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Originalarbeit

# The Sensitivity of the in Vitro Cytokinesis-Blocked Micronucleus Assay in Lymphocytes for Different and Combined Radiation Qualities

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**Purpose:** The dose-response relationship and the relative biological effectiveness (RBE) for the induction of micronuclei in lymphocytes was analyzed after irradiation in vitro with a 6-MeV neutron beam that was followed by 240-kV X-rays. The dose range of the combined exposure comprised 1 to 3 Gy. For reference, the dose-effect relationships found after X-ray (0.5 to 5 Gy)- and neutron (0.5 to 4 Gy) exposure applied separately are presented. The possibility of an interaction between the 2 radiation qualities is investigated by the method of isobole calculation termed "envelope of additivity".
**Methods:** Micronuclei were analyzed in PHA-stimulated, cytokinesis-blocked human lymphocytes.
**Results:** The dose-response relationships for the micronucleus frequencies induced by the neutron irradiation, as well as by the mixed exposure, were linear. A saturation effect was indicated after neutron doses higher than 3 Gy. After low LET exposure the dose-response curves were describable by a linear-quadratic model. For neutron-induced micronucleus frequencies, RBE-values of 2 to 3 and for the combined exposure RBE values of 1.5 to 2 were calculated for a range of effect of 0.5 to 1.5 micronuclei/binucleated lymphocyte. No indication was found for an interaction between the damage induced by X-rays and that produced by neutrons under our experimental conditions.
**Conclusions:** These studies demonstrate a clear dependence of micronucleus induction on radiation quality and emphasize the usefulness of the micronucleus assay in biological dosimetry, also in cases in which high LET radiation or a mixed beam is involved as the radiation source.

**Key Words:** Cytokinesis-blocked micronucleus assay · Lymphocytes · Neutrons · RBE · Additivity

**Die Sensitivität des In-vitro-Zytokinesis-Block-Mikronukleus-Assays für verschiedene Strahlenqualitäten und deren Kombination**

**Fragestellung:** Die Mikronukleusinduktion in Lymphozyten wurde nach In-vitro-Bestrahlung mit 6-MeV-Neutronen (0,5 bis 4 Gy), 240-kV-Röntgenstrahlung (0,5 bis 5 Gy) bzw. einer Kombination dieser Strahlenqualitäten (1 bis 3 Gy Gesamtdosis) untersucht. Anhand der Dosis-Wirkungs-Beziehungen für die einzelne und kombinierte Anwendung beider Strahlenarten wurde die relative biologische Wirksamkeit (RBW) für Neutronen bzw. für die Kombination von Neutronen und Röntgenstrahlen ermittelt. Mit Hilfe einer Isobolenkalkulation („envelope of additivity") wurde die Möglichkeit einer Interaktion zwischen den beiden Strahlenqualitäten analysiert.
**Methode:** Die Auswertung der Mikronuklei erfolgte in PHA-stimulierten humanen Lymphozyten, deren Zytokinese durch Cytochalasin B inhibiert worden war.
**Ergebnisse:** Für die Neutronen- wie auch für die kombinierte Bestrahlung ergaben sich jeweils lineare Dosis-Wirkungs-Beziehungen. Ein Sättigungseffekt stellte sich für Neutronendosen oberhalb von 3 Gy ein. Nach Niedrig-LET-Bestrahlung folgte die Dosis-Wirkungs-Beziehung dem linear-quadratischen Modell. Ein Vergleich der für die Induktion von 0,5 bis 1,5 Mikronuklei pro binukleärer Zelle isoeffektiven Dosen ergab für die Bestrahlung mit Neutronen RBW-Werte von 2 bis 3 und für die kombinierte Bestrahlung Werte von 1,5 bis 2 unter den von uns gewählten experimentellen Bedingungen. Es konnte kein Hinweis auf eine Interaktion zwischen den durch die Röntgen- bzw. Neutronenstrahlung induzierten Schäden gewonnen werden.
**Schlußfolgerungen:** Die Untersuchungen zeigen eine deutliche Abhängigkeit der Mikronukleusbildung von der Strahlenqualität. Die Ergebnisse verdeutlichen, daß der Einsatz des Mikronukleusassays in der biologischen Dosimetrie auch dann sinnvoll ist, wenn als Strahlenquelle eine Hoch-LET-Strahlung bzw. eine Mischung aus Hoch- und Niedrig-LET-Strahlen vorliegt.

