

Chromosomal Aberrations a Biological Dosimeter for Flight Personnel¹

Horst Kuni

M.D., University Professor, Clinical Nuclear Medicine,
Med. Centre of Radiology, Philipps-Universität Marburg, 35033 Marburg/Lahn

<http://staff-www.uni-marburg.de/~kuni/>

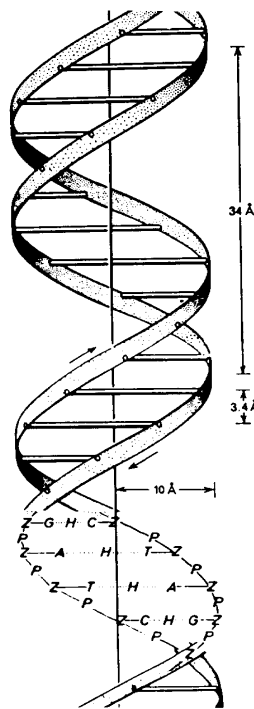
h.kuni@mail.uni-marburg.de

To begin with a short introduction in the use of certain alterations of chromosomes of human lymphocytes as a quantitative and very specific indicator of a radiation burden and therefore as a "biological dosimeter".

The human lymphocytes, a subgroup of the white blood cells, are circulating with the blood and also are staying for longer periods extravascularly in organs, in the system of lymphatic vessels and mainly

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in the lymphatic organs, the lymph nodes and the spleen. Normally they are in a stage without cell di-



vision (G_0 -phase).

Fig. 1: Model of the structure of DNA
 Z: deoxyribose A: adenine T: thymine
 P: phosphate G: guanine C: cytosine
 H: hydrogen bridge
 (from Laskowski 1981)

In the nucleus of the cell the DNA, deoxyribonucleic acid, exists as a long molecular chain in the shape of a double helix like a twisted rope-ladder (Fig. 1). The cords of the rope-ladder consist of a backbone of sugar (deoxyribose) and phosphate molecules. The steps connecting the two cords are two nucleic acids linked by a bridge of hydrogen: adenine - thymine or guanine - cytosine, representing the genetic code.

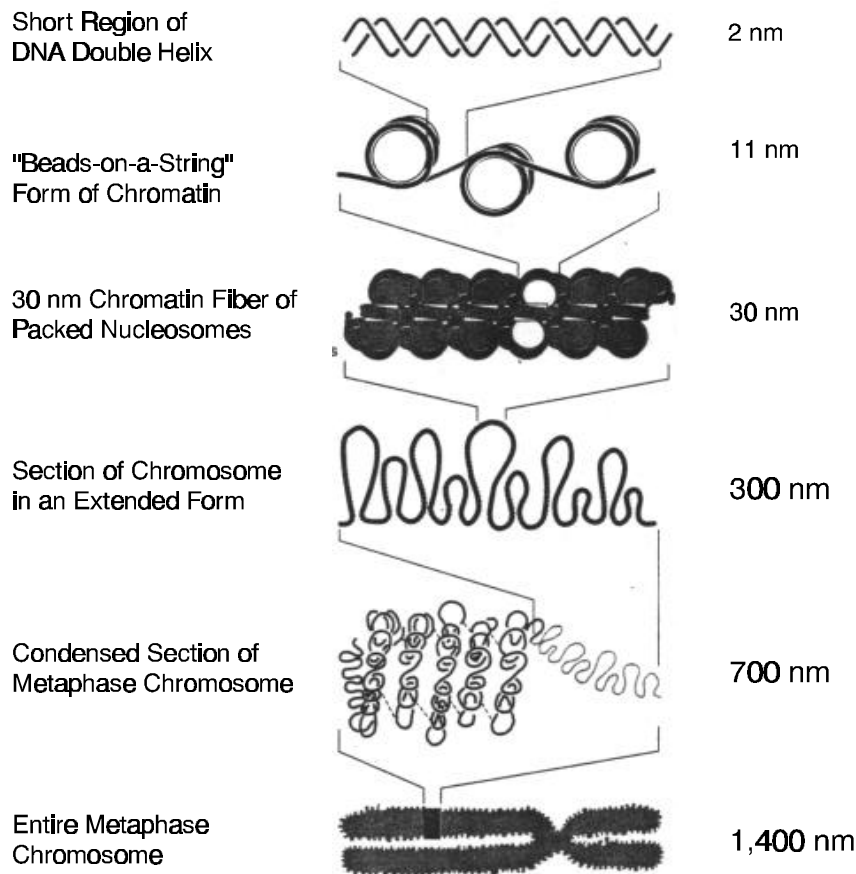


Fig. 2: Model of chromatin packing showing some of the many orders of chromatin packing postulated (from Alberts et al. 1994)

This chain is coiled two times around a little body building a nucleosoma (Fig. 2). The nucleosomes are attached to another building a chromatin fiber. This condensed structure is folded in loops. A certain part of the chain has a specialized function and is termed centromer.

A deposition of a large amount of energy in a short distance, termed clustogenesis, is able to cause a double break of the helix. Mostly the break will be repaired during about two hours.

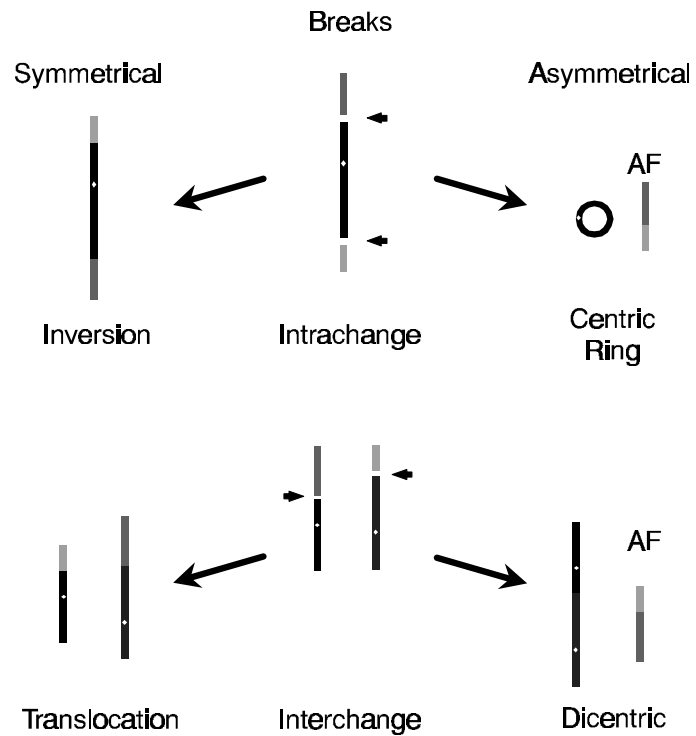


Fig. 3: Diagram of exchanges of chromosomal fragments

But, if the repair fails, the break persists and builds a fragment (Fig. 3). The production of multiple breaks in the critical space of time for repairing in a narrow neighbourhood gives the chance for new connections between the fragments. Two lesions in the same chain can lead to an intrachange: A symmetrical one with inversion of the sequence or an asymmetrical one forming a ring with the centromer and a fragment without the centromer. Two lesions in two various chains can lead to an interchange: A symmetrical one with translocation forming to new chains each with a centromer or an asymmetrical one forming a chain with two centromer, a dicentric, and a fragment without centromer, an acentric fragment (AF).

