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A REVIEW OF THE INTERNATIONAL SYMPOSIUM

SISTER CHROMATID EXCHANGES: TWENTY-FIVE YEARS OF EXPERIMENTAL RESEARCH

Held December 4-8, 1983, at Brookhaven National Laboratory, Upton, NY, USA

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INTRODUCTION

In 1957, J. Herbert Taylor and his colleagues in the Biology and Medical Departments at Brookhaven National Laboratory published their first contribution demonstrating the existence of sister chromatid exchanges (SCEs) detected cytologically in plant root-tip cells following the incorporation of tritiated thymidine into replicating DNA. The following year, the first contribution concerned with examining the molecular events involved in the formation of SCEs was published by J. Herbert Taylor. Twenty-five years later, scientific interest in the nature, significance, and utility of this cytogenetically observed phenomenon continues to expand. The purpose of this symposium, held on December 4-8, 1983, at Brookhaven National Laboratory and co-spousored by the Biology and Medical Departments, was to honor this initial research at Brookhaven by bringing internationally recognized leaders in the fields of genetics, cytogenetics, carcinogenesis, mutagenesis, radiation biology, toxicology, and environmental health together into an open forum to present and discuss: (i) current knowledge of the induction and formation of SCEs and their relationship to other biological endpoints, including carcinogenesis, mutagenesis, transformation, clastogenesis, DNA damage and repair, and cellular toxicity; (ii) the optimal strategies for the utilization of SCEs in genetic toxicology testing schemes involving in vitro and in vivo exposure situations; (iii) the most valid statistical methods for analyzing SCE data obtained from cells in culture, from cells in intact organisms, and from cells in humans; (iv) the relevance of SCEs as an indicator of human disease states, both inherited and acquired, and of progress in disease treatment; and (v) the use of SCEs as an indicator of human exposure to genotoxic agents and their relevance as a prognosticator of future adverse health outcomes.

To ensure a truly international program, three program committees representing the U.S., Japan, and Europe were formed, chaired by R. R. Tice and A. Hollaender, K. Morimoto, and B. Lambert, respectively, to invite scientists, expert in different aspects of SCE research and utilization, to make presentations and/or to contribute posters. This approach was extremely successful as demonstrated by the participation of 175 scientists from 20 countries. The participation of scientists from academia, industry, research laboratories, and regulatory agencies, all with a common interest in SCEs, provided for an extremely useful interchange of knowledge and facilitated the prioritization of future research directions.

The symposium program was divided into three areas of interest - the nature of SCEs, SCEs and genetic toxicology, and human studies. Each area included also an appropriate poster session. The symposium was highlighted by keynote addresses by J. Herbert Taylor and S. A. Latt. A general discussion on the relevance of SCE studies to public health, prevention, and intervention closed the meeting. Due to the number and variety of material presented at the symposium, the following summary does not include every presentation. A limited number of copies of the symposium abstract book are available and information on the symposium proceedings can be obtained from R. Tice.

THE NATURE OF SCE

These presentations were divided into four areas: (i) Characterization; (ii) Induction, Specificity, and Modulation; (iii) Mechanisms and Models; and (iv) Correlations.

Characterization

It is well-accepted that SCEs can be induced by the systems used for their visualization, i.e., by the beta rays from incorporated tritiated thymidine or by the presence of BrdUrd and other halogenated pyrimidines. The existence of spontaneous SCEs and the extent to which the SCEs induced by BrdUrd are due to nucleotide pool inbalances and/or to its incorporation into DNA remain subjects of controversy. Several presentations supporting the existence of spontaneous SCEs, either through extrapolation of halogenated pyrimidine dose: SCE response curves to zero dose (J. P. O'Neill, USA; H. Tsuji, Japan) by quantitatively analyzing the frequency of SCEs induced by BrdUrd in successive generations (C. Gutierrez, Spain), or through an assessment of SCEs in ring chromosomes (C. R. Geard, USA) were made. However, whether these SCEs arise as a consequence of normal DNA replication or are the result of DNA damage induced by routine cellular processes such as that which may occur from the formation of oxygen radicals during normal oxidative processes (I. Emerit, France) remains to be elucidated. The question of the relative contribution of incorporated BrdUrd versus the nucleotide pool imbalances on SCE induction was not resolved during this meeting. For example, O'Neill reported a linear SCE dose response curve for Chinese hamster ovary (CHO) cells which could be related directly to the degree of DNA substitution by BrdUrd and chlorodeoxyuridine. Tsuji, on the other hand, reported that the major factor in determining the frequency of BrdUrd-induced SCEs in the mouse lymphoma cell line, L5178Y, and two sublines, ES4 and ES4r1, was the concentration of BrdUrd in the medium and not the amount of BrdUrd in DNA. The results presented suggest that both effects are real and dependent on the cell type and BrdUrd concentration range.

