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# In Vitro and In Vivo Analysis of Sister Chromatid Exchange*

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## I. Introduction

5-Bromo-2'-deoxyuridine(BrdUrd)-dye techniques for sister chromatid exchange (SCE) analysis are being extensively used to characterized the impact of mutagens and carcinogens on chromosomes. A number of such agents, known to damage DNA, have been observed to cause significant increases in SCE frequencies, typically at doses below those necessary to induce an appreciable increase in chromosome aberrations. The validity of SCE analysis as a sensitive and convenient test for mutagen-carcinogens is currently being investigated both in vitro, with cultured cells, and in vivo, in different tissues of intact animals. In addition, the biological significance of SCE formation is being examined in studies comparing SCE induction with events such as mutagenesis at specific loci, and an increased amount of effort is being directed at characterizing chemical events associated with SCE formation. The present paper will review different techniques for SCE detection, some of the information derived from application of SCE methodology, and hypotheses about the mechanism and significance of SCE formation.

SCEs represent the interchange of DNA between replication products at apparently homologous loci. These exchanges, which are generally detected in cytological preparations of metaphase chromosomes, presumably involve DNA breakage and reunion, although the molecular basis of SCE formation, as well as the biological significance of exchanges, is not completely understood. In spite of these uncertainties, analysis of SCE formation in cytological systems has already provided information about chromosome structure and has been used to detect the effects of clastogens and to differentiate between chromosome fragility diseases.

SCEs were first described by J. Herbert Taylor et al. (185), who utilized autoradiography to detect differentially labeled sister chromatids in cells which had undergone one cycle of $^{3}$H-thymidine incorporation followed by a replication cycle in nonradioactive medium. Reciprocal alterations in labeling (SCEs) were detected along the chromatids of a number of metaphase chromosomes. Analysis of SCE formation in cytological chromosome preparations has been facilitated by recently developed BrdUrd-dye techniques for detecting DNA synthesis.

## II. BrdUrd-Dye Methodology for SCE Detection

BrdUrd substitution into DNA quenches the fluorescence of certain bound dyes, such as 33258 Hoechst (97, 103, 104), acridine

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orange (35, 38, 80), and, at pH 11, 4′,6-diamidinophenylindole (DAPI) (117). Light energy absorbed but not emitted by such dyes can also promote the selective degradation of BrdUrd-substituted DNA (58, 59, 109), leading to reduced staining by Giemsa (90, 141). Additional effects of BrdUrd on chromatin structure permit detection by yet other Giemsa protocols (9, 24, 94, 156, 181, 207), and immunological methods for BrdUrd detection have been introduced (60).

Sister chromatid differentiation (SCD), which is necessary for SCE detection, can be achieved by two related protocols. Both require one cycle of BrdUrd incorporation into chromosomal DNA; they differ in that only one involves the presence of BrdUrd during the second cycle (fig. 1). In vitro studies typically utilize two cycles of BrdUrd incorporation, primarily to avoid the difficulty of changing cell culture medium, to remove the BrdUrd at the appropriate time. In contrast, in vivo studies usually involve BrdUrd incorporation for the first cycle only. BrdUrd is rapidly degraded in intact animals, and levels of BrdUrd drop rapidly as soon as external sources of BrdUrd are removed. A second cycle of BrdUrd incorporation has only a small effect on the baseline level of SCEs (125). In vitro cultures must be protected from light (e.g., ≤313 nm) that can degrade BrdUrd-substituted DNA; such precautions do not appear to be necessary for most in vivo studies. For both protocols, cells are trapped at metaphase of the second cycle following initial exposure to BrdUrd, and cytological chromosome preparations are then prepared by standard techniques.

Staining protocols used for BrdUrd detection have been described in detail elsewhere (97, 100, 105, 141) and will be reviewed only briefly here. Steps utilized in one fluorescent and one Giemsa protocol are summarized in table 1. BrdUrd administration protocols can achieve greater than

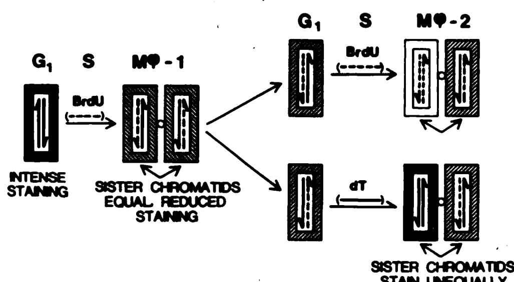


FIG. 1. Sister chromatid differentiation by 5-bromo-2′-deoxyuridine (BrdUrd)-dye techniques. Cells are allowed to incorporate BrdUrd (- - -) for one cycle, followed by a second cycle of replication in which the presence of BrdUrd is optional. Sister chromatids in metaphase chromosomes from such second division cells will exhibit unequal fluorescence, if stained, e.g., with 33258 Hoechst, or unequal intensity following Giemsa staining, reflecting different numbers of BrdUrd-substituted polynucleotide chains. Solid, hatched, and open areas surrounding each rectangle represent intense, intermediate, and pale staining, respectively.

TABLE 1
*Staining Protocols for Detecting BrdUrd Incorporation into Metaphase Chromosomes*

Fluorescence (33258 Hoechst) (97, 106)
1. Stain slides with 0.5 μg/ml dye in pH 7 phosphate buffer; mount at pH 7–7.5.
2. Excite fluorescence with near UV light, e.g., predominantly 365 nm Hg line, 400 nm dichroic mirror.
3. Observe fluorescence at or above 460 nm.
4. After fluorescence microscopy, slides can usually be incubated in 65, 2X SSC buffer (0.30 M NaCl and 0.03 M Na citrate, pH 7.0) and overstained with Giemsa to obtain a permanent preparation reflecting sister chromatid differentiation.

Fluorescence plus Giemsa (106, 141)
1. Stain slides with 33258 Hoechst or mount slides directly in excess dye (e.g., 50 μg/ml) in pH 7 phosphate buffer. Dilute dye into buffer from concentrated stock solution of dye in $H_2O$.
2. Expose slides, mounted in buffered dye solution, to light with appreciable intensity ≤ 400 nm, i.e., in a region absorbed by the dye. Exposure time is adjustable, typically a few hours if a standard 20 W cool white fluorescent light is used.
3. Incubate slides 15–30 min in 65°C 2X SSC buffer; rinse with $H_2O$.
4. Stain with Giemsa (e.g., 4% in 5 mM pH 6.8 phosphate buffer).

80% substitution of BrdUrd for thymidine in one or both DNA strands (8, 105). BrdUrd detection under these circumstances presents little problem, since appreciable fluorescence quenching occurs even at one third to one half maximal BrdUrd substitution levels (99, 105). When SCD is detected by 33258 Hoechst fluorescence, the contrast between chromatids can be optimized by mounting slides at a pH slightly above neutrality (e.g., pH 7.5) and an ionic strength of approximately 0.15 (104). Unfortunately, conditions promoting fluorescence contrast also lead to rapid fading of fluorescence. Conversely, use of a mounting medium at low pH or one containing glycerol stabilizes fluorescence but reduces BrdUrd-dependent contrast.

Of the many Giemsa protocols, one based on the procedure introduced by Perry and Wolff (141) is probably the most convenient. In this procedure, the dye (e.g., 33258 Hoechst) serves to photosensitize degradation of BrdUrd-substituted DNA. When bound to DNA, the dye exhibits very high absorbance in the near UV region ($\epsilon_{max} \sim 3 \times 10^4\ M^{-1}\ cm^{-1}$), so that staining with 33258 Hoechst should increase the sensitivity of BrdUrd-substituted chromosomes to DNA breakage by light of wavelengths between 350 to 400 nm by several orders of magnitude (compared with the sensitivity of BrdUrd-substituted but unstained chromosomes). This estimate is corroborated by observations of the ability of 33258 Hoechst to sensitize BrdUrd-substituted cells to killing with light (175, 176). Effective photosensitization requires dye to be complexed to DNA under conditions (e.g., pH 7.5 with 33258 Hoechst) such that BrdUrd quenches fluorescence (59). That is, light energy absorbed but not emitted as fluorescence is responsible for DNA degradation.

Experiments with appropriately labeled, synchronized cells (109) indicate that the procedure produces single strand breaks in BrdUrd-substituted DNA (fig. 2). Incubation of illuminated slides in warm (e.g., >65°C) buffer promotes elution of single stranded fragments. Importantly, the contrast in Giemsa-stained slides can be controlled by varying the time of illumination and hence DNA degradation (fig. 3).

Excellent SCD can be achieved with either fluorescence or fluorescence plus Giemsa protocols (fig. 4). For routine studies, Giemsa staining has the advantage of producing permanent chromosome preparations, permitting repeated examination by several observers, and brightfield rather than fluorescence microscopy can be used. Moreover, Giemsa-stained slides lend themselves to automated analysis, and detection of an SCE, with essentially redundant reciprocal information signaling exchange on sister chromatids, is probably

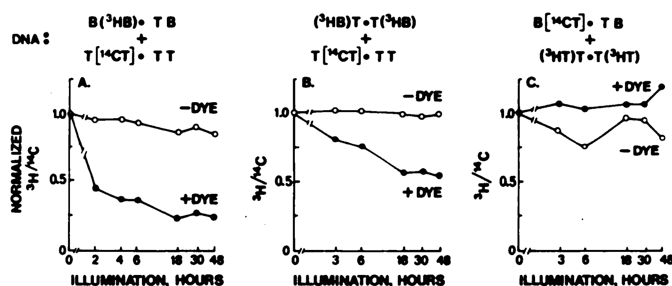


FIG. 2. DNA elution during a 5-bromo-2′-deoxyuridine (BrdUrd)-dye-Giemsa procedure. Synchronized Chinese hamster ovary (CHO) cells were cultured to produce DNA substituted as shown at the top of each frame. Mixtures of colcemid-treated cells (average mitotic index, approximately 40%) were applied to coverslips, mounted at pH 7 with or without prior staining with 33258 Hoechst, exposed 6 cm below a 20 W cool white lamp, for time periods indicated in the graphs and subsequently incubated 15 min in 2X SSC (0.30 M NaCl and 0.03 M Na citrate, pH 7.0) at 65°C. Relative elution of DNA species was estimated from the residual $^3H/^{14}C$ ratio (109).

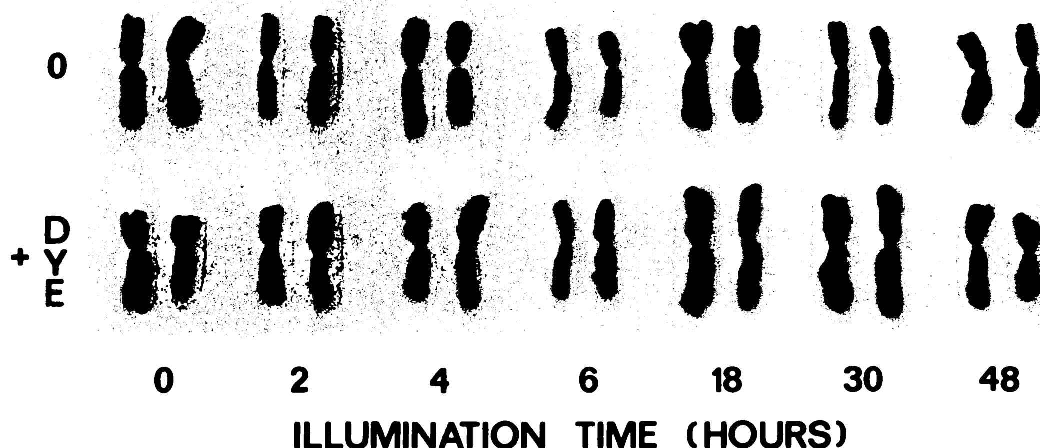


FIG. 3. Induction of sister chromatid differentiation in Chinese hamster ovary (CHO) chromosomes. The chromosomes shown are from synchronized CHO cells, described in figure 2 A, that were allowed to incorporate $^3$H 5-bromo-2′-deoxyuridine (BrdUrd) for one cycle followed by a cycle of nonradioactive BrdUrd. Slide treatment, including light exposure, was as described in the caption to figure 2, with or without 33258 Hoechst staining prior to light exposure. Photosensitization by 33258 Hoechst enhanced subsequent differential staining with Giemsa, and this increased with illumination time, up to 4 to 6 h.

