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.

[4] M. Winston, E. H. Johnson, J. K. Kelleher, S. Banerjee and L. Margulis, Cytobios. *9*, 237 (1974).
[5] R. Weichselbaum, M. Patel and T. K. Das Gupta, Nature, Lond. *254*, 349 (1975).
[6] G. Moellmann, J. McGuire and A. B. Learner, Yale J. biol. Med. *46*, 337 (1973).
[7] M. De Brabander, F. Aerts, R. Van de Veire and M. Borgers, Nature, Lond. *253*, 119 (1975).

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

B. Stavric, W. J. Johnson, S. Clayman, R. E. A. Gadd and A. Chartrand

Research Laboratories, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa (Ontario, Canada), 22 September 1975.

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%

[1] *AMA Drug Evaluation*, 1st edn. (American Medical Association, Chicago 1971), p. 126.

The Effect of fructose in the presence and absence of K-oxonate on serum urate levels

Day(s)	Treatment			Serum urate (mg/100 ml) at day 5 (blood taken $1^1/_2$ h after i.p. injection)
	1 to 3	4 and 5		
Group	K-oxonate (5% in diet)	K-oxonate (5% in diet; 250 mg/kg i.p.)	Fructose (5% in diet; 5 g/kg i.p.)	
A (control)	–	–	–	1.9 (1.7– 2.1)[a]
B	–	–	+	2.6 (2.2– 3.1)
C	+	+	–	3.7 (3.5– 4.1)
D	+	+	+	10.7 (9.7–11.4)

[a] Range of serum urate values obtained.

of industrial sugar consumption[2]. Moreover, the food industry is considering the substitution of cheaper 'high fructose corn syrup' for relatively expensive sucrose in soft drinks, dairy and frozen desserts, cooked goods and snacks[2,3].

Since the original observation of PERHEENTUPA and RAIVIO[4] that fructose administration to normal children or to children with congenital fructose intolerance causes a rise in serum urate levels, subsequent reports concerning serum urate levels after oral or intravenous fructose administration have been conflicting. STIRPE et al.[5] and HEUCKENKAMP and ZOLLNER[6] were able to demonstrate a hyperuricemic condition following fructose administration, while SAHEBJAMI and SCALETTAR[7] and CURRERI and PRUITT[8] did not find any significant change in serum urate levels in their studies. Morevover, FRANK and MÜLLER[9] have observed significant increases in serum urate levels during infusion of fructose only in patients with gout and hyperuricemia, but not in normal individuals.

Prior to the development of the hyperuricemic rat model by JOHNSON et al.[10] which simulates the human situation, it was not feasible to monitor the influence of drugs on serum urate levels in experimental animals. In this model the utilization of potassium oxonate (K-oxonate) to inhibit uricase activity prevents the conversion of urate to more soluble allantoin and thus allows the detection of abnormal serum urate levels in the rat arizing from drug therapy. In view of the conflicting reports involving human volunteers and patients, studies on the effect of fructose administration on serum urate levels in the uricase inhibited rat were undertaken.

Four groups of male Wistar rats (5 animals in each group) were treated as follows: Group A, no treatment; Group B, i.v. fructose infusion, 100 mg/kg; Group C, K-oxonate, 200 mg/kg, i.p. and Group D, 200 mg/kg K-oxonate 2 h prior to fructose administration. Serum urate was determined according to the method of ARCHIBALD[11] following precipitation of plasma proteins by the procedure of BUCHANAN et al.[12]. Compared to the untreated control animals (2.0 mg/100 ml serum urate), fructose or K-oxonate administration produced some elevation in serum urate levels (4.5 and 7.0 mg/100 ml respectively), while animals pretreated with K-oxonate showed a very marked elevation in serum urate levels (as high as 20 mg/100 ml, average 17.5%) 2 h after fructose infusion.

In another series of experiments with 4 groups of female Wistar rats (5 animals in each group) test compounds were introduced by addition to the basic diet of ground commercial rat cubes and by i.p. injection (Table). These results demonstrate that treatment with a combination of both K-oxonate and fructose produces much higher urate levels than either compound administered alone. This is in agreement with the results obtained in the i.v. studies. It is of interest to note that some deposits of uric acid were found in the kidney tubules of the rat in the K-oxonate/fructose treated group, thus possibly impairing normal renal function.

These results, utilizing the uricase inhibited rat, clearly indicate that fructose administration can result in markedly elevated serum urate levels. In spite of the production of these high urate levels, a study on the effect of fructose administration on hemostasis is feasible since STAVRIC et al.[13] have previously demonstrated that there is no significant change in such hematological parameters as the clotting time, hematocrit, platelet number or platelet aggregation in the uricase inhibited hyperuricemic rat. It is suggested that the indiscriminate use of fructose in foods be reappraised since one might expect that individuals with slightly elevated or borderline urate levels might be exposed unwittingly to large amounts of fructose daily, which could lead to hyperuricemia and its associated health problems.

[2] J. W. ROBINSON and Food Technical Service Staff (Food Engineering, May 1975), p. 57.
[3] An FE Research Report on *Food Sweeteners Enter a New Era* (Food Engineering, May 1975), p. 55.
[4] J. PERHEENTUPA and K. RAIVIO, Lancet *1*, p. 528 (1967).
[5] E. STIRPE, E. D. CORTE, E. BONETTI, A. ABBONDANZA, A. ABBATI and F. DE STEFANO, Lancet *2*, p. 1311 (1970).
[6] P. U. HEUCKENKAMP and N. ZOLLNER, Lancet *1*, 808 (1971).
[7] H. SAHEBJAMI and R. SCALETTAR, Lancet *1*, 366 (1971).
[8] P. W. CURRERI and B. A. PRUITT, Lancet *1*, 839 (1970)
[9] V. O. FRANK and M. M. MÜLLER, Wiener klin. Wschr. 86, 731 (1974).
[10] W. J. JOHNSON, B. STAVRIC and A. CHARTRAND, Proc. Soc. exp. Biol. Med. *131*, 8 (1969).
[11] R. M. ARCHIBALD, Clin. Chem. *3*, 102 (1957).
[12] M. J. BUCHANAN, I. C. ISDALE and B. S. ROSE, Ann. rheum. Dis. *24*, 285 (1965).
[13] B. STAVRIC, S. CLAYMAN, R. E. A. GADD and D. HÉBERT, Pharmac. Res. Commun. *7*, 117 (1975).