The use of other halogenated pyrimidines to characterize the formation of SCEs was of considerable interest during this symposium. R. J. DuFrain (USA) compared extensively the efficiency of different halogenated analogues of deoxycytidine and deoxyuridine to induce SCEs and reported that at equimolar concentrations the former analogues were more potent inducers than the latter. It was speculated that this difference might be due to a preferential incorporation of the halogenated deoxycytidines into DNA. Among the three different halogenated pyrimidines, the trend was toward higher SCE frequencies with greater deviation of the size of the halogen from the normal methyl group, i.e., Cl>I>Br. The data were interpreted to imply strongly a relationship between conformational changes in the chromatin and the formation of SCEs.

Another approach used to characterize SCEs was the use of mutant cell lines. L. E. Dillehay (USA) reported on an ethyl methanesulfonate (EMS)-sensitive mutant, EM9, isolated from CHO cells, Tsuji reported on a similarly isolated mutant, ES4, from L5178Y cells, and M. Siniscalco (USA) discussed cell lines derived from mouse L-A9 cells. These mutant cell lines exhibited an increased baseline frequency of SCEs and EM9 and ES4 also exhibited an increased sensitivity to various mutagens. These two properties appeared to be genetically linked because revertant cell lines which lost their increased sensitivity to mutagens also lost their increased baseline frequency of SCEs. In EM9 cells, the increased BrdUrd-dependent formation of SCEs appeared to result during DNA replication on a BrdUrd containing template, while in ES4, the increase appeared to be dependent on BrdUrd in the medium. This difference suggests that multiple gene defects may result in a phenotypically similar increased baseline level of SCEs, which is further supported by the finding of mutual complementation between the high SCE baseline L-A9 cells and cells

from individuals with Bloom syndrome (BS), an autosomal recessive condition characterized by a highly elevated frequency of SCEs. Siniscalco further reported that the short arm of chromosome 6 or autosome 19 was required in the hybrid cells for complementation to occur.

Induction, Specificity, and Modulation

The three central themes presented during these sessions were (i) the association between DNA strand(s) breaks (which can result in the formation of chromosomal aberrations) and SCE formation, (ii) the effects of various metabolic disturbances on the chemical induction of SCEs, and (iii) modulation of the chemical induction of SCEs by the BrdUrd incorporated into the DNA for sister chromatid differential staining.

T. Hori (Japan) presented data on the effect of thymidylate stress on SCE and chromosomal aberration induction by using thymidylate synthetase-negative mutants of the mouse FM3A cell line. Thymidylate stress led to a many-fold increase in SCEs and aberrations and was interpreted to indicate a possible relationship between the cessation of DNA replication at the growing forks of active replicons or their clusters, single strand gaps and their persistence, and SCE and aberration formation. J. L. Schwartz (USA) compared the effect of a poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide (3AB), on the baseline and chemically-induced frequencies of SCEs. Schwartz concluded that the primary lesion responsible for SCE induction was either a strand break or a lesion that could result in a strand break. A. T. Natarajan's group (The Netherlands) presented similar results and, in addition, data demonstrating an increase in 3AB-induced SCEs without a concommitent increase in mutations at the HGPRT locus in CHO cells. G. Speit (FRG), from studies in which Neurospora endonuclease was used to convert single-strand breaks to double-strand breaks in V79 cells exposed to various DNA damaging agents, concluded that single-strand breaks were not efficiently converted to SCEs. These data need to be reconciled and it may be that DNA conformational changes in combination with single-strand breaks are responsible for SCE formation.

Many studies were presented on the modulation of baseline and chemically-induced SCE frequencies by various metabolic pertubations. Inhibition of protein synthesis by cycloheximide (Y. Ishii, Japan) result in a decrease in baseline and chemically-induced SCEs while inhibition of DNA synthesis by hyroxyurea and aphidicolin (F. Palliti, Italy), of DNA methylation by 5-azacytidine (T. Ikushima, Japan) or of DNA topisomerase activity by noviobiocin (G. Renault, France) leads to an increase in baseline but not necessarily induced frequencies of SCEs. C. H. Ockey (UK) also discussed the possible influence of chemically-induced alterations in nucleotide pools on SCE frequencies. These data indicate that the induction of SCEs depends highly on the balance of intracellular enzyme activities and chromatin conformation.