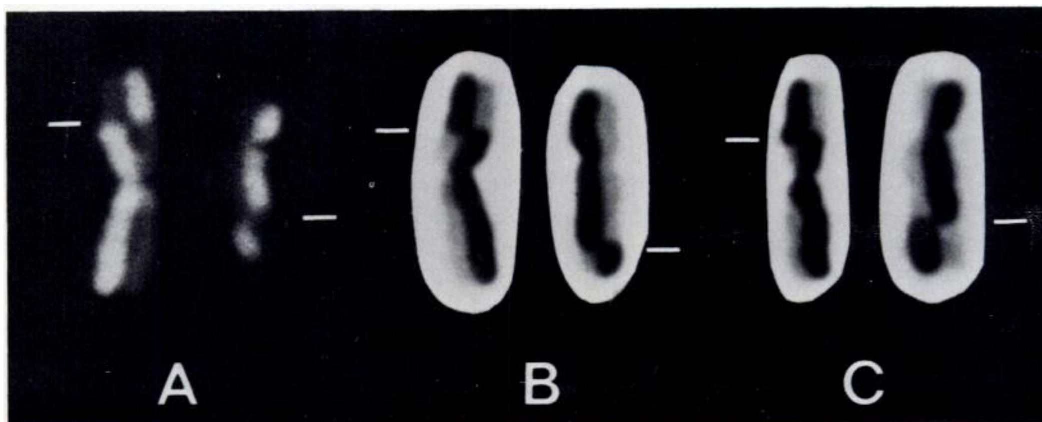


FIG. 4. Sister chromatid exchanges. The chromosomes in this figure are from human lymphocytes which replicated twice in medium containing $10^{-5}$ M 5-bromo-2′-deoxyuridine (BrdUrd), $6 \times 10^{-6}$ M U, and $4 \times 10^{-7}$ M 5-fluoro-2′-deoxyuridine (FdUrd). Those in figure 4A were stained with 33258 Hoechst and photographed under conditions described for fluorescence microscopy. Chromosomes in figure 4B were previously photographed to record fluorescence, as in figure 4A and then washed with $H_2O$, incubated 15 min at 60 to 65° C in 2X SSC, and stained with Giemsa (90). Chromosomes in figure 4C were exposed to fluorescent light while mounted in buffer containing $10^{-4}$ M 33258 Hoechst as described in table 1, incubated in 2X SSC, and stained with Giemsa. Chromosomes shown were chosen to demonstrate relatively unambiguous sister chromatid exchanges (indicated by short, horizontal lines) (106).

simpler than automated recognition of banded chromosomes. Automated detection of SCEs can now be accomplished at nearly the speed of manual studies, albeit with somewhat lower accuracy (206). However, this procedure is still at a fairly early stage of development. Some form of automation in SCE scoring may ultimately prove necessary, e.g., to screen hundreds of compounds or large numbers of individuals potentially exposed to clastogenic compounds.

## III. Basic Information About SCEs

Newer techniques for SCD have confirmed most of the conclusions about the overall features of SCE drawn from previous autoradiographic studies, e.g., that SCE is constrained by the polarity of the DNA helix (22, 183, 187), that segregation at mitosis of sister chromatids in pairs of homologues is random (33, 111), and that apposition of newly synthesized polynucleotide chains is external to old chains with respect to the centromere (67, 100, 164, 195, 199).

The position of SCEs detected by fluorescence or Giemsa can be reasonably well localized relative to chromosome banding patterns, for example, in human chromosomes, in Q-negative bands or at the junctions of Q-positive and Q-negative regions (34, 98, 127). Similar studies detected a clustering of SCE at junctions between heterochromatic and euchromatic regions in muntjac (27), kangaroo rat (21), microtus, and hamster chromosomes (69). The significance of these "junctional" regions is as yet unknown. Re-examination of the position of SCEs in highly extended chromosomes, prepared, e.g., as described by Yunis (204, 205), or by premature chromosome condensation (113) should help elucidate systematic characteristics of SCE localization.

The greater effective resolution of BrdUrd dye techniques has facilitated the detection of multiple, closely spaced SCEs (32, 93, 99, 199). This capability has increased the accuracy and simplicity with which SCE induction by many clastogenic agents can be quantitated (79, 99, 140). BrdUrd itself, like $^{3}$H-thymidine (23, 56, 121), induces SCEs (51, 80, 99, 198), and may be responsible for most of the baseline SCEs observed in the absence of additional clastogens. However, increments in SCEs can easily be scored and the extent of SCE induction [at least by mitomycin C (MMC)] does not seem to be very sensitive to BrdUrd levels to which cells are exposed (76).

## IV. Induction of SCE by Clastogens

Thus far, the most extensive use of SCE analysis has been to assess the impact of clastogens on chromosomes. Kato (79, 81) had originally employed autoradiography to demonstrate SCE induction by alkylating agents and proflavine. However, quantitation of high SCE frequencies was difficult with this method. BrdUrd-dye methodology was used to show that low doses of alkylating agents such as MMC (fig. 5) or nitrogen mustard induced large numbers of SCEs at concentrations well below those causing significant numbers of chromosome breaks (99). Numerous subsequent reports confirmed these observations and extended them to include other agents known to damage chromosomes either directly or after metabolic activation.

Dozens of mono- and bifunctional alkylating agents have been shown to induce SCEs (table 2). Since many of the agents initially used to induce SCEs were also well known mutagens and/or carcinogens, it was suggested that SCE analysis could be used as an assay for mutagens and carcinogens (140). Possible exceptions to this correlation include X-irradiation, which is only a weak SCE inducer but which is well known for its genetic and clastogenic effects, monomeric acrylamide, and bleomycin (table 3). Importantly, all of these agents induce chromosome breaks and/or rearrangements. The combination of SCEs *and* chromosome aberrations thus appears to give very few "false negatives" when examining mutagen-carcinogens. Moreover, there does not yet appear to be any convincing example thus far of an agent that is highly effective at inducing SCEs that is not also mutagenic or carcinogenic in at least some system. Of equal importance, a large number of agents that are not thought to be mutagenic or carcinogenic appear incapable of inducing SCEs (table 4).

## V. Extension of SCE Studies from in Vitro to in Vivo Systems

Bloom and Hsu (18) described the formation of SCEs in ovo in chick embryos

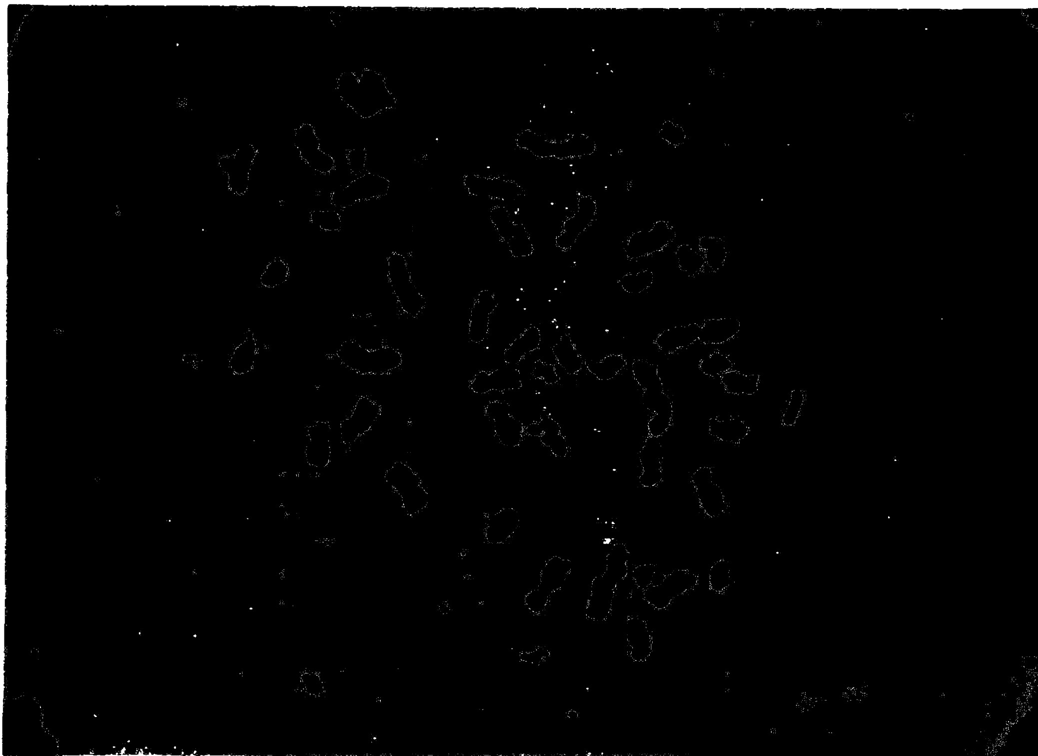

FIG. 5. Induction of sister chromatid exchanges SCEs in a human peripheral lymphocyte by mitomycin C. Mitomycin C (0.075 μg/ml) was present during the third and final day of cell culture. Slides of metaphase chromosomes were stained with 33258 Hoechst, exposed to light and 2X SSC, and then stained with Giemsa. More than 50 SCEs can be detected in this cell; untreated cells exhibit approximately 15 SCEs.

exposed to BrdUrd. The chick embryo system has excellent potential for examining tissue specific cytogenetic effects of mutagen-carcinogens during development (19). Subsequent reports described the induction by alkylating agents of SCE formation in marrow cells or spermatogonia of mice that received repeated doses of BrdUrd (5, 6, 192), and extension of in vivo SCE analysis to other rodent systems, as well as to the mudminnow (92), has been accomplished. The host mediated (10, 114, 119) aspects of in vivo systems, together with the obvious relevance of spermatogonial damage to germ cell formation, make this approach unique for studying environmental mutagenesis.

In contrast to combined in vivo-in vitro studies, in which a microsomal system capable of activating some agents is added directly to in vitro cultures (129, 173), or in which cultured cells are enclosed in porous chambers and implanted in animals (47, 70, 168), the in vivo systems permit examination of different processes in multiple tissues of a given organism. The in vivo system may prove particularly valuable, because recent data (17) suggest that the array of products produced by in vivo versus in vitro activation of potential clastogens may be different. Also, in vitro "activating" conditions are capable of actually reducing the SCE inducibility of some agents, such as N-acetoxyacetylaminofluorene (182), and in at least one instance (styrene) (37), a microsomal activating system was effective only if accompanied by cyclohexene oxide, an epoxide hydratase inhibitor.

In our laboratory, SCE formation has been detected in a number of tissues, including mouse spermatogonia, mouse bone marrow, thymus, and spleen cells (5–8). In-

TABLE 2
*Agents Capable of Inducing Sister Chromatid Exchanges (SCEs) (Strongly Positive)*

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Acetaldehyde | | | | | | | |
| | Human WBC | – | 4.0 | 24.0 | $4.5 \times 10^{-4}$ M | | 147 |
| | Chinese hamster ovary (CHO) | – | 4.7 | 28.4 | $2.3 \times 10^{-4}$ M | | 135 |
| N-acetylaminofluorene | | | | | | | |
| | CHO | + | 11 | 12 | $10^{-4}$ M | S9 | 182 |
| | CHO | +* | 12 | 34 | $10^{-4}$ M | S9 | 182a |
| | V-79 | – | 10.8 | 9.7 | $2 \times 10^{-5}$ M | | 143 |
| | *Mouse (CBA)* | | | | | | |
| | Marrow | + | 4.2 | 10.7 | 225 μg/g ($10^{-3}$ moles/kg) | In vivo | 161 |
| | Marrow | + | 5.9 | 22.7 | 225 μg/g | In vivo; partial hepatectomy | 161 |
| | Regenerating liver | + | 6.8 | 16.7 | 225 μg/g | In vivo; partial hepatectomy | 161 |
| N-acetoxyacetylaminofluorene | | | | | | | |
| | Human fibroblasts | – | 10 | 38 | $4 \times 10^{-6}$ M | | 158 |
| | V-79 | – | 10.8 | 32.4 | $2 \times 10^{-6}$ M | | 143 |
| | CHO | – | 11 | 33 | $10^{-7}$ M | ↓ With S9 | 182 |
| N-hydroxyacetylaminofluorene | | | | | | | |
| | V-79 | – | 10.8 | 41.3 | $2 \times 10^{-5}$ M | | 143 |
| | CHO | – | 11 | 33 | $10^{-4}$ M | ↓ With S9 | 182 |
| Adriamycin | | | | | | | |
| | Human WBC | – | 4.8 | 24 | $2 \times 10^{-7}$ M | | 130 |
| | Human WBC | +† | 4.8 | 16 | $1.6 \times 10^{-7}$ M | | 130 |
| | Human WBC | – | 10 | 24 | $10^{-7}$ M | | 48 |
| | CHO | – | 12.2 | 72 | $3 \times 10^{-7}$ M | | 140 |
| | *Mouse* | | | | | | |
| | Marrow (AKR) | + | 5.1 | 20 | 12 μg/g ($2.2 \times 10^{-5}$ moles/kg) | In vivo | 95 |
| | Marrow (C57) | + | 4.8 | 32 | 12 μg/g | In vivo | 95 |
| Aflatoxin $B_1$ | | | | | | | |
| | CHO | + | 11 | 37 | $10^{-4}$ M | ↑ With S9 | 182 |
| Alkeran | | | | | | | |
| | Human WBC | – | 10.8 | 23.5 | $1.3 \times 10^{-9}$ M | | 144 |
| Aminofluorene | | | | | | | |
| | CHO | – | 11 | 18 | $10^{-4}$ M | | 182 |
| 4-Aminoquinoline-1-oxide | | | | | | | |
| | DON | – | 3.5 | 24.1 | $5 \times 10^{-4}$ M | | 3 |

*Extended S9 exposure conditions.
†Treatment in vivo; cell culture in vitro.
‡Implanted in mice.
§Syrian hamster feeder layer.
¶Assumes chromosome #1 = 10% of genome.
‖Macrochromosomes.
#Assumes 0.1 g embryo.

