Triton-X-100, was also found to decrease. A decrease in the activities of these enzymes in the microsomal fractions with simultaneous inhibition in both the free and total activities in the synaptosomal fraction indicated a diminished level of the net amount of these enzymes under lithium-administered condition.

In vitro study indicates that lithium carbonate at 10 mM concentration could inhibit the activity of acid phosphatase to the extent of approximate 38 per cent and this inhibition was slightly lowered (from 38 per cent inhibition it is reduced to 25 per cent) by dialysis of the enzyme against 0.02 M acetate buffer (pH 5.0) containing 1 mM EDTA.

In the case of cholinesterase there was no alteration in the activity under the influence of lithium, which was also previously observed by other investigators [20].

Department of Biochemistry,
University College of Science,
35, Ballygunge Circular Road,
Calcutta-700019,
India

Miss HASI BERA
GORA CHAND CHATTERJEE

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Activation of nitrofurantoin to a mutagen by rat liver nitroreductase

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Many nitrofurans have been found to be mutagenic [1, 4, H. S. Rosenkranz, unpublished results] and to be endowed with oncogenic potential as well [5, 6]. Nitrofurantoin (1-[(5-nitrofurylidene)amino]-hydantoin, furantoin), the nitrofuran utilized most in antimicrobial chemotherapy is not carcinogenic [5, 6], yet it possesses mutagenic and DNA-modifying activity when tested in bacterial systems [2, 3 and H. S. Rosenkranz, unpublished results]. Because there has been a good correlation between mutagenicity in bacterial systems and carcinogenicity in animals [7–9], nitrofurantoin occupies a unique position in chemical carcinogenesis. In view of this situation and its probable relevance to the relationship between mutagens and carcinogens, we have investigated the mutagenicity of nitrofurantoin.

The Salmonella mutagenicity assay developed by Ames *et al.* [10] was used in this study. The indicator micro-organism (histidine auxotrophs), the test agent and when indicated the rat liver preparation (including the required co-factors) [7] were incorporated into the overlay. These mixtures were incubated at 37° in the dark [11] for 46 hr and revertants (mutants) to histidine-independence were enumerated. When anaerobic conditions were required, the plates were placed in a Gas-Pak system (BBL, Cockeysville, Md.), and incubated at 37° for 16 hr, whereupon they were incubated an additional 30 hr aerobically. Liver microsomes (actually the S-9 post-mitochondrial fraction) were prepared from Sprague–Dawley rats by a previously described procedure [7].

The indicator micro-organism was *Salmonella typhimurim* TA100 [12] and a mutant (TA100-FR1) derived therefrom which was deficient in nitro-reductase. TA100-FR1 was obtained by selection [13] of a strain capable of growth in the presence of nitrofurazone (20 μg/ml, 5-nitro-2-furaldehyde semicarbazone). The absence of nitroreductase (4 per cent of control) was confirmed colorimetrically [13]. TA100-FR1 was resistant to the growth inhibitory properties of other nitrofurans, nitro-heterocyclics and nitro-aromatic compounds which indicates that it is deficient in a non-specific nitroreductase.

It might be argued that the mutagenicity of nitrofurantoin in bacterial cells derives from its conversion by prokaryotes to an active hydroxylamino derivative but that this enzymic activity is lacking in mammals which could account for its lack of carcinogenicity if it be assumed [7] that the active mutagenic intermediate is also the metabolite responsible for the carcinogenic event. To test this possibility we have prepared a microbial indicator strain (TA100-FR1) deficient in nitroreductase. Unlike its parent (TA100), this strain is not mutagenized by nitrofurantoin (Table 1), although it does respond normally to a series of mutagens lacking the nitro-function (Table 1). Upon addition of a preparation derived from rat liver, the mutagenic activity of nitrofurantoin for TA100-FR1 was restored (Table 1). When the mixtures were incubated anaerobically for several hr, nitrofurantoin was mutagenic for TA100-FR1 even in the absence of the liver enzymes (Table 1); this appears to be due to the presence in these bacteria of a second, oxygen-labile, non-specific nitroreductase (unpublished results). However, upon supplementation with

Table 1. Mutagenicity of nitrofurantoin for *Salmonella typhimurium**

Additions	(μg/plate)	Post mitochondrial fraction	Revertants/plate			
			TA100		TA100-FR1	
			Aerobic	Anaerobic	Aerobic	Anaerobic
Nitrofurantoin	2		354 ± 18	303 ± 20	28 ± 3	53 ± 5
	2	+	409 ± 17	435 ± 21	47 ± 5	389 ± 18
	10		128 ± 4†	280 ± 12†	35 ± 3	597 ± 43
	10	+	102 ± 3†	280 ± 16†	214 ± 6	4875 ± 285
Ethyl methanesulfonate	7		5000	5000	5000	5000
2-Fluorenamine	25		0		0	
	25	+	2500		2500	
1,2-Epoxybutane	14		390 ± 20	410 ± 18	430 ± 26	450 ± 18
2-Bromoethanol	5.5		690 ± 32	710 ± 38	780 ± 44	750 ± 32

* The data given are the averages of replicate plates (minimum number = 6) which were subtracted from the spontaneous background, approximately 55 for TA100-FR1 and 190 for TA100.

† Decrease in number of revertants upon an increase in nitrofurantoin concentration is due to the lethal effect of this agent on TA100. This is not seen with TA100-FR1 which is resistant to this agent.

the post-mitochondrial fraction, this mutagenic activity was increased further (Table 1).

These results indicate the presence in rat liver of an enzyme activity capable of transforming nitrofurantoin into an active mutagen. There are a number of reports suggesting that nitrofurans and other nitro-heterocyclic substances can be reduced by rat liver enzymes; the enzymes implicated have been xanthine oxidase, NADPH-cytochrome *c* reductase and aldehyde oxidase [14–21]. In some instances, the isolation of the hydroxylamino derivatives has been reported [15, 19]. In others, although only the amino derivative was recovered, the hydroxylamino intermediate was implicated [18, 20, 21]. The difficulty in isolating the hydroxylamino derivates may be related to their extreme oxygen-lability [15]. It should be noted in this respect that the reduction of nitrofurans proceeds better anaerobically [17] and that the enzyme-mediated mutagenicity of nitrofurantoin was greatly enhanced by anaerobiosis (Table 1).

With respect to nitrofurantoin, specifically, it has been reported that it can be reduced by NADPH-cytochrome *c* reductase and milk xanthine oxidase [20]. The identity of the intermediate or final products had not been identified, although the hydroxylamino and amino derivatives, respectively, appear implicated [20, 21].

The present findings which indicate that nitrofurantoin can be metabolized by a mammalian enzyme to a mutagenic substance (presumably the hydroxylamino analog, see Ref. 8) lead to two alternate conclusions: (a) that it is possible to dissociate mutagenicity from carcinogenicity or (b) that the two functions cannot be dissociated but that the carcinogenicity of nitrofurantoin has not been tested under optimal conditions.

In view of the fundamental importance of the first conclusion and the immediate practical consequences of the second to human health due to the widespread use of this therapeutic agent, it would seem that further investigation of the biological properties of nitrofurantoin is a matter of urgency.

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* Present address: Department of Microbiology, New York Medical College, Valhalla, N.Y. 10595, U.S.A.

Departments of Microbiology and Pediatrics, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032, U.S.A.

HERBERT S. ROSENKRANZ*
WILLIAM T. SPECK

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