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Short Communication

MUTAGENICITY OF EXTRACTS OF URINE FROM RATS TREATED WITH AROMATIC AMINES

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Some aromatic amines are known to be carcinogenic. For instance, benzidine and β-naphthylamine, used in the dye industry, often caused bladder cancer of occupational origin (Clayson, 1964). Today industrial use of both chemicals is legally restricted in many countries.

The mutagenic activity of the urine of animals to which aromatic amines were given was investigated in the Salmonella/mammalian-microsome test developed by Ames et al. (1975). The results were as follows. (1) Urinary metabolites of some aromatic amines can be strongly mutagenic although the amines themselves are weak mutagens. (2) Even if an aromatic amine is not mutagenic, its urinary metabolites may show mutagenicity.

Urinary metabolites of 4 aromatic amines were tested. Benzidine was given by the Engineering Research Laboratory, Toray Industries Inc. *o*-Tolidine (3,3'-dimethylbenzidine), aniline, and *o*-toluidine were purchased from Wako Pure Chemical Industries Ltd., Osaka (Japan). In addition, 4-(*N*-acetylamino)-4'-aminobiphenyl, or *N*-acetylbenzidine, was synthesized as a benzidine metabolite to be found in urine. Each chemical, except *N*-acetylbenzidine, was administered orally to male Sprague-Dawley rats. The dose given was 80 mg/kg for benzidine and *o*-tolidine, and 300 mg/kg for aniline and *o*-toluidine. Following the administration of the chemicals, urine was collected during the first 24 h. The urine was adjusted to pH 7 and was extracted with ether. After the ether was evaporated, a dried residue was obtained as urine extracts (Table 1). The urine extracts were dissolved in dimethyl sulfoxide and were used for the mutagenicity assay. The mutagenicity of the urine extracts, as well as that of the chemicals, was tested on *Salmonella typhimurium* TA98 and TA100. S9 mix was prepared from male Sprague-Dawley rats treated with polychlorinated biphenyl. Further, the urine extracts from rats treated with benzidine were analyzed by high-pressure liquid chromatography and IR spectroscopy.

The mutagenic activity of the chemicals in vitro is presented in Table 2. Table 3 presents the mutagenic activity of the urine extracts for each test ma-

TABLE 1

AMOUNT OF URINE EXTRACTS FROM RATS TREATED WITH AROMATIC AMINES

Chemical	Given dose per rat (mg/kg)	24-h urine per rat (ml) [a]	Urine extract per rat (mg) [a]
None	—	23.6 ± 13.6	2.6 ± 1.2
Benzidine	80	24.7 ± 12.7	3.9 ± 1.7
o-Tolidine	80	22.3 ± 9.8	3.8 ± 1.4
Aniline	300	19.7 ± 10.6	3.4 ± 1.4
o-Toluidine	300	21.7 ± 11.2	3.4 ± 1.4

[a] Mean ± S.D. from 8–12 animals.

terial. The mutagenic activities of benzidine and *o*-tolidine were both considerably greater in urine than in vitro. Neither aniline nor *o*-toluidine showed mutagenic activity in vitro. However, urinary metabolites of both chemicals were mutagenic (Table 3). Chemical analysis showed that *N*-acetylbenzidine existed in the urine extracts from rats treated with benzidine. The mutagenicity of

TABLE 2

MUTAGENICITY OF AROMATIC AMINES IN VITRO

Chemical	Dose (μg/plate)	Histidine-positive revertants per plate [a]			
		TA98		TA100	
		−S9	+S9	−S9	+S9
None	—	24	40	136	141
Benzidine	50	19	96	119	184
	100	22	135	111	175
	200	21	222	122	206
	500	27	301	154	268
	1000	22	482	132	499
o-Tolidine	50	23	92	126	150
	100	17	118	134	146
	200	18	151	144	148
	550	25	225	132	151
	1000	20	290	140	160
Aniline	50	22	42	133	142
	100	21	48	126	127
	200	20	46	128	129
	500	19	50	130	136
	1000	22	45	126	128
o-Toluidine	50	25	45	137	130
	100	21	43	140	127
	200	22	42	142	142
	500	21	40	127	139
	1000	20	39	135	140
N-Acetylbenzidine	1	18	84	136	160
	5	17	350	140	189
	20	32	1140	127	422
	100	20	4530	135	1490

[a] Average of 2 separate Expts.

