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Limitations of the Salmonella/Mammalian Microsome Assay (AMES Test) to Determine Occupational Exposure to Cytostatic Drugs

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Abstract—*Urine samples of nursing personnel working in medical oncology divisions of several Swiss hospitals were examined for mutagenic activity. Urine samples were concentrated* 100 *times following XAD-2 chromatography and mutagenicity was determined using the* Salmonella/*mammalian microsome assay (Ames test). Apart from the urine samples of patients treated with cytostatic drugs and urine samples of nurses who are cigarette smokers, no mutagenic activity could be found. Also following exposure to an increased and defined quantity of cytostatic drugs no mutagenicity could be recovered from the urine. Four different nurses worked with cyclophosphamide, methotrexate, 5-fluorouracil, adriamycin and* cis-*platinum for* 3–4 hr *without using any protection such as gloves, masks or a vertical laminar airflow hood. Aqueous extracts of filters, through which air was pumped during the whole experiment (a personal air-sampler was fixed near the face of the test persons), were non-mutagenic. Parallel to the mutagenicity test chemical analyses were also done. The methothrexate content was determined in serum samples and the aqueous filter extracts and urine samples were examined for* cis-*platinum. All chemical determinations were negative. With the aid of urine concentrates of a patient treated with sub-therapeutic doses of cyclophosphamide as well as with normal urine to which single small amounts of different cytostatics (adriamycin, cyclophosphamide,* cis-*platinum) were added, the detection limits for the corresponding cytostatic drugs were determined and found to be in the range of* 2–10 mg *for cyclophosphamide and approx.* 10 μg *for adriamycin.* Cis-*platinum was lost during the passage through the XAD-2 columns. With these results at hand the sensitivity of the hitherto preferably used method (Ames test) for the monitoring of exposure to cytostatic drugs must be seriously questioned.*

INTRODUCTION

SEVERAL cytostatic drugs are mutagenic [1] and may have carcinogenic properties [2, 3]. Therefore, nursing and medical personnel handling such compounds regularly over a long period of time, especially in clinical oncology units, may represent a group with an increased occupational risk. Indeed several authors published data showing mutagenic activity in urine samples indicating uptake of cytostatic drugs [4–7]. These results were partly confirmed by the detection of chromosomal aberrations in cultured peripheral lymphocytes from exposed personnel [8, 9]. Detection of cyclophosphamide and *cis*-platinum in urine samples of nurses by chemical analysis further indicated uptake of antineoplastic agents [10, 11]. It was suggested that the compounds were to some extent taken up by inhalation or ingestion of aerosols [12, 13]. Aerosols can be formed during the transfer of the freshly dissolved drugs into the syringes as a consequence of an overpressure in the container [13]. Anderson *et al.* [5] and Kolmodin-Hedman *et al.* [14] showed that the use of an appropriate protection (gloves, vertical laminar flow cabinet) led to an abolition of the previously demonstrated mutagenic activity in the urine. Other studies however failed to demonstrate that urine samples of exposed personnel are mutagenic in the Ames test [11, 15, 16].

Due to the potential usefulness of the Ames assay for monitoring uptake of cytostatic drugs we have screened the urine of nurses exposed to different quantities of antineoplastic agents. In addition experiments were designed to define the detection limits of the assay.

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MATERIALS AND METHODS

Routine exposure to anticancer agents in Swiss medical oncology units

Twenty-four skilled nurses from six Swiss medical oncology divisions, (Zürich, St. Gallen, Bellinzona, Bern, Neuchâtel, Basel) participated in this study. In Switzerland no general recommendations concerning anticancer drug exposure existed at that time (1983/84). The use of gloves was allowed. Eleven nurses used gloves while 13 did not. The collection of the urine samples started with the morning urine to which the following further samples were added until at least 600 ml were reached. The samples of 1 day were pooled, frozen in aliquots of 200 ml and stored at −20°C. Each person under investigation collected urine during a working day and during a duty free day, preceded by at least 2 days without any possible exposure to cytostatic drugs. The working day was the third day of 3 consecutive days of work, during which as many as possible routine manipulations with commonly used cytostatic drugs had taken place.