**Schlüsselwörter:** Mikronukleustest · Binukleäre Lymphozyten · Neutronen · RBW · Additivität

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A considerable number of in vitro experiments with low LET radiation has been performed, revealing a clear dose dependence for induced micronucleus frequencies, thus emphasizing the suitability of the micronucleus assay for use in biological dosimetry [e. g., 8, 20, 26] and as a promising candidate in predictive assay research [7]. Meanwhile, the data base has become quite complex (for review, see [16]). After in vivo incorporation of the low-LET emitter radioiodine, micronuclei served reliably to estimate the absorbed dose, even in a dose range of 50 to 300 mGy [29]. However, there are instances in which, for example, a radiologist has to deal not only with low LET radiation but also with exposures due to high LET radiation qualities. The effect of high LET radiation on micronucleus induction in binucleated lymphocytes has been studied by a number of authors, and RBE values have been reported [3, 9, 11, 26, 28]. Micronucleus induction by combined high and low LET radiation, however, has not been studied extensively until now, although there is a definite chance of accidental exposure of this type in the nuclear industry. Therefore, we studied the dose-effect relationship, the values of RBEs and possible interactions between the 2 used radiation qualities with regard to the induction of micronuclei after in vitro exposure of lymphocytes to a combined exposure with identical absorbed doses of 6-MeV neutrons and 240-kV X-rays, respectively.

## Material and Methods

### *Donors of Lymphocytes*

Blood from 5 different healthy donors was obtained by cubital vein puncture. The donors were males and females of ages ranging from 31 to 40 years.

### Irradiation Conditions

Blood containing about 150,000 lymphocytes was resuspended in 1 ml RPMI 1640 medium. It was irradiated at room temperature before the addition of phytohemagglutinin (PHA).
For low LET irradiation, 240-kV X-rays were used (0.5 mm Cu filter, dose rate 1 Gy/min). The blood was irradiated in multiwell culture plates with doses of 0.5 to 5 Gy.

For high LET irradiation, neutrons produced by the beryllium reaction in the Cyclotron Essen (CV 28) were used [21]. The mean neutron energy is 6 MeV. The gamma-component of around 10% (complement to the 90% neutron component) was taken into account when the neutron dose range of 0.5 to 4 Gy was determined. The dose rate was 0.3 Gy/min. During exposure, the blood samples were placed at 2 cm depth in a phantom material (A 150), which is characterized by a hydrogen density equivalent to mammalian soft tissue for neutrons. The phantom was placed with its entrance surface at 125 cm distance from the neutron source. The radiation field was 20 × 20 cm.

The combined exposure with doses of 1 to 3 Gy consisted of equal absorbed doses of neutrons and X-rays, respectively. For practical reasons – because it was not possible to transport the samples within a shorter time from the neutron source to the X-ray machine – the time interval between neutron and subsequent X-ray exposure was 30 min. To avoid repair, the cells were kept on ice. PHA was added immediately after X-irradiation. Proliferation analysis showed that the delayed PHA stimulation did not change the proliferation capacity of the lymphocytes as compared to samples stimulated directly.

### *Lymphocyte Culture*

An aliquot of blood from cubital vein puncture with about 150,000 lymphocytes was transferred into 1 ml of RPMI 1640 medium (Gibco) containing 25% fetal calf serum and antibiotic/antimycotic solution (Gibco). After irradiation, 2.5% PHA (phytohemagglutinin; Gibco), was added to the culture medium. The cells were cultivated in multiwell tissue culture plates (Falcon) at 37 °C and 5% $CO_2$ in an incubator (Heraeus) [8].

### *Induction of Binucleated Cells*

Cytochalasin B (Sigma) at a final concentration of 5 µg/ml was added 44 hours after addition of PHA. Lymphocytes were harvested 24 to 26 hours after the addition of cytochalasin B: Erythrocytes were lysed by hypotonic treatment with a 125-mM KCl solution. The lymphocytes were then fixed by suspension in a mixture of ethanol and acetic acid (5.5 : 1). The fixation step was repeated until the supernatant was clear. The cell suspension was dropped on clean slides and air-dried.

### *Scoring of Micronuclei*

Slides were stained with Giemsa. Micronuclei were usually counted in 1000 binucleated cells per donor using a magnification of 500 ×.

