The dose-dependence of sister chromatid exchanges induced by 3 hydrocarbons, in the in vivo bone marrow test with Chinese Hamsters[1]

U. Bayer and T. Bauknecht

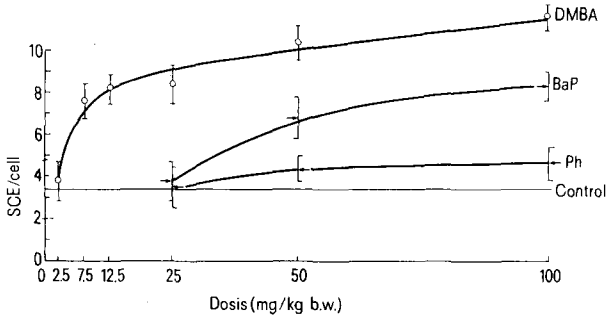
Forstbotanisches Institut der Universität, Bertoldstrasse 17, D–7800 Freiburg im Breisgau, and Institut für Humangenetik und Anthropologie der Universität, Albertstrasse 11, D–7800 Freiburg im Breisgau (Federal Republic of Germany, BRD), 16 June 1976

Summary. In accordance with their carcinogenic effects, 7,12-dimethylbenzanthracene and 3,4-benzo(a)pyrene induce sister chromatid exchanges in the bone marrow of Chinese Hamsters in vivo. Phenanthrene is inactive. A dose dependence of induced sister-chromatid exchanges can be shown.

Recent publications show that mutagenic substances induce not only chromosome aberrations but also sister chromatid exchanges. Differential staining of sister chromatid exchanges (SCE) was so far restricted to cultured cells[2–6].

Recently methods were developed to demonstrate the induction of SCE in vivo, i.e. in the bone marrow cells of treated animals[7]. With this new technique of Vogel and Bauknecht, the dose-dependence of SCE induced by 3 polycyclic hydrocarbons was examined: 7,12-dimethylbenzanthracene (DMBA); 3,4-benzo(a)pyrene (BaP) and phenanthrene (Ph).

Male Chinese Hamsters, 8–20 weeks old, received one dose either of 2.5, 7.5, 12.5, 25, 50 or 100 mg DMBA/kg b.w. or of 25, 50 or 100 mg BaP/kg b.w. or of 25, 50 or 100 mg/Ph/kg b.w. All 3 hydrocarbons were dissolved in Tricapryline, using a homogenizer for better solution. $2^1/_2$ h before fixation, 10 mg Colchicine/kg b.w. was given. The animals were sacrificed 24 h after the treatment with hydrocarbons, and 28 h after the first injection with BUdR. In the controls, untreated animals or animals treated with the pure solvent, the mean number of SCE/cell was 3.2 ± 1.2 and 3.4 ± 1.4 respectively. Compared with induced breaks and interchanges, the number of SCE per cell is much higher. Therefore much fewer cells need to be counted than in the case of chromosome aberrations. We found 50 cells adequate to get reliable results with hydrocarbons.



Dose response curve of sister chromatid exchanges induced by DMBA (top curve), BaP (middle curve), and Ph (bottom curve) in bone marrow cells of Chinese Hamsters. The horizontal line represents the control level (3.4). Abscissa: Dose (mg/kg b.w.). Ordinate: Mean number of sister chromatid exchanges ± standard deviation (vertical lines). Each point of the curves represents a sample of 50 cells from 2 animals.

The figure shows the dose-response curves obtained so far. DMBA, the most active compound, as well as BaP, show a non-linear increase of SCE. This may also be the case with Ph, but the number of SCE induced is not significant. The dose curve of DMBA is based on 6 different concentrations. A significant increase is obtained with 7.5 mg/kg b.w.; obviously no, or a very low, threshold value exists. The 3-point-curve of BaP rises from about 25 mg/kg b.w. with a significant increase between 25 and 50 mg/kg b.w. Here a distinct threshold value seems to exist. The increase of SCE induced by Ph is not significant with a dose of 100 mg/kg b.w.