The modulation of induced SCE frequencies by the incorporated BrdUrd needed for the visualization of SCEs was also an important topic at the symposium. W. F. Morgan (USA) and Ockey both presented data on the interaction between chemical mutagens, BrdUrd incorporation and SCE frequency. For some chemicals, e.g., methyl methanesulfonate (MMS), methyl nitrosourea (MNU), the frequency of SCEs was reduced by the presence of incorporated BrdUrd (Ockey), while for others, e.g., aphidicolin, the frequency was increased (Morgan). SCE frequencies for other chemicals such as bleomycin, proflavin, and nitrogen mustard (HN_2) were not modulated by the extent of BrdUrd substitution in DNA (Morgan; Ockey).

T. Cremer (FRG) used microbeam UV irradiation of restricted regions of interphase Chinese hamster cells to demonstrate that the induction of SCEs by UV-photoproducts, primarily dimers, within one region did not elicit an increase in SCEs within nonexposed

regions. The experimental protocol excluded the possibility that an imbalance in nucleotide pools rather than direct DNA damage induced SCEs. Similarly, A. Gentile (France) presented data from cell fusion studies in which one of the parent cells was exposed to UV light prior to hybridization and concluded that SCE formation was not mediated through inducible and diffusable recombinational enzymes.

Mechanisms and Models

Many models have been proposed for the formation of SCEs. Several of these models were discussed extensively at this symposium. These models were the Replication By-Pass Model of D. A. Shafer (USA), the Chromatin Conformation Model of DuFrain, the Path Probability Model of M. K. Conner (USA), and the DNA Crosslink-3 Cuts Model of Y. Fujiwara (Japan). Each model is based on specific (and sometimes limited) experimental evidence, and no general model or mechanism(s) for the formation of SCEs is so far confirmed. It has long been debated whether or not DNA cross-link induce SCEs. Fujiwara presented very elegant data with regard to crosslink formation and SCE induction in cells from normal individuals, xeroderma pigmentosum (XP), and Fanconi anemia (FA). After comparing the induction of SCEs by various alkylating agents across generations using the endoreduplicated cell method, Fujiwara concluded that 1 SCE occurred for about every 35 crosslinks in FA cells. Conner presented in vivo experimental data to support the hypothesis that SCEs occurred at the same site in successive generations if no repair of the responsible DNA lesion took place between the two generations. J. B. Schvartzman (Spain), using a three-way differential staining technique in human lymphocytes exposed to UV light between the first and second generations, could not demonstrate the cancellation of SCEs in two successive generations in spite of the persistence of SCE inducing damage. However, other types of lesions must be examined with this protocol before the phenomenon of SCE cancellation can be ruled out. The impact of SCE cancellation studies on the mechanisms of SCE formation are, of course, considerable and more experimental research needs to be conducted in this area. While the actual molecular steps involved in the formation of SCEs remains unresolved, the data presented at this symposium indicates that SCEs can be elicited in several different ways (see mutant cell discussion presented earlier). Plurality of cause suggests that the exchange between sister chromatids is a common, important function needed for cell survival.

Correlations to Other Biological Endpoints

Several studies were presented in which SCE induction was correlated with mutations, cell survival, cellulartransformation, specific DNA adduct formation, and tumor potency. Y. Nishi (Japan) presented data from 40 agents on cell survival and the induction of SCEs and mutations at the HGPRT locus. A high positive correlation between SCE and mutation induction was observed. Two classes of agents were identified: those, e.g., bleomycin, ethylating agents, x-rays, which induced more mutations than SCEs and those, e.g., methylating agents, bifunctional alkylators, which induced more SCEs than mutations. Nishi also presented data indicating a biphasic dose response relationship between SCE induction and toxicity. J. R. Connell (UK) and S. M. Morris (USA) presented two studies in which SCE formation was correlated with the induction of specific types of DNA damage, as well as with cell survival and chromosomal aberrations (Connell) or with mutations at the HGPRT locus and cell survival (Morris). Measuring levels of 7-methylguanine, O^6-methylguanine, and 3-methyladenine in DNA following the treatment of Chinese hamster V-79 cells with MNU and dimethyl sulphate (DMS), Connell reported that similar frequencies of SCEs and aberrations occurred at equitoxic doses of the two chemicals. However, since 20 times more O^6-methylguanine was produced in V-79 DNA by MNU than DMS at these doses, Connell concluded that the formation of this lesion was not primarily responsible for the induction of SCEs. Furthermore, no one specific methylated purine