### *Statistical Analysis*

Curve fitting was done by the least squares method, and the quality of the fit was tested by an analysis of variance. The possible interaction between the X-rays and neutrons in the mixed exposure was analyzed according to the mathematical model of the "envelope of additivity" [23].

## Results

Human peripheral lymphocytes of 5 donors have been exposed in vitro to 240-kV X-rays (0.5 to 5 Gy), 6-MeV neutrons (0.5 to 4 Gy) and to a combined exposure of both radiation qualities with equal absorbed doses (1 to 3 Gy).

Figure 1 shows the weighted means of the dose-response studies. For the analysis of the dose-response curves based on weighted means, the micronucleus frequencies were fitted by least squares weighted regression. The coefficients for the fits are given in Table 1 (y = micronucleus frequency; c = spontaneous micronucleus frequency; a = coefficient of the linear term; b = coefficient of the quadratic term, D = dose).

### *Dose-Response Relationship after X-Ray Exposure*

For X-ray-induced micronuclei (Figure 1), the linear-quadratic model ($y = c_x + aD_x + bD^2_x$) provided the best fit of the data, although a linear fit is mathematically also possible (Table 1). This is in agreement with earlier results [8].

### *Dose-Response Relationship after Neutron Exposure*

After irradiation with neutrons (Figure 1), we observed for each of the donors that the yield of micronuclei was higher as compared to X-rays. The frequency of micronuclei increased

linearly with dose up to 3 Gy ($y = c_N + aD_N$). At 4 Gy, no significant further increase is observed. This could indicate a saturation effect, which is reflected mathematically by the negative b-coefficient obtained for the linear-quadratic fit (Table 1). This plateau-like dose response correlated with a reduced proliferation ability of the cells determined as the ratio of binucleate cells to the total of bi- and mononucleate cells [27], which was diminished after irradiation with 4 Gy to 20% compared with the proliferative activity of the lymphocytes after exposure to 3 Gy. If the neutron data are fitted to the linear-quadratic fit by excluding the 4-Gy value, no significant negative b-value was found, indicating that saturation of the neutron-induced micronucleus frequency was obtained only for the highest neutron dose applied (4 Gy).

*Dose-Response Relationship after Combined Exposure*

The mixed exposure (Figure 1, Table 1) consisted of equal absorbed doses of neutrons and X-rays, respectively. For technical reasons, the exposure to different radiation qualities was spaced by a time interval of 30 min. The micronucleus frequency induced by the combined exposure was again higher than after X-rays alone and could best be described by a linear dose-response relationship ($y = c_c + aD_c$). Application of the linear-quadratic model yielded a negative b-coefficient, which is smaller than the b-term for the pure neutron exposition. The 95% confidence limits of the b-value include zero, indicating that the b-term can be neglected, so that a linear dose-response relationship can be accepted.

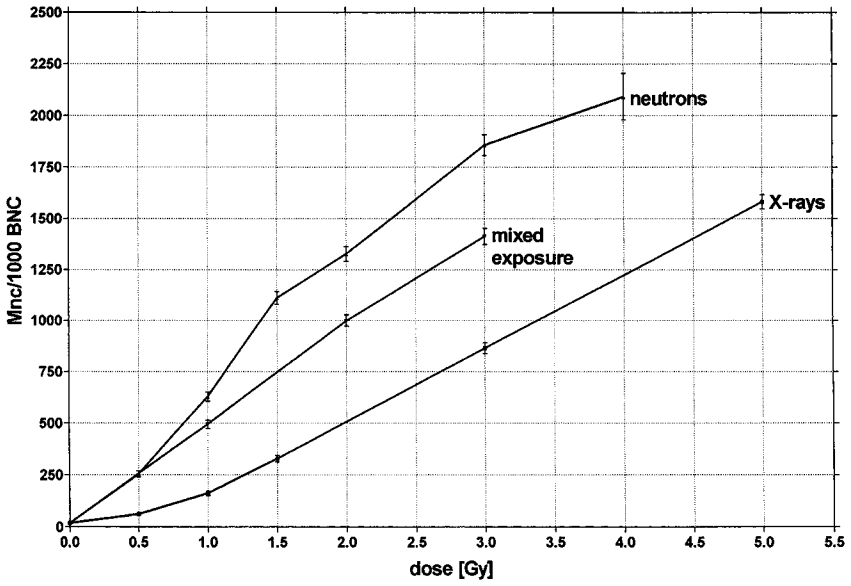



Figure 1. Comparison of the dose-response curves describing the in vitro micronucleus induction in lymphocytes after exposure to 240-kV X-rays, to 6-MeV neutrons and to a combined irradiation composed of equal absorbed doses of 6-MeV neutrons and 240-kV X-rays (e. g., a dose of 3 Gy means: 1.5 Gy neutrons + 1.5 Gy X-rays). The bars indicate the 95% confidence limits of the weighted means from 5 individuals.

