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# FURTHER ENVIRONMENTAL FACTORS CAUSING VARIATIONS OF SCE FREQUENCIES

## SUMMARY

The frequencies of spontaneously occurring sister chromatid exchanges (= SCE) were determined in control persons, persons exposed to very low doses of ionizing radiation and employees of a rubber factory. Besides smoking habits and the usage of oral contraceptives, background ultraviolet (= UV) radiation seems to exert the most pronounced effect on SCE levels in control persons.

## KEY WORDS

Sister chromatid exchanges, radiation exposure, occupational exposure, nitrosamines, cigarette smoking, oral contraceptives, ultraviolet radiation.

# VERÄNDERUNGEN DER SPONTANTEN SCE FREQUENZ DURCH UMWELTFAKTOREN

## ZUSAMMENFASSUNG

Die Frequenz der spontan auftretenden Schwesterchromatidaustausche (= SCE) wurde in den Lymphozyten von Kontrollpersonen, strahlenexponierten Personen und Arbeitern der Gummiindustrie untersucht. Neben den Rauchgewohnheiten und der Einnahme oraler Kontrazeptiva scheint vorallem die ultraviolette Umgebungsbestrahlung eine starke Wirkung auf die Anzahl der beobachteten SCEs auszuüben.

## SCHLÜSSELWORTE

Schwesterchromatidaustausche, berufliche Exposition, niedere Strahlendosen, Nitrosamine, Rauchen, orale Kontrazeptiva, ultraviolette Strahlung.

# FURTHER ENVIRONMENTAL FACTORS CAUSING VARIATIONS OF SCE FREQUENCIES

Studies in SCEs have been increasing in number, eversince the first demonstration of SCEs as indicators of the effect of chemical mutagens (Latt, 1974). Besides identifying potentially mutagenic chemicals, SCEs have been used to monitor exposure levels in occupationally exposed workers (Sorso et al., 1982; Stolley et al., 1984). Nevertheless controversy exists on the normal range and the significance of a raised frequency of SCEs. In an extensive study Soper et al. (1984) reported on SCE scores in 479 control persons and demonstrated an association of SCEs with reader, smoking, sex and age.

In our own laboratory we investigated exposed and unexposed persons for SCE frequency. Throughout the study the same experimental conditions were used; all evaluations were done by two readers, and their readings of each testperson pooled for statistical analysis. Thus inter-laboratory and reader-dependent differences can be excluded.

Soper et al. found by multiple observatioons, a within-person standard deviation for SCEs for 1.7/cell. In correct experimental settings this within-person standard deviation will be especially important. Parameters like sex, age, smoking habits and differences according to readers' or laboratories' practices - which already proved to influence the observed number of SCEs - should be taken into account before starting the experiment, and occupationally exposed individuals screened for all those influences.

In the present study the attempt was made to find a possible explanation for the individuals' variation of SCEs. Multiple examinations of the test persons showed relatively constant SCE

levels, except with very high SCE levels of persons who returned from vacation. In addition, three test persons were chosen who gave their personal consent to a whole-body UV-irradiation: a similar increase in SCE levels could be demonstrated, thus indicating that environmental UV radiation is most likely to affect SCE frequencies of lymphocytes to the same extent as an occupational exposure in a rubber factory.

Thus the significance of a raised level of SCEs as an indicator of damage caused by occupational exposure to potentially mutagenic substances has to be reevaluated.

MATERIAL AND METHODS

Blood was collected in sterile Vacutainers (Greiner) and cultures set up within two hours after sampling. The culture medium consisted of 10 ml RPMI 1640 (Gibco), 20% FCS, L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100µg/ml), PHA (Wellcome-Burroughs, 1%), 20µM BrUdR and 0.75 ml whole blood. Cultures were protected from light and incubated at 37°C for 70 hours. Colcemid (0.1µg/ml) was added, and two hours later cells were treated hypotonically (0.75 M KCl), fixed (methanol/glacial acetic acid 3:1), washed and slides prepared. For demonstration of SCEs, a modifyed fluorescence-plus Giemsa technique was used (Perry & Wolff, 1974). For each determination at least 20 well spread metaphases were scored.