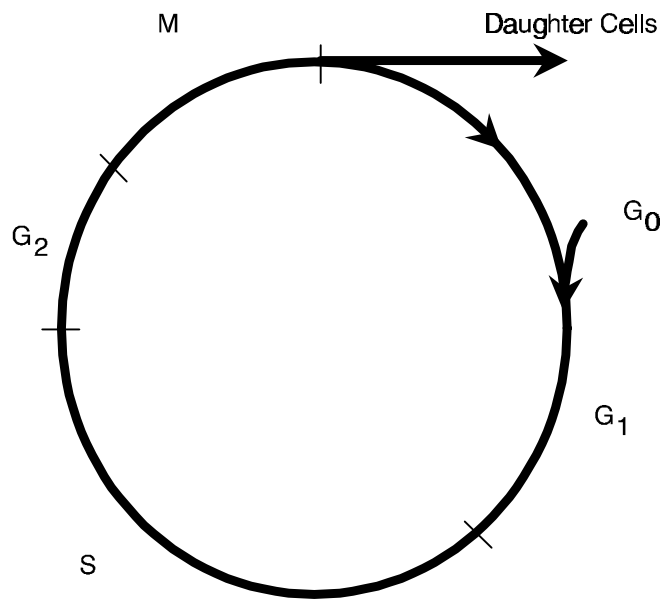


Fig. 4: Cell division cycle

G: gap phases

S: DNA synthetic phase

M: mitosis

If the cell is stimulated to enter the cell dividing cycle, in the case of lymphocytes e.g. by an immunological stimulus, the protein synthesis increased and the cell volume will be enlarged, the cell is in the G_1 -phase (Fig. 4).

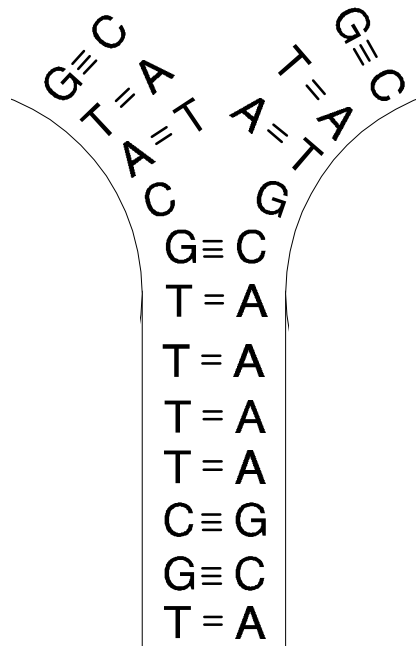


Fig. 5: Schematic diagram of DNA synthesis

The cell now moves to the S-phase, the stage of synthesis of DNA (Fig. 5). In the S-phase the two chains are separated at the hydrogen bridges. Each nucleotide is completed by attaching the complementary nucleic acid. Finally on each of the new chains a new backbone is synthesized. After a short rest, termed G_2 -phase, the cell enters the M-phase, in somatic cells termed mitosis.

In the prophase of the mitosis the looped chains begins a spiralisation. This further condensation is completed in the metaphase with a spiralisation of first and second order (Fig. 2, p. 3).

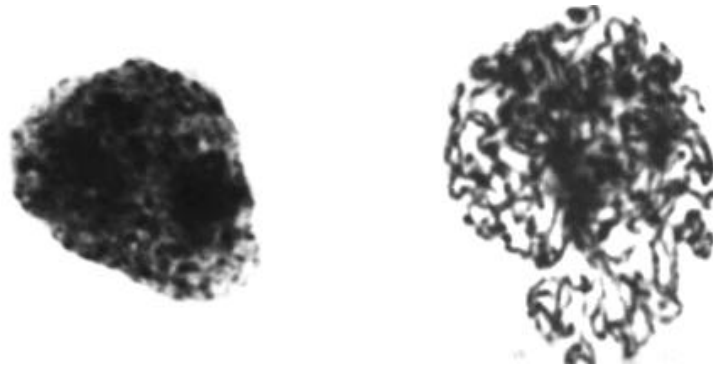


Fig. 6: Interphase (left) and prophase (right) in the light microscope
(Photos: G. Obe, K. Sperling, H.J. Belitz, from Laskowski 1981)

The chain of DNA becomes visible in the light microscope (Fig. 6). The membrane of the nucleus begins to break down. After this prophase the condensation of the chromosomes continues.



Fig. 7: Metaphase (left) and anaphase (right) in the light microscope (ref. 6, p. 7)

The chromosomes become shorter and a double structure becomes visible. The chromosomes are arranged in an equatorial plane (Fig. 7). Each is composed of two rod-like parallel structures termed chromatids, attached to each other at the centromer. In this metaphase two sets of individual chromosomes can be identified, in human 2×23 , and defects of their structure, the aberrations, can be recognized. In the next phase, termed anaphase, a spindle mechanism, attached to the chromosomes at the kinetophore of the centromer, separates each chromosome in two, mostly identical, daughter chromosomes.



Fig. 8: Telophase in the light microscope (ref. fig. 6, p. 7)

In the final phase, termed telophase, the chromosomes begin to decondense. The DNA is despiralized. New nuclear membranes appear. The cell dividing cycle is completed by the final division of the binucleated parent cell in two mononucleated daughter cells (Fig. 8).

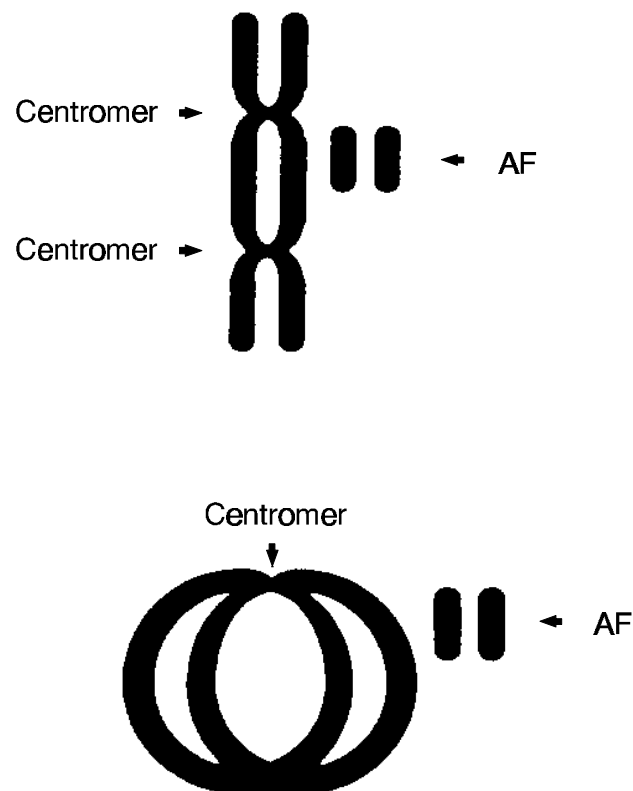


Fig. 9: Schematic diagram of chromosomal aberration types in the metaphase

A fragment as a result of the double chain break, described above, was doubled in the S-phase also and does not possess a centromere. Mostly it is not separated during the anaphase and is completely found in one of the two daughter cells. In the metaphase the described aberrations look also as double structures (Fig. 9).