Three additional nurses collected 24-hr urine during 2 weeks (4 duty free days, 10 working days). Each urine sample was kept at 0–4°C in the dark until the end of the collection period (start with the first urine in the morning). The pooled samples were finally frozen in aliquots of 200 ml in dark plastic bottles and stored until analysis at −20°C.

Control urine was collected from non-exposed office-workers (non-smokers).

Defined exposition

Four experienced oncology nurses (non-smokers; no medications) working in four different medical oncology divisions had to prepare within approximately 3–4 hr the working solutions for 5 'CAP' and 5 'CMF' therapies. Each CAP-solution consisted of 1000 mg cyclophosphamide, 50 mg adriamycin, 100 mg *cis*-platinum. The doses for the CMF-solutions were 1000 mg cyclophosphamide, 50 mg methotrexate, 1000 mg 5-fluoro-uracil. Vials with the smallest dose were used and the content for the different drugs was: cyclophosphamid 100 mg, adriamycin 10 mg, *cis*-platinum 25 mg, methotrexate 50 mg, 5-fluoro-uracil 250 mg. No protection (gloves, gowns, masks, safety cabinets) were used. Twenty-four-hour urine samples were collected during 5 consecutive days starting 2 days before the day of the experiment.

Air sampling filter. During the whole experiment the test persons wore an air sampling pump (capacity 2 l/min) fixed near the neck for the collection of potential aerosols and small drug particles (present in the inspired air) on the surface of the filter (glassfiber; diameter 25 mm; Spectro Glass Fiber; Gelman, MI, U.S.A.).

Extraction of the air sampling filter. The glassfiber filters were extracted with 4.0 ml 0.1 M phosphate buffer, pH 7.4 during 1 hr at room temperature (shaking) followed by sterile filtration (Millex-GS; Millipor, Molshejm, France). Of the filtrate 3.0 ml were used for the mutagenicity test and 1.0 ml for the determination of the methotrexat content.

Determination of the methotrexate and of the cis*-platinum content*

The methotrexate content was determined in blood samples (collected immediately after the experiment and the next morning) and in the aqueous extracts of the air filters with the aid of a fluorescence polarisation immunoassay [24]. The analysis for *cis*-platinum was made in the 24-hr urine samples of days 1 and 3 (the experimental exposition took place at day 3), measuring the atomic absorption of platinum with a Varian AAS 875 equipment (Varian, Mulgrave, Australia; detection wavelength 259.5 nm; maximum temperature 2650°C). The samples were pretreated with ammoniumbromide to avoid the formation of double-peaks during the atomisation process [25].

Determination of the detection limit

Urine of a tumor patient. An informed patient under active treatment with cytostatic drugs received fractioned small doses of cyclophosphamide prior to his definitive therapy. After a > 2 week interval of no treatment (during which the control urine sample was taken) the following increasing doses were given in 2-day intervals: 1, 10 and 100 mg. Immediately before the injection of cyclophosphamide the urinary bladder was cleared and approx. 3–4 hr later a spot urine sample was collected.

Addition of cytostastic agents to control urine. Small amounts of various commonly used cytostatic drugs were added as single doses to pooled control urine samples of a non-smoker (with proven absence of mutagenic activity). The following amounts were separately dissolved in 200 ml urine samples: cyclophosphamide (0.5, 2, 8, 32 mg), adriamycine (1.25, 2.5, 10, 40 μg) and *cis*-platinum (0.06, 0.125, 0.25, 0.5, 1.0 mg).

Concentration of the urine samples

The urine samples were thawed and their pH adjusted with 2 N NaOH to pH 7.4. Afterwards the samples were filtrated through four layers of paper filter (no. 595; Schleicher und Schüll, Feldbach, Switzerland). The filtrate (200 ml) was loaded on a XAD-2 column (15 × 1 cm; XAD-2 particle size 0.31–1.0 mm; Serva, Heidelberg, West Germany). The resin was washed several times with acetone before packing and chromatographed following the

method of Yamasaki and Ames [17]. After the whole amount of the urine sample had run through the column the resin was washed with 50 ml H_2O bidest. and finally the apolar compounds were eluted with two-times 15 ml acetone. The two acetone extracts were separately evaporated to dryness under N_2 (passage through sterile cotton plugs) at 60°C and the residues were stored in the dark at −20°C.