Abbildung 1. Dosis-Wirkungs-Kurven für in vitro induzierte Mikronuklei in Lymphozyten nach 240-kV-Röntgen-, 6-MeV-Neutronenbestrahlung und nach einer Exposition, die sich aus einer Kombination von jeweils 50% Energiedosis Röntgen- und Neutronenstrahlung zusammensetzt. Die Balken geben den 95%-Vertrauensbereich des aus fünf Versuchen bestimmten Mittelwertes der Einzelstichproben an.

| Radiation quality | c* | a* | b* |
|---|---|---|---|
| a) Curve fit linear-quadratic ($y = c + aD + bD^2$) | | | |
| X-rays | -0.027 (-0.157/0.103) | 0.213 (0.065/0.361) | 0.022 (-0.006/0.051) |
| Neutrons | -0.064 (-0.264/0.136) | 0.850 (0.603/1.097) | -0.076 (-0.135/-0.017) |
| X-rays + neutrons | 0.014 (-0.320/0.349) | 0.514 (-0.023/1.051) | -0.015 (-0.187/0.156) |
| b) Curve fit linear ($y = c + aD$) | | | |
| X-rays | -0.095 (-0.226/0.037) | 0.326 (0.274/0.379) | |
| Neutrons | 0.105 (-0.152/0.361) | 0.546 (0.427/0.665) | |
| X-rays + neutrons | 0.029 (-0.070/0.129) | 0.468 (0.415/0.521) | |

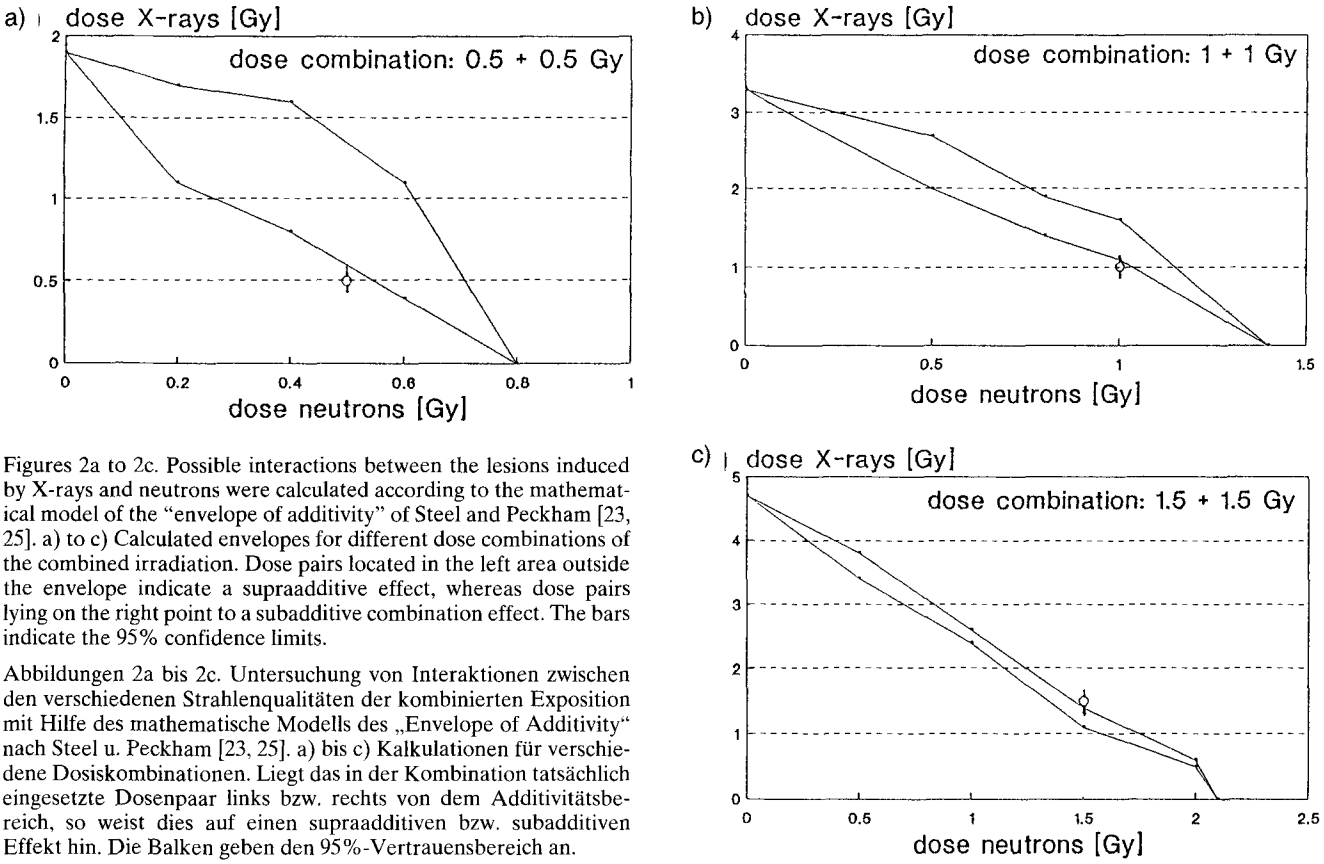
Table 1. Fitted coefficients for X-ray, neutron and combined exposure calculated from the values of the weighted mean dose-response data from 5 individuals, respectively. The c-value represents the mathematically calculated spontaneous micronucleus frequency, the a- and b-coefficients refer to the linear and quadratic term of the dose-response equations, respectively. *The values in parentheses are the 95% confidence limits.