Multiple hits in one nucleus can be followed by more than one interchange and the formation of a tricentric chromosome or multicentric chromosomes of higher order. This kind of aberration, the cumulation of more than one dicentric in one cell and centric rings are indicators of a clustogenesis by high densely ionizing radiation with a linear energy transfer (LET) of more than $3.5 \text{ keV}/\mu\text{m}$, e.g. by a neutron.

To investigate for chromosomal aberrations some millilitres of blood are withdrawn together with heparin preventing coagulation in a sterile syringe. The blood is cooled and carried to the laboratory as soon as possible. The lymphocytes, of interest are the little lymphocytes with origin from the thymus, the T-cells, are isolated and incubated in a cell culture for about two days. They were activated by exposure to an extract of beans, phytohaemagglutinine (PHA), and stimulated to enter a cell dividing cycle. After 48 hours the majority of the about five percent of dividing cells are in the first mitosis, M1.

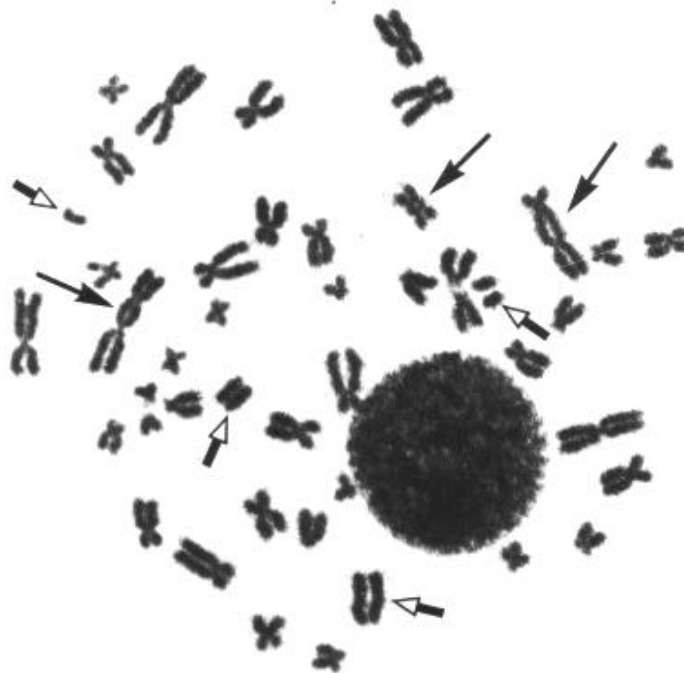


Fig. 10: Dicentric chromosomal aberrations (full arrows) and acentric fragments in the metaphase of a lymphocyte (empty arrows), 52 h after irradiation in the G_0 -phase with 3 Gy X-ray

(Photo: G. Mindek, from Fritz-Niggli 1988)

Finally the culture is treated for a few hours with colcemid, a colchicine-derivative, a spindle poison to accumulate cells in the metaphase. The cells are now treated with a hypotonic solution for swelling up the metaphases, fixed, spread on glass microscope slides and dried. The slides are screened by an automatic metaphase finder which stored the coordinates of each metaphase. The human observer retrieves the coordinates from the computer storage and evaluates the metaphases for chromosome aberrations (Fig. 10).

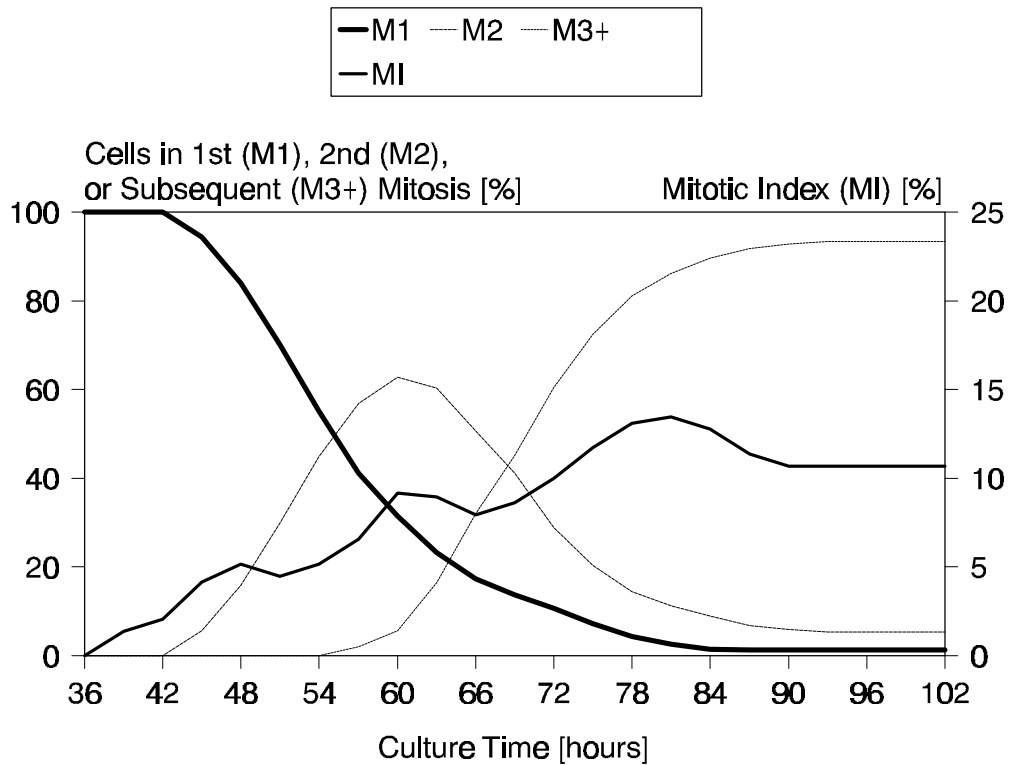


Fig. 11: Mitotic frequency (MI) of PHA-stimulated lymphocytes and relative proportion of cells in first (M1), second (M2), third and later (M3+) mitoses in relation to the duration of blood culture
(data from Bender et al. 1987)

Some cells have very short cell cycle times and are after 48 hours already in the second mitosis, M2 (Fig. 11).