Mutagenicity test

The *Salmonella*/mammalian microsome assay was performed following the methods of Ames *et al.* [18]. *Salmonella typhimurium* strains TA1535, TA98 and TA100 were obtained from B. N. Ames (University of California, Berkeley, U.S.A.).

As an activation system 0.5 ml of 20% (v/v) rat liver homogenate (S9) per plate was added. It was prepared from a batch of frozen S9 (kept in liquid nitrogen at −196°C) from livers of 200 g male Sprague–Dawley rats treated with Aroclor 1254 [18]. The liver slices were homogenized in 0.01 M Tris–HCl buffer with 0.25 M sucrose. The S9 contained 46 mg/ml protein as was determined by the method of Lowry *et al.* [19]. For the experiments without activation 0.25 M sucrose in 0.01 M Tris–HCl buffer, pH 7.4 instead of S9 was used.

The preincubation method of Yahagi *et al.* [20] was used for the samples to which S9 was added. For the tests without activation the normal plate and treat test was run. Only for the mutagenicity tests of the air filter extracts (50–200 µl aqueous extract per plate) the preincubation method was used for all tests, with and without addition of S9.

The evaporated acetone eluates of the urine samples loaded on the XAD-2 columns were dissolved in 2.0 ml dimethylsulfoxide (DMSO; Fluka, Buchs, Switzerland) corresponding to a concentration by a factor of 100. Normally 50, 100 and 200 µl of this urine concentrate were added per plate. Two replicate plates per dose were incubated.

As solvent controls 50–200 µl DMSO alone was added. The following standard mutagens were used as positive controls (amounts per plate): without activation — 2.5 µg sodium azide (TA1535, TA100), 7.5 µg nitrofluorene (TA98); with activation — 5.0 µg benzo(a)pyrene (TA98, TA100), 2.0 mg cyclophosphamid (TA1535).

Sodium azide and cyclophosphamide were dissolved in 100 µl 0.1 M phosphate buffer, pH 7.4, benzo(a)pyrene and nitrofluorene in 50 µl DMSO. With the exception of cyclophosphamide (Serva, Heidelberg, F.R.G.) the standard mutagens were obtained from Fluka (Buchs, Switzerland).

For the determination of the colony forming units the cell suspension was diluted 10^6 times with 0.85% NaCl. Aliquots of 0.3 ml were mixed with 6.0 ml top agar supplemented with histidine (0.05 mM biotin, 60 µg/ml *L*-histidine-HCl) and 2.0 ml of this suspension was spread over the surface of each of three replicate minimal medium plates.

The mutagenic effects of the positive controls were within the expected range. The density of the bacterial cultures was between 1 and 3 × 10^9 cells/ml.

All plates were incubated for 2 days at 37°C. The revertant colonies were marked and counted. In cases of more than 200 colonies per plate characteristic sectors of 1/3 to 1/32 were chosen containing between 80 and 120 colonies. The mutation factor (F) was calculated as the quotient between the total number of revertants on the treated plates divided by the number of spontaneous revertants (solvent controls). Plates with F values > 2 were designated as positive whereas F values < 0.7 were denoted as the result of bactericidal effects. In addition the background growth of the bacteria was checked under a stereo-microscope. An inhibition of the background growth was related with bactericidal effects.

RESULTS

Routine exposure

Six hundred-millilitre urine samples from 24 nurses working in six different medical oncology units were collected on free days and on working days. Because smoking individuals excrete considerable activities of frameshift mutagens in their urine, only the results of non-smokers are presented (Table 1). Of 19 urine concentrates tested only two were positive (TA98 with activation); the samples were collected by two different nurses during a duty-free day. Approximately one third of the 18 urine-specimen collected on working days had bactericidal effects in case of TA98 (±S9). Neither with TA98 nor with TA100 could a distinct mutagenic activity be found in the urine samples of all non-smoking nurses collected during working days. Only the urine concentrates of cigarette smoking oncology nurses induced significant mutagenic effects with TA98 (Table 2). Out of 10 urine samples obtained from smoking nurses, five showed positive mutagenic effects with the addition of S9 and 2 without activation; one sample was also positive using TA100 with rat-liver homogenate. Again about 25% of the tested urine concentrates were bactericidal (TA98, TA100; +S9). Also the urine samples of patients treated with cyclophosphamide (1500 mg) or daunomycin (75 mg) were positive (data not shown). Cyclophosphamid treatment correlated with mutagenic effects in strains TA1535 and TA100 (±S9; the maximum mutation factor (F) was 50 for

