

FIG. 5. (a) to (f).—Pollen grains of *T. bracteata* at metaphase fixed after different intervals with a dose of 360 r at 25° C. $\times 1500$.

- (a) 4 days : a dicentric, two telocentrics (cross-hatched), an acentric and a minute.
 (b) 5 days : a pentacentric and several acentrics and minutes. The cell has an extra centromere.
 (c) 6 days : a hexacentric and several acentrics and minutes.
 (d) 6 days : a dicentric, a monocentric ring, deferred SR (one C_1 SR, one C_0 SR) and many acentrics and minutes.
 (e) 7 days : a diploid cell with a C_2 , a C_3 ring, a C_1 ring, a triradial and many acentrics and minutes. The triradials arise by $2B''$ (at meiosis) $3R'$ (in the pollen grain).
 (f) 7 days : a diploid cell with a C_2 , seven C_1 and C_2 rings of which five are interlocked. There are many minutes and some acentrics longer than the longest chromosome of the normal complement.



(Obe)

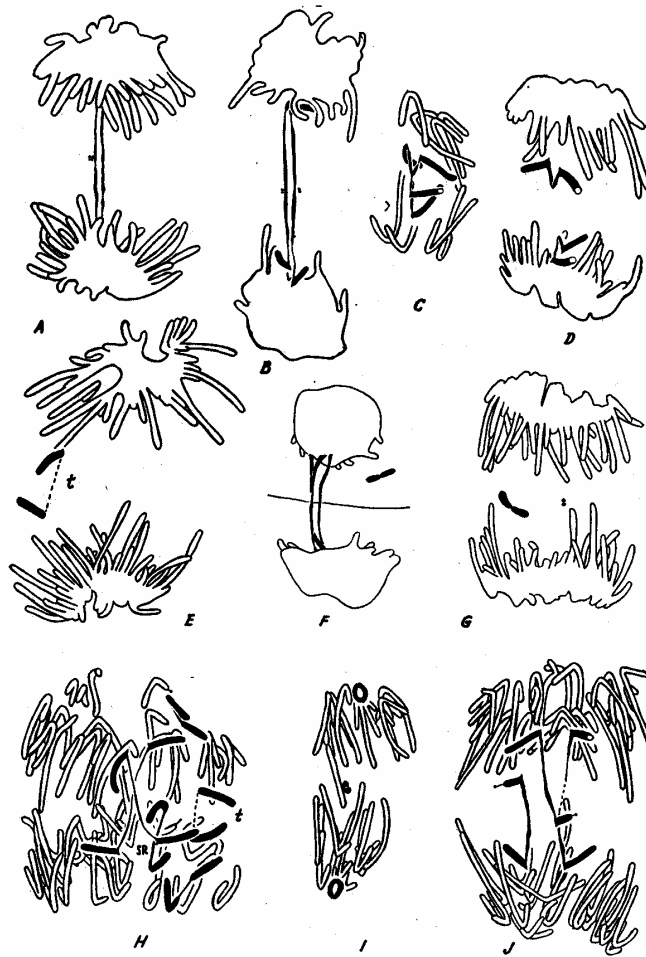


FIG. 5.—

- A and B:* Telophases with a normal C_2^c forming two bridges, together with small paired fragments at the equator, the result of multiple breakage at the previous division.
- C and D:* Anaphases in which the centromeres of each chromatid have gone to the same pole; there has been a barrier to free separation at an earlier stage and the chromatids may originally have been interlocked.
- E:* Telophase with a lagging telocentric, formed by breakage of a bridge at the centromere in a previous division.
- F and G:* Telophases with misdividing telocentrics: in *F*, there are also three bridges (*cf.* fig. 8C) and in *G*, a small fragment.
- H:* Anaphase with three dicentrics: see text.
- I:* Anaphase in which the dicentric has been replaced by a ring chromosome and a small fragment at the equator.
- J:* Anaphase with three bridges, following the union of broken and unbroken chromosome ends: see text.