3 main conclusions can be drawn from the results:
1. The induction of SCE corresponds to the results obtained from chromosome aberrations (unpublished).
2. It reflects the sequence of carcinogenic effects by the 3 hydrocarbons with DMBA as the most active compound.
3. The SCE test is much more sensitive than the induction of chromosome aberrations. A significant increase of SCE can be observed with concentrations much lower than those which are necessary to induce chromosome aberrations. The test may be used as a quick method to screen for the induction of chromosome aberrations and to examine the existence of a threshold value.

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Mouse sex vesicle. C-band and pairing at the light and electron microscope[1]

Maria Luiza Beçak, Sylvia M. Carneiro and W. Beçak[2]

Serviço de Genética, Instituto Butantan, C. Postal 65, São Paulo (Brazil), 23 June 1976

Summary. C banded mouse pachytene chromosomes were studied with the light and electron microscopes by the whole mount technique. The X and Y chromosomes show pairing by the long, by the short or by both long and short arms. Assuming Lyon's hypothesis, the latter suggests that the Y segment transferred to the X is intercalar. With the light microscope, a negative image of the synaptonemal complex is evidenced.

Chromatin free synaptonemal complexes (S.C.) resulted from water spreading of mouse pachytene nuclei pretreated with saline solution[3]. In this paper we report C banding of the same material at the electron and light microscopes after some modifications in the technique described.

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The preparations were obtained from normal male Swiss albino mice with $2n = 40$ acrocentrics. For electron microscopy, the preparations were made as follows: The seminiferous tubules were placed in 0.8% or 0.7% NaCl solution for 4–10 min, squashed on a slide, the cell suspension obtained being spread over distilled water in a plastic tray with Teflon bars. Collected on collodium (1.5%) covered grids, this material was treated with phosphate buffered trypsin pH 6.9 (0.01%, 5 min at 37 °C), stained in aqueous PTA (1%, 5 min at room temperature), then washed in distilled water and air dried. The electron micrographs were obtained in a Siemens Elmiskop I, at 60 kv and low magnification.

For light microscopy, the cell suspensions were obtained as for the electron microscope. But the dispersion was made on distilled water in a Buchner funnel, containing about 6 slides at the bottom. A rubber tube adapted to the lower end of the funnel was used for the control of the water outflow. By water decantation (5 min), the nuclei were deposited on the slides. After air drying, the cytological preparations were selected at the phase microscope. They were stained for 30 min in

