Table II. Effects of magnetic field on adjuvant-induced arthritis in rats[a]

Days after adjuvant injection	Treatment days	Hindpaw volume in ml[b] (% increase ± SE)[c]		Arthritis score of forepaw (score ± SE)[d]		Body weight gain (%)[c]	
		Control	M.F.[e]	Control	M.F.[e]	Control	M.F.[e]
14	0	2.45 (0)	2.56 (0)	0	0	0	0
18	4	3.84 (60.0 ± 8.2)	3.72 (47.0 ± 5.0)	3.3 ± 0.39	2.4 ± 0.24	2.0	4.5[f]
21	7	3.67 (52.6 ± 7.4)	3.38 (33.7 ± 4.4)[f]	3.4 ± 0.43	2.8 ± 0.48	4.1	8.8[f]

[a] Average values for 10 rats. [b] Average values for both paws. [c] Values relative to those of treatment day 0. [d] 0 None; 1 mild; 2 moderate; 3 severe; 4 very severe. [e] M.F. Magnetic field. [f] $p < 0.05$ in relation to control.

in the carrageenan edema test. A dose of 0.1 ml of liquid paraffin (Merck, Germany) containing 0.6 mg of heat-killed *Mycobacterium butyricum* (Difco) was injected intradermally into the basal part of the tail. The volume of the hindpaws was measured as described above. The arbitrary arthritis score in the forepaws was also recorded.

Treatment by a magnetic field: An apparatus to produce a 50 Hz alternating magnetic field was provided by Kawasaki Electric Industry Co., Ltd., Yushima 3, Bunkyo-ku, Tokyo. The intensity of the magnetic field was about 1,200 gauss in a wooden cage where the animals were placed. The control animals were also placed in the box, but the current was not switched on. The temperature in both the boxes was adjusted to about 20 °C. In the carrageenan edema test, the rats were placed in the magnetic field for 3 h from just after the carrageenan injection. In the experiment of adjuvant arthritis, the rats were treated in the magnetic field for 3 h every day from 14 days after the adjuvant injection.

As shown in Table I, the carrageenan edema in the treated group was significantly suppressed as compared with that in the control group. The behavior of the treated rats was like that of control animals. 14 days after the adjuvant injection, the rats' hindpaws had swollen moderately (about 2.5 ml). As shown in Table II, the swelling increased further for the next 4 days and then decreased gradually. The effects of the magnetic field on adjuvant-induced arthritis are summarized in Table II. The arthritis in forepaws was not seen after 14 days, but was intense after 18 and 21 days in the control animals. As summarized in Table II, the arthritis in the hind- and fore-paws in the rats exposed to the magnetic field was weaker than that in the controls. No changes in behavior were observed in either group, and the body weight gain was greater in the treated group than in the controls. It should be clarified whether or not the effects of the magnetic field on adjuvant arthritis are only due to its anti-inflammatory effects.

As described above, the alternating magnetic field used in this study has an anti-inflammatory effect in rats. LIKHACHEV[4] reported that the action of a constant magnetic field on central nervous system led to a rapid normalization of erythrocyte sedimentation rate in experiments with turpentine-induced inflammation in rabbits. DEGAN[5] used magnetic fields in the treatment of traumatic edema in humans. The mechanisms of the biological effects of magnetic field were discussed also by LIKHACHEV and other investigators, but the mechanism of anti-inflammatory effects observed in this study is as yet unknown.

Summary. The effects of a 50 Hz magnetic field on experimentally-induced inflammation in rats were studied. Carrageenan edema was inhibited significantly by exposure to magnetic field for 3 h. Adjuvant-induced arthritis in rats was also suppressed by the magnetic field.

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Energy Linked Ion Accumulation in Mitochondria from Alloxan Diabetic Rats

We reported earlier[1] that the maximal respiration rate, stimulated by ADP or Ca^{+2}, is lower in mitochondria from diabetic animals (diabetic mitochondria) than in normal mitochondria, but the total amount of ATP produced or Ca^{+2} accumulated will eventually equal that for normal mitochondria. These results appeared to be an ion related phenomenon. It is known that variations in cellular K^+ and Ca^{+2} contents do affect many cellular processes[2-4]. The purpose of this study was to compare ion accumulation in normal and diabetic mitochondria for the first time.