Loss.—In cells with a short intercentric segment, with a criss-cross separation, anaphase lagging and consequent loss occur. Micronuclei consequently appear later (fig. 6). They are either single or double

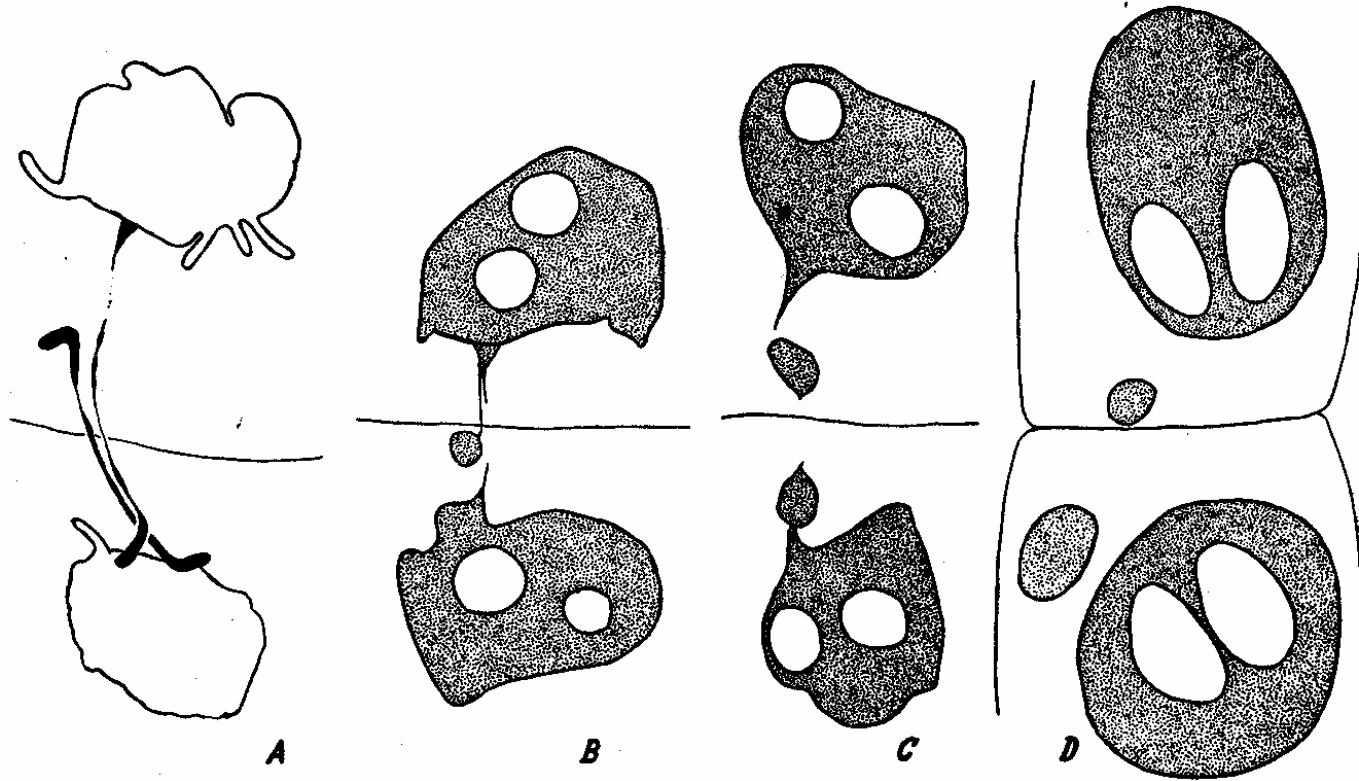


FIG. 6.—Loss of parts of the dicentric.

A: Late telophase with one centromere in one cell excluded.

B-D: Resting stages with micronuclei corresponding to the loss of: in *B*, one centromere in one cell; in *C*, one centromere in both cells; in *D*, both centromeres in one cell and one in the other. $\times 1,420$.

