was appreciated using the 'hot plate test'[11]. This test was performed 30 min after pilocarpine (16 mg · kg^{-1}) or oxotremorine (0.125 mg · kg^{-1}) administration.

Palpebral ptosis was induced by i.p. administration of reserpine (2.5 mg · kg^{-1}) 4 h before the administration of the cholinergics (pilocarpine, 16 mg · kg^{-1} or oxotremorine, 0.25 mg · kg^{-1}). The ptosis was appreciated[12] 30 min after the cholinergic drug.

Results and discussion. The results are indicated on the Figure. The cholinergic-induced hypothermia was not antagonized either by atropine (1 mg · kg^{-1}) or by methylatropinium (1 mg · kg^{-1}). Pilocarpine-induced hypothermia was not antagonized by higher doses (4 mg · kg^{-1} i.p.) of atropine ($-2.8\,°C \pm 0.3$) or of methylatropinium ($-3.4\,°C \pm 0.2$). Oxotremorine-induced hypothermia was antagonized significantly more by atropine ($-2.0\,°C \pm 0.4$) than by methylatropinium ($-4.2\,°C \pm 0.3$).

The decrease in activity induced by the two cholinergic drugs was clearly antagonized by atropine but was not modified by methylatropinium. The increase in the reaction time to the nociceptive stimulus induced by pilocarpine or oxotremorine was suppressed by atropine, whereas it was not significantly modified by methylatropinium. Atropine or methylatropinium inhibited the antagonism of the two cholinergic drugs towards the palpebral ptosis induced by reserpine. The two effects which seem related to the sedative action of cholinergics (decrease in locomotor activity, increase of the reaction time to pain) were antagonized by atropine and not by methylatropinium, and the results are in keeping with those of other authors (vide supra). Identical doses of atropine and methylatropinium suppressed the antagonism of oxotremorine and pilocarpine towards reserpine-induced palpebral ptosis. Therefore this antagonism seems to be at a peripheral level.

On the other hand, the oxotremorine- and pilocarpine-induced hypothermia was not modified by the same doses of atropine or methylatropinium which can be active in the other tests. However, the doses of oxotremorine and pilocarpine were higher than the doses necessary to obtain the three other effects. It was possible partly to antagonize the oxotremorine-induced hypothermia but not the pilocarpine-induced hypothermia, with 4 times higher doses of atropine (4 mg · kg^{-1} i.p.) but not with methylatropinium (4 mg · kg^{-1} i.p.). Thus the hypothermia induced by pilocarpine or oxotremorine does not seem to be due completely to a cholinergic mechanism.

However, the possibility of pharmacological or metabolic interactions between quaternary derivatives of atropine-like drugs and cholinergics must be kept in mind. For instance, KARLEN, TRÄSKMAN and SJÖQVIST[13] showed that the peripheral effects of oxotremorine induced marked changes in the blood flow to different tissues. The antagonism of this peripheral effect by atropine methylnitrate results in lower brain concentration of oxotremorine compared to controls because of a greater effective peripheral volume of distribution.

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Melatonin Antagonizes Colchicine-Induced Mitotic Arrest[1]

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Summary. Melatonin, in concentrations up to 10^{-3} *M*, showed no effect on mitosis in cultures of HeLa or KB cells. However, when melatonin at 10^{-4} *M* was preincubated with HeLa cells prior to addition of 10^{-7} *M* colchicine, a reduction in the mitotic index, in comparison to colchicine alone, was observed.

It has been recently reported that the pineal hormone, melatonin, exhibits colchicine-like antimitotic activity in *Allium cepa* root hair cells[2]. We have attempted to determine whether this activity would also be displayed in mammalian cell lines.

HeLa cells and KB cells were purchased from Flow Laboratories and grown in Eagle's medium with 10% fetal calf serum. Logarithmically growing cells were exposed to the drug in monolayer for 7 h at 37 °C. At the end of the incubation period the cells were trypsinized off the culture dish, fixed (acetic acid:methanol/1:3), treated with Geimsa stain, and examined for mitotic fugures (500 cells per slide; 2 slides from separate cultures per experiment).