could be identified as being the sole cause of the induced SCEs. Morris measured the levels of different DNA adducts following the exposure of CHO cells to four simple alkylating chemicals and three aromatic amines. Eleven different purine and pyrimidine adducts and phosphotriesters were measured following the treatment of the cells with the ethylating alkylating agents, while the methylating alkylating agents induced adducts at eight nucleic acid sites and phosphotriestes. Each of the three aromatic amines induced only one adduct at the C8-position of deoxyguanosine. From these studies, Morris concluded that while a strong linear correlation was observed between O^6-alkylation and mutations, neither SCE induction nor cloning ability related to the formation of a single specific alkylation product. The aromatic amines did induce SCEs. However, a quantitative correlation between the number of adducts formed and SCE response did not exist. These data are especially interesting and may indicate the involvement of secondary processes between adduct formation and SCE induction.

N. C. Popescu (USA) presented data on the correlation between SCE induction, the persistence of the damage leading to the formation of the SCEs, and cellular transformation in Syrian hamster cells exposed to a variety of chemical and physical agents. A positive correlation between transformation and the induction of SCEs was reported, even though the ratios of induced SCEs relative to transformation varied with the agent. This finding is very similar to that obtained for mutation data and SCE induction. The SCE response and the induction of transformation also directly correlated after various split dose protocols, suggesting that the DNA lesions involved in the formation of SCEs were essential to the initial stage of neoplastic development. No correlation was found between those agents which induced the most persistent DNA lesions capable of eliciting a DNA response and transformation.

Finally, S. Parodi (Italy) discussed, for 49 chemicals for which both sets of data were available, the correlation between the in vivo induction of SCEs in rodent bone marrow and rodent tumor induction. Parodi concluded that the correlation ($r=0.57$, $p<0.001$) was rather good, especially when the same tumor data base was compared with other short-term bioassay data. For example, a correlation of only about 0.4 was obtained when tumor potency was compared with the response of the same chemicals in the Ames Salmonella test, liver DNA adduct formation, liver cell alkaline elution patterns, and in liver cell UDS. While this study provides compelling evidence for the applicability of the SCE test to identifying carcinogenic chemicals, Parodi also noted that for certain classes of compounds, such as aromatic amines and azoderivatives, SCE predictivity was almost zero.

SCEs AND GENETIC TOXICOLOGY

Although early autoradiographic efforts by H. Kato (e.g., Kato, 1974) indicated that mutagens induced SCEs, it was not until the introduction of the BrdUrd-dependent technique of Latt (1973) that SCEs as an indicator of mutagenic and carcinogenic potency became widely considered. This increased interest in SCEs as a genotoxic endpoint was largely initiated by the extensive study conducted by Perry and Evans (1975). The second group of presentations dealt with the utility of SCEs as an indicator of genotoxic damage.

Nonmammalian Systems

H. R. Zakour (USA) and S. E. Bloom (USA) presented two different nonmammalian systems for assessing the induction of SCEs by chemical and physical agents. The first system involved the in vitro culturing of peripheral blood leukocytes obtained from the coldwater marine fish, the Pacific staghorn sculpin (Leptocottus armatus), as a system for detecting the presence of marine pollutants. Zakour discussed the methods involved in the culturing of fish leukocytes and presented preliminary data on the sensitivity of

these cells to MNNG treatment in vitro. Bloom summarized SCE and chromosomal aberration data on 53 chemicals tested in the chick embryo system, and for a more limited number of chemicals correlated the SCE response with subtle teratogenic effects in the developing chick.