TABLE 3

MUTAGENICITY OF URINE EXTRACTS FROM RATS TREATED WITH AROMATIC AMINES

Urine extract (UE)	Dose (μg/plate)	Histidine-positive revertants per plate [a]			
		TA98		TA100	
		−S9	+S9	−S9	+S9
UE (control) [b]	100	27	36	120	136
	1000	26	40	132	128
UE (benzidine) [c]	10	24	165	118	124
	25	25	323	124	224
	50	18	740	137	288
	100	22	1720	139	445
	200	23	3800	161	806
UE (o-tolidine)	10	40	210	125	130
	25	45	306	140	150
	50	41	524	197	310
	100	76	1140	280	570
	200	88	2240	310	840
UE (aniline)	50	25	60	123	124
	100	35	130	134	143
	200	40	250	142	137
	500	26	561	150	166
	1000	28	424	139	169
UE (o-toluidine)	50	21	76	136	118
	100	20	100	124	112
	200	28	150	124	124
	500	26	259	130	133
	1000	24	303	116	123

[a] Average of 2 separate Expts.
[b] Urine extract obtained from rats to which no chemical was given.
[c] Urine extract obtained from rats to which benzidine was given.

N-acetylbenzidine itself was very strong in the presence of S9 mix (Table 2).

Coupling urine samples to the Salmonella mutagenicity assay was initiated by Durston and Ames (1974) and Commoner et al. (1974). Although the technique has not been used so widely, it has brought about useful information for the study of metabolism and carcinogenesis of aromatic amines. The urine extract made with ether includes not only metabolites of a given chemical but also normal metabolites of substances other than the test material. How much of the urine extract is due to the metabolites of the given chemical is not clear, partly because all the metabolites of the chemical could not be identified in the present study and partly because the amount of the urine extracts from individual control rats varied considerably (Table 1). However, if the urine extract as a whole showed stronger mutagenicity than the corresponding chemical, we could imagine that there was at least a metabolite whose mutagenicity was more active than the original chemical. The phenomenon was observed for both benzidine and *o*-tolidine.

Today *N*-hydroxylation is regarded as the initial and essential process for the carcinogenesis of aromatic amines (Miller, 1970). *N*-Hydroxy-4-acetylaminobiphenyl, a metabolite of 4-aminobiphenyl, was found as a strong carcinogen by

Miller et al. (1961). The knowledge that *N*-acetylated compounds are main metabolites of benzidine has accumulated in recent years (Kellner et al., 1973; Morton et al., 1979). Our experimental results are interesting in the light of those findings. *N*-Acetylbenzidine found in urine might conceivably be metabolized to *N*-hydroxyacetylbenzidine in the presence of S9 mix thereby becoming a strong mutagen.

Aniline was once considered a causative agent of bladder cancer (Rehn, 1895). Although this was denied later, the National Cancer Institute (U.S.A.) reported that aniline could produce tumors in rats (Federal Register, 1979). It is interesting that neither aniline nor *o*-toluidine was mutagenic when tested in vitro, while urinary metabolites of both substances were mutagenic. In consideration of the carcinogenicity of aromatic amines the mutagenic activity of urine samples is important and full of suggestions.

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Short Communication

AIRBORNE MUTAGENS EXTRACTED FROM PARTICLES OF RESPIRABLE SIZE

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Direct-acting frameshift mutagens have been detected in air-pollution samples from urban localities in the United States, Europe, and Japan [4,5,7,10,11]. These substances, yet to be identified chemically, are borne on aerosolized mixtures of variously-sized soot particles. It is of interest to investigate the relationship between airborne particle size and mutagenic content because of the tendency of the smaller particles to become entrapped in the lung parenchyma [6]. Preliminary data collected in a Los Angeles study indicated that respirable-sized particles may be more mutagenic than the larger-sized particles [3]. We have completed a study of the mutagenicity of variously-sized pollutant particles collected in Durham NC and will present the results in this report.

13 samples of airborne particulate matter were collected between June 1978 and January 1979 by Lester Spiller and his associates at the U.S. EPA air sampling site located in Durham NC. Each sample consisted of the mass collected by 4 Andersen 2000 samplers [2,8] operated for 120—168 h at flow rates of 20 cubic feet of air per minute. The particulate matter was separated into 5 fractions and deposited on the stages of the samplers: Stages 1—4, loaded with aluminum foil filters coated with propylene glycol, collected the particles ranging from 7—11 microns (stage 1), 4.7—7 microns (stage 2), 3.3—4.7 microns (stage 3), and 2.1—3.3 microns (stage 4); backup filters (glass fiber or Teflon-coated fiber) collected the smaller-sized particles. Electron-microscopic examination of particles deposited on the filters confirmed that the samplers were operating according to manufacturer's specifications. Freshly collected samples were sealed in tight containers and shipped to Riverside for extraction and bioassay. The filters from corresponding stages were combined and extracted 6 h with acetone in Soxhlet apparatuses [10]. The extracts were concentrated in vacuo, weighed, and redissolved in volumes of DMSO appropriate for mutagenicity testing.

Initial experiments were done to select the Ames' *Salmonella typhimu*