Mitochondria were isolated from livers of normal and alloxan diabetic rats as described previously[1]. Ionic contents were determined as described by MALBICA and HALL[5].

1. Ion contents in a resting state. After isolation in 0.25 *M* sucrose the normal and diabetic mitochondria contained similar amounts of Na^+, 6.7 and 7.8 nmoles/mg protein; K^+, 82.0 and 85.7 nmoles/mg protein; Mg^{+2}, 63.0 and 51.3 nmoles/mg protein; and Ca^{+2}, 10.0 and 8.6 nmoles/mg protein, respectively. These are similar to literature values, under similar isolation conditions, for normal mitochondria[5,6].

2. Ionic contents after 3.0 m*M* Ca^{+2} incubation with respiratory substrate and ATP present. After a 20 min incubation with 3.0 m*M* Ca^{+2} the diabetic and normal

Table I. Ionic contents of normal and diabetic mitochondria after incubation in 3.0 m*M* Ca^{+2} medium

Treatment	*n*	nmoles/mg mitochondrial protein ± SE				
		Na^+	K^+	Ca^{+2}	Mg^{+2}	PO_4^{-3}
Normal	7	443 ± 89	107 ± 11	1371 ± 223	360 ± 55	1091 ± 263
Diabetic	7	268 ± 46	127 ± 17	1272 ± 173	343 ± 32	1012 ± 252

Medium contained: 1 mg mitochondrial protein/ml, 5.5 m*M* Na-KH_2PO_4, 75.0 m*M* *Tris* HCl (pH 7.4), 10.0 m*M* $MgCl_2$, 7.0 m*M* adenosine triphosphate (ATP, 2 Na, Sigma), 25.0 m*M* sucrose, 5.0 m*M* NaCl 3.0 m*M* $CaCl_2$, and 5.0 m*M* Na succinate. The final volume was 12 ml.

mitochondria contained similar amounts of the ions assayed (Table I). These correspond to reported values for normal mitochondria[6,7]. There was a significant positive correlation[8] between K^+ and Mg^{+2} contents in the normal ($r = 0.784$) and no significant correlation in the diabetic ($r = 0.426$). A positive correlation between the amount of Mg^{+2} and K^+ is observed in normal liver mitochondria[9–11].

3. Ion contents after 0.187 m*M* Ca^{+2} incubation with no adenine nucleotides and active respiration. The protocol for the 0.187 m*M* Ca^{+2} studies was explained previously[1]. Briefly, the incubation media contain either 80 m*M* K^+ or 120 μ*M* K^+ (Na^+ medium), Na^+ replacing K^+. Each of these is paired with succinate, a substrate whose accumulation requires and is regulated by K^+ or β-hydroxybutyrate, a substrate which is not regulated by K^+ in this way[11]. After a 5 min incubation there was a significant difference in the ionic content (Table II): a lower accumulation of Ca^{+2} in the diabetic in K^+ medium with succinate as substrate.

The results demonstrate that liver mitochondria from alloxan diabetic rats display some significant differences from normal mitochondria in patterns related to energy linked ion accumulation. The role K^+ plays in regulating energy generation could account for the results in this study and other reports[1].

There is some evidence supporting this hypothesis. 1. Potassium and calcium levels in mitochondria or cells depend upon the integrity of the limiting membrane and adequate energy (for a summary see Caswell[12]). 2. In freshly isolated normal and diabetic mitochondria, where respiration and energy generation are minimal and no active transfer is occurring, the K^+ and Ca^{+2} levels are the same. 3. When there is active respiration and ATP is the energy source, there is no difference in ion contents after accumulation studies but K^+ and Mg^{+2} contents, which are usually positively correlated in mitochondria, are not correlated in the diabetic. Magnesium levels are stable in cells but K^+ levels vary in different metabolic conditions[9,11]. 4. In normal mitochondria K^+ and Ca^{+2} compete for energy from the electron transport chain for uptake during active respiration with no external energy source[13]. Under these conditions K^+ content does not change in the diabetic but Ca^{+2} contents decrease in K^+ medium with a K^+ requiring substrate.