In Vitro Mammalian Systems

S. Abe (Japan) examine ility of 20 cultured cell lines to activate a variety of promutagens to genotc apable of inducing SCEs. Several cell lines isolated from human heptomas, rat epatomas, and rat esophagael tumors were found to be highly competent in the activation of promutagens. These results indicate the availability of cell lines for cytogentic studies which do not require the addition of a metabolic activation system. A. D. Bloom (USA) presented an interesting approach for assessing SCE inducing capability in a CHO in vitro system in which successive shake-offs from the same culture were used to obtain second generation metaphase cells for analysis. H. Norppa (Finland) discussed the importance of erythrocyte mediated metabolic activation of styrene to a reactive metabolite capable of inducing SCEs in human lymphocytes in vitro and possibly in vivo and compared these results with that obtained with other vinyl compounds. J. L. Wilmer (USA) presented similar data for aniline and suggested that oxyhemoglobin was the main component of the erythrocyte responsible for the activation of aniline to a reactive species. Wilmer also showed data demonstrating that isolated hemoglobin induced SCEs in human lymphocytes. A. L. Brooks (USA) compared the ability of various complex environmental pollutants (extracts of particles from various automobile and coke oven emissions) to induce SCEs in Chinese hamster primary lung cultures, HGPRT mutations in CHO cells, and reverse mutations in the Ames Salmonella test. The data indicated that while metabolic activation was required for the induction of bacterial mutations by these complex mixtures, it was not required for the inducation of SCEs or mutations in mammalian cells. Furthermore, the relative potencies of the complex mixtures in inducing these different genotoxic effects were better correlated between SCEs and mutations in mammalian cells than between mutations in mammalian cells and mutations in bacteria.

In Vivo Mammalian Systems

A number of presentations dealt with various aspects of different in vivo mammalian systems for the analysis of SCEs. S. B. Neal (USA) assessed SCE induction in bone marrow spermatogonia, and intestinal epithelium cells of Chinese hamsters using the agar-coated pellet technique of King et al. (1982). Different chemicals and different routes of exposure were used to compare the levels of induced SCEs in different tissues. Following an intraperitoneal treatment, several agents (busulfan, hycanthone, TEM) induced SCEs in bone marrow cells but not spermatogonia while others (MMS, CP, triethylenethiophosphoramide) induced SCEs in both cell populations. An oral treatment of Chinese hamsters with the nitroaromatic compounds metronidazole and 2 nitro-p-phenylenediamine induced SCEs in the intestinal epithelium but not the bone marrow. P. Morales-Ramirez (Mexico) favorably compared the use of BrdUrd-adsorbed activated charcoal as a means for ensuring an adequate supply of BrdUrd in mice with BrdUrd pellet implantation, multiple injections, and infusion techniques. Included in this presentation was a discussion of techniques for the analysis of SCEs in isoproternol-stimulated salivary gland tissue as well as for bone morrow and spermatogonia cells. J. W. Allen (USA) presented techniques for analyzing SCEs in primary and secondary spermatocytes of the Armenian hamster and discussed the applicability of this system for investigating the relationship between recombination and SCE formation. J. L. Ivett (USA) demonstrated the use of BrdUrd-dependent replicative history analysis to determine the optimal dose and termination time for evaluating SCEs and chromosomal aberrations in vivo. A. D. Kligerman (USA) summarized the utility of

rodent peripheral blood cultures for the analysis of SCEs induced by chemicals in vivo. The rodent peripheral blood culture system was considered to be especially useful in that it allowed for a direct comparison of SCE data obtained in humans with that obtained in animals.

A variety of other animal studies were presented. The findings of these studies included a synergistic enhancement of alkylation-induced SCE frequencies in the bone marrow cells of mice with plastic implants (R. D. Benz, USA), the accumulation of SCE-inducing lesions in the peripheral blood lymphocytes of pigs and sheep exposed chronically to dimethylbenzanthracene (A. F. McFee, USA), the persistence of SCE-inducing lesions caused by CP (T. Takeshita, USA) and gamma radiation (P. Morales-Ramirez, Mexico) in the bone marrow of mice, or by CP in the peripheral blood lymphocytes of rats (A. Basler, FRG), the induction of SCEs in the bone marrow of mice exposed to tritiated water (A. L. Carsten, USA) or the involvement of bone marrow metabolism in the induction of SCEs by benzene (Hook, USA).