TABLE 2 (*continued*)

| Agent | Cell Type | Acti-vation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Refer-ence |
|---|---|---|---|---|---|---|---|
| Aniline | | | | | | | |
| | DON | – | 7.7 | 21.8 | $2 \times 10^{-3}$ M | | 2 |
| Benzo($\alpha$)pyrene | | | | | | | |
| | Human WBC | – | 15 | 28 | $10^{-6}$ M | | 150 |
| | Human WBC | – | 15 | 27 | $10^{-6}$ M | | 160 |
| | DON | – | 3.5 | 6.5 | $10^{-4}$ M | | 3 |
| | CHO | + | 11 | 26 | $10^{-4}$ M | S9 | 182 |
| | V-79 | +‡ | 5.5 | 11.3 | 150 $\mu$g/g ($6 \times 10^{-4}$ moles/kg) | | 168 |
| | V-79 | – | 10.8 | 9.2 | $4 \times 10^{-6}$ M | | 143 |
| | V-79 | +§ | 10.8 | 37.9 | $4 \times 10^{-6}$ M | | 143 |
| $\beta$-Propriolactone | | | | | | | |
| | DON | – | 7.7 | 32.7 | $10^{-4}$ M | | 2 |
| | CHO | – | 12.2 | 83.4 | $3 \times 10^{-4}$ M | | 140 |
| BrdUrd + light | | | | | | | |
| | Human WBC | – | 4.6 | 17 | ($3 \times 10^{-6}$ M + $5 \times 10^{3}$ ergs/mm$^{2}$) | Near UV (unfiltered) | 194 |
| | CHO | – | 1.6 | 10.2 | ($10^{-5}$ M + several light flashes) | Light at end of S | 75 |
| | DON | – | 4¶ | 32¶ | ($3 \times 10^{-6}$ M + light) | 20′, 20 W, bulb | 82 |
| | Vicia faba | – | 22 | 65 | ($10^{-4}$ M + light) | 30′, 40 W near UV | 87 |
| BrdUrd | | | | | | | |
| | Human WBC | – | | 15 | $10^{-4}$ M | ($4 \times 10^{-7}$ M FdUrd) | 97 |
| | Human WBC | – | | 37 | $4 \times 10^{-4}$ M | ($4 \times 10^{-7}$ M FdUrd) | 101 |
| | Human WBC | – | | 27 | $7 \times 10^{-4}$ M | | 39 |
| | Human WBC | – | | 42 | $5 \times 10^{-4}$ M | | 96 |
| | Human fibroblasts | – | | 5.6–5.8 | $10^{-5}$ M | One cycle | 125 |
| | Human fibroblasts | – | | 6.5–7.8 | $10^{-5}$ M | Two cycles | 125 |
| | CHO | – | | 16 | $2 \times 10^{-5}$ M | | 198 |
| | DON | – | | 5¶ | $10^{-4}$ M | | 80 |
| | Chick embryo | + | | 0.75 ‖ | 250 $\mu$g/g# | | 18 |
| | Mudminnow | + | | 2.5 | 500 $\mu$g | In vivo | 92 |
| | Allium cepa | – | | 2.8 | $10^{-4}$ M | One cycle | 162 |
| | Allium cepa | – | | 5.5 | $10^{-4}$ M | Two cycles | 162 |
| Busulfan | | | | | | | |
| | Human WBC | – | 10.8 | 27.7 | $2 \times 10^{-7}$ M | | 144 |
| Butylbutanolnitrosamine | | | | | | | |
| | DON | – | 3.5 | 5.8 | $1.5 \times 10^{-3}$ M | | 3 |
| N-n-butylurea | | | | | | | |
| | DON | – | 3.5 | 6.8 | $1 \times 10^{-3}$ M | | 3 |
| N-n-butyl-N-nitrosourea | | | | | | | |
| | DON | – | 3.5 | 21.9 | $1 \times 10^{-3}$ M | | 3 |

TABLE 2 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| N-n-butyl-N-nitrosourethane | | | | | | | |
| | DON | − | 8.8 | 18.4 | $10^{-4}$ M | | 2 |
| Chlorambucil | | | | | | | |
| | Human WBC | − | 5.1 | 56 | $10^{-5}$ M | | 170 |
| | Human WBC | − | 10.8 | 33.4 | $3 \times 10^{-7}$ M | | 144 |
| Cyclophosphamide | | | | | | | |
| | Human WBC | − | 19.6 | 22.9 | $2 \times 10^{-3}$ M | | 6 |
| | Human WBC | − | 10.8 | 11.8 | $4 \times 10^{-8}$ M | Very low dose; no activation | |
| | Human WBC | +‡ | 2–5 | 20–35 | 30 μg/g ($1.2 \times 10^{-4}$ moles/kg) | | 70 |
| | *Mouse* | | | | | | |
| | 3T3 | − | 23.1 | 34.0 | $2 \times 10^{-3}$ M | | 6 |
| | Marrow (CBA) | + | 7.4 | 22.4 | 5 μg/g ($2 \times 10^{-5}$ moles/kg) | In vivo | 8 |
| | Marrow (AKR) | + | 5.1 | 50 | 5 μg/g | In vivo | 95 |
| | Marrow (C57) | + | 4.8 | 90 | 5 μg/g | In vivo | 95 |
| | Marrow (NMRI) | + | 3.7 | 13.7 | 10 μg/g ($4 \times 10^{-5}$ moles/kg) | In vivo | 14 |
| | Marrow (NMRI) | + | 4 | 24 | 25 μg/g ($10^{-4}$ moles/kg) | In vivo | 192 |
| | Marrow (CBA) | + | 7.7 | 57.5 | 20 μg/g | In vivo | 7 |
| | Marrow (AKR) | + | 1.4 | 17.7 | 25 μg/g | In vivo | 146 |
| | Thymus (CBA) | + | 9.1 | 33.1 | 5 μg/g | In vivo | 8 |
| | Spleen (CBA) | + | 6.8 | 25.1 | 5 μg/g | In vivo | 8 |
| | Spleen (CBA) | + | 6.7 | 46.3 | 20 μg/g | In vivo | 7 |
| | Spermatogonia (CBA) | + | 3.4 | 8.1 | 5 μg/g | In vivo | 8 |
| | Spermatogonia (CBA) | + | 1.7 | 8.8 | 20 μg/g ($8 \times 10^{-5}$ moles/kg) | In vivo | 6 |
| | Regenerating liver (CBA) | + | 5.4 | 22.8 | 10 μg/g | In vivo; partial hepatectomy | 161 |
| | CHO | − | 12.2 | 21.2 | $10^{-3}$ M | | 140 |
| | CHO | + | 11 | 55 | $10^{-3}$ M | S9 | 173 |
| | V-79 | +‡ | 4 | 30 | 15 μg/g ($6 \times 10^{-5}$ moles/kg) | | 47 |

*Extended S9 exposure conditions.
†Treatment in vivo; cell culture in vitro.
‡Implanted in mice.
§Syrian hamster feeder layer.
¶Assumes chromosome #1 = 10% of genome.
‖Macrochromosomes.
#Assumes 0.1 g embryo.

TABLE 2 (*continued*)

| Agent | Cell Type | Acti-vation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Refer-ence |
|---|---|---|---|---|---|---|---|
| | V-79 | + | 5.5 | 16 | 15 μg/g | Implanted in mice | 168 |
| | Chinese hamster cheek pouch | + | 4.8 | 10.1 | 5 μg/g | In vivo | 168 |
| | Rabbit WBC | +† | 5.7 | 20.7 | 35 μg/g (1.4 × $10^{-4}$ moles/kg) | | 172 |
| | Chick embryo | + | 1.2 ‖ | 13.6 ‖ | 50 μg/g# (2 × $10^{-5}$ moles/kg) | In vivo | 19 |
| Deoxythymidine | | | | | | | |
| | Chinese hamster lung | − | 10 | 50 | $10^{-2}$ M | | 191 |
| | Chick embryo | + | 1.2 ‖ | 7.5 ‖ | 50 mg/g# (0.22 moles/kg) | In vivo | 19 |
| Dibutylnitrosamine | | | | | | | |
| | DON | − | 3.5 | 6.0 | 7 × $10^{-4}$ M | | 3 |
| Diethylnitrosamine | | | | | | | |
| | CHO | + | 11 | 25 | 0.1 M | S9 | 129 |
| | V-79 | +‡ | 5.5 | 8.8 | 600 μg/g (6 × $10^{-3}$ moles/kg) | | 168 |
| | Mouse (AKR) marrow | + | 1.4 | 3.3 | 100 μg/g ($10^{-3}$ moles/kg) | In vivo | 146 |
| | Mouse (NMRI) marrow | + | 3.7 | 4.2 | 200 μg/g (2 × $10^{-3}$ moles/kg) | In vivo | 14 |
| Dimethylnitrosamine | | | | | | | |
| | CHO | + | 11 | 100 | 0.04 M | S9 | 129 |
| | DON | − | 3.5 | 25.7 | 0.12 M | | 3 |
| | V-79 | +‡ | 5.5 | 9.1 | 30 μg/g (4 × $10^{-4}$ moles/kg) | | 168 |
| | Mouse (NMRI) marrow | + | 3.7 | 10.6 | 2 μg/g | In vivo | 4 |
| 7,12-Dimethylbenzanthracene | | | | | | | |
| | CHO | − | 10.2 | 13.7 | $10^{-3}$ M | | 50 |
| | CHO | + | 10.5 | 17.1 | $10^{-3}$ M | S9 | 50 |
| | Chinese hamster cheek pouch | + | 6.7 | 11.5 | 0.5 mg in mineral oil | In vivo | 168 |
| | DON | − | 3.5 | 10.0 | $10^{-3}$ M | | 3 |
| | V-79 | +‡ | 5.5 | 11.1 | 150 μg/g (6 × $10^{-4}$ moles/kg) | | 168 |
| | Rat gliosarcoma | − | 15.5 | 26.9 | $10^{-4}$ M | | 50 |

TABLE 2 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| | Rat gliosarcoma | + | 15.2 | 34.8 | $10^{-4}$ M | S9 | 50 |
| Dimethylphenyltriazine | | | | | | | |
| | Mouse (NMRI) marrow | + | 3.7 | 6.9 | 6 μg/g (3 × $10^{-5}$ moles/kg) | In vivo | 14 |
| N-dibutylamine | | | | | | | |
| | DON | − | 3.5 | 7.1 | $10^{-3}$ M | | 3 |
| Dibutylphthalate | | | | | | | |
| | DON | − | 8.8 | 13.6 | $10^{-4}$ M | | 2 |
| Dimethylamine | | | | | | | |
| | DON | − | 3.5 | 6.2 | 1.2 × $10^{-3}$ M | | 3 |
| Diphenyl | | | | | | | |
| | DON | − | 7.7 | 13.1 | $10^{-3}$ M | | 2 |
| Diepoxybutane | | | | | | | |
| | CHO | − | 12.2 | 91 | 3 × $10^{-6}$ M | | 140 |
| Ethylmethane sulfonate | | | | | | | |
| | Human WBC | − | 15 | 25 | 2 × $10^{-3}$ M | | 110 |
| | Human WBC | − | 10 | 27 | $10^{-3}$ M | | 48 |
| | Human fibroblasts | − | 10 | 31 | 4 × $10^{-4}$ M | | 158 |
| | CHO | − | 12.2 | 103 | 3 × $10^{-3}$ M | | 140 |
| | CHO | − | 7.0 | 82 | 2 × $10^{-3}$ M | | 26 |
| | CHO | + | 11 | 26 | 5 × $10^{-3}$ M | S9 | 173 |
| | DON | − | 4¶ | 42¶ | 5 × $10^{-3}$ M | | 83 |
| | BHK | − | 21.1 | 88.4 | 4.2 × $10^{-3}$ M | | 78 |
| | THK (clone A) | − | 9.8 | 65.3 | 4.2 × $10^{-3}$ M | | 78 |
| | THK (clone B) | − | 21.5 | 80.7 | 4.2 × $10^{-3}$ M | | 78 |
| | THK (clone E) | − | 18.6 | 113.9 | 4.2 × $10^{-3}$ M | | 78 |
| | THK (clone G) | − | 9.4 | 41.5 | 1.7 × $10^{-3}$ M | | 78 |
| | Vicia faba | − | 20 | 85 | 4 × $10^{-2}$ M | | 89 |
| | Rabbit WBC | +† | 5.7 | 13.6 | 0.2 mg/g (1.6 × $10^{-3}$ | | 172 |
| | Chick embryo | + | 1.2 ‖ | 8.6 ‖ | 3 μg/g# (2.5 × $10^{-2}$ moles/kg) | In vivo | 19 |
| Ethylnitrosourea | | | | | | | |
| | CHO | − | 7.0 | 62 | 1.5 × $10^{-3}$ M | | 26 |
| Methylnitrosourea | | | | | | | |
| | DON | − | 8.8 | 50.6 | $10^{-3}$ M | | 2 |

* Extended S9 exposure conditions.
† Treatment in vivo; cell culture in vitro.
‡ Implanted in mice.
§ Syrian hamster feeder layer.
¶ Assumes chromosome #1 = 10% of genome.
‖ Macrochromosomes.
# Assumes 0.1 g embryo.