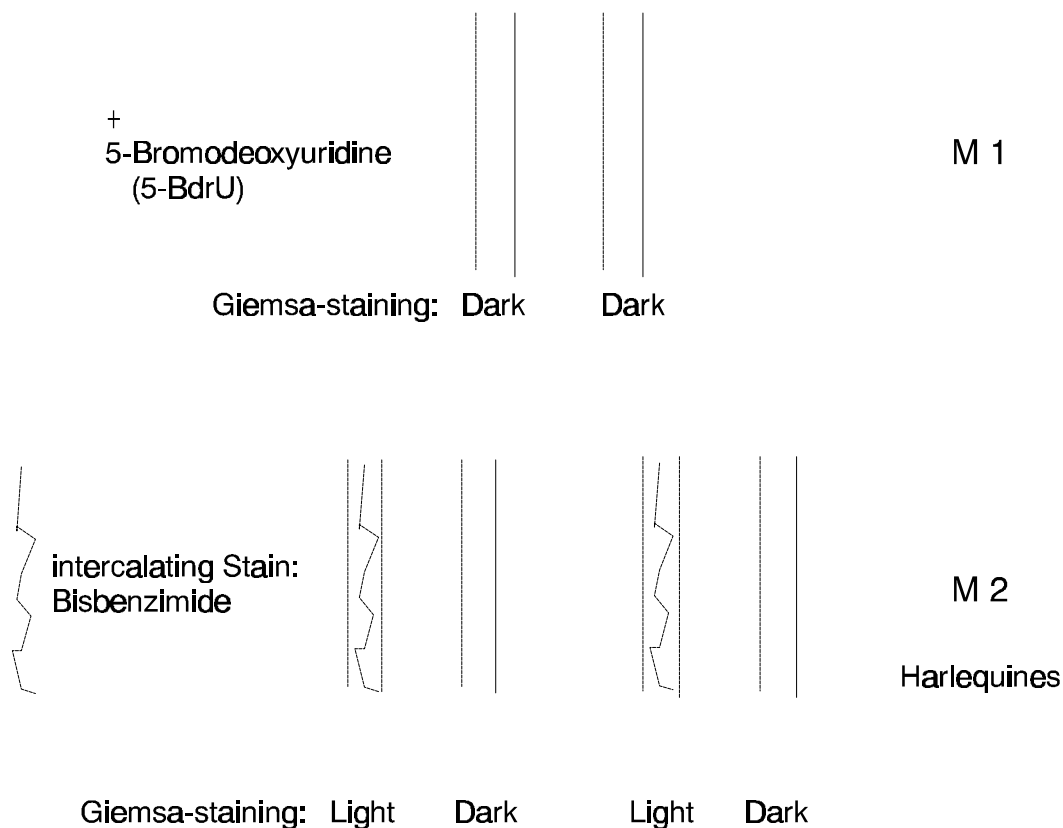


Fig. 12: Schematic diagram of the differential staining method for differentiation of mitosis of various stages

To discriminate the first mitosis from mitoses of a higher order, 5-bromodeoxyuridine is added to the cell culture medium. This substance is incorporated in the DNA in the S-phase preceding each mitosis. After a second S-phase in this medium the chromosomes contain one chromatid, which has both chains build up with the artificial building stone. An intercalating staining, bisbenzimidazole, can be attached to these chromatids. After Giemsa-staining these chromatids look not so dark as the others. This picture is called Harlequin (Fig. 12). Only metaphases with chromosomes presenting two dark chromatids, M1 cells, are scored. This is important because during mitosis about the half of dicentric are lost, termed unstable aberrations. Ignoring this differentiation of M1-cells gives false low results.

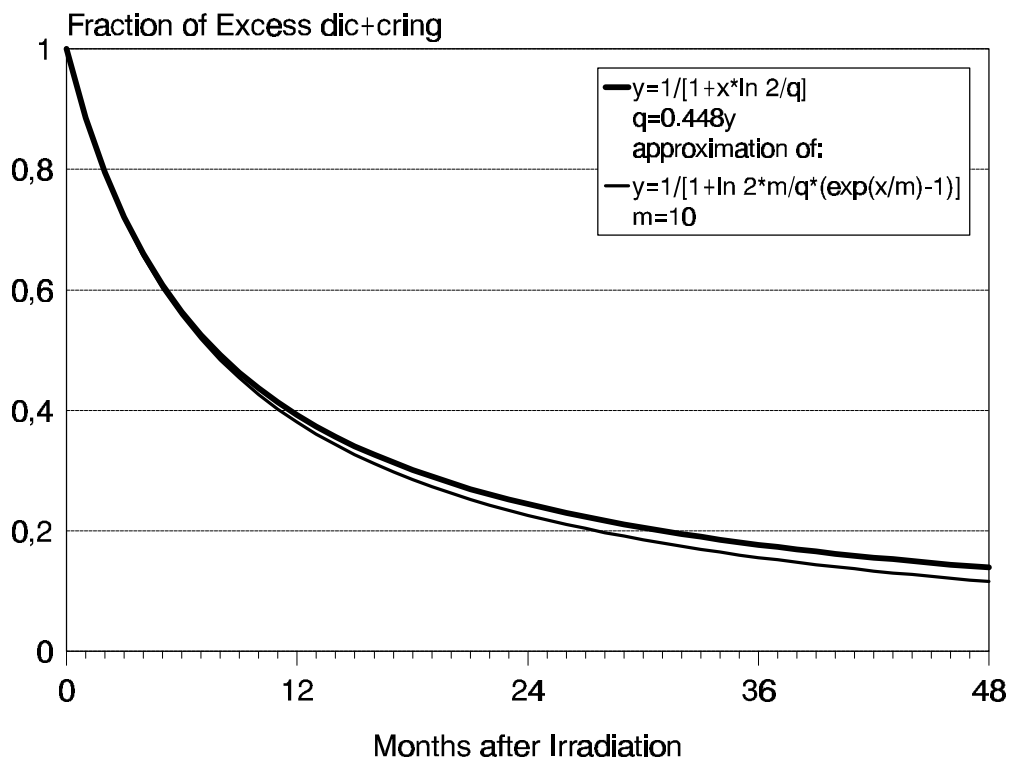


Fig. 13: Decline of the frequency of chromosomal aberrations after a single radiation burden (Formulas: Bauchinger et al. 1989)

Also in vivo there is a disappearance of unstable chromosomal aberrations due to this process and additionally due to death of the lymphocytes after a mean life span of about ten years. The time course of the frequency of dicentrics and centric rings, the decline, is a complex function like an exponential curve with a continuous increasing half time (Fig. 13). According to Bauchinger et al. [1989] in the first years the decline can be approximated by a time-hyperbolic model.