Colchicine produced maximum accumulation of metaphases (22–25%) at 5×10^{-7} *M* in both cell lines. Melatonin, however, even at concentrations of 10^{-3} *M* did not alter the mitotic index from control values (control cultures of both cell lines had a mitotic index of $4 \pm 1.5\%$). It was felt that perhaps melatonin might affect some other aspect of the mitotic cycle. It has been shown, for instance, that cytochalasin B inhibits cytokinesis (mitosis just prior to cell cleavage) through interaction with microfilaments[3]. Therefore, differential counts on the various phases of mitosis were performed, including telophase 1 (complete, bipolar separation of chromosomes) and telophase 2 (pinching of the cytoplasm and reconstruction of the nucleus), for colchicine,melatonin and control cultures. The values obtained are displayed in Table I and show that melatonin has no effect at this concentration (10^{-4} *M*) on any phase of the mitotic cycle in these cells.

In order to assess the effect of melatonin on the antimitotic action of colchicine both agents (colchicine,

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Table I. Effect of colchicine and melatonin on the distribution of stages of mitosis in HeLa cells

Compound	Prophase	Percent of all cells in mitosis					Mitosis (%)
		Late prophase	Equatorial plates	Anaphase	Telophase		
					1	2	
Colchicine (10^{-7} *M*)	4	96	0	0	0	0	11.8
Melatonin (10^{-4} *M*)	5	46	25	5	1.2	18.8	2.6
Control	8	44	17	6	3.0	22.0	4.8

Table II. Effect of melatonin on colchicine-induced mitotic arrest in HeLa cells

Compounds and concentrations	Mitosis (%)
Colchicine (10^{-7} *M*)	9.0
Melatonin (10^{-4} *M*)	4.2
Melatonin (10^{-4} *M*) [a]	9.2
Colchicine (10^{-7} *M*) [a]	
Melatonin (10^{-4} *M*) [b]	4.4
Colchicine (10^{-7} *M*) [b]	
Control	4.8

[a] Added simultaneously. [b] Preincubated with melatonin 1 h before adding colchicine.

10^{-7} *M*; melatonin, 10^{-4} *M*) were added simultaneously to HeLa cultures. No difference between the mitotic index of these cultures and those treated with colchicine alone was seen. However, when cultures were incubated for 1 h with 10^{-4} *M* melatonin before adding 10^{-7} *M* colchicine, a decrease in the mitotic index with respect to colchicine alone was observed (Table II). This is consistent with the observation that high concentrations of melatonin can displace colchicine from tubulin, the protein subunit of microtubules which comprise the mitotic spindle[4]. Melatonin is unique, however, in its ability to displace colchicine from tubulin without causing mitotic arrest itself. The 1-hour preincubation time necessary for melatonin to have an observable effect on colchicine's antimitotic action may reflect time required to become bound to or alter a cellular receptor such as tubulin.

Two analogs of melatonin, tryptamine and 5-methyltryptamine, were examined for their ability to influence mitotic inhibition by colchicine. Neither agent influenced colchicine's antimitotic activity, nor did either agent alone have any effect on mitosis. Thus, the ability of melatonin to reduce colchicine's effectiveness as a mitotic inhibitor appears to have some structural specificity.

Melatonin has been implicated in a number of processes which involve microtubules. For instance, it has been found that pinealectomy results in slow wound healing and that this effect can be reversed by melatonin as reflected in an increased number of mitotic figures at the wound site in melatonin-treated sujects[5]. Movement of melanin granules in melanocytes appears to be dependent on reversible interconversion between microtubules (24 nm) and filaments (10 nm)[6]. Microtubules appear to be associated with aggregation of granules; filaments appear to be associated with dispersion of granules. Granules are found to disperse on treatment with colchicine, possibly due to conversion of microtubules into filaments. Melatonin, on the other hand, causes the aggregation of granules as does cytochalasin B[6]. In light of these reports, then, it is not especially surprizing that an interaction between colchicine and melatonin occurs in microtubule-mediated mitotic division. Evidence against interconversion between microtubules and filaments has also been presented[7]. Thus, the interrelationship among these various observations involving melatonin, colchicine, filaments and microtubules awaits elucidation.

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Effect of Fructose Administration on Serum Urate Levels in the Uricase Inhibited Rat

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Summary. Fructose administration to the uricase inhibited rat produces a very marked elevation in serum urate levels.

Fructose, a monosaccharide, is currently available to the medical profession for use as a nutrient whenever a rapidly metabolizable source of calories is required. It is especially valuable in treating hypoglycemia in newborn infants because no hypoglycemic rebound occurs. Furthermore, it is better tolerated than dextrose in diabetics because it is metabolized in the absence of insulin[1]. Following its introduction as a sugar substitute in a variety of food preparations, fructose has elicited increasing attention. It now appears that in the near future, fructose as 'high fructose corn syrup' could account for up to 40%

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