Table 1. *Urine mutagenicity of non-smoking nurses and controls**

Strain	Controls			Nurses					
				Days off work			Working days		
	Pos.†	Tox.‡	Tot.§	Pos.	Tox.	Tot.	Pos.	Tox.	Tot.
+S9‖									
TA98	0	0	2	2	3	19	0	6	18
TA100	0	0	2	0	3	19	0	7	18
−S9‖									
TA98	0	0	2	0	0	19	0	1	18
TA100	0	0	2	0	1	19	0	0	18

* As controls urine samples from non-exposed office workers were collected (non-smokers).
† A urine sample is designated as positive if the mutation factor is > 2.
‡ A urine sample is designated as toxic if the mutation factor is < 0.7 or if the bacterial background lawn is inhibited.
§ Total number of tested urine samples.
‖ +S9: with activation; −S9 without activation.

Table 2. *Comparison of the urine mutagenicity of smokers and non-smokers (nurses and controls)**

Strain	Smokers			Non-smokers		
	Pos.†	Tox.‡	Tot.§	Pos.	Tox.	Tot.
TA98						
+S9‖	5	3	10	2	9	39
−S9‖	2	0	10	0	1	39
TA100						
+S9	1	2	10	0	10	39
−S9	0	0	10	0	1	39

*†‡§‖ See footnotes to Table 1.

TA1535 with S9; compare Figs. 2 and 3) and treatment with daunomycin with mutagenicity in TA98 (±S9; the maximum F was 20 with S9).

In addition 24 hr urine portions were collected daily over a period of 2 weeks (working days and days off) from three nurses. No clear correlation between the exposure to cytostatic drugs and the urine mutagenicity could be found. As an example the mutagenic activity (reported as mutation-factor F; without and with the addition of rat liver S9) of the urine samples of one nurse starting with Sunday and followed by the 5 consecutive working days is shown in Fig. 1. Only on working-day 5 a weak mutagenic activity was detected with strain *S.typhimurium* TA100 (without S9). There was a

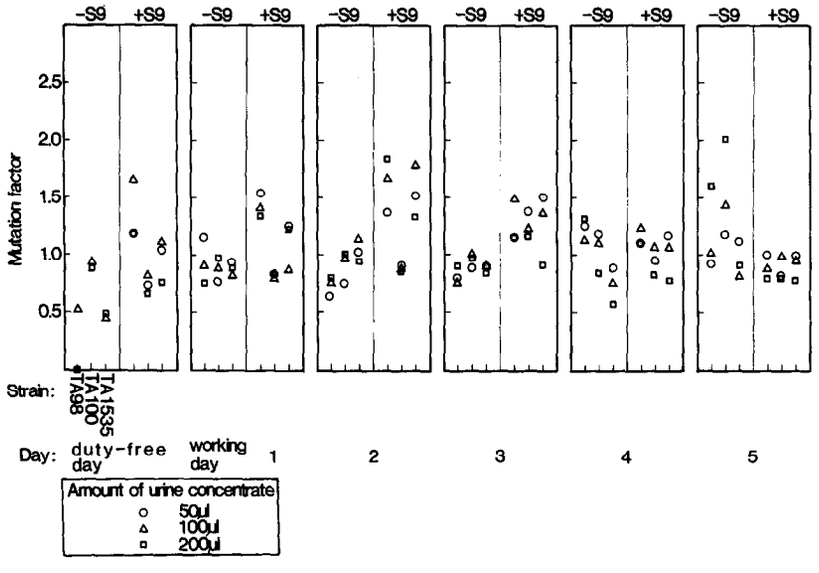

Fig. 1. *Urine mutagenicity data of a nurse working in a medical oncology department. Twenty-four-hour urine samples were collected on each day and were stored, concentrated and tested as described in Materials and Methods.*