The spontaneous frequency of 0.3 dicentrics per 1,000 M1 is the equilibrium of an continuous generation of chromosomal aberrations, nearly completely by radiation from natural and artificial sources, mainly medical radiation, and the elimination procedure described just now. A single radiation burden leads to an increase of dicentrics approaching back to the spontaneous frequency with the decline.

A continuous elevation of radiation dose, as it is typical in some occupations, causes an elevation of the equilibrium frequency proportional to the doses, provided

- that the spontaneous frequency of dicentrics is exclusively caused by ionizing radiation and
- the radiation of the occupational environment has the same biological effectiveness in producing dicentrics.

Important reasons prove that the first assumption is valid [Hoffmann, Schmitz-Feuerhake 1993]. In a short interaction time ab about 120 min., in which the effectiveness decreases with a short half time of 15 min., two times a double chain break must be induced in a short distance of 10-100 nm. This needs two times the energy of about 100 eV, an energy which is one order of magnitude over the range of ordinary chemical reactions. Thus deposition of energy is the typical result of a penetration of the nucleus by ionizing radiation. With the exception of some so called radiomimetic chemicals, clustogens, the majority of chemical substances which are able to impair the DNA in such manner without cell killing cause only breaks of one chromatid resulting from a single chain break.

The second assumption certainly is not valid in the case of radiation burden to flight personnel. The main part of the dose results from neutrons with a much higher relative biological effectiveness than gammarays and electron rays which cause the spontaneous frequency of dicentrics.

Only neglecting this fact it is possible to calculate a dose using the method of Traut [Traut 1990]. According to Traut the dose per year of a continuous radiation exposure in relation to the dose of the background radiation can be calculated as the relation of the frequency of dicentrics found in the investigated person to the spontaneous frequency.

I will try building up a calibration curve for the biological dosimeter. I use a sensitivity of $4.11 \cdot 10^{-5}$ dicentrics per mGy X-rays, derived as an unweighted mean from the literature of the eighties published from laboratories working with the differential staining method (Tab. 1). Using the decline model of Bauchinger in the case of chronic exposure I integrate the time course to 2.38 years of equivalent effectiveness to a single burden (Integration up to ten years using the time-hyperbolic model, thereafter the complex formula of Bauchinger, ref. Fig. 13, p. 13). By multiplying both figures I get the gradient of the calibration curve (Fig. 16, p. 18).

Tab. 1: Summary of data of the sensitivity producing chromosomal aberrations (dicentrics and central rings) by various kinds of ionizing radiation and derived means.
Newer results from the eighties, published from laboratories working with the differential staining method
(Data: NCRP 1990)
All values $\cdot 10^{-2}$ per Gy (α)

	X-rays (220-250kV)	gammarays (Co-60)	n (fission)
	4,04	1,07	79,7
	4,34	2,97	36,9
	4,15	1,42	60,0
	4,37	1,57	
	3,64	0,93	
		4,20	
means	4,11	2,03	58,87

In samples of controls, selected from non-smokers and persons without extraordinary radiation burden, e.g. by radiological examinations in medicine, a spontaneous frequency of 0.3 dicentrics per 1,000 M1 is found [Hoffmann, Schmitz-Feuerhake 1993] and, paying attention to the dynamic of decline, can be associated to about 3 mGy/a of a radiation which is equivalent to X-rays inducing dicentrics (Fig. 16, p. 18).

The frequency of dicentrics in the group of flight personnel investigated by Scheid et al. shows a highly significantly unhomogenous result [Scheid et al. 1993]. This is also true for the results of Heimers et al., which are published after my oral presentation [Heimers et al. 1994]. In the following analysis the data of both working groups are put together. Also after calculation of the relative frequency of excess dicentrics per 100 block hours the inhomogeneity of the data remains. I have got the block hours of the personnel analyzed in Münster over three years before the chromosomal analysis [Gabriel 1994]. Allowing for decline I have used a weighting factor of 1 for the actual year 1991, of 0.52 for 1990 and of 0.35 for 1989. One person (BD 117) must be excluded because of missing data. The block hours per year of the personnel analyzed in Bremen is published in the poster. Only one persons (F) is exposed to intercontinental flights for only one year before the analysis. Their block hours are weighted therefore by 1/1.87.

Ordering the individual results by magnitude and calculating sliding mean and standard deviation I have separated two groups (Fig. 14)²:

² The criterion of separation of the data of Scheid et al. was a clearly visible steeper increase of the sliding standard deviation to the magnitude of the sliding mean. After the later addition of the results published by Heimers et al. the criterion can be maintained.

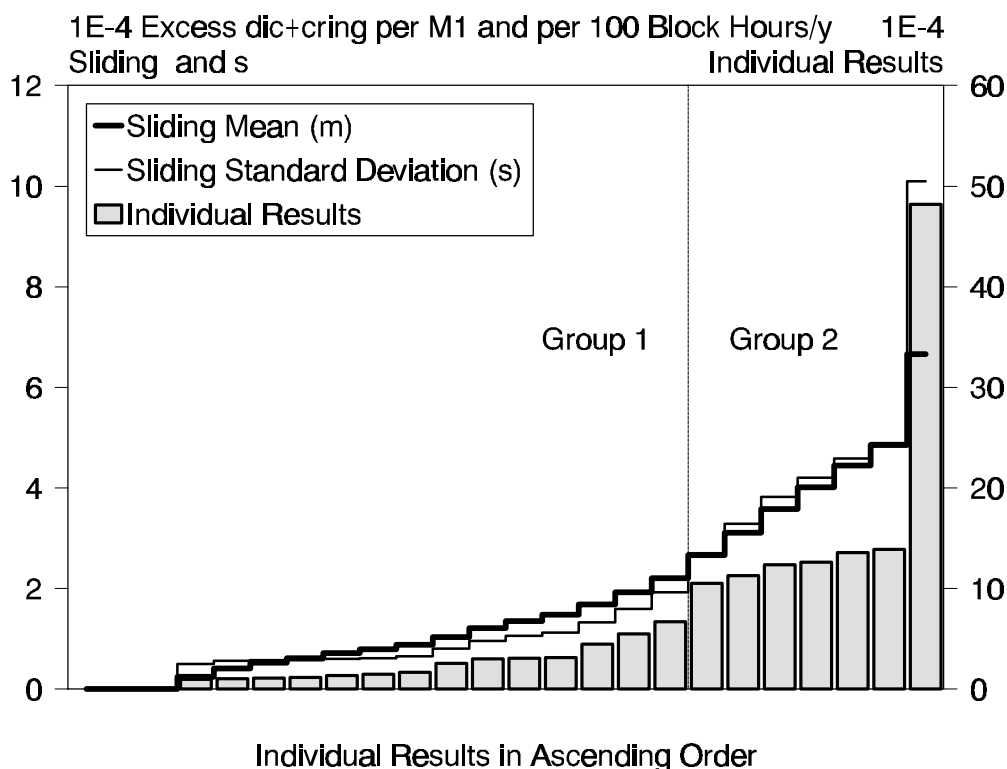


Fig. 14: Individual results of the analysis for chromosomal aberrations of German pilots and stewardesses, expressed as excess dicentric (dic) and central rings (cring) per M1 and per 100 block hours per year, ordered by magnitude, and sliding means and sliding standard deviation, each from the first up to the actual value.

