medium, with 20 times the K^+ present in this study, K^+ accumulation took place in both normal and diabetic mitochondria after a 5-min-incubation from the time of calcium addition[2]. The ion levels were measured by flame photometry. The net accumulation of K^+ in the 80 mM



Change in medium K^+ with the addition of Ca^{2+}. Calcium was added at the arrow, a downward deflection indicates uptake by the mitochondria with a decrease in medium K^+. The upper tracing is with mitochondria from a normal animal (N) and a total uptake of 10 nmoles of K^+/mg which equals 92 nmoles/mg and the lower tracing from a diabetic animal (D) with an uptake of 121 nmoles/mg which equals a total content of 200 nmoles/mg.

K^+ medium was 247 nmoles/mg protein in the diabetic and 244 nmoles/mg in the control[2]. The diabetic mitochondria accumulated approximately the same amount of K^+ in either 4 mM or 80 mM K^+ medium while the control accumulated considerably less K^+ in the 4 mM medium. In addition, we also reported that the amount of Ca^{2+} accumulation is lower in the diabetic immediately after cessation of state 3 respiration[1] and 5 min later[2], but only when K^+ is present in the medium. Potassium movement is also increased in the diabetic compared to the normal when valinomycin is present in the incubation medium (J.C. Hall, personal communication). The addition of zinc-free insulin (5.9×10^{-9} M) to the mitochondrial preparations had no effect on ion movements in the control or diabetic mitochondria as reported previously[2].

2) Volume changes. Uptake of osmotically active substances into mitochondria is accompanied by passive water movement and swelling[3,4,6,7]. Both Ca^{2+} and K^+ produce an osmotic response in the mitochondria under specific conditions[7]. Volume changes after Ca^{2+} addition or after K^+ (as K-phosphate) addition were monitored as changes in optical density at 520 nm. With Ca^{2+} addition both preparations swelled (change of 0.47 OD U) as has been reported for normal mitochondria[3,7]. After K^+ addition the control mitochondria swelled (0.080 U) to a greater extent than the diabetic mitochondria (0.051 U). These results agree with a recent report that diabetic mitochondria undergo damped oscillations of swelling and contraction associated with K^+ movement in mitochondria[4]. This would also explain the lesser extent of swelling in the diabetic despite higher K^+ accumulation.

It is apparent that the differences in ion movement that we measured in the diabetic and normal mitochondria are only observed when Ca^{2+} and K^+ are both present and when these ions can be accumulated by the mitochondria. Potassium and calcium levels in mitochondria depend upon the integrity of the limiting membrane and an adequate energy supply[9]. My results suggest a competition between K^+ and Ca^{2+} accumulation and/or maintenance in diabetic mitochondria which occurs in normal mitochondria only under specific experimental conditions[8,9]. There are various factors that could cause this which include altered fatty acid levels in the diabetic mitochondria[4] or the altered Mg^{2+}/K^+ ratios in the diabetic that are linked to the role of Mg^{2+} in mitochondrial integrity[2,9–11]. These can lead to the lower efficiency in energy production observed in the mitochondria from the diabetic animals[1].

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