Tabelle 1. Mathematisch ermittelte Gleichungsparameter für die Dosis-Effekt-Beziehung nach Röntgen-, Neutronenbestrahlung sowie nach kombinierter Exposition (c = spontane Mikronukleusfrequenz; a bzw. b = Koeffizient des linearen bzw. des linear-quadratischen Terms). *Die Zahlen in Klammern geben den 95%-Vertrauensbereich an.

| Effect | dose [Gy] | | | RBE | |
|---|---|---|---|---|---|
| | X-rays | neutrons | combined exposure | neutrons | combined exposure |
| 0.5 MN/BN | 2.0 | 0.7 | 1.0 | 2.9 | 2.0 |
| 1.0 MN/BN | 3.4 | 1.4 | 2.0 | 2.4 | 1.7 |
| 1.5 MN/BN | 4.8 | 2.3 | 3.1 | 2.1 | 1.5 |

Table 2. Doses needed for the induction of specific effects and corresponding RBE values for the neutron exposure and the combined irradiation. The RBE-values are calculated by dividing the X-ray dose needed for the induction of a specific effect by the isoeffective neutron or mixed exposure dose.

Tabelle 2. Vergleich isoeffektiver Dosen und entsprechender RBW-Werte nach Neutronenbestrahlung und kombinierter Exposition. Die RBW-Werte wurden als Quotient aus der für einen bestimmten Effekt benötigten Röntgendosis und der isoeffektiven Dosis der Neutronen- bzw. Kombinationsexposition ermittelt.




Figures 2a to 2c. Possible interactions between the lesions induced by X-rays and neutrons were calculated according to the mathematical model of the "envelope of additivity" of Steel and Peckham [23, 25]. a) to c) Calculated envelopes for different dose combinations of the combined irradiation. Dose pairs located in the left area outside the envelope indicate a supraadditive effect, whereas dose pairs lying on the right point to a subadditive combination effect. The bars indicate the 95% confidence limits.

Abbildungen 2a bis 2c. Untersuchung von Interaktionen zwischen den verschiedenen Strahlenqualitäten der kombinierten Exposition mit Hilfe des mathematische Modells des „Envelope of Additivity" nach Steel u. Peckham [23, 25]. a) bis c) Kalkulationen für verschiedene Dosiskombinationen. Liegt das in der Kombination tatsächlich eingesetzte Dosenpaar links bzw. rechts von dem Additivitätsbereich, so weist dies auf einen supraadditiven bzw. subadditiven Effekt hin. Die Balken geben den 95%-Vertrauensbereich an.

The comparison of the dose-response curves after exposure with X-rays, neutron irradiation and the combined exposure shows that the frequency of micronuclei and the shape of the dose-response curves appear to correlate clearly with the contribution of high LET to the exposure.