Excess: Original result minus spontaneous frequency ($0.3 \text{ E-}3$ dic and cring per M1).

Block hours per year weighted: Last year before analysis: 1.0; year before: 0.52; further year before: 0.35.

Vertical dotted Line: Borderline between group 1 and group 2

Combined data from Scheid et al. 1993 and Heimers et al. 1994

- Group 1 of 17 persons with a mean of 1.29 dicentric and central rings per 1,000 M1. The mean of the weighted block hours per year in this group is 478.6. The group consists in the data of Scheid et al. exclusively of males, in the combined data the overweight of 14 males to 3 females remains. (Total 19,067 metaphases were analyzed).
- Group 2 of 7 persons with a mean of 4.6 dicentric and central rings per 1,000 M1 and 254.1 weighted block hours per year. This group consists of four females and three males (Total 8,205 metaphases).

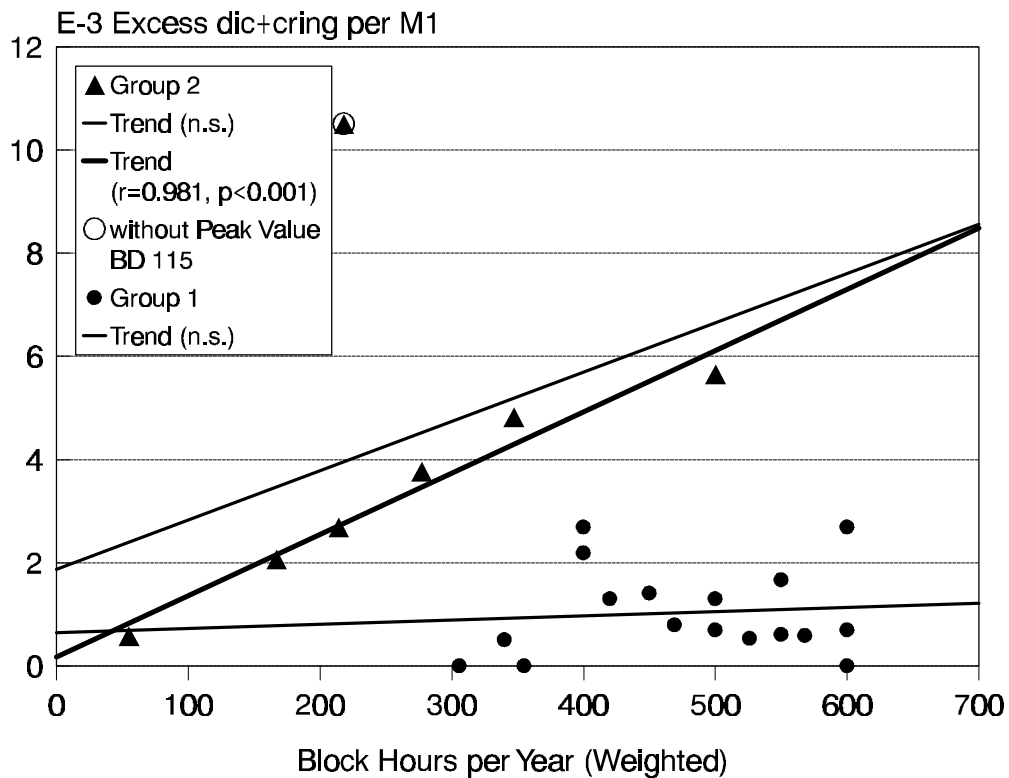


Fig. 15: Frequency of chromosomal aberrations of German pilots and stewardesses, expressed as excess dicentric (dic) and central rings (cring) per M1, in relation to weighted block hours per year
 Excess: Original result minus spontaneous frequency (0.3 E-3 dic and cring per M1).
 Block hours per year weighted: Last year before analysis: 1.0; year before: 0.52; further year before: 0.35.
 Combined data from Scheid et al. 1993 and Heimers et al. 1994

The correlation of excess dicentric and central rings per M1 and the weighted block hours per year is quite different in both groups (Fig. 15). In group 1 the correlation is very weak and not significant. In group 2 the correlation is clearly better, but also not significant. After exclusion of the extraordinary result of BD 115 the correlation becomes highly significant ($r=0.98$, $p<0.001$).

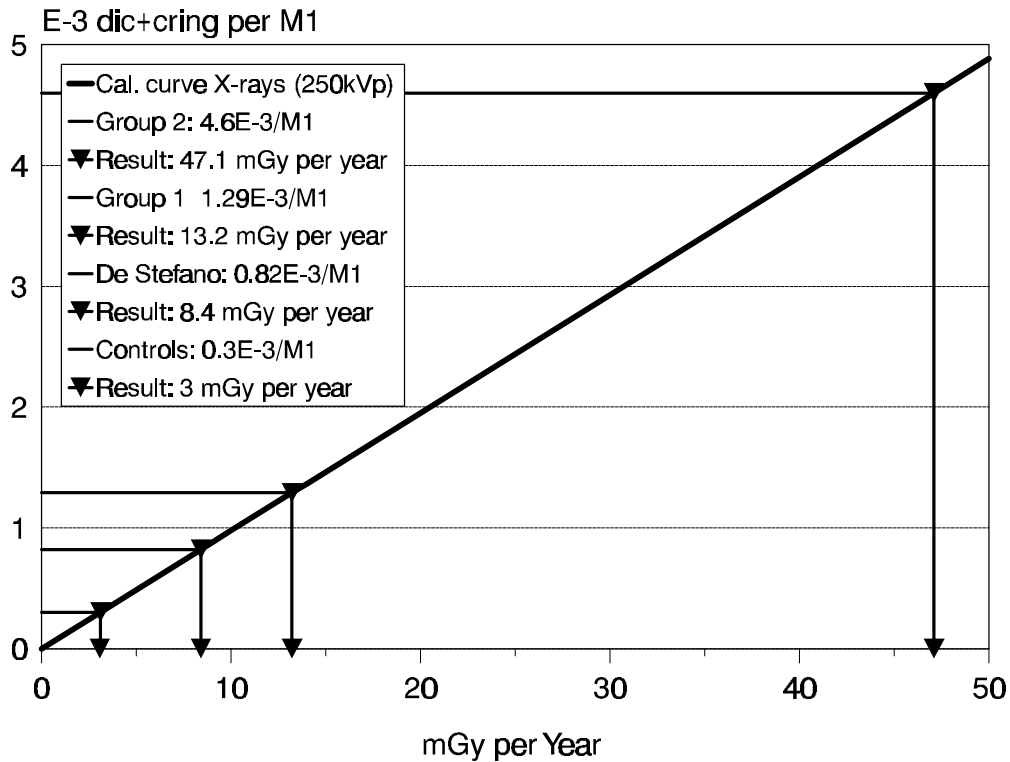


Fig. 16: Calibration curve of the biological dosimeter for a radiation equivalent to X-rays producing dicentrics (dic) and central rings (cring)

Applying the calibration curve to the mean of group 1, 1.29 per 1,000 M1 including the spontaneous frequency, a dose rate of 13.2 mGy/y of radiation equivalent to X-rays can be calculated (Fig. 17).