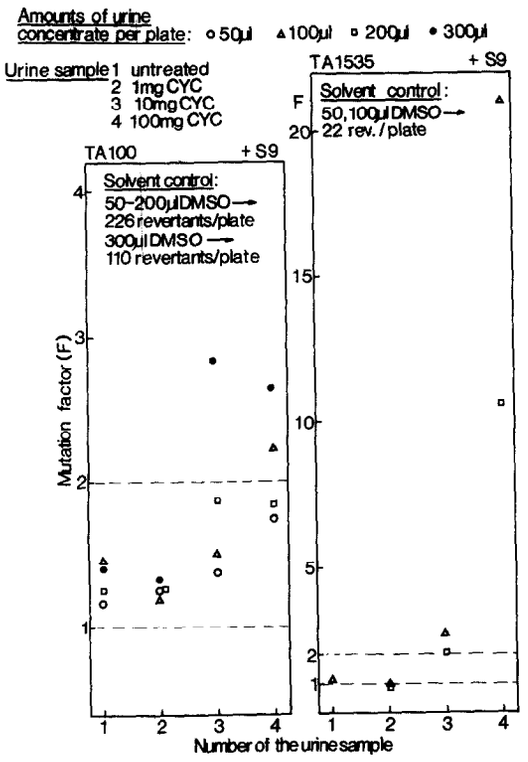

Fig. 2. Detection limit of the mutagenicity assay for cyclophosphamide (CYC) using urine samples of a patient. A patient was treated with different subtherapeutic doses of CYC. Spot urine samples were collected after the treatment and tested for mutagenic activity with S. typhimurium TA100 *and* TA1535 *with addition of rat liver homogenate. Up to* 300 µl *urine concentrate dissolved in DMSO was added per plate. The maximum amount showed slightly bactericidal effects (reduced background growth; reduced number of spontaneous revertants). For details see Materials and Methods.*

dose-dependent increase (50–200 µl urine concentrate per plate) with a maximum F of about 2. TA98 showed only a weak increase of the mutagenic activity (F was approx. 1.5 with 200 µl urine concentrate) and TA1535 was negative. With the addition of S9 all three used strains remained negative for the same working day 5, in contrast to working days 2 and 3 with maximum F values between 1.5 and 1.8 for TA98 and TA100. Maximum F values of approx. 1.5 for TA98 were also detected on the duty free day and on working day 1.

Defined exposure

Mutagenicity tests. All tested urine samples of the four nurses who prepared 10 standardized intravenous chemotherapy combinations during a relatively short period of time and with a potentially high risk of exposure were negative (TA100 and TA98; without and with activation; all F values were < 2). In addition the aqueous extracts of the air filters, worn during the test period, did not show any detectable mutagenic activity using the same strains and activation conditions (test run with preincubation).

Chemical analysis. Methotrexate: No methotrexate was detected (detection limit: 2 nmol/l) in the nurses blood samples (taken immediately at the end and 14–16 hr after the preparation of the drug solutions) and in the aqueous extracts of the air filters. *Cis*-platinum: None of the four urine samples of day 1 (no exposure) and day 3 (preparation of the drug solutions) contained any measurable amount of *cis*-platinum (detection limit: 2µg/l).

Detection limit for selected cytostatic drugs

Cyclophosphamide (CYC). Patient urine: The urine samples of a patient collected about 3 hr after treatment with subtherapeutical doses of 1, 10 and 100 mg of CYC were tested for mutagenic activity with TA1535 and TA100 (Fig. 2). After treatment with 10 and 100 mg of CYC both strains showed positive mutagenicity; TA1535 was more sensitive compared to TA100 (the mutation factor with 200 µl urine concentrate per plate was 2 and 10 in case of 1 and 10 mg CYC respectively for TA1535 and nearly 2 with both doses for TA100). The urine sample of the smallest dose (1 mg CYC) showed no induction of revertants in comparison to the control. *Direct addition to control urine*: The mutagenic activity of a urine sample with no mutagenic activity (control urine) to which 0.5–32 mg CYC were added is shown in Fig. 3. With 50 µl urine

