chemical modifications of the structure, since such modifications, even in the collagens of organisms subjected to environmental stress, have long been recognized[9]. Recently TRELSTAD[10] and HAY[11] have described 4 different molecular species of collagens which represent different structural gene products. It stands to reason therefore that the 'loss of trophic influence' in the cremaster muscle due to 60-day-chronic-transection of the nerve brings forth the quantitative and qualitative changes in the collagens, which imply the impairment of the action of specific structural genes[12].

Summary. Denervation of genitofemoralis in the bonnet monkey for 60 days resulted in a significant increase in neutral salt-soluble, alkali-soluble and insoluble collagens as well as glycoproteins. The hydroxyproline content of the salt-soluble and insoluble collagens in the muscle increased on denervation. These changes are discussed to imply the impairment of the action of specific structural genes.

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Mutagenic Effect of Ethionine on *Candida lipolytica*

Ethionine, the structural analogue of methionine, was first synthesized by DYER in 1938[1] and was shown to be detrimental to rats, and later by other workers, to many microorganisms at a level of about 2 mg per ml. Its effects, as so far known, is that it can compete with methionine for enzyme-binding sites and thereby interfere with methionine metabolism. Regulatory mutants of microorganisms have been obtained with resistance to ethionine which overproduce methionine[2-4]. During our attempts to isolate ethionine resistant strains of *Candida lipolytica*, we observed that ethionine itself can induce mutations in this organism to auxotrophy, morphological variation and possibly resistant to the analogue itself.

Table I. Effect of UV-irradiation on survival and ethionine resistance in *Candida lipolytica*

UV-dose (min)	Survivors (%)	Frequency of ethionine resistant strains ($\times 10^{-4}$)	
		3 days	6 days
0	100	0	0.178
2	100	0.355	0.550
4	6.980	0.822	1.031
6	0.021	—	—
8	0.003	6.950	8.130

Table II. Effect of ethionine on survival and mutagenesis in *Candida lipolytica*

Ethionine concentration (mg/ml)	Survivors (%)	Petite colonies ($\times 10^{-4}$)	Auxotrophs (%)
0	100	0	0
1	66.6	0.6	0
2	59.2	4.0	0
4	48.1	7.0	0
8	7.4	8.5	1.8
12	3.7	9.8	1.0
16	3.7	9.0	2.0

Ethionine mutagenesis in this case was suspected when UV-irradiation was employed to mutagenize the culture for isolation of ethionine-resistant strains. The non-irradiated control samples of the culture when plated out on ethionine began to show not only the morphological variations but also a significant number of ethionine-resistant colonies. The following procedure was adopted: 5 ml cell suspension of *C. lipolytica*, containing about 10^5 cells per ml from exponential growth phase, was irradiated with UV of 10 Ergs per mm^2 per sec for different periods of time ranging from zero (control) to 8 min in open petri dishes. After overnight refrigeration, the cultures were plated out on malt agar containing 10 mg DL-ethionine (Koch Light Labs. Ltd.) per ml. They were also separately plated out without ethionine for viable count. All the plates were incubated at 30 °C.

The inactivation of cells due to UV and the corresponding ethionine-resistant mutants among the survivor can be discerned from data in Table I. The ethionine plates were observed for colonies and data are presented for observations made on the 3rd and 6th days of incubation. The control without UV-irradiation showed no ethionine resistant colonies on the 3rd day, but on the 6th day they were seen at a frequency of 0.178×10^{-4}. In the irradiated samples, ethionine resistant colonies were observed on the 3rd day itself, but in all cases a significant increase in their frequency was recorded on the 6th day. All the ethionine plates, both UV-irradiated and non-irradiated showed morphological variants of wrinkled, petite and coloured colonies.

The UV-induced ethionine resistance is clearly evident by its proportionality to UV-dose (Table I). However, the emergence of ethionine resistant colonies in the case of non-irradiated control culture over prolonged incubation, as well as the occurrence of morphological variants, could not be satisfactorily explained. It was probable that these mutations were caused by ethionine itself after prolonged periods of contact with the cells.

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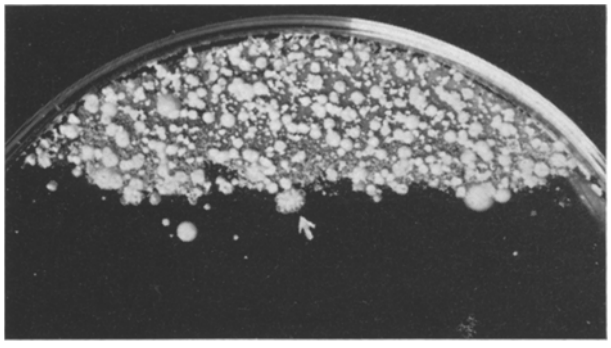

Gradient plating of *Candida lipolytica* on ethionine after 10 days incubation at 30 °C. A few types of colony variants can be seen. Arrow indicates a rough colony variant.

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

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