DNA is the primary target for the production of CA

UV of 260 nm

Photoreactivation

Hereditary diseases (Repair)

BrdU substitution

Inhibitors of DNA synthesis

Ionizing radiation (RBE, LET)

Mutagens react with DNA

Endonucleases

Cytogenetic tests

- All investigations must be conducted following GLP
- Dose-effect relationships (at least 3 doses)
- Evaluation of more than one genotoxic endpoint
- The test agent may only be active when metabolized (S9)
- At least three independent experiments should be performed
- Cell cycle parameters and survival of cells have to be checked

Assays and endpoints

- **Comet assay**: Indicator test for DNA damage but not for mutations
- **SCE assay**: Indicator test for repaired DNA damage. Substitution with BrdU could be a problem
- **Chromosomal aberration (CA) assay**: Indicates that the tested agent is mutagenic (chromosome mutations)
- **Micronucleus assay (MN)**: Indicates that the tested agent induces chromosome mutations or leads to misdistribution of whole chromosomes (genome mutations). These types can be differentiated using FISH for centromeric DNA.
- The assays can be performed **in vitro and in vivo**.
- **Cell cycle** parameters have to be taken into account
- It is recommended to use more than one assay, the results should point in the same direction

Spontaneous frequencies of CA and SCE in 1000 metaphases of human peripheral lymphocytes; in brackets half-life in years

DIC:

0.5-1 in 1000 metaphase (3)

Limit of detection in biological dosimetry: 0.1 Gy

Translocations:

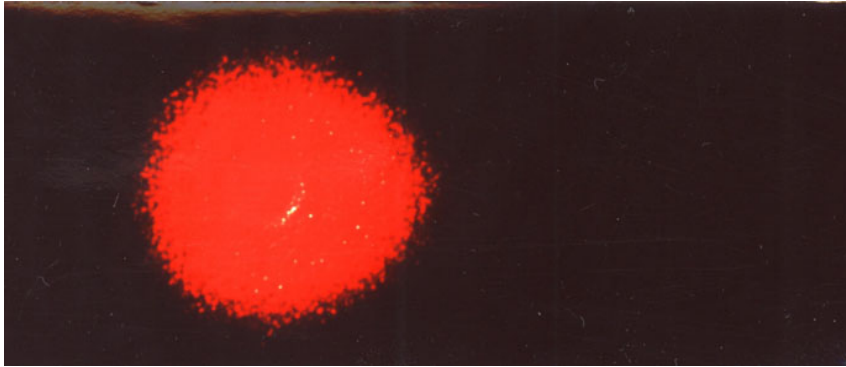
reciprocal 2.5-8 (7) ; one way 3-11 (5.2)

Limit of detection in biological dosimetry for age <40 years 0.3 Gy and for age >40 years 0.5 Gy

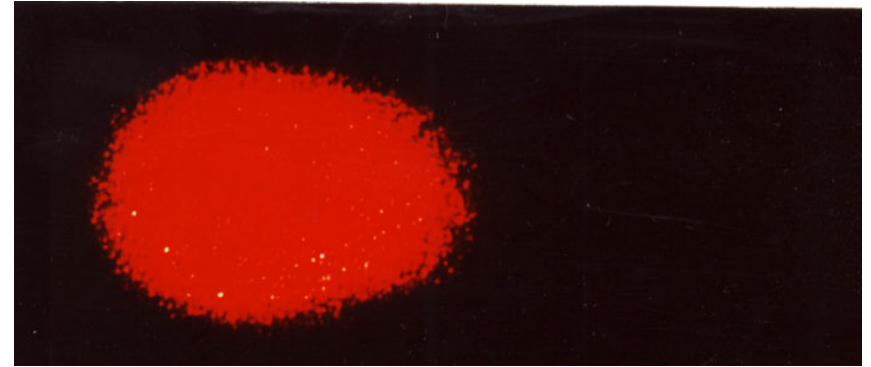
From Pressel et al. (1999; 2000) and Lindholm et al. (2002)

Essen „Comet Assay“:

Typische Kometen nach Röntgenbestrahlung
Propidiumiodid-Färbung, 400fache Vergrößerung



0 Gy



1 Gy



5 Gy



10 Gy

Interpretation of cytogenetic test results: one endpoint

- **Comet positive**: Indication that the test agent induces lesions in DNA.
- **CA positive**: The test agent induces DNA lesions which by mis-repair lead to the formation of CA. CA are the most reliable cytogenetic endpoint to proof that an agent is mutagenic/carcinogenic.
- **MN positive**: MN without centromeres indicate that a test agent induces CA. MN with centromeres indicate that the agent induces misdistribution of chromosomes by disruption of the spindle mechanism (aneugenic action).