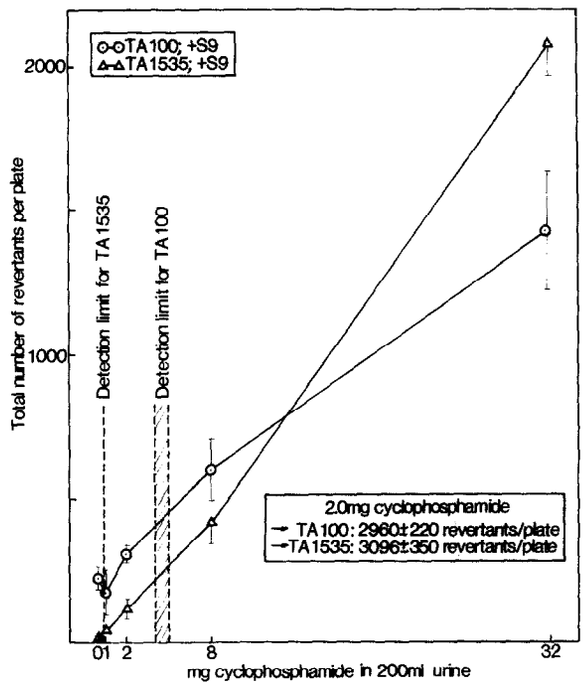

Fig. 3. Detection limit of the mutagenicity assay for cyclophosphamide (CYC) using control urine with different amounts of CYC. Different amounts of CYC were dissolved in 200 ml *urine with no mutagenic activity (control urine). The mutagenicity of urine concentrates (total numbers of revertants per plate) dissolved in DMSO* (50 µl *per plate) was determined with* S. typhimurium TA1535 *and* TA100 *with addition of rat liver homogenate following the methods described in Materials and Methods. The mutagenic activity of* 2.0 mg *CYC which was directly added to the plates is also shown.*

concentrate per plate the detection limit for TA1535 is smaller than 0.5 mg (F value 2.9 with 0.5 mg CYC) and is in the range of 2.0–8.0 mg CYC for TA100 (F approx. 1.5 for 2.0 mg CYC). A comparison with the mutagenic activity of 2.0 mg CYC which was directly added to the plates (Fig. 3) shows that practically no CYC is lost during the urine concentration.

Adriamycin (ADM). The urine samples to which 10 and 40 μg of ADM were added, yielded positive results (TA98; without the addition of S9; Fig. 4). The detection limit therefore must be in the range of 10–40 μg ADM dissolved in 200 ml control urine. With 200 μl urine concentrate in DMSO (control urine containing 10 μg ADM) a mutation factor of 1.6 was reached; with 400 μl the factor was 3.4. The recovery of ADM after the XAD-2 chromatography was nearly 100%. 2.0 μg ADM freshly diluted induced about 100 revertants per plate whereas on plates with 200 μl urine concentrate of a sample to which 10 μg A had been added (this corresponds to 1.0 μg A per plate) 60 revertants were counted.

Cis-*platinum (DDP).* The urine samples containing 0.06–1.0 mg DDP showed no mutagenic activity with TA98 and TA100 (25–200 μl urine concentrate per plate). One hundred microlitres of urine concentrate of the sample with 0.06 mg DDP should contain theoretically 6.0 μg DDP, an amount yielding a distinct mutagenic effect with TA98 and TA100. Negative results were also obtained with a urine concentrate of a patient treated with 100 mg DDP. Two hundred millilitres of undiluted urine or the same amount of samples diluted 10- or 100-times with non-mutagenic control urine was chromatographed on the XAD-2 column. DDP directly added to the plates served as a positive control: 5 μg DDP per plate led to a 4- to 6-fold increase over the control with TA98 and TA100.