Statistical Evaluation of SCE Data

The five different presenters (E. B. Whorton, Jr., USA; H-C Wulf, Denmark; P. Vercauteren, Belgium; D. H. Moore, II, USA; and G. Pesch, USA) were in complete agreement that SCE data were not normally distributed and, thus, not amenable to parametric statistical analysis. Whorton compared several methods of transforming SCE data, including square-root and log normal transformations, obtained from in vitro and in vivo genetic toxicology studies and from human biomonitoring studies. He concluded that the square-root transformation would permit parametric analyses. Wulf, using human lymphocyte data, and Pesch, examining data taken from studies involving the bethnic worm, Neanthes Arenaceodentata, support this conclusion. Whorton and Wulf identified the sources of variability in their respective human data samples and attempted to estimate, for a human in vivo study, the number of cells to be analyzed per individual and the size of the test groups, using various alpha and beta values to set the statistical parameters. Both presenters were in close agreement. Statistical sensitivity would be increased by increasing the number of individuals in each group rather than increasing the number of cells analyzed per individual. The number of cells to be analyzed per person ranged from 25 to 50 and the number of individuals to be included in each group was estimated to be approximately 10, depending on the level of sensitivity required. Vercauteren discussed an additional statistical method involving the analysis of SCE data on an individual chromosome basis which was postulated to offer increased sensitivity for detecting a significant difference between control and treatment data. Moore discussed the impact of "outlier" or high frequency cells on the analysis of SCE data obtained from biomonitoring studies and concluded that Student's t test was more powerful for detecting individual differences from a pool of normals while Chi square analysis of HFC data was more powerful for detecting differences between groups of individuals.

HUMAN STUDIES

The Human Lymphocyte System

The persistence of SCE-inducing DNA damage in human lymphocytes in vitro were studied by several contributors, using different experimental protocols. In general, the results presented were in agreement that the persistence of the SCE-inducing lesions depended strongly on the mode of action of the chemicals on DNA. B. Lambert (Sweden) showed that the SCE-inducing HN2 lesions were removed more rapidly in the late than in the early G_1 phase of PHA-stimulated human lymphocytes. SCE-inducing damage caused by MMS and adriamycin was found to be more persistent than damage caused by HN2 and melphalan. L. G. Littlefield (USA) reported that lesions induced by EMS gave rise to SCEs

during each of three successive cell cycles, whereas SCEs induced by MMC appeared to arise mainly during the first round of DNA replication after exposure. For ThioTEPA and cis-PDD, the observations indicated that a small proportion of the lesions persisted and continued to elicit SCEs during successive cell cycles. K. Morimoto (Japan) showed that higher frequencies of SCEs were obtained in cells pulse-treated with MMC, 4NQO or MMS at stages shortly before the first S phase than in cells treated earlier and related these results to the involvement of DNA excision repair. Morimoto also noted that cells with more damage had longer cell cycle times, which suggests that multiple harvest times may be necessary in some of these studies.

Other potential sources of variability with regard to SCE assessment were reported by J. L. van Staden (Republic of South Africa), Wiencke (USA), and Deknudt (Belgium). Van Staden demonstrated that the rate of SCE formation in PHA-stimulated human peripheral blood lymphocytes depended upon the length of the cell cycle (which was variable across culture time) and the time of exposure to BrdUrd. Deknudt examined baseline and chemically-induced SCEs in vitro in human lymphocyte stimulated with four different mitogens differing in lectin valency and/or sugar specificity. While baseline SCE frequencies were not altered by the use of different mitogens, MMC- and CP-induced SCE frequencies were dependent upon the mitogen used, as was the proliferative rate within the culture. Wiencke presented data indicating that various plasma and serum supplements had a profound effect on the ability of benzo(a)pyrene to induce SCEs in human lymphocytes in vitro. The findings indicate the complexity of comparing data from different studies with dissimilar experimental protocols.

It was concluded that differences in cell cycle stage at the time of exposure, the chemical nature of the lesions induced as well as specific rate of removal and degree of persistence of different types of SCE-inducing damage in human lymphocytes can greatly influence the final yield of SCEs. The importance of this conclusion for the practical use of SCE analysis in biomonitoring studies is obvious, and it was suggested that in vitro studies of the persistence of SCE-inducing lesions caused by a particular chemical should be conducted before in vivo studies in human populations are initiated.

Genetic Diseases

Attempts were made by several contributors to characterize the abnormal SCE response that has been reported to occur in some inherited human disorders (for review see Ray and German, 1982). The elevated SCE-frequency in Bloom syndrome (BS) has been an unexplained observation which has attracted much interest because of its obvious importance for the understanding of the mechanism of SCE-formation. Y. Shiraishi (Japan) analyzed SCEs in three way differentially stained chromosomes in third generation cells to demonstrate that most SCEs in BS cells occurred during the second cell cycle when BrdUrd-containing DNA was used as the template for replication. Data from somatic cell hybridization studies, presented by M. Siniscalco (USA), demonstrated mutual complementation between BS fibroblast cells and a rodent cell line (L-A9) with a high spontaneous rate of SCE. Eventually, isolation of mutant cells which are non-complementary to BS-cells will aid in clarifying the genetic defect(s) responsible for this condition and its characteristic increase of baseline SCEs.