TABLE 2 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Methylazoxymethanol acetate | | | | | | | |
| | Human WBC | – | 5.3 | 11.6 | $7.6 \times 10^{-5}$ M | | 40 |
| | DON | – | 7.7 | 51.9 | $10^{-4}$ M | | 2 |
| 4-Methyl-N'nitro-N-nitrosoguanidine | | | | | | | |
| | CHO | – | 12.2 | 106.2 | $10^{-6}$ M | | 140 |
| | V-79 | – | 10.8 | 59.1 | $4 \times 10^{-6}$ M | | 143 |
| | Mouse (NMRI) marrow | + | 3.7 | 7.2 | 0.3 μg/g ($2.3 \times 10^{-6}$ moles/kg) | In vivo | 14 |
| | Vicia faba | – | 20 | 85 | $2 \times 10^{-5}$ M | | 89 |
| 3-Methylcholanthrene | | | | | | | |
| | V-79 | +‡ | 5.5 | 9.3 | 100 μg/g ($3.7 \times 10^{-4}$ moles/kg) | | 168 |
| | V-79 | – | 10.8 | 8.9 | $3.7 \times 10^{-6}$ M | | 143 |
| | V-79 | +§ | 10.8 | 38 | $3.7 \times 10^{-6}$ M | | 143 |
| | DON | – | 3.5 | 4.4 | $10^{-4}$ M | | 3 |
| Methylmethane sulfonate | | | | | | | |
| | CHO | – | 12.2 | 98 | $3 \times 10^{-4}$ M | | 140 |
| | Chinese hamster marrow | + | 3.3 | 9.0 | 10 μg/g ($10^{-4}$ moles/kg) | In vivo | 122 |
| | Rabbit WBC | +† | 5.7 | 11.4 | 25 μg/g ($2.5 \times 10^{-4}$ moles/kg) | | 172 |
| | Mouse (AKR) marrow | + | 1.4 | 9.8 | 100 μg/g ($10^{-3}$ moles/kg) | In vivo | 146 |
| | Vicia faba | – | 20 | 88 | $1.5 \times 10^{-3}$ M | | 89 |
| | Chick embryo | + | 1.2 ‖ | 9.5 ‖ | 1.5 mg/g# ($1.5 \times 10^{-2}$ moles/kg) | | 19 |
| 33258 Hoechst | | | | | | | |
| | CHO | – | 12.2 | 67 | $10^{-5}$ M | | 140 |
| | CHO | – | 4.4 | 7.0 | $3.4 \times 10^{-6}$ M | | 171 |
| Mitomycin C | | | | | | | |
| | Human WBC | – | 12 | 120 | $9 \times 10^{-7}$ M | | 99 |
| | Human WBC | – | 15 | 29 | $9 \times 10^{-8}$ M | | 110 |
| | Human WBC | – | 10.8 | 48.6 | $1.2 \times 10^{-7}$ M | | 144 |
| | Human WBC | – | 11 | 47 | $4.5 \times 10^{-7}$ M | | 76 |
| | Human WBC | – | 10 | 92 | $3 \times 10^{-7}$ M | | 48 |
| | Human lymphoblasts | – | 10 | 39 | $4.5 \times 10^{-7}$ M | | 158 |
| | CHO | – | 12.2 | 128 | $10^{-7}$ M | | 140 |
| | | – | 7 | 77 | $7.5 \times 10^{-8}$ M | | 26 |
| | DON | – | 7¶ | 30¶ | $2 \times 10^{-6}$ M | Autoradiography | 81 |
| | | – | 2.4 | 28.6 | $10^{-6}$ M | | 86 |
| | Vicia faba | – | 20 | 70 | $2.2 \times 10^{-6}$ M (0.75 μg/ml) | | 89 |
| | BHK | – | 21.3 | 138.3 | $9 \times 10^{-8}$ M | | 78 |

TABLE 2 (*continued*)

| Agent | Cell Type | Acti-vation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Refer-ence |
|---|---|---|---|---|---|---|---|
| | THK (clone A) | − | 7.9 | 79.5 | $9 \times 10^{-8}$ M | | 78 |
| | THK (clone B) | − | 18.9 | 116.6 | $9 \times 10^{-8}$ M | | 78 |
| | THK (clone E) | − | 21.4 | 178.1 | $9 \times 10^{-8}$ M | | 78 |
| | THK (clone G) | − | 14.0 | 102.3 | $9 \times 10^{-8}$ M | | 78 |
| | Muntjac | − | 8.0 | 35 | $3 \times 10^{-6}$ M | | 72 |
| | Muntjac | − | 6 | 52 | $6 \times 10^{-8}$ M | | 25 |
| | Mouse (AKR) marrow | + | 5.1 | 50 | 5 μg/g ($1.5 \times 10^{-5}$ moles/kg) | In vivo | 96 |
| | Marrow (C57) | + | 4.8 | 90 | 5 μg/g | In vivo | 95 |
| | Spermatogonia (CBA) | + | 1.8 | 7.2 | 0.5 μg/g ($1.5 \times 10^{-6}$ moles/kg) | In vivo | 5 |
| | Chick embryo | + | 1.2 ‖ | 5.2 ‖ | 1 μg/g# ($3 \times 10^{-6}$ moles/kg) | In vivo | 19 |
| 8-Methoxy-psoralen | | | | | | | |
| | Human WBC | − | 12.1 | 34.2 | $5 \times 10^{-7}$ M + $2.3 \times 10^{5}$ ergs/mm² near UV light | | 28 |
| | Human WBC | − | 4.6 | 25 | $2.5 \times 10^{-4}$ M + $5 \times 10^{3}$ ergs/mm² near UV light | | 194 |
| | Human WBC | − | 7 | 20 | $2 \times 10^{-5}$ M + $4 \times 10^{4}$ ergs/mm² near UV light | | 128 |
| | CHO | − | 15.0 | 88.7 | $6 \times 10^{-6}$ M + $1.7 \times 10^{4}$ ergs/mm² near UV light | | 102 |
| 4,5′8-Trimethyl-psoralen | | | | | | | |
| | Human WBC | − | 11 | 34 | $5 \times 10^{-6}$ M + $4 \times 10^{4}$ ergs/mm² near UV light | | 101 |

* Extended S9 exposure conditions.
† Treatment in vivo; cell culture in vitro.
‡ Implanted in mice.
§ Syrian hamster feeder layer.
¶ Assumes chromosome #1 = 10% of genome.
‖ Macrochromosomes.
# Assumes 0.1 g embryo.

TABLE 2 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| 4-Nitroquinoline-1-oxide | | | | | | | |
| | CHO | – | 12.2 | 57 | $10^{-6}$ M | | 140 |
| | V-79 | – | 10.8 | 46.9 | $2.6 \times 10^{-6}$ M | | 143 |
| | DON | – | 7¶ | 32¶ | $4 \times 10^{-6}$ M | Autoradiography | 81 |
| | DON | – | 3.5 | 35.6 | $10^{-6}$ M | | 3 |
| Nitrogen mustard | | | | | | | |
| | Human WBC | – | 12 | 45 | $2 \times 10^{-8}$ M | | 99 |
| | CHO | – | 12.2 | 109 | $3 \times 10^{-6}$ M | | 140 |
| N-nitrosodiphenylamine | | | | | | | |
| | DON | – | 7.7 | 13.8 | $2.5 \times 10^{-5}$ | | 2 |
| Procarbazine | | | | | | | |
| | Mouse (AKR) marrow | + | 1.4 | 6.5 | 0.2 μg/g ($9 \times 10^{-7}$ moles/kg) | In vivo | 146 |
| Proflavine | | | | | | | |
| | CHO | – | 7.0 | 12 | $1.6 \times 10^{-6}$ M | | 26 |
| | V-79 | – | 10.8 | 16.5 | $3.3 \times 10^{-6}$ M | Fewer SCEs at 2.5 μg/ml | 143 |
| | DON | – | 7¶ | 21¶ | $4 \times 10^{-6}$ M | Autoradiography | 81 |
| Propane sulfone | | | | | | | |
| | DON | – | 7.7 | 21.0 | $10^{-3}$ M | | 2 |
| 1-(Pyridyl)-3,3-dimethyltriazine | | | | | | | |
| | V-79 | +‡ | 5.5 | 17.3 | 100 μg/g ($5.3 \times 10^{-4}$ moles/kg) | | 168 |
| Quinacrine mustard | | | | | | | |
| | Human WBC | – | 5.1 | 85 | $2 \times 10^{-6}$ M | | 170 |
| | CHO | – | 12.2 | 121.1 | $10^{-6}$ M | | 140 |
| | Vicia faba | – | 20 | 105 | $2.5 \times 10^{-6}$ M | | 89 |
| Saccharin | | | | | | | |
| | Human WBC | – | 9.8 | 17.0 | $2.2 \times 10^{-2}$ M | | 200 |
| | CHO | – | 8.8 | 12.0 | $5.5 \times 10^{-2}$ M | | 200 |
| | DON | – | 7.7 | 15.2 | $10^{-3}$ M | Fewer SCEs at $5 \times 10^{-2}$ M saccharin | 2 |
| Sodium nitrite | | | | | | | |
| | DON | – | 3.5 | 12.0 | $3 \times 10^{-3}$ M | | 3 |
| Styrene | | | | | | | |
| | CHO | + | 14.1 | 28.0 | $10^{-5}$ M | S9 + cyclohexene-oxide to inhibit epoxide hydratase | 37 |
| Styrene oxide | | | | | | | |
| | CHO | – | 11.9 | 62 | $8 \times 10^{-7}$ M | ↓ With S9 | 37 |
| Thiotepa | | | | | | | |
| | Vicia faba | – | 20.6 | 76.0 | $2 \times 10^{-4}$ M | | 88 |

TABLE 2 (*continued*)

| Agent | Cell Type | Acti-vation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Refer-ence |
|---|---|---|---|---|---|---|---|
| Triaziquone | | | | | | | |
| | Human WBC | – | 7.3 | 89.9 | $2.2 \times 10^{-7}$ M | | 64 |
| | Human WBC | – | 4.1 | 14.7 | $10^{-8}$ M | | 16 |
| | Human WBC | – | 5.4 | 47 | $10^{-7}$ M | | 52 |
| | Human fibroblasts | – | 9.3 | 79.6 | $2.2 \times 10^{-9}$ M | | 64 |
| | V-79 | – | 13.4 | 40.3 | $4.3 \times 10^{-10}$ M | | 193 |
| | Mouse (NMRI) marrow | + | 4 | 30 | 0.125 μg/g (5.5 $\times 10^{-7}$ moles/kg) | In vivo | 192 |
| | Marrow | + | 3.7 | 17 | 0.125 μg/g | In vivo | 14 |
| Tritiated deoxythymidine | | | | | | | |
| | Kangaroo rat | – | | 9 | 18 C/mM | Autoradiography | 56 |
| Tris (2,3-dibromopropyl) phospohate | | | | | | | |
| | V-79 | – | 5 | 25 | $2.9 \times 10^{-5}$ M | | 47 |
| | V-79 | +‡ | 3 | 16 | 0.5 mg/g (7.2 $\times 10^{-4}$ moles/kg) | | 47 |
| UV light (254 nm) | | | | | | | |
| | CHO | – | 6.6 | 13.2 | 26 ergs/mm$^2$ | Autoradiography | 201 |
| | DON | – | 7¶ | 38¶ | 80 ergs/mm$^2$ | autoradiography; ↓ with caffeine | 81 |
| | V-79 | – | 13.4 | 50.2 | 50 ergs/mm$^2$ | | 193 |
| X-ray | | | | | | | |
| | Human WBC | | 5.1 | 10 | 150 rads, G1 | | 170 |
| | Human WBC | | 5.2 | 15.5 | 200 rads, G1 | ↓ by L-cysteine | 4 |
| | Human WBC | | 10 | 10.9 | 200 rads, S | | 48 |
| | CHO | | 12.2 | 27 | 200 rads, G1 | | 140 |
| | CHO | | 12.2 | 35 | 200 rads, S | | 140 |
| Virus (SV-40) | | | | | | | |
| | Human fibroblasts | – | 7.5 | 8.3 | | T-antigen negative | 131 |
| | | – | 7.5 | 18.0 | | T-antigen positive | 131 |
| Vaccinia | | | | | | | |
| | Human WBC | – | 7.9 | 9.8 | | | 93 |

* Extended S9 exposure conditions.
† Treatment in vivo; cell culture in vitro.
‡ Implanted in mice.
§ Syrian hamster feeder layer.
¶ Assumes chromosome #1 = 10% of genome.
|| Macrochromosomes.
\# Assumes 0.1 g embryo.