The preliminary result of De Stefano with a mean of 0.82 dicentrics and central rings per 1,000 M1 (4,889 metaphases were analyzed) gives a dose rate of 8.4 mGy/y [De Stefano 1994]. The mean of group 2, 4.6 dicentrics and central rings per 1,000 M1, gives a result of 47.1 mGy/y for a mean work load of 254.1 block hours!

But in the flight environment there are mainly gamma rays and neutrons, not X-rays. Now I will try to make a roughly estimation of the neutron dose rate. First I have to estimate the influence of the Gamma rays. Gamma rays of high energy have an effectiveness for producing dicentrics, which has about the half value of X-rays. I apply a gradient derived in the same manner as for X-rays (cf. tab. 1, p. 14).

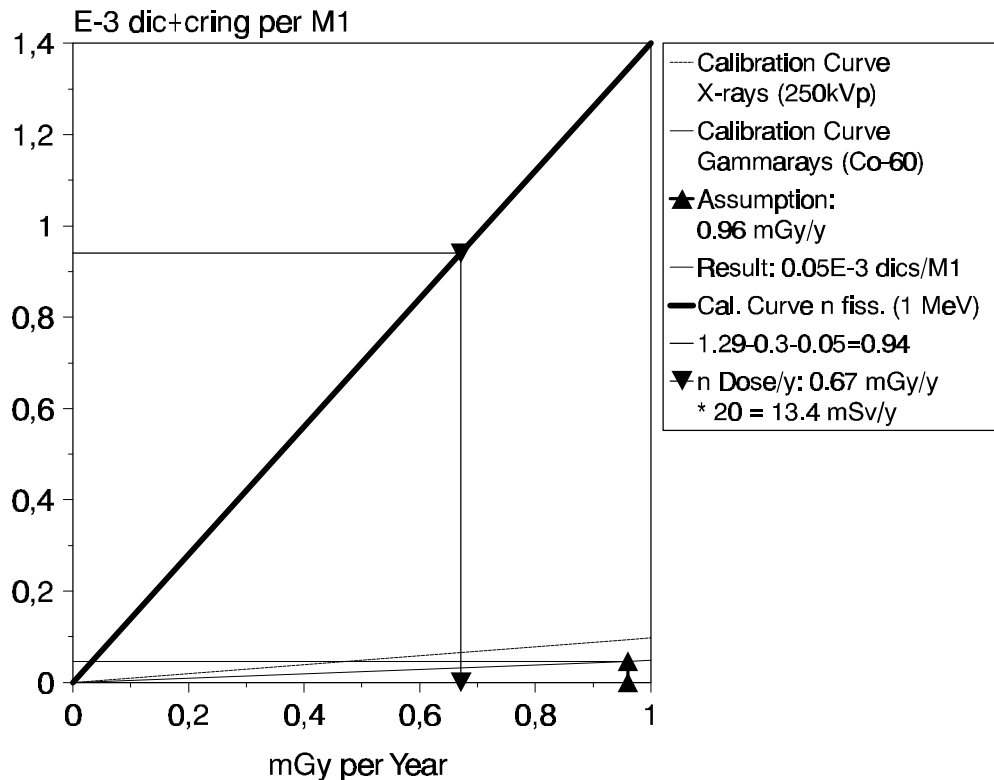


Fig. 17: Calibration curves of the biological dosimeter for gamma rays (Co-60) and neutrons (fission) and application for the data of group 1 (cf. fig. 14, p. 16)

Also using a high dose rate of $2 \mu\text{Gy/h}$, which leads to a dose rate of 0.96 mGy/y in group 1 (478.6 weighted block hours per year), only 0.05 dicentric and central rings per 1,000 M1, three percent in group 1, can be explained by the exposure to gamma rays (Fig. 17).

Fission neutrons of about 1 MeV energy are 14 times more effective than X-rays producing dicentric (cf. tab. 1, p. 14). The rest of dicentric and central rings of group 1, not explained by gamma rays and the spontaneous frequency, 0.94 per 1,000 M1, gives a dose rate of 0.67 mGy/y . Multiplied with the radiation weighting factor recommended by ICRP, an equivalent dose of 13.4 mSv/y is calculated [ICRP 60 1991].

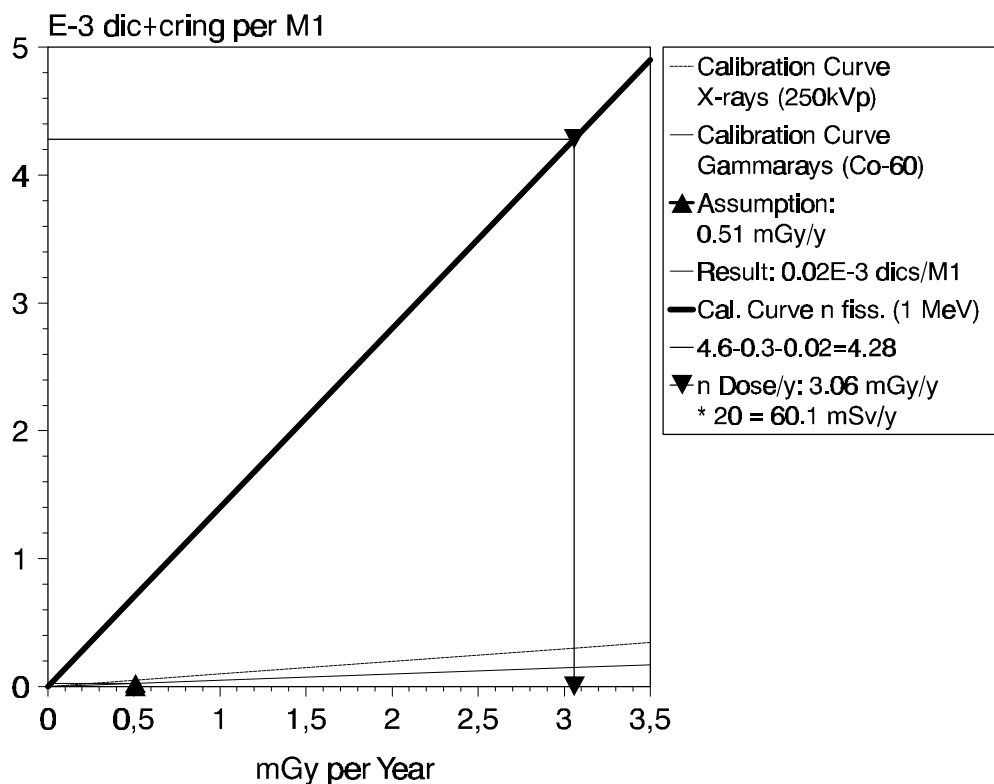


Fig. 18: Calibration curves of the biological dosimeter for gamma rays (Co-60) and neutrons (fission) and application for the data of group 2 (cf. fig. 14, p. 16)