Fibroblasts from patients with Fanconi anemia (FA) and xeroderma pigmentosum (XP) had been studied by Y. Fujiwara (Japan). The reduction of MMC-induced SCEs as a function of repair time was shown to follow a biphasic curve in normal cells and in XP cells. The first rapid decline (2 hours) was similar in normal and XP cells, whereas the second component was much slower in XP cells (50 hours) than in normal cells (14 hours). FA cells exhibited only the slow decline component. It was suggested that XP cells are

defective in monoadduct repair, and FA cells in an initial step of cross-link repair of MMC-induced damage.

Less comprehensive data were presented from studies of a number of other human disorders. The SCE frequency in lymphocytes from patients with Alzheimer disease was found to be 2-3 times lower after exposure to 4NQO and 2 times higher when exposed to MMC compared to lymphocytes from normal donors. In contrast, lymphocytes from patients with familial polyposis coli and FA showed only slightly higher SCE levels than normal cells after exposure to MMC or 4NQO (Morimoto). Fibroblasts from patients with basal cell nevus syndrome (BCNS) were reported to show an elevated SCE frequency during confluent holding for up to 24 hours after UV-exposure. In contrast, the UV-induced SCE levels declined to near baseline levels within that time period in normal cells under similar conditions, suggesting that BCNS fibroblasts may have a diminished capacity for repair of SCE-inducing UV damage (H. Nagasawa, USA).

Human Variability and Cancer

It is a general observation that normal human or malignant cells of different origin or from different tissues may show consistent differences in the baseline frequency of SCEs. However, attempts to correlate SCE levels with predisposition to cancer, or to the malignant phenotype in tumors or leukemias have generally failed. S. Knuutila (Finland) reported a variable decrease of the baseline SCE levels in virus- or ENU-transformed epithelial rat cells as compared to untransformed cells, and concluded that the frequency of SCEs does not change dramatically after transformation in vitro. A study presented by E. Gebhart (FRG) on SCE frequencies in a number of metastatic breast tumor cell lines suggested that the differences observed between these cell lines was rather a facultative than an obligatory feature of the cells.

Attempts to ascertain whether increased levels of SCEs were related to a predisposing genetic background in cancer patients have so far given conflicting results. A. Oikawa (Japan) reported on a study of the SCE frequency induced by MMS, 4NQO and Trp-P-2 in vitro in lymphocytes from 53 healthy individuals and 10 cancer patients with different types of neoplasm. The proportion of individuals whose lymphocytes showed an increased SCE response to MMS and Trp-P-2 was higher among the cancer patients than in the control population, suggesting a possible relationship between sensitivity to these agents and development of malignancy. K. Morimoto (Japan) also conducted an in vitro stress analysis (using MMC) of lymphocytes obtained from normal individuals and observed an increased response in lymphocytes of smokers and passive smokers over nonsmokers. While smokers had elevated baseline SCE frequencies, passive smokers and nonsmokers did not differ. Similarly, J. Hopkins (UK) detected the greatest in vitro SCE response to cigarette smoke condensate in lymphocytes obtained from smokers with lung cancer, the next greatest in lymphocytes obtained from smokers, and the lowest response from nonsmokers. Furthermore, this SCE response was not dependent upon exogenous metabolic activation, suggesting that the SCE-inducing chemical was not benzo(a)pyrene. A. Ghidoni (Italy) presented a study of SCE levels in PHA-stimulated peripheral lymphocytes of sporadic and familial cases of cutaneous malignant melanoma (CMM). The mean SCE rates were found to be significantly higher and heterogenously distributed among the CMM patients as compared with closely related or unrelated subjects. In addition, the rate of lymphocyte proliferation was found to be slower in the CMM patients than in the controls.

Results presented by R. Becher (FRG) and A. A. Sandberg (USA) demonstrated a progressive decline in the baseline frequency of SCEs from normal lymphocytes to bone marrow cells from healthy subjects, patients with chronic myelocytic leukemia and acute leukemia. It was suggested that stem cells have the lowest SCE rates and that the SCE

frequency is inversely related to the degree of differentiation. Moreover, a significantly higher rate of cell proliferation was found in normal lymphocytes and bone marrow cells than in neoplastic cells. It was pointed out that it is still an unsettled question whether there is an increased sensitivity of cancer cells to SCE inducing agents, and that variability in growth patterns as well as other systemic effects may partly be responsible for this uncertainty.