TABLE 3
*Agents Exhibiting Mixed or at Most Weak SCE Induction Behavior*

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Acridine orange | | | | | | | |
| | V-79 | – | 10.8 | 16.9 | $3.3 \times 10^{-6}$ M | | 143 |
| | | – | 10.8 | 11.8 | $8.3 \times 10^{-6}$ M | | 143 |
| Acrylamide | | | | | | | |
| | Mouse (DDY) marrow | + | 2.9 | 3.7 | 0.1 mg/g ($1.4 \times 10^{-3}$ moles/kg) | Chromosome breakage | 166 |
| | Spermatogonia | + | 3.1 | 4.2 | 0.1 mg/g | Chromosome breakage | 166 |
| Anthracene | | | | | | | |
| | V-79 | +* | 5.5 | 4.3 | 150 μg/g $5.6 \times 10^{-4}$ moles/kg) | | 168 |
| | DON | – | 3.5 | 5.4 | $1 \times 10^{-4}$ M | | 3 |
| | DON | – | 3.5 | 4.8 | $1 \times 10^{-4}$ M | | 3 |
| Barbital | | | | | | | |
| | DON | – | 7.7 | 9.7 | $8 \times 10^{-3}$ M | Inconsistent concentration dependence | 2 |
| Bleomycin | | | | | | | |
| | Human WBC | – | 5.4 | 5.4 | $3.2 \times 10^{-6}$ M | Chromosome breakage | 52 |
| | Chinese hamster ovary (CHO) | – | 12.2 | 23.6 | $3 \times 10^{-6}$ M | Chromosome breakage | 140 |
| | Vicia faba | – | 20 | 21 | $2 \times 10^{-6}$ M | Chromosome breakage | 89 |
| Butylhydroxyanisole | | | | | | | |
| | DON | – | 7.7 | 11.0 | $10^{-4}$ M | | 2 |
| Butylbutanolamine | | | | | | | |
| | DON | – | 3.5 | 5.0 | $3 \times 10^{-4}$ M | | 3 |
| Caffeine | | | | | | | |
| | Human WBC | – | 4.6 | 5.5 | $10^{-3}$ M | | 194 |
| | | – | 11 | 22 | $1.5 \times 10^{-3}$ M | | 77 |
| | | – | 25 | 32 | $10^{-3}$ M | 8-Methoxypsoralen + light | 194 |
| | | – | 6.1 | 8.0 | $5 \times 10^{-4}$ M | Also potentiates alkylating agents | 41 |
| | Human WBC | – | 47 | 80 | $1.5 \times 10^{-3}$ M | + Mitomycin C | 77 |
| | Human fibroblasts | – | 2.9 | 6.8 | $1.5 \times 10^{-3}$ M | | 154 |
| | Vicia faba | – | 76 | 77.3 | $5 \times 10^{-4}$ M | + Thiotepa | 88 |
| | | | | | | ≤10% increase with 5 alkylating agents | 89 |
| | V-79 | – | 13.4 | 14.6 | $10^{-3}$ M | | 193 |
| | | – | 40.3 | 32.4 | $10^{-3}$ M | + Triaziquon | 193 |

* Implanted in mice.
† Exposure in vivo; culture in vitro.
‡ BrdUrd, 5-bromo-2′-deoxyuridine; FdUrd, 5-fluoro-2′-deoxyuridine.
§ Syrian hamster feeder layer.

TABLE 3 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| | | – | 40.3 | 27.9 | $2 \times 10^{-3}$ M | + Triaziquon | 193 |
| | | – | 50.2 | 34 | $10^{-4}$ M | + UV light | 193 |
| Cytosine arabinoside | | | | | | | |
| | Human WBC | – | 10.8 | 14.3 | $8.2 \times 10^{-6}$ M | Marked intersample variation | 144 |
| | Human WBC | +† | 10.9 | 8 | $8.2 \times 10^{-6}$ M | | 144 |
| Deoxycytidine | | | | | | | |
| | Human WBC | – | 10 | 15 | $10^{-4}$ M ($10^{-5}$ M BrdUrd‡ + $4 \times 10^{-7}$ M FdU) | | 110 |
| | Human WBC | – | 10.6 | 11 | $10^{-4}$ ($10^{-4}$ M BrdUrd but no FdU) | | 48 |
| Di-(2-ethylhexyl)-phthalate | | | | | | | |
| | DON | – | 8.8 | 11.0 | $10^{-3}$ M | | 2 |
| Fluorescent brightener 24 (Kayaphor SN) | | | | | | | |
| | DON | – | 7.7 | 11.3 | $10^{-4}$ M | | 2 |
| Fluorescent brightener 225 (Kayaphor LSK) | | | | | | | |
| | DON | – | 7.7 | 10.6 | $10^{-4}$ M | | 2 |
| Maleic hydrazide | | | | | | | |
| | Vicia faba | – | 20 | 98 | $5 \times 10^{-5}$ M | | 89 |
| | CHO | – | 12.2 | 15 | $10^{-3}$ M | No change in SCE between $10^{-5}$ M and $10^{-3}$ M | 140 |
| | CHO | – | 11 | 11 | $10^{-3}$ M | ± S9 | 173 |
| 2-Methyl-4-dimethylaminobenzene | | | | | | | |
| | DON | – | 7.7 | 11.3 | $10^{-4}$ M | | 2 |
| Phenanthrene | | | | | | | |
| | V-79 | – | 7.7 | 11.3 | $10^{-4}$ M | | 143 |
| | | +§ | 11.8 | 11.1 | $5.6 \times 10^{-5}$ M | | 143 |
| | DON | – | 8.8 | 10.6 | $10^{-3}$ M | | 2 |
| Potassium metabisulfite | | | | | | | |
| | DON | – | 7.7 | 10.6 | $10^{-3}$ M | | 2 |
| Potassium sorbate | | | | | | | |
| | DON | – | 7.7 | 12.4 | $2 \times 10^{-2}$ M | | 2 |
| Pyrene | | | | | | | |
| | V-79 | +* | 5.5 | 4.7 | $7.5 \times 10^{-4}$ M | | 143 |
| | | – | 10.8 | 12.7 | $5 \times 10^{-5}$ M | | 168 |
| | | +§ | 10.8 | 16.6 | $5 \times 10^{-5}$ M | | 168 |

TABLE 3 (*continued*)

| Agent | Cell Type | Activation | Baseline SCE/Cell | Induction SCE/Cell | Conditions | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Pyridine | | | | | | | |
| | DON | – | 7.7 | 10.9 | $5 \times 10^{-3}$ M | Inconsistent concentration dependence | 2 |
| Sodium benzoate | | | | | | | |
| | DON | – | 7.7 | 12.7 | $10^{-2}$ M | | 2 |
| Sunset yellow FCF (food yellow #5) | | | | | | | |
| | DON | – | 7.7 | 10.5 | $2 \times 10^{-3}$ M | Highly toxic | 2 |
| 4-O-Tolylazo-O-toluidine | | | | | | | |
| | DON | – | 7.7 | 10.0 | $10^{-5}$ M | | 2 |
| Urethane | | | | | | | |
| | DON | – | 7.7 | 14.4 | $8 \times 10^{-2}$ M | | 2 |
| Vincristine | | | | | | | |
| | Human WBC | – | 10.8 | 25.5 | $6.1 \times 10^{-8}$ M | | 144 |
| | Human WBC | – | 11.7 | 3.7 | $3 \times 10^{-6}$ M | | 177 |
| Virus (vaccinia) | | | | | | | |
| | Human WBC | | 7.9 | 9.8 | | In vivo | 93 |

terestingly, spermatogonia have a lower baseline SCE level than the other tissues, and SCE induction by MMC or cyclophosphamide is also lowest in spermatogonia. Very recently, a system for detecting SCE induction in regenerating liver has been developed (8, 161) (fig. 6, 7). Since the liver contains the highest level of microsomal activating activity (20, 61, 66), chemical activation and SCE induction should be possible within the same cell. This system increases the sensitivity of detection of SCE induction by agents (e.g., acetylaminofluorene) that require activation but which have thus far appeared to be relatively ineffective at SCE induction (161, 181, 182), perhaps, because once they are activated in the liver, they react without reaching more peripheral tissues. A liver system for SCE detection should also facilitate studies (Schreck, unpublished data) of the relative sensitivity of mice with different genetically determined basal and inducible liver arylhydrocarbon hydroxylase activity levels to clastogens requiring metabolic activation.

A major methodological difficulty with in vivo studies has been the requirement for multiple BrdUrd injections (5, 6, 193) or continuous BrdUrd infusion (138, 157, 159) because of rapid host metabolism of BrdUrd. The BrdUrd infusion method may prove especially valuable in studies in which sustained, known concentrations of clastogens must be administered to animals. We have introduced a simplified procedure, involving the use of BrdUrd in the form of a small tablet that can be implanted subcutaneously (7). Tablets can be prepared with a small, commercially available pill press (e.g., Parr Co., Moline, Ill.). Nearly 100% unifilar replacement of thymidine by BrdUrd during a single cycle can thus be effected, and tablets with different release kinetics have been prepared (8). The tablets will probably be more useful for large scale in vivo SCE studies in tissues such as bone marrow, the replication of which is apparently not seriously inhibited by the high BrdUrd levels provided by the tablets. However, relative to BrdUrd tablets, multiple BrdUrd injections give better results (e.g., a higher mitotic index) with regenerating liver cells and result in lower baseline SCE levels (161).

In vivo SCE analysis has now been per-

TABLE 4
*Agents Found Not to Induce S.C.E.s**

| Agent | Cell Type | Activation | S.C.E. Baseline | Treatment Limit | Comments | Reference |
|---|---|---|---|---|---|---|
| Acetone | | | | | | |
| | DON | – | 3.7 | $7 \times 10^{-5}$ M | | 3 |
| | Chick embryo | + | 1.2† | 50 μl/g‡ ($7 \times 10^{-4}$ moles/kg) | | 19 |
| Alcohols | | | | | | |
| Butanol | | | | | | |
| | CHO | – | 5.0 | $1.4 \times 10^{-5}$ M | | 135 |
| | Chick embryo | + | 1.2† | 100 μl/g‡ ($1.1 \times 10^{-3}$ moles/kg) | | 19 |
| Ethanol | | | | | | |
| | DON | – | 3.7 | $8.7 \times 10^{-5}$ M | | 3 |
| | CHO | – | 5.0 | $2.2 \times 10^{-5}$ M | | 135 |
| | Chick embryo | + | 1.2† | 150 μl/g‡ ($2.6 \times 10^{-3}$ moles/kg) | | 19 |
| Methanol | | | | | | |
| | CHO | – | 5.0 | $3 \times 10^{-5}$ M | | 135 |
| Propanol | | | | | | |
| | CHO | – | 5.0 | $1.7 \times 10^{-5}$ M | | 135 |
| Aminopyrine§ | | | | | | |
| | DON | – | 7.7 | $10^{-4}$ M | | 2 |
| Arochlor 1254¶ | | | | | | |
| | V-79 | + ‖ | 5.5 | 0.5 mg/g | | 168 |
| Bilirubin | | | | | | |
| | Human WBC | – | 16.5 | $3.4 \times 10^{-7}$ M | | 163 |
| | Human WBC | – | 16.2 | $3.4 \times 10^{-7}$ M + light | 17 J/cm$^2$ | 163 |
| | Human WBC | +# | 9.0 | $2.3 \times 10^{-7}$ M + light | | 163 |
| Dibutylhydroxy-toluene | | | | | | |
| | DON | – | 7.7 | $10^{-3}$ M | | 2 |
| N-n-butylure-thane | | | | | | |
| | DON | – | 8.8 | $10^{-3}$ M | | 2 |
| ε-Caprolactone | | | | | | |
| | DON | – | 7.7 | $10^{-3}$ M | | 2 |
| Cycloheximide | | | | | | |
| | Human fibro-blasts | – | 2.9 | $1.8 \times 10^{-6}$ M | | 154 |
| Diethylstilbes-terol | | | | | | |
| | DON | – | 7.7 | $10^{-4}$ M | | 2 |

* Negative results based only on a single test system, especially one that does not involve metabolic activation, should be viewed as tentative.