*RBE-Values*

To compare the effects of the various radiation qualities, values for the RBE of neutron and combined irradiation were calculated. Due to the different shapes of the dose-response curves, no single RBE value for neutrons or combined exposure can be given with X-rays as reference. Consequently, RBE values for different effect levels have been calculated by dividing the absorbed X-ray dose by the absorbed neutron or combined exposure dose needed for the induction of a specific micronucleus frequency. Table 2 shows that the RBE decreases with increasing neutron or combined doses. Within the observed effect range (0.5 to 1.5 micronuclei/binucleated lymphocyte), the RBE-values ranged between 2 and 3 for neutrons and 1.5 and 2 for combined irradiation.

*Statistical Distribution*

In order to test the conformity of micronucleus frequencies observed after different radiation qualities to the Poisson distribution, values of the $u$-parameter were calculated and are presented in Table 3. This $u$-parameter equals one standard normal deviate if the distribution is Poissonian. Negative values of $u$ indicate underdispersion and positive values overdispersion of the measured distribution compared with the Poisson distribution. If the magnitude of $u$ exceeds ± 1.96 the under- ($< -1.96$) or overdispersion ($> +1.96$) is significant on the 95% level.

The spontaneous micronucleus frequency showed a small degree of overdispersion. Overdispersion was found also for the micronucleus frequencies induced by the different radiation qualities up to a dose of 1 Gy. After neutron exposure, the amount of overdispersion was higher as compared to X-rays alone or combined irradiation. Beyond 1 Gy, the overdispersion observed after X-ray exposure was changed into a Poissonian distribution at 1.5 Gy and to an underdispersion at $> 1.5$ Gy. In contrast, underdispersion was apparent for doses $> 1$ Gy for neutrons alone and mixed exposure.

*Possible Interactions between X-Rays and Neutrons*

Possible interactions between the X-rays and neutrons in the combined exposures were calculated according to the mathematical model of the "envelope of additivity" [23, 25]. This model allows the decision whether the observation of a combined effect either above or below the sum of the single effects is merely due to the shape of the dose-response curves or to true interaction between both agents under study. This model suggests that dose pairs located in the left area outside of a calculated envelope of additivity indicate a supraadditive effect, whereas dose pairs lying on the right point to a subadditive effect.

As Figure 2 shows, we observed no statistically significant deviations from the additive model. After a dose combination of 0.5 Gy neutrons and 0.5 Gy X-rays, we found a

| Dose | BN | MN/BN | Dispersion Index | $u$ | Statistical distribution |
|---|---|---|---|---|---|
| Before irradiation | | | | | |
| 0 Gy | 4373 | 0.017 | 1.07 | 3.30 | Overdispersion |
| b) After X-ray irradiation | | | | | |
| 0.5 Gy | 4992 | 0.062 | 1.05 | 2.50 | Overdispersion |
| 1.0 Gy | 4978 | 0.162 | 1.04 | 2.00 | Overdispersion |
| 1.5 Gy | 5000 | 0.328 | 0.99 | -0.50 | Poissonian dispersion |
| 3.0 Gy | 4886 | 0.865 | 0.89 | -5.44 | Underdispersion |
| 5.0 Gy | 5071 | 1.581 | 0.78 | -11.08 | Underdispersion |
| c) After neutron-irradiation | | | | | |
| 0.5 Gy | 4319 | 0.254 | 1.24 | 11.16 | Overdispersion |
| 1.0 Gy | 4730 | 0.628 | 1.19 | 9.24 | Overdispersion |
| 1.5 Gy | 4309 | 1.111 | 0.90 | -4.64 | Underdispersion |
| 2.0 Gy | 3759 | 1.327 | 0.86 | -6.07 | Underdispersion |
| 3.0 Gy | 2877 | 1.856 | 0.73 | -10.24 | Underdispersion |
| 4.0 Gy | 650 | 2.091 | 0.62 | -6.85 | Underdispersion |
| d) After combined irradiation | | | | | |
| 0.5 + 0.5 Gy | 4866 | 0.495 | 1.10 | 4.93 | Overdispersion |
| 1.0 + 1.0 Gy | 4738 | 0.999 | 0.93 | -3.41 | Underdispersion |
| 1.5 + 1.5 Gy | 3606 | 1.413 | 0.77 | -9.77 | Underdispersion |

Table 3. The statistical distribution of micronuclei among lymphocytes after exposure with the different radiation qualities ($u$-test). (BN = number of analyzed binucleated lymphocytes; MN/BN = mean of micronuclei/binucleated lymphocyte; Dispersion Index = variance/mean of micronucleus-frequency; $u$ = parameter of the test.)