It appears that these results relate more to energy for maintenance of K^+ levels than K^+ permeability of the mitochondria[12]. Therefore, altered mitochondrial K^+

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Table II. The ionic contents of the normal and diabetic mitochondria after 0.187 m*M* Ca^{+2} accumulation

Treatment	Substrate	Medium	*n*	nmoles/mg mitochondrial protein ± SE		
				Na^+	K^+	Ca^{+2}
Normal	Na succinate	Na^+	10	127.74 ± 8.78	127.30 ± 11.69	53.36 ± 7.94
Diabetic	Na succinate	Na^+	10	147.42 ± 8.18	114.05 ± 20.82	48.28 ± 6.23
Normal	Na-DL-BOH	Na^+	4	155.67 ± 42.06	164.00 ± 26.03	42.87 ± 12.35
Diabetic	Na-DL-BOH	Na^+	3	156.43 ± 3.91	153.17 ± 15.46	33.41 ± 10.65
Normal	Na succinate	K^+	7	54.58 ± 6.38	244.07 ± 14.92	54.51 ± 7.67
Diabetic	Na succinate	K^+	5	49.60 ± 5.88	247.00 ± 24.03	[a]27.00 ± 3.35
Normal	Na-DL-BOH	K^+	3	45.86 ± 11.92	280.00 ± 32.63	38.13 ± 10.61
Diabetic	Na-DL-BOH	K^+	3	63.87 ± 14.16	279.00 ± 32.00	42.15 ± 12.67

Medium contained: 3–4 mg of mitochondrial protein/ml, 75.0 m*M* *Tris* HCl (pH 7.4), 80.0 m*M* NaCl (Na^+ medium) or 80.0 m*M* KCl (K^+ medium), 0.187 m*M* $CaCl_2$, and one of the following substrates: 5.0 m*M* Na succinate, or 10.0 m*M* Na-DL-β-hydroxybutyrate. [a] $p = 0.05$.

levels or movements in our study would account for all observed results. Measurement of K^+ movements are necessary before this is actually shown and such studies are in progress. We can conclude, at this time, that normal and diabetic mitochondria differ in energy metabolism and this difference is reflected in ion accumulation patterns.

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Summary. Ionic accumulation related to energy production was determined to be less efficient in diabetic mitochondria compared to normal mitochondria.

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Hemmung der Sulfataufnahme durch *Lemna minor* L. durch SO_2 in subletalen Konzentrationen

Inhibition of Sulfate Uptake by *Lemna minor* L. during Aeration with Sublethal Concentrations of SO_2

In Gebieten, welche durch SO_2-Immissionen belastet sind, stellt sich die Frage nach möglichen Veränderungen des Schwefelstoffwechsels höherer Pflanzen[1].

Material und Methoden. Die Versuchspflanzen wurden während 6 Wochen in einer Wachstumsanlage unter SO_2-Begasung kultiviert[2]. Je 6 Kulturgefässe wurden mit verschiedenen SO_2-Konzentrationen begast. Kulturbedingungen: Dauerlicht 4500 Lux, 27 °C, Nährlösung E-NO_3^- pH 6,4[3]. Nach einer Kulturdauer von 6 Wochen wurden Sulfataufnahmeexperimente und Sulfatschwefelbestimmungen durchgeführt. Die Organismen für die kinetischen Experimente (Figuren 2 und 3) stammten aus der Kontrollkultur. Aus jedem Kulturgefäss wurden je 2 Proben von ca. 130 Gliedern in eine spezielle Fütterungsküvette übertragen[4]. Nach einer Akklimatisationszeit von 2 h wurde $^{35}SO_4^{2-}$ (0,3 μCi/ml Nährlösung) zugegeben und für die Bestimmung der Aufnahmerate während 2 h bzw. im ersten kinetischen Experiment (Figur 2) während der angegebenen Zeiten gefüttert. Anschliessend wurden die Organismen während 2 h auf inaktiver Nährlösung bei 4 °C ausgewaschen. Die Bestimmung der Aktivität erfolgte nach nasser Veraschung mit 30% H_2O_2 im Flüssigkeits-Scintillationszähler. Zur Analyse der Begasungsluft diente ein UV-Spektrometer (Spektrometrics III d) und die chemische Analyse nach WEST und GAEKE[5]. Die Sulfatschwefelbestimmung erfolgte nach Gefriertrocknung des Pflanzenmaterials nach der Methode von JOHNSON und NISHITA[6], die Sulfitbestimmung ebenfalls nach West und GAEKE[5].