The same procedure, applied to group 2 (254.1 weighted block hours per year), gives a gamma ray dose rate of 0.51 mGy/y, corresponding to only 0.02 dicentrics and central rings per 1,000 M1. From the rest of dicentrics and central rings of 4.28 per 1,000 M1, not explained by gamma rays and the spontaneous frequency, a dose rate of 3.04 mGy/y and an equivalent dose of 60.1 mSv/y can be derived (Fig. 18).

In the persons analyzed by Scheid et al. in single cases a additional exposure to medical sources of radiation can not be excluded. Catheterism of the coronary vessels, an important diagnostic tool in examination persons with a high risk of coronary diseases, is the investigation with the highest medical radiation dose. The positive intercept of the Y-axis in the correlation between aberration frequency and block hours per year seen in group 2 may be an indication of the higher medical radiation dose of flight personnel in average due to the yearly medical check.

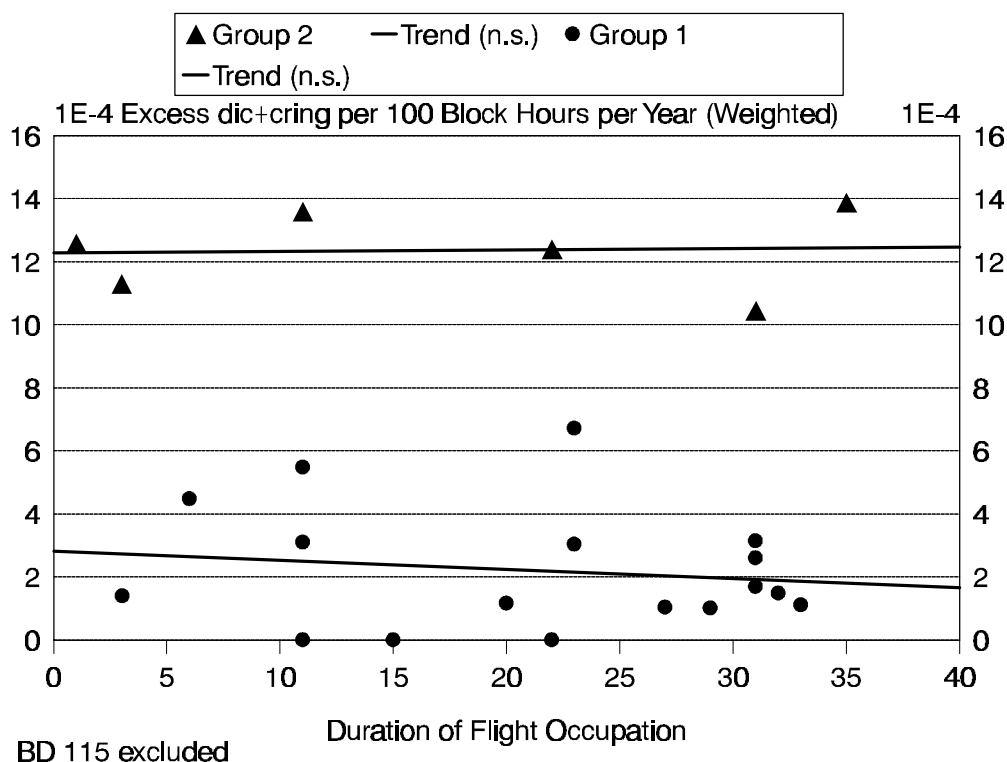


Fig. 19: Frequency of chromosomal aberrations of German pilots and stewardesses, expressed as excess dicentric (dic) and central rings (cring) per M1 and per weighted 100 block hours per year in relation to the duration of flight occupation
 Excess: Original result minus spontaneous frequency (0.3 E-3 dic and cring per M1).
 Block hours per year weighted: Last year before analysis: 1.0; year before: 0.52; further year before: 0.35.
 Duration of flight occupation:
 Data of Scheid et al.: Difference between year of first flight and year of analysis (1992). BD 83, 84, 86, 87: set globally to eleven years [Gabriel 1994].
 Data of Heimers et al.: Difference between year of first flight and year of analysis (1993). C set to 23 years.
 Combined data from Scheid et al. 1993 and Heimers et al. 1994

There are some possible explanations for the disparity of the results between the two groups, which are not excluding one another.

- Heimers et al. discuss an increasing inability of heavy damaged cells to proliferate. The impression discussed by Heimers et al. may be the result of a tendency of a lower amount of block hours per year in the personnel with a longer membership in the air crew. But in fact, the data of group 1 show a weak, but not significant inverse correlation between the increasing amount of years flying and the excess aberrations per block hour (Fig. 19).

- It is remarkable, that the mean value of weighted block hours per year is much lower in group 2 than in group 1 and as usual in flight personal. This may be an indication of a greater susceptibility to some medical problems in this group connected with a higher medical radiation dose. But, the strong correlation of the aberration frequency and the block hours per year in the entire group contradicts this explanation.
- Scheid et al. discuss the possibility of individual differences in the biological sensitivity to a radiation dose. In fact, this explanation have to discuss referring to group 1, which shows not only lower results in average, but also a lack of correlation with the block hours. But, a difference of sensitivity in this magnitude must be reproducible in other exposure conditions. Up to now in the literature there is no indications of a higher sensitivity of woman, which are more frequent seen in the group 2.
- The higher frequency of woman in group 2 can be the indication of an inhomogeneity of the radiation field in the air plane. Neutrons of high energy may be moderated to lower and biologically more effective energies by an environment of kerosine and passengers rich of protons.
- The dose rate may be very inhomogeneous during the time and events like solar flares may significantly contribute to some single individual radiation doses. The individuals affected by such conditions, e.g. flying certain routes, may be exposed in excess proportionally to their block hours per year.

Finally I will emphasize that the relative effectiveness of neutrons inducing cancer is higher than the radiation factor of 20 suggests and much higher than the relative effectiveness producing dicentrics [Kuni 1991, 1993]. Therefore it is also important to look at experiences with morbidity and mortality of flight personnel [Blettner 1994, Kuni 1994].

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