Statistical analysis:

Rank statistics according to Kruskal & Wallis were performed, to study the effect of nitrosamine exposure and smoking in the rubber factory group, and differences according to UV-exposure in the probands of the 1st control group. In addition, pair-wise comparisons of groups according to Scheffé and pair-wise comparisons of ranks according to Nemenyi were calculated, unless the analysis of variance did not show any significant difference.

Probands:

78 control persons without any known exposure to radiation or chemicals were compared with persons occupationally exposed to low levels of ionizing radiation and personnel of a rubber industry, mainly exposed to volatile nitrosamines. An anamnesis for health status, medication, medical X-ray exposure, drinking and smoking habits was performed. Only healthy persons without foregoing medical treatment or X-ray examinations were allocated for the experiments. 10 of the test persons gave their consent to monthly blood sampling (October to January and July to September). Of further 9 probands blood could only be taken at irregular intervals within the duration of the two years. Control persons were employees of the Austrian Research Centre Seibersdorf, staff of two Viennese hospitals and unexposed personnel of an Austrian rubber factory, with ages ranging from 20 to 43 years.

Radiation exposed persons came from the same Research Centre working at the Austrian ASTRA reactor, with ages varying from 30 to 47 years. Their mean monthly dose, as measured by TL-dosimetry, varied from 1.4 to 9.6 $10^{-4}$ Gy/month.

Probands exposed to chemical pollutants were 22 employees of a rubber factory working at sites where significant concentrations of N-nitrosodimethylamine and N-nitroso-morpholine had been measured (Stehlik et al., 1984). Those latter test-persons were compared with 18 unexposed controls working in the same factory. Their ages ranged from 22 to 57 years.

UV-B exposure:

The solarium (Hanon 6030) consisted of 3 UV-B lamps, each of 150 W; irradiations were performed in a distance of 150 cm for 5 min every second day. The UV dose, actually applied, was about 100J/m² per irradiation.

RESULTS

As indicated by table 1, SCE scores for control persons ranged from 5.0 to 12 SCEs/cell. The mean value obtained for all non-smokers was 6.8 ($\pm$ 0.8) (tab. 1).

TABLE 1: SCE levels observed for persons with radiation or chemical exposure, and control persons without any exposure.

| Type of exposure | total no of test pers. | female | male | smoker | | | non-smoker | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No | SCE/cell | range | No | SCE/cell | range |
| no evident exposure | 79 | 35 | 44 | 20 | 8.1$\pm$1.2 | 6.9-12.0 | 59 | 6.8$\pm$0.8 | 5.0-8.5 |
| >1.4.10$^{-4}$ Gy month | 16 | - | 16 | no smokers available | | | 16 | 6.9$\pm$2.8 | 4.8-8.3 |
| volatile nitrosamines | 22 | - | 22 | 11 | 11.1$\pm$1.4 | 8.3-13.0 | 11 | 10.2$\pm$1.4 | 8.8-12.8 |

By further analysis of control values of female testpersons we recognized a significant effect of oral contraceptives on the number of SCEs. 17 women taking combined contraceptives (ethenylestradiol/levanergestrel, ethenyl estradiol/levanergestrel/lynestrenol and norethisteron/mestranol) were compared with 17 controls, and SCE levels proved significantly different (tab. 1a).

TABLE 1a: SCEs in lymphocytes of female, unexposed persons.
1 = oral contraceptive users
2 = no oral contraceptives

| | 1 | 2 |
|---|---|---|
| mean | 8.4 | 6.3 |
| standard deviation | 1.3 | 0.7 |
| number of persons tested | 17 | 17 |

rank statistics: P = 0.000
signif.level = 5%, diff. signif.

Exposure to very low doses of ionizing radiation does not affect the level of spontaneously occurring SCEs, though it exerts some influence on the frequency of inducible SCEs, (Tuschl et al., 1983) (tab. 2).

TABLE 2: SCE levels observed in lymphocytes of radiation exposed persons and controls.

| No of test persons (only males, non-smokers) | unexposed (exposure $<1.4.10^{-4}$Gy/ month) 21 | exposed (exposure $>1.4.10^{-4}$Gy/ month) 16 |
|---|---|---|
| no of metaphases scored | 538 | 428 |
| m (SCE/cell) | 6.8 | 6.9 |
| s.d. | 2.9 | 2.8 |

difference not significant

Exposure in the rubber factory raises the number of SCEs found in peripheral lymphocytes, an effect that proved statistically significant by comparing exposed and unexposed persons from the same factory (tab. 3).