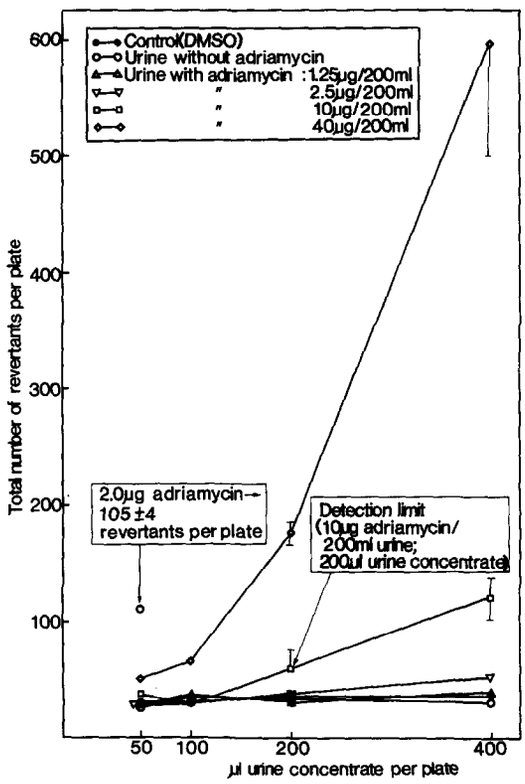

Fig. 4. Detection limit of the mutagenicity assay for adriamycine (ADM). Different amounts of ADM were dissolved in 200 ml *control urine without mutagenic activity. The direct acting mutagenicity (no addition of rat liver homogenate) of urine concentrates dissolved in DMSO* (200 μl *per plate) was determined with* S. typhimurium TA98 (*see Materials and Methods*).

DISCUSSION

In a series of consecutive studies, we were unable to demonstrate a correlation between urine mutagenicity and exposure to cytostatic drugs in a group of over 20 Swiss oncology nurses. Neither in the urine samples collected during normal working periods (spot urine and 24-hr urine) nor in 24-hr urine samples taken during and following a defined drug exposure, mutagenic activity was detected. The weak single positive effect detected in the pilot study (Fig. 1; TA100 on day 5) cannot be related to the preparation of cytostatic drugs of the corresponding day, because TA1535 which is more sensitive towards cyclophosphamide (2 g of this drug were prepared on working-day 5) was negative. Only smoking nurses excreted mutagenic urine. Corresponding to the finding of Yamasaki *et al.* [17] the mutagenic activity of smokers was restricted to TA98 (frameshift mutagens). The presence of frameshift mutagens in the urine of non-smokers (Table 2) could be related to mutagenic pyrolysates which are formed during heat-treatment of food [21, 22]. Also other authors [11, 15, 16] were not able to find a positive correlation between the occupation with cytostatic drugs and mutagenic activity in urine samples of exposed personnel, while others [4–7] presented opposite data.

There are different possible explications for these discrepancies.

1. Falck *et al* [4] who first published data showing an enhanced urine mutagenicity of nurses working with cytostatic drugs used a fluctuation assay (growth of the bacteria in suspension) instead of the plate assay in the present study for the detection of the mutagenic activity. Especially for promutagens, which have to be metabolized by the microsomal enzymes of the S9, the incubation and growth of bacteria in suspension together with the test compound and S9 increases the mutagenic effects [20]. Therefore, a fluctuation assay could be also more sensitive for the detection of urine mutagens. To test this possibility coded urine

samples of the defined exposure study (day 1 and day 3 of each of the four test persons; concentration by XAD-2 chromatography) were examined also with the fluctuation method used by Falck *et al.* [4] in the latter authors laboratory (P. Einistö at the Institute of Occupational Health, Helsinki, Finland). The following test strains were used: TA98 (+S9), TA100 (−S9), and *E. coli* WP2 uvrA (±S9). Only one sample (day 3) out of the eight tested samples was slightly positive with TA98 (+S9; possible relationship to nutritional conditions). This signifies that the negative data of the defined exposure study reported in this paper are not a result of the selection of the urine mutagenicity test method.