Tabelle 3. Prüfung der statistischen Verteilung der Mikronuklei auf Poisson-Verteilung. (BN = Gesamtzahl ausgewerteter Zweikerner; MN/BN = mittlere Mikronukleuszahl pro Zweikerner; Dispersion Index = Varianz/mittlere Mikronukleusfrequenz; $u$ = Prüfgröße.

slight trend to a supraadditive and after 1 Gy + 1 Gy or a 1.5 Gy + 1.5 Gy combination a nearly additive effect.

## Discussion

At present, there is no biological indicator that can be used for monitoring dose limits of occupational radiation exposure. This indicator can definitely be found in the field of physical dosimetry. In radiation accidents involving higher doses, several biological indicators are available [15]. One of the most prominent prerequisites of a biological indicator used in dose reconstruction is its ability to estimate radiation dose for many people within a short time. Most such studies are based on the analysis of dicentric chromosomes. At present, the micronucleus assay using the cytokinesis-block method is discussed as a simpler cytogenetic dosemeter, a less expensive and less time-consuming alternative to the traditional scoring of dicentric chromosomes [16]. Difficulties exist for assessing radiation doses of past exposures because of the temporal decline of cells containing "unstable" aberrations. A very attractive method as an alternative means for retrospective biodosimetry is the chromosome painting of "stable" aberrations such as symmetrical translocations and insertions [e. g., 24]. However, in cases when an acute whole-body exposure has occurred and the screening of many victims is necessary, micronuclei are particularly useful because an automatization of the assay is possible and considerable progress has already been made in this direction [4].

After exposure to low LET radiation, the linear-quadratic model is most frequently used to describe the dose-response relationship for micronucleus induction in lymphocytes [e. g., 8, 12, 20]. As micronuclei are derived mainly from acentric fragments after radiation exposure [e. g., 6], one should expect a dose-response relationship with a marked linear component. Micronuclei, however, are not only produced by this one-track mechanism, but also by 2-track actions, which become more important at higher doses of low LET radiation. Thus, the inclusion of a quadratic term (starting at about 1 Gy) is both biologically and statistically justifiable (Figure 1, Table 1) as already described before [8].

In contrast to the effect of X-rays on micronucleus induction, the effect of high LET radiation is not well documented. After exposure to 6-MeV neutrons, we found a linear shape of the dose-response relationship up to 3 Gy, suggesting a predominance of one-track events [1, 13]. Exceeding 3 Gy, a tendency to saturation was indicated in our experiments, which is also reflected by a significantly negative b-value derived from fitting to the linear-quadratic model (Figure 1, Table 1). Only one single report in the literature [11] found a marked dose-squared component for the dose-response equation describing the micronucleus induction in lymphocytes by fast-neutron irradiation. Other investigators who studied the effect of high LET radiation on micronucleus expression reported a linear dose dependence of the induction of micronuclei in lymphocytes for high LET radiation [3, 10, 26]. Such a linearity in the dose response was described not only for lymphocytes, but also for the exposure of the early stages of mouse embryos [13, 19] and of hepatocytes [18].

The indication of a saturated micronucleus induction after exposure to higher doses (Figure 1) could be attributed to different phenomena: At higher neutron doses, a reduction of cell proliferation of highly damaged cells occurs and less binucleated cells are formed. Although a sufficient number of binucleated lymphocytes has been scored, one could argue that particularly cells with multiple aberrations have already died before or during the first karyokinesis. Such heavily damaged cells escape from scoring as micronucleated binucleates. Furthermore, the majority of radiation-induced micronuclei originates from acentric fragments. Since with densely ionizing

radiations substantially higher numbers of acentrics will be induced than with sparsely ionizing radiation qualities, many of the micronuclei scored at higher neutron doses can be assumed to contain more than one acentric; in another study of the authors, the inclusion of 2 fragments derived from different chromosomes into the same micronucleus was shown by FISH-analysis (unpublished results). This effect of several aberrations resulting in only one single micronucleus scored may also contribute to a possible saturation effect.