TABLE 3: SCEs in lymphocytes of nitrosamine exposed and control persons.
significance level = 5%

| | smoker exposed | non-smoker exposed | smoker non exposed | non-smoker non exposed |
|---|---|---|---|---|
| m (mean) | 11.1 | 10.2 | 8.3 | 7.6 |
| s (standard dev.) | 1.4 | 1.4 | 0.8 | 0.7 |
| n (number of test persons) | 11 | 11 | 9 | 10 |

P <2% factor A (smoker/non smoker)
P <0.1% factor B (exposed/non exposed)

Despite the significant difference between exposed and unexposed workers in the present investigation, we wanted to examine the constancy and reliability of control values. In a long term study lymphocytes of ten persons were investigated repeatedly with blood taken in Oct, Nov, Dec, Jan, July, Aug and Sept. Unfortunately, some of the test persons withdrew during the series of experiments. Further nine test persons were investigated in irregular intervals during two years.

Interestingly, intraindividual variations were rather high (tab. 4 and 5), a fact that could neither be attributed to methodological differences nor to different evaluations by individual readers.

TABLE 4: SCE levels observed in lymphocytes of 10 unexposed control persons, blood sampled in October to January and July to September.

| Person no | SCE's per cell | | | | | | |
|---|---|---|---|---|---|---|---|
| | Oct | Nov | Dec | Jan | Jul | Aug | Sept |
| 1 | 6.6 | 6.3 | 5.4 | 5.0 | 6.4 | 5.8 | o |
| 2 | 7.0 | 6.5 | 7.0 | 8.5* | o | o | o |
| 3 | 6.4 | 5.8 | 5.2 | 5.3 | 6.3 | 5.6 | 6.8 |
| 4 | 8.9* | 6.9 | 7.1 | 5.8 | 6.6 | 6.3 | 6.2 |
| 5 | 7.7 | 7.6 | 6.3 | 6.5 | 6.2 | 5.3 | 6.0 |
| 6 | 6.7 | 7.0 | 6.4 | 7.0 | 6.5 | 6.2 | 7.0 |
| 7 | 6.6 | 6.7 | 6.4 | 6.9 | 6.4 | 7.1 | 6.8 |
| 8 | 5.4 | 5.8 | 5.4 | 5.8 | 5.9 | 5.3 | 9.3* |
| 9 | 5.6 | 5.7 | 5.0 | 5.7 | 5.8 | o | o |
| 10 | 6.9 | 7.3 | 6.8 | 6.6 | 6.2 | 5.4 | 5.0 |

o withdrew during experiment
* blood taken after vacation

TABLE 5: SCE levels observed in lymphocytes of 9 unexposed control persons, blood taken in irregular intervals.

| Person no | SCE's per cell | | | | | |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th sampling |
| 1 | 6.1 | 7.6 | 8.8* | 6.9 | 7.9 | 7.9 |
| 2 | 6.2 | 7.1 | 7.2 | 8.6* | | |
| 3 | 6.9 | 5.7 | 7.8+ | 5.4 | 7.7 | 6.2 |
| 4 | 6.1 | 6.0 | 7.1 | 7.5 | 8.9* | |
| 5 | 7.6 | 6.4 | 9.1* | 7.6 | | |
| 6 o | 7.2 | 8.7 | 8.9* | 7.9 | 7.1 | |
| 7 o | 8.5 | 8.2 | 7.1 | 8.0 | 8.1 | |
| 8 o | 8.1 | 8.1 | 7.4 | 8.2 | | |
| 9 o | 8.4 | 10.1 | 8.7 | | | |

+ herpes simplex infection
o oral contraceptive user
* blood sampled after vacation

At comparing anamneses of the test persons it became evident that increases of SCE frequencies were regularly found with persons who just returned from vacation. Most of them spent their holiday at the seaside, one person went skiing to the mountains. Only in one case (tab. 2) a herpes simplex infection could be correlated with an increase of the SCE frequency. The statistical evaluations by variance analysis revealed a significant effect of vacation on the SCE level observed: In 11 test

persons the highest value of SCEs was observed immediately after vacation and was significantly different from all other values obtained before or more than two weaks after holidays (tab. 6).