† Macrochromosomes only.

‡ Assumes 0.1 g embryo.

§ This agent has been described as being mutagenic (119).

¶ Arochlor 1254 is a potent inducer of mono-oxygenase activating enzymes (10), in addition to any direct genetic effect it might have.

‖ Implanted in mice.

\# WBC from infants receiving phototherapy.

TABLE 4 (*continued*)

| Agent | Cell Type | Activation | S.C.E. Baseline | Treatment Limit | Comments | Reference |
|---|---|---|---|---|---|---|
| Dimethylsulfoxide | | | | | | |
| | Human WBC | – | 15.5 | $1.3 \times 10^{-2}$ M | | 163 |
| | V-79 | + ‖ | 5.5 | Not given | | 168 |
| | Mouse (NMRI) marrow | + | 3.7 | 0.7 mg/g ($9 \times 10^{-4}$ moles/kg) | | 14 |
| 8-Ethoxycaffeine | | | | | | |
| | Vicia faba | – | 20.6 | 10 mM | | 87 |
| Ethylene glycol | | | | | | |
| | Chick embryo | + | 1.2† | 100 μl/g‡ ($1.7 \times 10^{-3}$ moles/kg) | | 19 |
| Fluorescent brightner (#260) | | | | | | |
| | DON | – | 7.7 | $10^{-4}$ M | Highly toxic | 2 |
| Fluorescent light | | | | | | |
| | Human WBC# | – | 15.5 | 17 J/cm$^2$ | | 163 |
| Hydroxyurea | | | | | | |
| | V-79 | – | 11.8 | $1.3 \times 10^{-5}$ M | | 143 |
| Lead acetate | | | | | | |
| | Human WBC | – | 4.1 | $10^{-5}$ M | | 16 |
| N-methylurea | | | | | | |
| | DON | – | 7.7 | $10^{-3}$ M | | 2 |
| Methylene blue | | | | | | |
| | V-79 | – | 11.8 | $10^{-4}$ M | | 143 |
| 8-Methoxypsoralen | | | | | | |
| | Human WBC | – | 12.1 | $5 \times 10^{-7}$ M | No light | 28 |
| | Human WBC | – | 8 | $2 \times 10^{-5}$ M | No light | 128 |
| | CHO | – | 16 | $5 \times 10^{-5}$ M | No light | 102 |
| Near UV light | | | | | | |
| | Human WBC | – | 12.1 | $2.3 \times 10^{5}$ ergs/mm$^2$ | | 28 |
| | Human WBC | – | 7 | $1.5 \times 10^{5}$ ergs/mm$^2$ | At most a 20% increase | 128 |
| | CHO | – | 16 | $1.5 \times 10^{4}$ ergs/mm$^2$ | | 102 |
| Penicillin | | | | | | |
| | Chick embryo | + | 1.2† | 3 mg/g‡ ($1.3 \times 10^{-2}$ moles/kg) | | 19 |
| Perylene | | | | | | |
| | V-79 | – | 11.8 | $4 \times 10^{-5}$ M | | 143 |
| | V-79 | + ‖ | 5.5 | 150 μg/g ($6 \times 10^{-4}$ moles/kg) | | 168 |
| Quinoline | | | | | | |
| | DON | – | 7.7 | $10^{-3}$ M | | 2 |
| Salt solutions | | | | | | |
| Sodium acetate | | | | | | |
| | Human WBC | – | 4.1 | $10^{-5}$ M | | 16 |
| Hanks' balanced salt solution | | | | | | |
| | Chick embryo | + | 1.2† | 1 ml/g‡ | | 19 |

TABLE 4 (*continued*)

| Agent | Cell Type | Activation | S.C.E. Baseline | Treatment Limit | Comments | Reference |
|---|---|---|---|---|---|---|
| 0.3 M NaCl-0.03 M citrate | | | | | | |
| | Chick embryo | + | 1.2† | 200 μl/g‡ | | 19 |
| 0.2 M phosphate-0.1 M citrate | | | | | | |
| | Chick embryo | − | 1.2† | 200 μl/g‡ | | 19 |
| Sodium dehydroacetate | | | | | | |
| | DON | − | 7.7 | $10^{-3}$ M | | 2 |
| Streptomycin | | | | | | |
| | Chick embryo | + | 1.2† | 5 mg/g‡ (8 × $10^{-3}$ moles/kg) | | 19 |

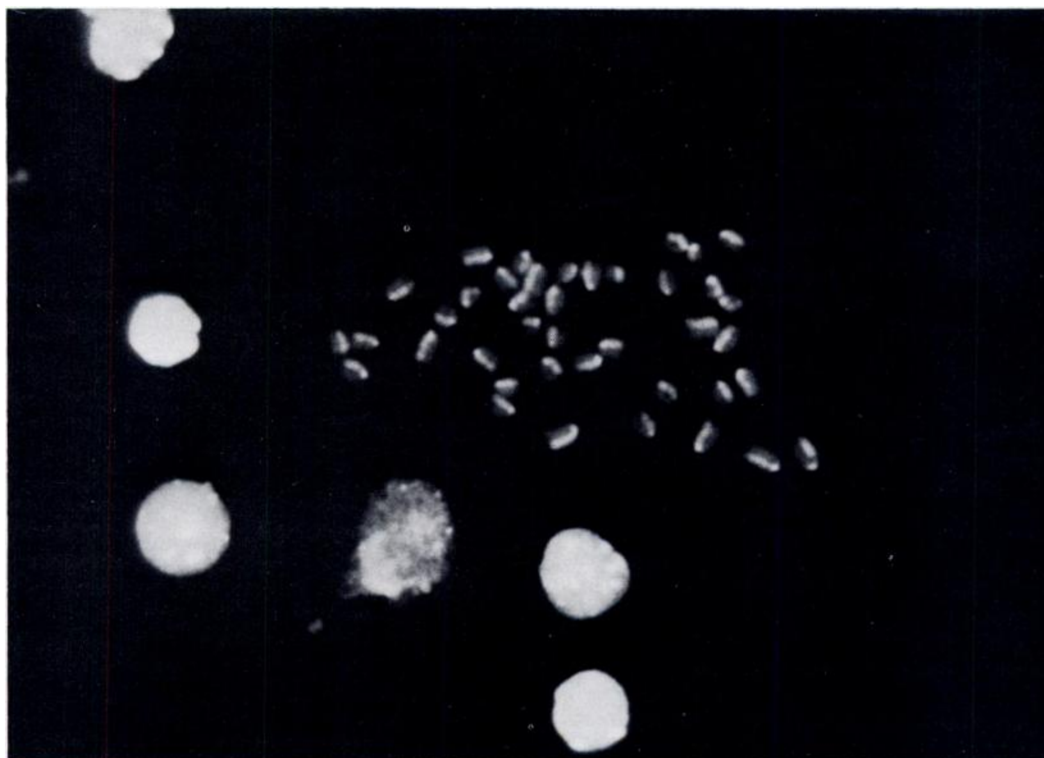

FIG. 6. Sister chromatid exchanges SCEs in a regenerating mouse liver cell. An 8-week-old male CBA mouse was subjected to partial (~65%) hepatectomy. Thirty-two hours later, the animal received a series of 13 half-hourly intraperitoneal injections of $10^{-2}$ M 5-bromo-2′-deoxyuridine (BrdUrd). Cell harvest, 55 h after partial hepatectomy, was preceded (4 h) by i.p. injection of 160 μg of colcemid. Slides were stained with 33258 Hoechst, and SCEs were detected by fluorescence microscopy. Three SCEs can be seen in this cell; controls on the average had 7 SCEs per cell (161).

formed on cells from Chinese hamster marrow (8, 15) and cheek pouch mucosa (169). This latter tissue is accessible not only to systemic exposure but also to topically applied agents such as 7,12 dimethylbenzanthracene. In the latter situation, one cheek pouch can be exposed to clastogens, with the other serving as an internal control.

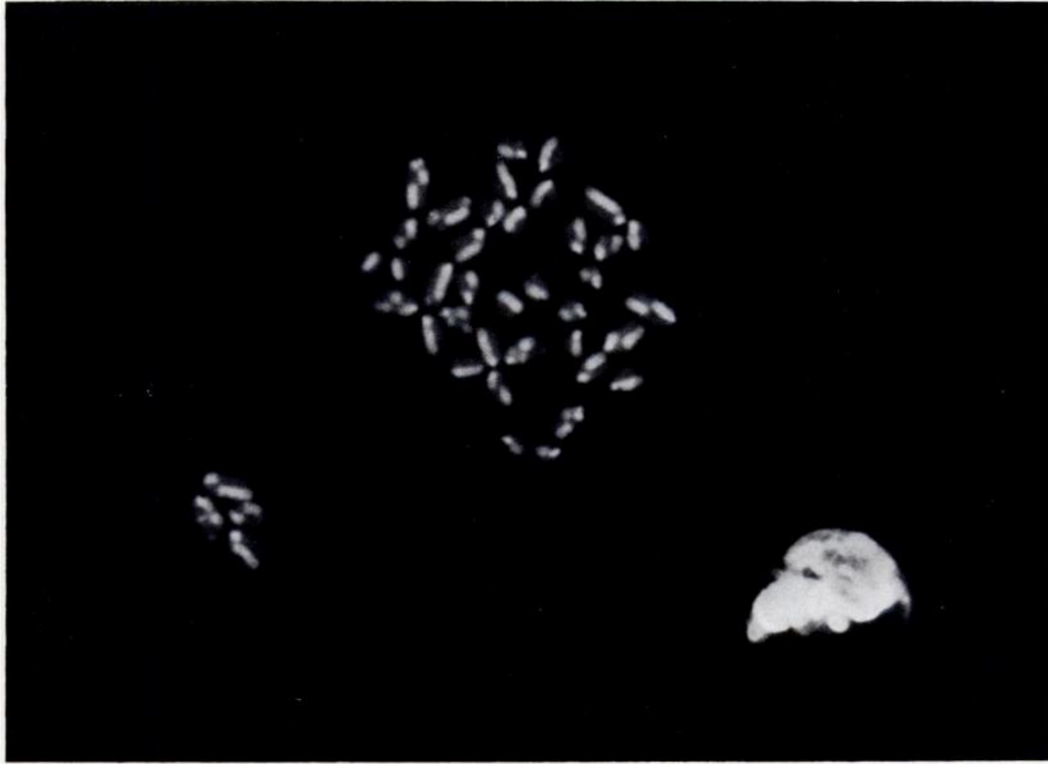

FIG. 7. Induction of sister chromatid exchanges SCEs in regenerating mouse liver cells by cyclophosphamide. The experimental protocol was essentially that of figure 4, except that cyclophosphamide (5 mg/kg) was injected i.p. 1 h after the final 5-bromo-2′-deoxyuridine (BrdUrd) injection. This cell exhibits more than 35 SCEs; cells treated by this protocol had, on the average, 20 SCEs per cell (161).

This system should be especially useful for cytogenetic evaluation of putative topical carcinogens.

A different type of "in vivo" SCE analysis involves the use of SCE frequencies to assess the cytogenetic impact of clastogenic agents administered to patients, usually in the course of chemotherapy (1, 144). Peripheral lymphocytes withdrawn from patients exposed to various drugs are cultured for two cycles in medium containing BrdUrd prior to SCE analysis. Nevstad (130) utilized this approach to detail the time course of SCE elevation due to adriamycin, a compound previously stated to induce SCE in patients (140). Perry (139) has continued this type of study. Widespread use of this procedure will require means to account for variations in the persistence of SCE elevation following treatment, as well as lymphocyte toxicity, which compromises the yield of analyzable metaphases.