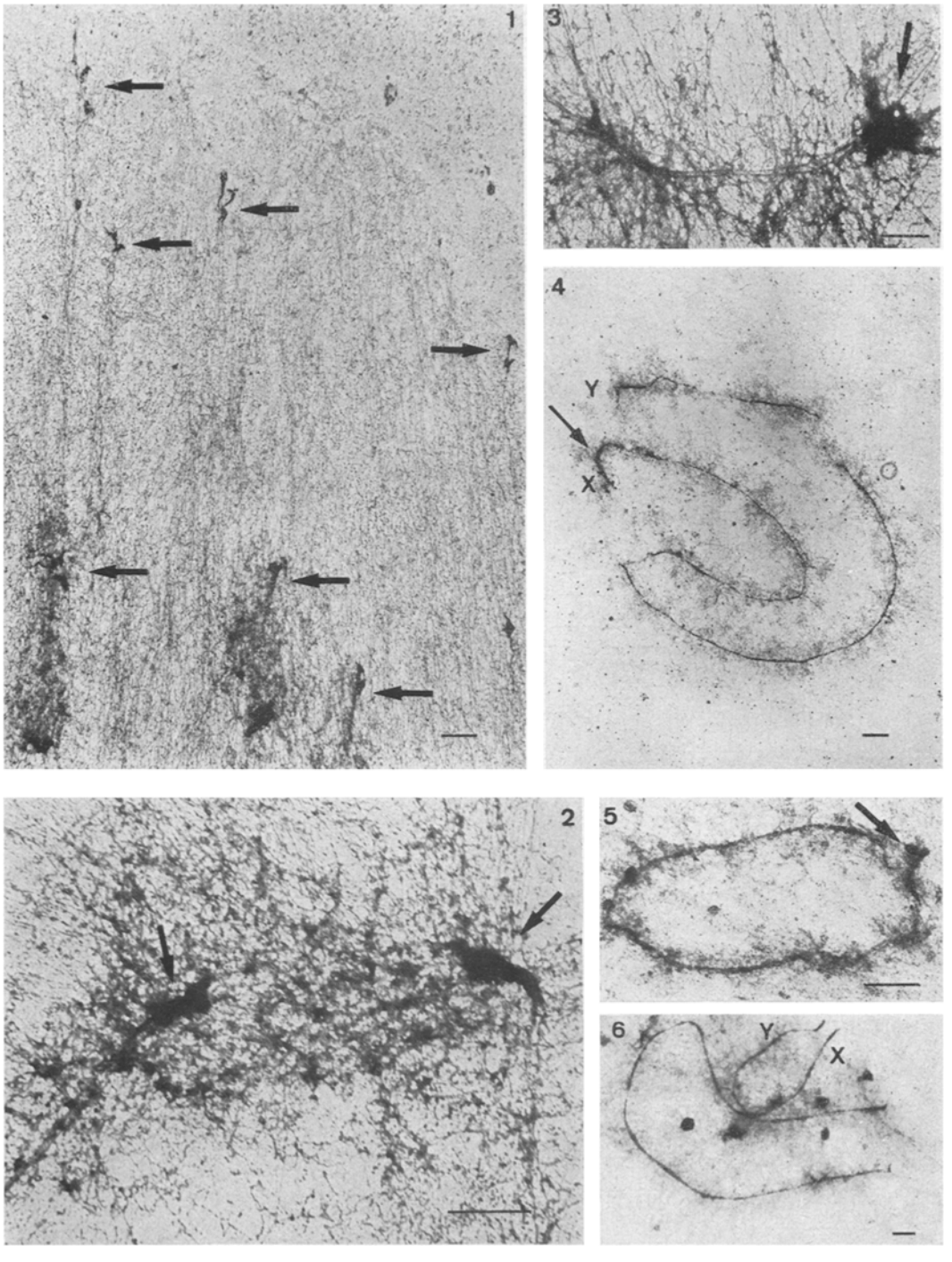

Fig. 1–6. Electron micrographs showing the C band (arrows) in the autosomal bivalents (1–3) and in the X (4,5); XY pairing at the long arm (4) and by both telomeres (5); overlapping of X and Y cores (6). Bars (1 μm).

Giemsa diluted in Sörensen phosphate buffer pH 6.8, washed in distilled water and the coverslips mounted with Permount.

The C band was observed in the autosomal bivalents and in the X chromosome at the electron microscope (Figures 1–5). Rarifaction of the non-centromeric chromatin and partial or total removal of the S.C. were also detected (Figure 1). X and Y pairing by the long arms (Figure 4) and by both long and short arms in a ring-like configuration (Figure 5) were evidenced. The most frequent XY configuration is shown in Figure 6.

With the light microscope, we found similar aspects. The C band appeared in the autosomal bivalents (Figure 7) and in the X chromosome (Figure 8). A narrow and regular slit at the synaptic surface of the autosomal and sexual bivalents was seen (Figures 9, 10). We interpreted this empty space as rarifaction of the non-centromeric chromatin and S.C. digestion, as already described in the electron microscope study.

The water spread technique resulted in sex vesicle distension. This and the C banding facilitated the analysis of the XY pairing with the light microscope. From 34 vesicles selected, 3 showed X and Y univalents (Figure 11), 25 showed pairing by the long arms (Figure 12), 1 exhibited pairing by the short arms (Figure 13). But in 5 the X and the Y were simultaneously paired by the long and short arms (Figures 14–16 and 8) like the ones seen under the electron microscope (Figure 5). Besides the pairing by both ends, the X shows a bending at its middle portion (Figures 15, 16). The nucleolus at the long X arm was also frequently observed (Figures 11 and 14) as already reported[4].