TABLE 6: SCE values in lymphocytesof 11 unexposed control persons. several observations in irregular intervals.
Factor A: before vacation (1)/ after vacation (2)
significance = 5%

| | 1 | 2 |
|---|---|---|
| mean | m 6.9 | 9.0 |
| standard deviation | s 2.6 | 3.0 |
| n (number of metaphases) | n 1928 | 316 |

P <0.1. difference highly significant

One of the most potent inducers of SCEs in vitro is UV-irradiation (Wolff et al., 1974; de Weerd-Kastelein et al., 1977). Since all probands spent their vacation in areas with a high background UV-irradiation and all of them agreed to have basked in the sun for several hours per day, UV-irradiation seemed to be the main course of the observed increase in SCEs. In a special experiment we achieved the informed consent of three test persons to whole body UV-irradiation by a solar lamp. Blood was sampled immediately before irradiation, after 5 and after 10 irradiations. Five times 100J/m² already induced a significant increase in spontaneously occurring SCEs; obviously this increase could no more be stimulated by a further bunch of five irradiations (tab. 7).

TABLE 7: Total body UV-B-irradiation.

| Test persons | | SCE/cell before irradiation | after 5 irradiations | after 10 irradiations |
|---|---|---|---|---|
| 1 (oral contraceptive user ) | o | $8.1^{+}3.0$ | $10.4^{+}3.3$ | $9.6^{+}3.4$ |
| 2 | o | $7.5^{+}2.9$ | $8.5^{+}2.9$ | $8.8^{+}1.0$ |
| 3 | o | $7.8^{+}2.1$ | $9.0^{+}2.8$ | $8.8^{+}2.3$ |

Non-parametric H-test:
Significance level: 5%
factor A: before irradiation (1)/after UV-B irradiation (2)

| | 1 | 2 |
|---|---|---|
| mean | 7.8 | 9.2 |
| standard deviation | 2.7 | 2.9 |
| number of metaphases | 63 | 126 |

P <0.1%, difference highly significant

## DISCUSSION

The results of the present investigations are in agreement with previous studies on SCE frequencies with regard to the range for control persons (5 - 17.5 SCE/cell, Soper et al., 1984); the effects of smoking habits (Lambert et al., 1982; Obe et al., 1982), the use of oral contraceptives (Husum et al., 1982; Murthy and Prema, 1979, 1983) and exposure to nitrosamines (Sorsa et al., 1982). Unlike Soper et al. we could only demonstrate a minute effect of sex, which was statistically not significant. Differences according to age could not be considered, since the persons investigated in the present study were within a relatively narrow age range. Repeated examinations of the same test persons revealed a striking effect of environmental conditions on SCE frequencies. Probably, the most important source of elevated SCE levels in healthy persons is environmental UV-radiation. While occupational exposure to low doses of ionizing radiation ($1.4 - 9.6.10^{-4}$ Gy/month) does not exert any significant effect on the number of spontaneously occurring SCEs, UV-B whole-body irradiation stimulates the formation of SCEs. A significant

fraction of UV-radiation has the capacity to penetrate into mammalian skin, even into cutaneous vessels, thus a remarkable number of lymphocytes can be affected by whole body irradiation. The effect of UV-radiation on immunologic responses is well established (for ref. see Elmets & Bergstresser, 1982). Klein & coworkers (1983) investigated the effect of whole-body UV-B irradiation on DNA synthesis, DNA repair and nucleoid sedimentation characteristics, and found an increase in the rate of DNA synthesis and repair replication and an enhancement of DNA breaks, presumably during repair of UV-induced pyrimidine dimers. Unlike those studies, the present investigation points to an impedement in the velocity of DNA synthesis or the retardation of the cell cycle after UV-B irradiation: While in controls, 72 hours after PHA stimulation 50% second and 30% third mitoses were observed, still 80% second and only 10% third mitoses were found in irradiated persons (results not shown).