Interpretation of cytogenetic test results: Two endpoints

- **Comet negative, CA positive:** Primary DNA damage is repaired, misrepair leads to CA.
- **Comet negative, MN positive:** Primary DNA damage is repaired, misrepair leads to CA which give rise to MN. MN could also be the result of misdistribution of whole chromosomes which can be checked with FISH probes for centromere sequences.
- **Comet negative, SCE positive:** Primary DNA damage is repaired. SCE could be the result of an interaction of the test agent with BrdU.
- **CA positive, MN negative:** This result is inconclusive.

Interpretation of cytogenetic test results: two endpoints

- **CA negative, SCE positive**: SCE indicate damage in DNA. The dose of the tested agent may be so low that SCE but not CA are induced. An interaction of the test compound with BrdU may lead to SCE.
- **MN negative, SCE positive**: The amount of DNA damage may be so low that SCE but not MN have been induced. An interaction of the test compound with BrdU may lead to the positive SCE test.

Intepretation of cytogenetic test results: Three endpoints

- **Comet positive, CA and MN negative:** The damage leading to comets may have been repaired efficiently.
- **Comet negative, CA and MN positive:** DNA damage leading to comets may have been repaired. Misrepair of damage leads to CA and MN. It has to be checked whether MN contain whole chromosomes or centromere-free chromosomal fragments.

Control frequencies of CA (percent aberrant cells: 10 sets of data)

- Human lymphocytes, healthy donors 21-45 years (Hilliard et al., 2007):
- 0.0-1.0; 0.0-2.0; 0.0-2.0; 0.0-0.5; 0.0-0.5; 1.0-1.5; 0.0-0.5; 1.0-1.5; 0.0-1.0; 1.0 (historical mean: 0.25%, range 0.0-2.0%)
- 26 males, 40-58 years, 1.3%, SD 0.9, spread: 0.3-4.1
- Wolf et al., 2004)
- CHO cells (Hilliard et al., 2007):
- 0.5-2.5; 0.5-3.0; 1.0-3.0; 1.0-2.0; 1.0-2.0; 0.0-2.0; 1.0-4.5; 0.0-2.5; 0.5-1.5; 1.0-2.5
- (Hilliard et al., Mutation Res. 616 (2007) 103-118)
- (Wolf et al., Int J Radiat Biol. 80 (2004) 147-153)

Frequencies of CA in HPL are **age dependent**

- CA analyses in **HPL of 128 persons** between 9 and 58 years:
- Significant positive **correlation between CA frequencies and age** with respect to **dicentric and centric ring chromosomes and percent aberrant cells**
- **At age of 70 this would result in about 1 additional DIC or centric ring per 1000 metaphases and to 1.6 (+/- 0.4) % aberrant cells**
- (Wolf et al., 2004)

MN in HPL

Very high variability: Laboratory methods (culture medium, concentration of CytB, fetal calf serum, scoring)

Clear **age effect**.

Gender: Femals 19% higher level of MN

MNC (number of MN per 1000 binucleated cells): 1.75 (SD: 1.45) – 53.61 (SD: 39.58):
Overall value 6.5.

Bonassi et al. (2001) *Environ Molec Mutagenesis*, 37, 31-45.

Genetic polymorphisms may influence MN formation in exposed people:

Iarmarcovai et al. *Mutat. Res.: Rev. Mutat. Res.* (2007), doi:10.1016/j.mrrev.2007.10.001

MN with and without centromeres in HPL of controls

- Number of micronucleated binucleated cells **with centromeres** (C+MN): **58%** (95% CI: 52-65).
- Number of persons analyzed: **58**
- **60,563** MNCB: **541** MN, **180** without and **361** with centromere.
- Iarmarcovai et al. (2007) Mutation Res. 615, 18-27.

CA in CHO cells and in HPL

- Compounds tested for CA: 10
- CHO: 10+ (2 of these induced polyploidies in addition to CA)
- HPL: 3+; 6- (2 of these induced polyploidies in addition to CA); 1+/-
- Inhibition of DNA synthesis: 6+; 2+/-; 2 not done.
- No difference between **isolated HPL** or **HPL in whole blood**
- „...the differential aberration induction in **CHO** cells and **HL** may be related to effects on **cell cycle checkpoints** and **apoptosis**.“
- „...studies in lymphocytes or other normal human cells may play a useful part in the **follow-up strategy** to assess weight of evidence about genotoxic risk for humans for compounds that are positive in the Chinese hamster cell aberration assays.“

- Hilliard C. et al. (2007) Chromosome aberrations in Chinese hamster and human cells: A comparison using compounds with various genotoxicity profiles, Mutation Res. 616, 103-118.