2. A comparison of the daily amount of different cytostatic agents prepared by personnel working in a centralized hospital pharmacy [7] with medical oncology units, investigated by the present study shows several important differences. Considerably less cytostatic drugs are normally prepared by one person in decentralized units. As an example, the following total amounts were prepared per week by eight hospital pharmacists [7]: 33 g adriamycin, 440 g cyclophosphamide, 11 g *cis*-platinum. The respective amounts in the units under investigation by the present study were at least 10 times smaller. Therefore the negative results obtained with this study might partly be a result of a smaller exposure, possibly below the detection limit. For this reason an additional experiment was carried out, during which the nurses had to dissolve a relatively high amount of five different cytostatic drugs within a short period of time without any protection. In this experiment plasma and air filter extracts (for methotrexate) as well as untreated urine samples (for *cis*-platinum) were chemically analyzed in addition to the urine mutagenicity. The fact that none of these two compounds could be detected in any sample supports the negative urine mutagenicity data, representing more indirect evidence for a lack of exposure. The limits of detection for *cis*-platinum (2 μg/l) and for methotrexate (2 nmol/l) should be low enough to detect even a percutaneous or respiratory absorption of small amounts of these compounds.

3. The negative urine mutagenicity especially in relation to the defined exposure study also raises the question whether during the XAD-treatment of the urine, mutagens might be lost. No mutagenic activity could be detected in the concentrated samples of urine to which *cis*-platinum had been added and with patient urine (treatment with 100 mg *cis*-platinum) although the pure compound was positive with TA98 and TA100 [1]. This is most probably a result of a loss of the relatively small polar DDP molecule upon the XAD-2 treatment as described by Venitt *et al.* [11]. Therefore the exposure of nursing personnel working with *cis*-platinum cannot be adequately detected by the urine-mutagenicity method of Yamasaki *et al.* [17]. In addition, the detection limit as it was determined with this routine-method for cyclophosphamide was rather high: approx. 0.5–8.0 mg for the urine to which cyclophosphamide had been added; about 10 mg in case of patient urine. Taking into account that only part of the unmetabolized compound is excreted by the renal pathway, the higher detection limit for the patient urine can be explained. But independently from the method to determine the detection limit it can be stated that the mutagenicity test procedure used for this study is not sensitive enough to detect a cyclophosphamid exposure: taking into account, that only about 10% of the compound is renally excreted in an unmetabolized form, between 5 and 80 mg of CYC had to be taken up. For adriamycin on the other hand the sensitivity of the test is higher because the compound has a stronger mutagenicity. The detection limit for adriamycin was in the range of 10 μg per 200 ml urine. Because only about 5% of the test compound is excreted unmetabolized with the urine within 7 days between 0.2 and 2.0 mg adriamycin must be completely absorbed to obtain a positive urine. This amount in fact may be reached as a result of work exposure. For other mutagenic cytostatics such as chlorambucil and melphalan (L-PAM) we calculated a detection limit which is approx. 10 mg based on the sensitivity of the mutagenicity test and the excretion kinetics. This indicates that small amounts of these drugs absorbed in an aerosol form most probably cannot be detected with the urine-mutagenicity method.

4. Another methodological problem relates to the mode of urine sampling because the excretion kinetics of the different cytostatic drugs varies from several hours to some days. Falck *et al.* [4] used spot urine collected immediately after the work shift whereas Nguyen *et al.* [7] tested 24-hr urine. Using spot urine some slowly excreted drugs (as for example adriamycin) may be missed whereas with 24-hr urine the excreted drugs are diluted. We collected 24-hr urine portions of three nurses during the routine exposure and of the four nurses taking part at the defined exposure study; for the detection of the mutagenicity following routine exposure, the morning urine and the two or more following spot urine samples of 24 nurses were pooled. We speculated that the drugs excreted slowly would be found in the morning urine and that the cytostatics with faster excretion kinetics would be collected in the spot urine at the same day.

Considering the different methodological problems of the used urine-mutagenicity assay (also the

fact that certain nutritional factors as well as smoking can enhance the urine-mutagenicity) we feel that the measurement of urine mutagenicity is not sensitive enough to detect very small amounts of cytostatic drugs potentially absorbed or ingested during work in the form of aerosols. In agreement with Venitt *et al.* [11] we suggest that this test system should be replaced by more sensitive methods such as refined direct chemical analyses.

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