Additionally, this latter mechanism may explain the underdispersion of observed micronucleus frequencies encountered with increasing radiation doses. This hypothesis is underlined by the fact, that the appearance of underdispersion after exposure to different radiation qualities showed different patterns of dose dependence: the higher the LET, the lower the dose after which underdispersion was observed (Table 3). In contrast to our observations, the micronucleus distributions obtained by Vral et al. [26] after gamma and neutron irradiation with doses similar to those used in the present study showed an overdispersion compared with the Poisson distribution. However, their results displayed a decrease of overdispersion for higher doses of both gamma- and neutron irradiation.

The plateau-like course of the dose-response curve in the higher dose range and the corresponding negative coefficient for the quadratic term of the dose-response equation yielded after weighted least squares analysis for the linear-quadratic model was also found by Huber et al. [9]. Vral et al. [26], who used a neutron beam with comparable mean energy (5.5 MeV) to the one used by us (6 MeV), emphasized that no saturation effect occurred in their experiments. However, saturation was indicated in our study by the 4 Gy data point, while Vral et al. [26] did not use doses higher than 3 Gy.

The RBE values calculated for the neutron exposure ranged between 2 and 3 within the observed effect area (dose range of 0.7 to 2.5 Gy for neutrons and 2.0 to 4.8 Gy for X-rays; see Table 2). This result is comparable with the data published by Bilbao et al. [3] for alpha-particles and Ono et al. [18; binucleated hepatocytes] for fast neutrons. The RBE values published by Vral et al. [26], whose neutron beam has a comparable mean energy to that in the present study, are almost identical with the RBE values of this study.

In radiation accidents, in which high LET radiation is involved, one has to expect a certain contribution by low LET radiation. One investigation dealing with mixed irradiation determined the combined effect of alpha-particles ($^{238}$Pu) and X-rays on micronucleus expression in rat lung epithelial cells [5]. So far, we are aware of only one report dealing with the effect of an approximately mixed beam on micronucleus induction in human lymphocytes, in which the gamma-contamination of fission spectrum neutrons amounts to 24% [10]. The combined exposure used in our experiments was composed of the same absorbed doses of neutrons and X-rays, respectively. In agreement with the results of Brooks et al. [5] and Huber et al. [10], the dose-response relationship found by us after combined exposure could be best described by a linear dose-response relationship (Figure 1, Table 1). If the RBE values for the mixed exposure are estimated from the dose-effect curves published by Huber et al. [10], slightly higher values result for the low-dose range, whereas, in the higher dose range, the RBE is quite comparable with our results (Huber et al. [10]: for 0.5 MN/BN, RBE = 3; for 1.0 MN/BN: RBE = 1.6; this paper: for 0.5 MN/BN, RBE = 2; for 1.0 MN/BN, RBE = 1.7). The differences can be probably attributed to the higher neutron component within the mixed beam used by Huber et al. [10] (76%) as compared to our beam which was composed of equal absorbed doses of neutrons and X-rays.

Brooks et al. [5] who worked on the rat lung epithelial cells exposed to $^{238}$Pu/280 kV X-rays, found for each investigated dose of the mixed exposure (0.8 to 3 Gy) a higher frequency of induced micronuclei than expected from the sum of the single effects. The authors postulated an interaction of the lesions which were induced by the different radiation qualities with a synergistic effect. A synergistic effect of simultaneous exposure to Co-60 gamma rays and Po-210 alpha rays with regard to cell survival, which should correlate with the expression of micronuclei [2, 22], was described by Murthy et al. [17] for yeast cells. In contrast, our results showed no obvious deviation from the additive model, which may be due to the different cell system or high-LET source used by us. Furthermore, the time interval between the 2 different radiation qualities in our experiments may have lead to a reduction of a potential interaction effect by repair-mechanisms. However, due to the similarity of the underlying damage mechanism, interaction between different types of ionizing radiation is expected to be, in general, of the iso-additive type. Thus, the one type of radiation substitutes for a certain dose of the other radiation type [25].

In conclusion, the results reveal a clear sensitivity of the micronucleus dosemeter for different radiation qualities. As the comparison of the weighted mean dose-response curves after X-ray, neutron and combined exposure show (Figure 1), the slope of the dose-response curves, and consequently the amount of induced micronuclei correlate clearly with the contribution of high LET to the exposure: X-rays with the lowest, the combined exposure with an intermediate and the neutron irradiation with the highest efficiency in inducing micronuclei.

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