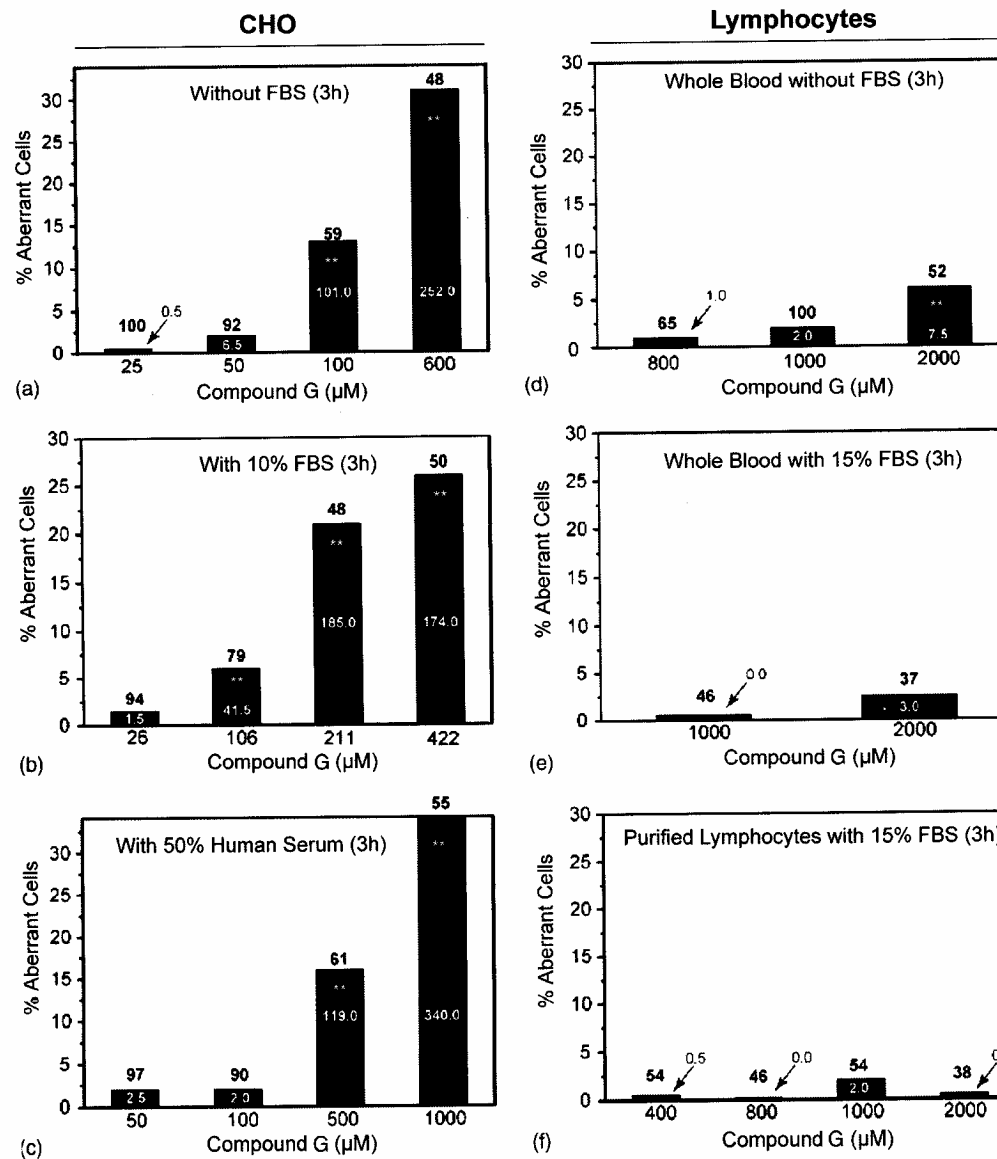


Fig. 1. The percentage of cells with chromosomal aberrations in cultures treated with Compound G (concurrent controls for CHO: 0.5–2.5; concurrent controls for lymphocytes: 0.0–1.0). Numbers on tops of columns are population doublings taken at harvest, as percentages of controls (CHO cells) or mitotic indices, as percentages of controls (lymphocytes). Numbers inside columns are frequency of aberrations per 100 cells as a cell may have more than one aberration. No precipitate observed in CHO culture medium at doses scored; precipitate observed at 2000 μM and above in lymphocyte culture medium. For lymphocytes without FBS, 800 μM was from a separate experiment. ** Statistically significant increase over concurrent control ($P \leq 0.01$). * Statistically significant increase over concurrent control ($P \leq 0.05$). CHO positive not markedly affected by serum (even 50% human serum). Very weak aberration induction in lymphocytes when compared with CHO even at doses with marked mitotic suppression.

Indirect genotoxicity

- Enzyme inhibition
- Topo I and II
- Imbalance of DNA precursors
- Energy depletion
- Production of oxygen species
- Lipid peroxidation
- Sulphydryl depletion
- Nuclease release from lysosomes
- Inhibition of protein synthesis
- Protein denaturation
- Ionic imbalance
- Scott et al. (1991), from Kirkland et al. (2007)

Table III. Some indirect mechanisms of genotoxicity from Scott *et al.* (13)

Mechanisms	Cellular target	Examples
Enzyme inhibition	Enzymes of DNA synthesis	Hydroxyurea, fluorodeoxyuridine, aphidicolin, 2-deoxyadenosine (155), methotrexate (156)
	Enzymes of DNA repair	Cytosine arabinoside, aphidicolin, 3-aminobenzimide (155)
	Topo I	Camptothecin (157)
	Topo II	amsacrine (<i>m</i> -AMSA) (158), formaldehyde (159), VP-16 (160)
	Na ⁺ /K ⁺ ATPase	Ouabain (131)
Imbalance of DNA precursors	DNA precursors	DNA bases and nucleosides (161)
