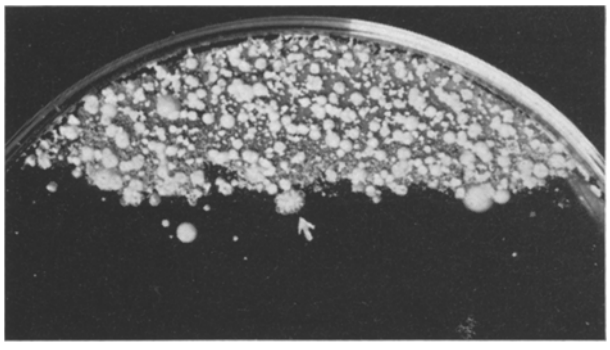

Gradient plating of *Candida lipolytica* on ethionine after 10 days incubation at 30 °C. A few types of colony variants can be seen. Arrow indicates a rough colony variant.

In order to study the effect of ethionine alone on the cells, actively growing culture was treated with ethionine as follows: 4 ml of minimal salts medium (K_2HPO_4 0.7%; NH_4Cl 0.5%; $MgSO_4$ $7H_2O$ 0.02%; NaCl 0.01% and glucose 1.0%) containing different levels of ethionine ranging from zero (control) to 16 mg/ml in broad test tubes was inoculated with 0.5 ml cell suspension from exponential growth phase containing 10^8 cells/ml. The tubes were fixed at an angle of 45° and agitated in a reciprocatory shaker at 30 °C for 4 days. It has previously been observed that in the presence of even low levels of ethionine (i.e., 1 mg/ml) the culture showed a growth lag of about 4 days and the cells which grew after this period were entirely resistant to inhibitory levels (i.e., 10 mg/ml) of ethionine. Hence, after 4 days of incubation, when the growth was not yet evident in the above tubes, the cells were washed twice with physiological saline and plated out on malt agar for viable count and observation of mutants. Auxotrophy was scored by plotting the colonies on minimal salts and malt agar media. It could be seen from data in Table II that cell inactivation was proportional to ethionine concentration. It could be further seen that both petite colonies and auxotrophs were scored only for the ethionine treated cells, their frequencies in both cases being proportional to ethionine concentration. Auxotrophs were, however, found only in those cultures treated with ethionine at concentration higher than 8 mg/ml. Although a number of various other types of colony variants were observed on the ethionine plates (Figure) or in the ethionine treated cultures, for convenience of counting only the petite colonies were considered.

The above observations indicated that ethionine was mutagenic to *Candida lipolytica*, when to be in contact with the cells for a fairly long period of 4 days. Most conventional chemical mutagens such as mustards, alkane sulphonic esters etc. have, however, been known to be mutagenic at normal dose levels by very much shorter durations of contact with the microbial cells. Several factors such as possible chemical change in ethionine, permeability, site of interaction with the cell, cellular components etc., will be of interest for understanding the mechanism of ethionine mutagenesis in this system.

Summary. Ethionine, was found to induce auxotrophic and petite colony variants in *Candida lipolytica* after prolonged contact with the cells.

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Effects of Magnetic Field on Inflammation

Treatment of some rheumatic and inflammatory diseases by magnetic fields is common in Japan as a home therapy. A series of experiments on the biological effects of magnetic fields have been done mainly in the Soviet Union. These were summarized in two recent monographs[1,2]. It was pointed out that magnetic fields had some influence on inflammation. But the anti-inflammatory effects of magnetic fields has not been assessed in the usual experimental modes widely used. We tested the effects of a magnetic field on carrageenan edema and adjuvant-induced arthritis in rats.

Table I. Effects of magnetic field on carrageenan edema in rats

Time after carrageenan injection (h)	Increase in paw volume (% ± SE)[a]	
	Control	In magnetic field
1	37.0 ± 2.8	24.5 ± 2.6[b]
2	42.5 ± 3.6	34.6 ± 2.4
4	66.4 ± 4.1	48.9 ± 2.1[b]
6	65.1 ± 3.0	43.8 ± 2.7[b]

[a] Average values for 14 animals. [b] $p < 0.01$ in relation to control.

Carrageenan edema test: random-bred male rats of the Wistar strain weighing about 160 g were used. They were housed in 2 cages, and fed with the same food and running water, in a laboratory at a temperature of 20 °C. The animals in each cage were divided just before the experiments into 2 groups, one for the magnetic treatment and the other for controls. A 0.1 ml of 1% carrageenan (Viscarine 402) suspension in saline was injected in the subplantar region of the left hindpaw. The volume of the left hindpaw was measured before and 1, 2, 4 and 6 h after the carrageenan injection according to Van Arman et al.[3].

Adjuvant-induced arthritis: random-bred male rats of the Sprague-Dawley strain aged from 8 to 10 weeks and weighing about 220 g were used. The method of feeding and grouping of the animals were as described

[1] M. F. Barnothy, *Biological Effects of Magnetic Fields* (Plenum Press, New York, London 1969), vol. 2.

[2] A. S. Pressman, *Electromagnetic Fields and Life* (Plenum Press, New York, London 1970).

[3] C. G. Van Arman, J. Pharm. exp. Ther. *150*, 328 (1965).

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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[4] A. I. LIKHACHEV, *Biological Effects of Magnetic Fields* (Plenum Press, New York, London 1969), vol. 2, p. 137.
[5] I. L. DEGEN, Orotop. Travm. Protez. *11*, 47 (1970).
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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