## VI. SCD in Meiotic Cells

In vivo administration of BrdUrd has permitted SCD in meiotic cells. Previous studies of SCD in meiosis had utilized autoradiography (137, 184), which afforded limited resolution. Initial success with BrdUrd was achieved in the X-Y bivalent of the mouse (6), in which SCD was detected. However, meiotic interchange is not known to occur in the mouse X-Y pair, and only very limited SCD was effected in autosomes, perhaps because of marked BrdUrd sensitivity. Allen et al. (8) have investigated meiosis in the Armenian hamster, an animal in which meiotic interchange presumably occurs in the X-Y bi-

valent (112) and have detected nonsister chromatid exchange, most likely due to meiotic recombination. BrdUrd-dye techniques have also been used to study meiotic interchange in locust chromosomes (186).

## VII. Interpretation of SCE Induction Tests

A number of potentially confounding variables and other limitations must be kept in mind when interpreting the results of SCE tests. For example, exposure to BrdUrd must be high enough to permit good SCD but not so high that it produces a variable and unacceptably high background level of SCEs. Moderate (e.g., $<10^{-4}$ M) doses of BrdUrd do not appear to alter the response of cultured cells to other clastogens, but much higher levels of BrdUrd induce a sharp increase in SCEs, out of proportion to additional BrdUrd incorporation (99, 101), which might compromise the sensitivity with which additional SCE induction can be detected. Also, while most early studies of SCEs were done with alkylating agents, chosen primarily to exemplify efficient SCE induction, it is desirable that future studies be capable of examining agents for which clastogenic activity is less certain. In these instances, at most a small increment in SCEs might be observed, and variables, such as effects due to the vehicles used to dissolve the agent or the time required for metabolic activation, may become important. Agents for which information on SCE induction is conflicting, or for which induction is at most minimal and examined in only one system, are tabulated separately (table 3).

A major problem in arriving at a decision about the clastogenicity of a new compound is the upper limit of the concentration to be tested before negative results are to be accepted. Typically, this upper limit will be a treatment level that is sufficiently toxic to cells that proliferation for one or two cycles, which is necessary for SCE detection, is inhibited. Such toxicity may become evident either in chromosome breakage or in alteration of specific cell kinetic parameters (and a reduced mitotic index). In any case, particularly in view of the approximately linear dependence of SCE induction on clastogen concentration observed by Carrano et al. (26) in Chinese hamster ovary (CHO) cells, it would seem meaningful ultimately to describe results in terms of the SCE increment per cell per concentration of specific agent. A minimal requirement would seem to be the acquisition of data in a range over which the SCE frequency showed progressive increase with increasing treatment levels. Since agents to be tested may perturb the cell cycle, the most accurate estimate of SCE induction would probably require several collections of metaphases, to include all cells exposed, as utilized by Carrano et al (26).

Finally, the limitations of the test system employed must be considered. Most frequently, one wishes to know whether an unknown agent will cause genetic damage to a variety of human tissues. If this agent is active without metabolic modification, a human peripheral lymphocyte test system may be adequate, subject primarily to the possibility that different human tissues might have different repair capacities or drug metabolism rates. If metabolic activation of an agent is required, a rodent test system is most frequently used. However, DNA repair in rodents is known to differ from that in man (189) and interpretation of results with rodent cells should consider this difference. If microsome preparations are used to activate the agent to be tested, differences between the modifications effected in vivo and those caused by isolated microsomes may prove important. Typically, most artifacts due to particular test systems will tend to produce false negative rather than false positive results. Because of the former possibilities, utilization of multiple test systems is probably advisable. However, comparison of test data on different substances would be facilitated if the plethora of test systems currently utilized (e.g., tables 2–4) was reduced to a standard

set, which was then applied to each compound.

It is possible that "positive" results might depend on the use of an unrealistically high treatment dosage. This problem is inherent in many short term tests, for which high level short term exposure is used to estimate the effect of low dose exposure over an interval of many years. Quantitative estimates of SCE induction efficiency per unit exposure will be important, both to characterize the potential hazard of an individual chemical and to estimate the possible additive effects of many agents, each present in low amounts. Introduction of *quantitative,* rather than *qualitative* evaluations of chemicals may prove to be very important in large-scale mutagen-carcinogen testing. SCE induction tests are very well suited for such a quantitative analysis.

## VIII. Relationship of SCE Induction to DNA Damage, Repair, and Synthesis

A variety of chemical and physical agents, exhibiting diverse modes of interaction with DNA (table 2) as well as transformation of cells SV40 virus (131), are capable of inducing SCEs. Alkylating agents, of many different types, seem to be especially effective. SCEs can also be induced by irradiation of BrdUrd-substituted DNA (75, 82, 84), a treatment causing predominantly (although not exclusively) single strand breaks (71). Only fragmentary information exists, however, about the quantitative relationship between the number and types of alkylation products or DNA strand interruptions, the efficiency of their repair, and the number of SCEs produced. Quantitation of DNA alkylation and removal can be accomplished by chemical analysis of reaction products or, if suitable isotopic derivatives can be obtained, by measurement of radioactivity in newly formed DNA adducts.

We have obtained evidence that SCE may account for only a small fraction of DNA damage by 8-methoxypsoralen plus near UV light (Cassel and Latt, unpublished data). The combination of 8-methoxypsoralen plus 365 nm light, but not either agent alone, is effective in inducing SCE in human and CHO chromosomes (28, 42, 101, 102, 106, 106a, 107, 128, 194). The dependence of SCE on either light or 8-methoxypsoralen, keeping the other agent fixed, has been quantitated (101) and an assay for measuring the binding of tritiated 8-methoxypsoralen developed, so that the ratio between these two quantities can be compared. Data thus far indicate that one SCE is induced (in the two cycles following DNA damage) per approximately 200 8-methoxypsoralen-DNA adducts (Cassel and Latt, unpublished data). This result is currently being analyzed into components due to mono- and bifunctional adducts.

We have thus far obtained both cytological and biochemical evidence for the persistence of alkylation by 8-methoxypsoralen during at least a few replication cycles. The cytological data (102) consist of the observation of reciprocal interchanges of dark chromatids in third cycle metaphases, indicative of SCE formation after the second cycle (125); SCEs formed during the first two cycles appear as isolated segments of darkly staining chromatids in third division metaphases. Similar data implicating SCE induction during the third cycle following DNA damage have now been described by Ishii and Bender (76) in cells treated with MMC. Thus, alkylation damage might be persistent and cumulative. Persistence of DNA damage might underlie the observation of Stetka et al. (174) that repeated exposure of rabbits to MMC ultimately leads to persistently elevated SCE levels (in peripheral lymphocytes cultured in vitro).

Shafer (165) has recently postulated that SCE formation involves the bypass of DNA crosslinks during replication. This model is compatible with the observation that 8-methoxypsoralen adducts are slowly removed by cells. It will now be important to determine whether, as predicted by Shafer, those adducts remaining after replication are still in the form of crosslinks.

It is instructive to note that, since SCEs

reflect less than 1% of DNA adducts and chromosome breaks are less than 1% as frequent as SCE (98, 99), chromosome breaks may detect $10^{-4}$ to $10^{-5}$ or less of the total DNA damage in a cell. The disparity between the numbers of DNA adducts, SCEs, and chromatid breaks might contribute to the multiplicity of results obtained by investigations comparing SCE to break ratios and the relative location of chromosome breaks and incomplete SCE following exposure of cells to different clastogens.

Consistent with an earlier suggestion by Heddle et al. (65), nearly half of the breaks in chromosomes in lymphocytes from Fanconi's anemia patients treated at the start of S with MMC occur at incomplete SCE sites (110), as do 25 to 50% of the breaks induced at the end of the DNA synthesis (S) phase by UV-irradiation of BrdUrd-substituted Chinese Hamster cells (84). Also, treatment of rat cells with dimethylbenzanthracene a few hours prior to harvest, i.e., at the end of S for the metaphases scored, gives a similar distribution of SCEs and breaks (190). In other systems, breaks occur in the absence of SCE (74, 154). Although an explanation of these divergent observations is not apparent, there is ample room within the confines of observed stoichiometry for a given combination of damage and cell response to cause SCE and chromosome breaks by completely or largely divergent paths.

SCE formation appears to be tightly coupled to DNA synthesis. Wolff et al. (201) demonstrated that UV-damaged rodent cells needed to pass through S phase for SCE induction to be detected. Variation in SCE inducibility within the S phase was investigated by Kato (82, 85), who used near-UV light to induce SCEs in unsynchronized BrdUrd-substituted, Chinese hamster cells. The position of cells within S at the time of irradiation was estimated from the time between irradiation and metaphase collection and by the extent of incorporation of an $^{3}$H-thymidine pulse that was administered at the time of irradiation and then detected at metaphase. Although SCEs induced at the end of S were observed to occur preferentially in late replicating regions, the efficiency of SCE induction appeared to be maximal near mid-S, coinciding with the maximum in the rate of DNA synthesis.

Analysis of SCE induction by 8-methoxypsoralen plus light in synchronized cells (102, 106a) led to a different conclusion, namely that SCE induction was maximal at the start of S and decreased progressively throughout the S phase. The difference between this result and that of Kato (82) may be due to lack of cell synchrony in Kato's experiment or to a difference in the type of DNA damage effected. Preliminary evidence for the latter possibility has recently been presented by Shafer (165). This possibility is especially easy to test, e.g., by treating synchronized cells with BrdUrd plus light. Loss of coherence in cell phasing during S would tend to broaden the SCE versus S phase traverse curve, especially for data attributed to the start of S. For data obtained at the end of S, there was better agreement between the two studies; a need for DNA synthesis in a given chromosome region, subsequent to DNA damage, appeared necessary for SCE induction. The molecular events accounting for the coupling between SCE induction and DNA synthesis remain to be determined, however.

## IX. Biological Significance of SCE Formation

Implicit in many of the above studies is the assumption that SCE formation bears a direct relationship to DNA damage, repair, and mutagenesis. Certain evidence lends support to this idea. Carrano et al. (26) have observed an increase in mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) [and adenine phosphoribosyl transferase (APRT) (26a)] locus of the CHO cells in proportion to concentrations of ethylmethanesulfonate, ethylnitrosourea, MMC, and proflavine, in ranges also causing a linear response in SCE induction. Relatively fewer mutations were observed with the bifunctional agent

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MMC, or with its monofunctional decarbamyl analogue. Assuming the existence of 50,000 genes per cell, all with the same mutagenic susceptibility as HGPRT, Carrano et al. (26) estimated that 0.01 to 10 mutations per SCE occurred during the first two S phases. It would seem desirable, although admittedly difficult, to develop a method of measuring SCE induction and mutagenesis in the same cells, to rule against the possibility that these two phenomena reflect disparate effects of alkylation in different members of a cell population.

Alkylation by psoralen derivatives plus light, a powerful inducer of SCE formation (28, 101, 102, 106, 106a, 107, 128, 194), is known to stimulate DNA strand interchange in recombination-proficient but not in recombination-deficient (Rec A) bacteria (31). This observation prompted the suggestion (99) that SCE formation in metaphase chromosome was somehow analogous to recombinational repair (151, 152) in bacteria. A feature complicating this analogy is the possible difference between DNA repair processes in bacterial and mammalian cells (115, 116). Recombinational repair in bacteria may be error-prone; e.g., it has been reported that induction of mutations in bacteria by UV or psoralen plus light requires a functional Rec A system (73). The relationship of these observations to the error-prone S.O.S. repair system (196) in bacteria remains to be determined. Of potential interest in this regard are the claim (91) that the tumor promoter TPA (12-0-tetradecanolyphorbol-13-acetate) can induce SCEs, and the data of Matsushima et al. (123) that the protease inhibitors, e.g., antipain and elastatinal, which are capable of blocking S.O.S. functions (124), have produced effects suggesting inhibition of SCE induction.

Another event in addition to mutagenesis paralleling SCE induction by clastogens is the release of SV40 virus from transformed cells. This has been demonstrated (78) in a number of different hamster kidney cell lines, using MMC and ethylmethane sulfonate (EMS). A 10,000-fold greater concentration of EMS (relative to MMC) was needed both for SCE induction and for virus induction.

## X. SCE Formation in Human Chromosome Fragility Diseases

Analysis of SCE formation has been used to differentiate between various inherited human diseases characterized by chromosome fragility and a predisposition for the development of neoplasia (53). These diseases, which include Bloom's syndrome, Fanconi's anemia, and ataxia telangiectasia, presumably involve defects in DNA repair. The diseases potentially constitute test systems, with specific DNA repair defects, for dissecting the SCE process, and cells from other diseases (e.g., xeroderma pigmentosum, see below) may permit extraordinarily sensitive clastogen detection. All three conditions listed above are rare, but they follow an autosomal recessive inheritance mode, and the respective heterozygotes amount to 1 to 2% of the total population (178, 179, 180). Since these heterozygotes also appear to be at an increased risk for certain forms of cancer (178), they make up several percent of all individuals with those conditions.