Energy depletion	Energy metabolism systems	Dinitrophenol (99), cyanide (162,163)
Production of active oxygen species	Oxygen and superoxide radicals	Paraquat (164), hydrogen peroxide (165)
Lipid peroxidation	Membranes	Phorbolmyristate acetate, asbestos (166), chromium chloride (167)
Sulphydryl depletion	Sulphydryls	Diethylmaleate (131)
Nuclease release from lysosomes	Lysosomes	NDI (132), hypotonic medium (168)
Inhibition of protein synthesis	Nuclear proteins	Cycloheximide (131)
Protein denaturation	Nuclear proteins	Calcium hypochlorite (169), <i>N</i> -chloropiperidine (145)
Ionic imbalance	Chromatin? Enzymes?	Ethylenediaminetetraacetic acid, salts of saccharin, nitrilotriacetic acid, secalonic acid (170)

Kirkland, D.J. et al. (2007)

Chromosomal Aberrations (CA) result from DNA Double-Strand Breaks (DSB)

- (1) The effectivity of ionizing radiations to induce CA is positively correlated with their capacity to induce DSB
- (2) Transformation of single strand breaks to DSB in living cells with Neurospora endonuclease leads to an elevation of CA frequencies
- (3) Endonucleases induce CA
- (4) Neocarzinostatin and bleomycin induce CA

Origin of DSB

- (1) From **single strand lesions** during DNA replication (S-phase)
- (2) During **repair of single strand lesions**
- (3) **Direct induction** by ionizing radiation, endonucleases, some antibiotics
- **CA induced by DSB** in **G1**: Chromosome type; in **S**: chromosome and chromatid type; in **G2**: chromatid type

Origin of chromosomal aberrations

- Breakage and reunion theory (Sax, 1941):
 - No break-no exchange (Savage, 1998)
- Exchange theory (Revell, 1963):
 - No exchange-no break (Savage, 1998)
- Molecular theory (Chadwick, Leenhouts, 1981):
 - One DSB-one exchange (C and L, 2002)
 - No DSB-no aberration (Pfeiffer et al, 2000)
- Signal model for chromatid breakage
 - (Bryant, 2004)