Since little is known about the biological significance of SCEs, interpretation of a raised SCE level must be done very carefully. Nevertheless, an increase of SCEs is an indeniable indirect measure of mutation resulting from DNA damage, and SCE analysis should have its application in the prevention of hereditary disease and cancer in public health work. Environmental pollutants caused by life-style, i.e. smoking, UV irradiations, etc. are to some extent easier to avoid than pollutants produced by industries, and besides issuing orders on the industrial management, a lot of information of the public is the need of the moment.

REFERENCES

de Weerd-Kastelein, E.a., W. Keijzer, G. Rainaldi and D. Bootsma (1977) Induction of sister chromatid exchanges in Xeroderma pigmentosum cells after exposure to ultraviolet light, Mut.Res. 45, 253-261.

Elmets, C.A. and P.R. Bergstresser (1982) Ultraviolet radiation effects on immune processes, Photochem.Photobiol. 36, 715-719.

Goyanes-Villaescasa, V.J., M. Ugarte and A. Vazquez (1977) Sister chromatid exchanges in babies treated by phototherapy, Lancet, Nov. 19, 2, 1084-1085.

Husum, B., H.C. Wulf and E. Nieburg (1982) Normal sisterchromatid exchanges in oral contraceptive users, Mut.Res. 103, 161-164.

Klein W., F. Kocsis, H. Altmann, R. Günther und W. Rauschmeier (1983) Über die Beeinflussung des DNA-Metabolismus von Lymphozyten aus dem Blut von Ganzkörper UV-bestrahlten Probanden. OEFZS-Ber.No. A-0390, BL-414/83.

Lambert, B., A. Lindblad, K. Holmberg et al. (1982) The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. In: Sister Chromatid Exchange. J. Wolff (ed.), J. Wiley & Sons, N.Y., 1982, pp. 149-182.

Latt, J.A. (1974) Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by Mitomycin C. Proc.Natl.Acad.Sci.(USA), 71, 3162-3166.

Murthy, P.B.K. and K. Prema (1979) Sister chromatid exchanges in oral contraceptive users, Mut.Res. 68, 149-152.

Murthy, P.B.K. and K. Prema (1983) Further studies on sister chromatid frequency in users of hormonal contraceptives, Mut.Res. 119, 351-354.

Obe G., H.-J. Vogt, S. Madle, A. Fahring and W.D. Heller (1982) Double blind study on the effect of cigarette smoking on the chromosomes of human peripheral blood lymphocytes in vivo, Mut.Res. 92, 309-319.

Perry, P. and S. Wolff (1974) New Giemsa method for the differential staining of sister chromatids, Nature (Lond.) 251, 156-158.

Schwartz, A.L., F.S. Cole, F. Fiedorek, et al. (1978) Effect of phototherapy on sister chromatid exchange in premature infants, Lancet, July 15, 2, 157-158. Soper, K.A., P.D. Stolley, J. Galloway et al. (1984) Sister chromatid exchange (SCE) report on control subjects in a study of occupationally exposed workers, Mut.Res. 129, 77-88.

Sorsa, M., J. Maki-Paakkanen and H. Vainio (1982) Identification of mutagen exposures in the rubber industry by the sister chromatid exchange method, Cytogenet.Cell Genet. 33, 68-73.

Stehlik, G., O. Richter, H. Tausch, H. Wihlidal, H. Begutter und H. Klus (1984) Arbeitsplatzüberwachung hinsichtlich flüchtiger Nitrosamine, OEFZS-Ber.No. A-0543, BL-465/84.

Stolley, P.D., K.A. Soper, S. Galloway et al. (1984) Sister chromatid exchanges in association with occupational exposure to ethylene oxide, Mut.Res. 129, 89-102.

Tuschl, H., R. Kovac and H. Altmann (1983) UDS and SCE in lymphocytes of persons occupationally exposed to low levels of ionizing radiation. Health Phys. 45, 1-7.

Wolff, S., J. Bodycote and R.B. Painter (1974) Sister chromatid exchanges induced in Chinese hamster cells by UV irradiation of different stages of the cell cycle: The necessity for cells to pass through S, Mut.Res. 25, 73-81.

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