Resultate. In zwei voneinander unabhängigen Versuchsserien konnte gezeigt werden, dass die mit SO_2 begasten Lemnen eine signifikante Hemmung der Sulfataufnahme gegenüber der Kontrolle zeigen (Figur 1). Kontrollexperimente zeigten, dass diese Hemmung nicht auf die Einwirkung von Sulfit, welches durch gelöstes SO_2 in der Nährlösung auftreten könnte, zurückzuführen ist. Ebenso liess sich eine durch Oxidation dieses Sulfits zu Sulfat bedingte Isotopenverdünnung zur Erklärung des Phänomens ausschliessen[4]. In zwei kinetischen Experimenten zeigte sich, dass diese Hemmung der Sulfataufnahme sehr rasch eintritt (Figuren 2

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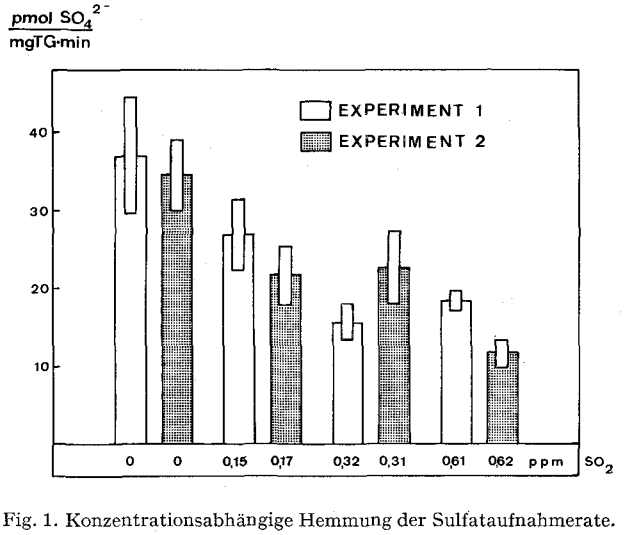

Fig. 1. Konzentrationsabhängige Hemmung der Sulfataufnahmerate. Mittelwerte und Standardabweichungen von je 6 parallelen Proben. Der *t*-Test ergibt eine Signifikanz gegenüber der Kontrolle auf $p < 0{,}01$ bei allen Gaskonzentrationen.

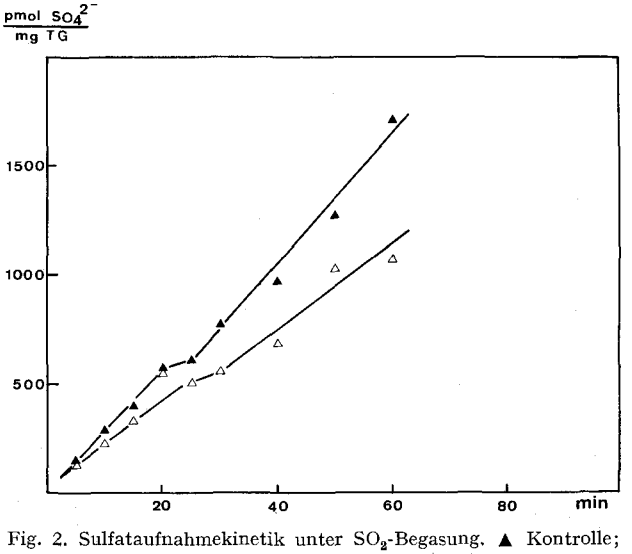

Fig. 2. Sulfataufnahmekinetik unter SO_2-Begasung. ▲ Kontrolle; △ 0,5 ppm SO_2. Begasung: 0,5 ppm SO_2 zu Versuchsbeginn.