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Detection of Mutagenic Activity in the Urines of Anesthesiologists: A Preliminary Report

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While halothane was without mutagenic activity in the *Salmonella* mutagenicity assay, even in the presence of microsomal fractions, the urines of anesthesiologists induced mutations of the base-substitution type.

On the basis of laboratory as well as epidemiological studies, anesthetic gases have been implicated as responsible for an increased incidence of spontaneous abortions, congenital malformations, and cancer among anesthesiologists (*1–4*). In view of the established relationship between the ability to induce genetic changes in bacteria and cancers in mammals (*5, 6*), a number of investigators have attempted to identify the anesthetic agent responsible for the unwanted side-effects by determining their ability to induce mutations in the *Salmonella* mutagenicity assay developed by Ames and his associates (*7*). However, even though this system can be adapted to the determination of the mutagenic activity of gases (*8*), none of the commonly used anesthetics was found to be mutagenic, even in the presence of microsomal mixtures derived from rat livers (*8–10*), although one of the anesthetic gases, a vinyl chloride analog which is not commonly used, was shown to possess genetic activity for *Salmonella typhimurium* (*11*). It is known, however, that in man anesthetics are metabolized and that some of these metabolites are recoverable in the urine (*12*). Thus in humans, halothane is biotransformed to trifluoroacetric acid, *N*-trifluoroacetyl-2-aminoethanol, *N*-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine as well as to several other as yet unidentified urinary metabolites (*12*). The presence in urines of the ethanolamide and cysteine conjugates suggests the existence of very reactive metabolic intermediates. Some of these could, conceivably, still be present in the urine. For these reasons we undertook an examination of the urines of persons exposed to anesthetic gases. The immediate question which arose was a decision whether to investigate the urines of surgical patients receiving a single–albeit heavy–dose of these agents or to select a population of anesthesiologists who are exposed to these gases chronically and who can be considered to be "saturated" with three agents (*13*). In view of the report that administration of halothane to anesthesiologists resulted in a more rapid metabolism and in the formation of more metabolite when compared to controls (*14*), it seemed that perhaps the liver of these physicians are "induced" with respect to the enzymes responsible for the biotransformation of halothane. This result led us to investigate the mutagenic activity of the urines of a group of anesthesiologists. The present is a very preliminary report of our findings. The data indicate that the urines of these persons do contain material endowed with genetic activity.

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Experimental Procedures

The *Salmonella typhimurium* mutagenicity procedure devised by Ames and his associates was followed (*7*). Strains TA1535, TA1538, and TA100 (*7*) were used in this study. The liver microsomes used in this study were obtained from rats induced with the polychlorinated biphenyl Arachlor 1254 (*7*).

The anesthesiologists who participated in this study were on the staff of two of our teaching hospitals. Some of them had been anesthesiologists for less than 1 year (residents) while others had several years of continuous exposure to anesthetic gases. All participants supplied histories which included smoking habits, consumption of "ethnic" foods, etc.

Urines were collected, sterile-filtered, and either used immediately or stored at −20°C. Storage at that temperature had no effect on mutagenic activity. Portions of unconcentrated urines were incorporated into the agar overlay.

Results

None of the urines tested exhibited mutagenic activity for strain TA1538, the indicator strain for frameshift mutations. On the other hand, all of the urines derived from anesthesiologists exhibited some mutagenic activity for strains TA1535 or TA100. An analysis of the results indicates the possible presence in the urines of more than one type of mutagenic substance (Table 1). Thus some of the specimens exhibited activity only for strain TA100 (e.g., A2, A3, A8, A9, A12, A13). Of these, some lost activity upon incubation in the presence of microsomes (e.g., A3, A13). Some of the specimens induced mutations in strain TA100 as well as TA1535 (e.g., A1, A4, A5, A6, A7, A10). In each instance the presence of rat liver microsomes greatly diminished or eliminated altogether mutagenicity for strain TA1535 (Table 1).

Samples obtained following 2 days of absence from the operating room still possessed mutagenic activity (i.e., A4 and A4a, A10 and A10a, Table 1).

The mutagenic response of those urines with considerable activity was dependent upon the amount of urine added (Table 2, specimen A4). There was no apparent correlation between smoking habit and extent of mutagenic response in these nonconcentrated specimens.

Table 1. Mutagenic activity in the urines of anesthesiologists.[a]

	Revertants per plate[b]			
	TA1535		TA100	
Specimen	−S9	+S9[c]	−S9	+S9
A1	24	0	48	34
A2	4	0	15	48
A3	5	4	156	30
A4	127	10	143	119
A4a	64	3	146	28
A5	109	0	148	72
A6	90	20	108	29
A7	185	29	112	136
A8	3	11	47	145
A9	0	0	71	93
A10	24	0	236	215
A10a	60	0	59	44
A11	23	5	121	109
A12	0	0	103	119
A13	5	0	60	0
A14	42	0	54	60
A15	12	0	277	115

[a]0.2 ml of sterile urine was incorporated into the agar overlay. The values shown are the averages of replicate plates Representative colonies from each plate were selected and their histidine-independent character confirmed.

[b]Results are expressed as revertants per plate above the internal controls. Thus for strain TA1535 and TA100, a background of approx. 14 and 150 revertants per plate was subtracted from each experimental value.

[c]S9 refers to the microsomal preparation derived from rat livers.

Table 2. Concentration dependence of mutagenic activity present in the urines of an anesthesiologist.[a]

	Revertants per plate			
	TA1535		TA100	
Amount added, μl per plate	−S9	+S9[b]	−S9	+S9
50	10	0	33	20
100	49	2	100	45
200	127	10	143	119

[a]Results are expressed as revertants per plate above control.

[b]S9 refers to the microsomal preparations derived from rat livers.

Discussion

These results raise a number of interesting questions. Should the mutagenic activity present in the urines of anesthesiologists indeed be due to active metabolites of anesthetic gases, then it would be expected that this activity was due to the reactive intermediates that are formed presumably in the liver. It would be expected that only a trace of these will be excreted in the urines, the majority would react with body fluid and cellular constituents. Indeed it has been shown that only a small proportion of these gases are recoverable in the urine, feces and exhaled air (*13, 15*). The fact that microsomal preparations reduced mutagenic activity is consistent with the finding that these substances bind to microsomes and are metabolized by such cell-free preparations (16–18).

The finding that the urines from anesthesiologists remained mutagenic even after 2 days of absence from the operating room is compatible with the re-

ports that anesthesiologists excrete anesthetics or their metabolites for as long as 6 days following their last day of exposure to these gases (*14, 15*).

The present findings require considerable elucidation, they do, however, indicate the feasibility of using the mutagenic index of urines for epidemiological purposes and for the detection and elucidation of the chemical nature of potential mutagens and carcinogens present in body fluids.

Studies to identify the nature of the mutagenic substance(s) are underway. We are very fortunate in this respect that we are able to monitor our study populations prior to their occupational exposure. Thus because our medical students spend their first two years on our Westchester County campus without significant contact with the hospital, we are able to follow them for periods prior to and during their clinical years. Similarly, because the prospective anesthesiologists can be identified during their senior year in medical school and/or during their one-year of (medical) internship, we are able to monitor their urines for one year prior to their chronic exposure and subsequently. In this connection it is significant that extent of mutagenicity is correlated with the number of years of exposure. This may indicate an induction of specific enzymes capable of metabolizing anesthetics. This is consistent with the finding that anesthesiologists were more efficient metabolizers of halothane (*14*).

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