Cells from patients with Fanconi's anemia have been shown to be highly susceptible to killing (44, 45) and to chromosome breakage (11, 153, 155) by bifunctional alkylating agents, and they appear to exhibit reduced ability to excise UV (142) and *gamma* irradiation products (145), and DNA crosslinks (46).

Lymphocytes from Fanconi's anemia patients, while exhibiting essentially normal SCE frequencies in the presence of BrdUrd, respond to MMC treatment with a subnormal increase in SCE formation (110). This observation has now been confirmed in two other laboratories (quoted in references 48 and 167). The reduced stimulation of SCE formation by MMC in Fanconi's anemia is associated with increased chromatid breakage. However, the relative contribution of MMC monoadducts and crosslinks to the

SCE and chromosome breakage results has not yet been determined. Interestingly, approximately half of the breaks induced in Fanconi's anemia lymphocytes by MMC occurred at sites of incomplete SCE formation (101, 110), which is compatible with the hypothesis that the break increment and at least some of the exchange deficit are causally related.

Our initial studies of lymphocytes from four patients with Fanconi's anemia have been repeated with similar results on two other patients with this disease. Fibroblasts from Fanconi's anemia patients show only a marginal deficit in SCE response, although chromosome breakage in the presence of MMC is elevated, and the response in cells from different sources is heterogenous. The results can be interpreted to suggest that Fanconi's anemia cells are defective in a form of DNA repair.

We have not detected abnormalities in short term SCE induction in cells from Fanconi's anemia heterozygotes. However, extended exposure of carriers to low levels of the potentially bifunctional alkylating agent diepoxybutane (12) does seem to elicit abnormally high chromosome breakage in both diseased and heterozygote cells. This latter observation may reflect accumulation over several cell cycles of incompletely repaired DNA damage.

In Bloom's syndrome, the baseline SCE frequency is greatly elevated (29). It is not yet apparent how this relates to retarded rate of DNA replication fork progression (55, 62) or increased sensitivity to ultraviolet light (55) in these cells. Tice et al. (188) have observed an approximately 50% elevation in SCE frequencies in normal fibroblasts cocultivated with cells isolated from patients with Bloom's syndrome. One interpretation of these data is that a humoral factor is responsible for the SCE elevation in Bloom's syndrome. German et al. (54) reported that, in certain Bloom's syndrome patients, a subpopulation of lymphocytes does not exhibit elevated SCEs, perhaps suggesting that, if such a humoral factor exists, not all cells are equally susceptible. Recently, Shiriashi and Sandberg (167) have shown that lymphocytes from a patient with Bloom's syndrome undergo a modest additional increase in SCEs upon exposure to MMC. This increase may in part be limited by the high baseline level of SCEs (≥100/cell) and the existence of a saturation level of SCE formation (or detection) in a given cell.

Patterson et al. (136) reported that cells from patients with ataxia telangiectasia exhibited a reduced ability to excise DNA bases damaged by high energy radiation. More recent studies (30) have indicated that the X-ray survival of cells from ataxia telangiectasia patients is well below normal, while survival of cells from heterozygotes was intermediate between that of cells from normal and diseased individuals. However, cells from ataxia telangiectasia patients show normal baseline SCE levels (49, 63) as well as a normal SCE response after exposure to X-irradiation, MMC, EMS, and adriamycin (48).

Cells from patients with xeroderma pigmentosum, another hereditary disease with a predisposition for neoplasia, with the exception of the postreplication repair deficient variant of this disease (36), hyperreact to UV irradiation (13) or alkylating agents (192, 202, 203), undergoing a much greater increase in SCEs than do identically treated normal cells. Xeroderma pigmentosum cells that exhibit SCE hyperinducibility also have a reduced ability to excise alkylation products (e.g., 6-0-methylguanine) (57). This is compatible with the idea that SCE results from DNA damage that has not been removed. However, as shown by Wolff et al. (197, 203), the relative inducibility of SCE and chromosome breaks in xeroderma pigmentosum cells depends strongly on the type of DNA damage involved.

It is interesting to note that in xeroderma pigmentosum a hyperinducibility in SCEs correlates with a hyperinducibility of mutations by similar agents (120). Conversely, in Fanconi's anemia, the hypoinducibility in SCEs, more marked with MMC than with EMS, is accompanied by a decrease in

the ability of both of these alkylating agents to induce mutations (43). Thus, even though SCE may reflect only a small fraction of the total damage caused to DNA, it is intriguing to speculate that the SCE-inducing component of this damage might ultimately prove to be an important component biologically.

## XI. The Mechanism of SCE Formation

Various approaches have been used to investigate the mechanism of SCE formation. Kato (84) has examined SCE inducibility in unsynchronized Chinese hamster cells that were allowed to incorporate BrdUrd for one cycle and grow a second cycle in the presence or absence of BrdUrd. SCE induction at a time approximating the last few hours of the second S phase was effected by irradiation with near UV light. Only a small additional increase in SCE was observed in those cells that had incorporated BrdUrd for the second S phase, prompting the suggestion that SCE induction might have multiple pathways, at least one of which was independent of the degree of BrdUrd substitution. However, if SCE induction in a particular chromosome region requires DNA synthesis following damage (111), then only the regions that had not replicated a second time at the time of irradiation would be susceptible to SCE induction. These would be unifilarly substituted with BrdUrd, independent of the growth protocol used, and no difference in SCE induction would be expected in the two types of cells, whatever the specific mechanisms involved.

Kato (84) also examined the effect of caffeine on SCE induction and found it to inhibit induction in cells that had undergone one round of BrdUrd incorporation but to stimulate SCE induction, during the second S phase, in cells that incorporated BrdUrd for two cycles. Interpretation of this result will depend on the chromosomal location of these additional SCEs. Kato (79) has previously reported that caffeine inhibited SCE induction by UV in Chinese hamster cells, prompting analogy with post-replication repair, while other workers have observed either a potentiation (194) or an inhibition (193) of SCE frequencies with caffeine (also table 3). Vogel and Bauknecht (193) stressed the importance of the toxicity of caffeine and its effect on selection of metaphases for scoring. Recently Ishii and Bender (77) have determined that SCE potentiation by caffeine requires that the caffeine be added with or soon after the SCE inducer. Caffeine may well exert multiple effects that might be very difficult to dissect.

SCE induction, like mutagenesis (149), may also be influenced by agents, e.g., cysteine (4), capable of trapping free radicals. However, these results, like those in which the enzymes superoxide dismutase and catalase protect cells from chromosome breakage (132–134), probably deal more with the chemistry of the inducing agent than with alterations in cellular response to the damage induced.

## XII. Evidence for Sister Chromatid Interchange at the DNA Level

Two types of experimental approaches have been used to search for DNA exchanges that correspond to SCEs. Both utilize cells that have incorporated BrdUrd for less than one cycle, and thus contain DNA substituted in only one strand. Following sister strand exchange, junctions of substituted and unsubstituted polynucleotide should result and appear as material of intermediate density in alkaline CsCl gradients (148). The Holliday model (68) for DNA recombination also predicts segments of heavy-heavy (and light-light) DNA at interchange sites in neutral CsCl gradients.

Rommelaere and Miller-Faures (148) reported the detection of Chinese hamster DNA in alkaline CsCl gradients with intermediate density. However, most of this material exhibited rapid renaturation following neutralization, a result expected for crosslinked DNA. If DNA from the Chinese hamster cells was centrifuged in neutral CsCl, approximately 0.1% of the material exhibited density greater than that of hy-

brid, heavy-light (HL) DNA and interpretable as containing segments of bifilarly substituted, heavy-heavy (HH) DNA. The amount of this DNA was increased 4-fold by UV irradiation (100 ergs/mm$^2$) prior to BrdUrd incorporation, but this amount was more than 10 times that expected from the number of SCE in these cells.

Moore and Holliday (126) similarly detected 0.1% HH DNA from rapidly growing CHO cells cultured not quite one cycle in medium containing BrdUrd. MMC (1 $\mu$g/ml), when administered in highly toxic amounts 5 h prior to harvest, appeared to increase both HH DNA and SCE. Again, the amount of HH DNA was much more than expected for the number of SCE observed.

Loveday has repeated and extended the Moore and Holliday experiments, but was not able to reproduce their observations, either under the conditions they described or after introducing a number of methodological refinements (118). Synchronized CHO cells that had incorporated BrdUrd for one cycle exhibited a small amount (0.4 ± 0.2%) of DNA banding with a density expected for HH DNA, but this was not increased by addition of sufficient MMC (0.03 $\mu$g/ml, at the start of S) to more than triple the SCE frequency (fig. 8). Significantly, the dense DNA persisted after a subsequent round of replication in the absence of BrdUrd (calling its bifilar substitution into question), and material with a similar density shift from the main band DNA was seen in cells that incorporated $^3$H thymidine (but not BrdUrd). While Loveday's data do not rule out the existence of the HH DNA predicted by the Holliday model, they suggest that the biochemical evidence thus far claimed for this DNA is very weak, and that additional experiments are necessary to clarify the chemical events associated with DNA interchange during SCE formation.

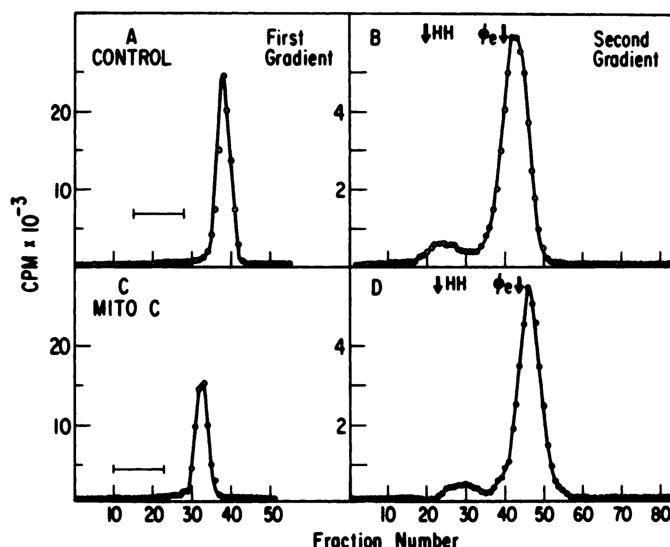


FIG. 8. Density gradient analysis of Chinese hamster ovary (CHO) DNA after one cycle of 5-bromo-2′-deoxyuridine (BrdUrd) incorporation. Synchronized CHO cells were released into medium containing $^3$H-BrdUrd (5 mCi/ml, 2 × 10$^{-5}$ M) and colcemid (0.45 $\mu$g/ml) was added after 8 h. DNA was isolated from metaphase cells 4 h later, sheared, and centrifuged to equilibrium in CsCl. Three drop fractions were collected and aliquots (10%) were counted: figure 8A, control; figure 8C, 0.03 $\mu$g/ml mitomycin C added at the time of release from G1-S. The indicated fractions were recentrifuged with $^{14}$C-labeled DNA and 2 drop fractions were collected onto filters. The second gradients are shown in figure 8B, no mitomycin C; figure 8D, + mitomycin C. The arrows mark the expected position of heavy-heavy (HH) DNA and the actual position of DNA $\phi$e DNA ($\phi$e). Total radioactivity (cpm): figure 8A, 1.1 × 10$^6$, with 6.6 × 10$^3$ in dense DNA (0.65%); figure 8C, 6.7 × 10$^5$, with 4.9 × 10$^3$ in dense DNA (0.73%) (118).

## XIII. Summary

Methodology for SCE detection is now well developed. BrdUrd-fluorochrome or Giemsa techniques have greatly simplified cytological SCE analysis. SCE formation can be studied in cultured cells, in intact animals, or in combined systems in which cells from treated animals are cultured in vitro or chemicals are activated by microsomal preparations before exposure to cultured cells. Alterations in SCE frequencies in any of these systems can serve as sensitive indices of the interaction of mutagen-carcinogens with chromosomes.

Most mutagen-carcinogens are potent inducers of SCEs, while a few others increase chromosome breakage. The combination of SCE and chromosome aberration as a test for clastogens thus has few "false negatives." Conversely, there is no convincing example yet of a compound which is highly effective at inducing SCEs that is not mutagenic and/or carcinogenic in at least some system.

In vitro SCE analysis can also be used clinically for differentiation of human chromosome fragility diseases, and it may prove useful for monitoring chromosome damage in cells from patients exposed to clastogenic agents during chemotherapy. Information about the mechanism of SCE induction by chemical and physical agents is still rudimentary, and little is known about the molecular abnormalities underlying most human chromosome fragility diseases or about the causes for alterations in SCE formation in these diseases. Present empirical applications of SCE analysis should increase as more is understood about the basic mechanism of SCE formation.

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