XY pairing by the long arms was shown by serial sections under the electron microscope[4], in whole-mount preparations[3,5] and in C banded squash preparations under the light microscope[6]. The high frequency of pairing just by the long arms suggests early terminalization at the short arms. This could result from absence of chiasmata in the short arms. Hypotonic treatment could also influence the early separation of the achiasmatic arms.

The presence of the X and Y univalents in pachytene nuclei can be attributed to failure of pairing, late pairing or early terminalization:

Failure of pairing of the sex chromosomes should result in post-reduction. But according to Ohno et al.[7], only pre-reduction occurs. Late pairing of the X and Y is also improbable because the sex chromosomes and the autosomes begin their pairing process synchronically[7]. According to Solari[4], the decrease in size of the long arms' S.C. may be interpreted as an early repulsion of the sex chromosomes.

According to Lyon[8], the X-chromosome inactivation in mammals was preceded by the appearance of a small differential segment, carrying the Y genes for the testis. This was followed by the transfer of a large segment from the Y to the X chromosome. According to this scheme, the transference would have occurred at the terminal portion of the homologous X arm.

Accepting this hypothesis, in the mouse a transference of an intercalate segment of the long Y arm to the middle of the long X arm could also be assumed. Hence, X and Y can pair by both proximal and distal extremities. The anomalous S.C.[4] and the bending of the long X arm may be associated to the translocation[8].

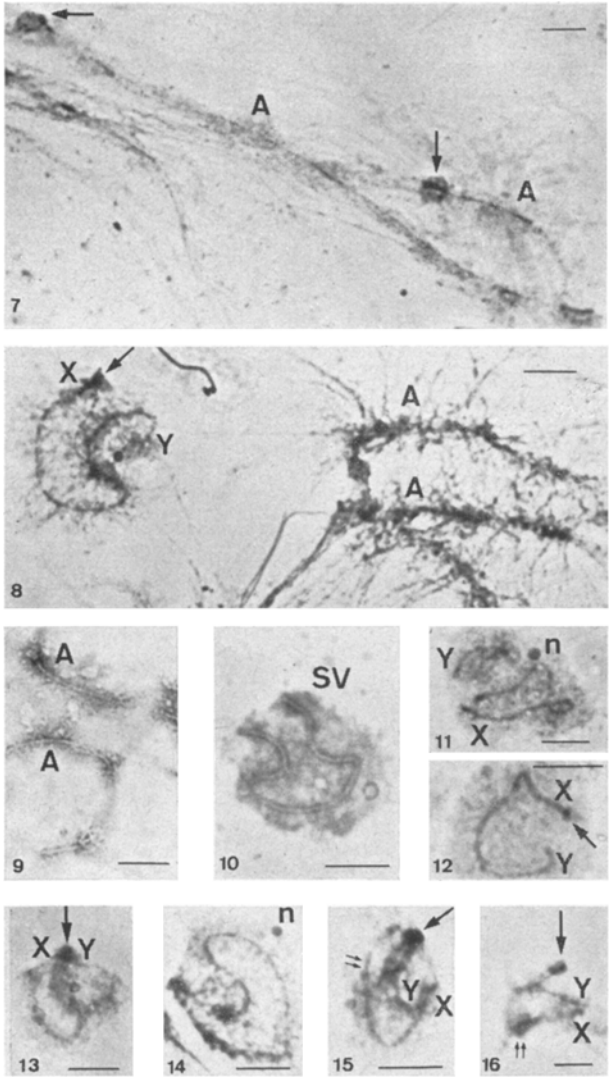

Fig. 7–16. Light microscope aspects of pachytene nuclei prepared by the water spreading technique. C band (arrows) in the autosomes (A) (7). Localization of the distended sex vesicle in relation to the autosomes and C band in the X (8). Negative image of the S. C. in the autosomes (9) and in the sex vesicle (10); X and Y univalents and the nucleolus (n) (11); X and Y paired by the long X arm (12); by the short X arm (13); by both the telomeres (14–16 and 8); bending of the X (double arrows) (15, 16). Bars (5μm).

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