Biomonitoring of Patients Treated with Cytostatic Agents

The use of SCE analysis in evaluating in vivo-induced genotoxic damage has been encouraged by the dramatic increase of SCEs observed in cancer patients treated with certain cytostatic agents (for ref see Lambert et al., 1982). Data presented by N. P. Singh (USA) demonstrated that cultured lymphocytes from patients treated with CP, MMC and/or cis platinum in combination with other drugs exhibit 3-5 fold higher levels of SCE as compared to control individuals. Other cytostatic drugs, e.g. vincristine, adriamycin, methotrexate and dihydro-azacytidine, were not found to increase the frequency of SCEs. D. P. Fox (UK) found no evidence of increased SCE levels in peripheral lymphocytes from diabetic patients subjected to chlorpropamide treatment or in hypertensive patients receiving atenolol, whereas patients treated with azathioprine for immune suppression and sulphasalazine against ulcerative colitis showed significantly increased SCE levels.

The use of SCE analysis in directing anti-cancer chemotherapy towards more effective drug regimens was addressed by D. Mourelatos (Greece). Lymphocytes from leukemic patients treated in vivo with CP or cytosine arabinoside were found to respond with a synergistically increased rate of SCEs when exposed to the methylxanthines, diphylline or theobromine, during cultivation in vitro. In some contrast to this approach was the implications of the study of T. Raposa (Hungary), in which the potential of various anti-cancer drugs to induce secondary neoplasm was compared with their SCE-inducing characteristics. A striking correlation was observed between drugs shown to induce an increase in SCEs in vivo and drugs reported to increase the frequency of secondary acute leukemias. It was suggested that DNA lesions which lead to a persistent elevation of SCEs in human lymphocytes are related to those which are important in the etiology of secondary acute leukemias, and consequently, that SCE analysis should be useful in the development of new therapeutical protocols in order to minimize the risk for secondary malignancy.

Biomonitoring of Occupational Exposure

The importance of considering smoking as a confounding factor in population studies of SCE frequencies in lymphocytes was discussed in several presentations. Other confounding factors, such as culture conditions, gender and the life-style of the donor were outlined by V. F. Garry (USA). Convincing data, implying an SCE inducing effect in vivo of the occupational exposure to ethylene oxide (EO) was presented by C. Laurent (Belgium) and S. M. Galloway (USA). Their studies also indicated that the increased SCE frequency in subjects exposed to EO remains for a considerable time after cessation or reduction of the exposure. A study by T. Watanabe (Japan) did not demonstrate an increased frequency of SCEs in styrene-exposed workers, but a slight increase in SCEs was found in styrene-exposed smokers as compared to non-exposed smokers. Styrene exposure had also been studied by L. Camurri (Italy), who reported an increased SCE frequency only in workers exposed to styrene air concentrations higher than 200 mg/mc. K. Kinnainmaa (Finland) presented results from a study of SCE frequencies in herbicide sprayers exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), both hypolipidemic peroxisome proliferators. Linnainmaa pointed out that both of these chemicals may induce genotoxic damage via the excessive production of hydrogen peroxide

and active oxygen radicals. Experimental studies favoring this mode of action were presented, and it was concluded that SCE analysis may not be an appropriate technique for the detection of in vivo genotoxicity caused by these agents.

The effects of occupational exposure to inorganic metal compounds on the SCE frequency in lymphocytes were presented by H-C Wulf (Denmark). A positive correlation was found between SCE frequency and blood levels of mercury and cadmium, but not for lead and selenium. A second study showed a correlation between SCE levels and increased levels of zinc protoporphyrin in lead-exposed battery plant workers, indicating that zinc protoporphyrin may be a better estimate of lead-exposure than lead-blood levels.

KEYNOTE ADDRESSES

Keynote addresses were given by J. Herbert Taylor (USA) and Samual A. Latt (USA). Taylor discussed his work conducted during 1956 and 1957 while visiting the laboratory of Phil Woods in the Biology Department at Brookhaven National Laboratory which, in collaboration with W. L. "Pete" Hughes of the Medical Department, was responsible for the initial investigations into the nature and significance of SCEs. Taylor obtained tritiated thymidine from Hughes (who had been preparing it as a possible tool for killing cancer cells) for the purpose of examining DNA replication at the chromosomal level in root-tip cells of Vicia faba. Unfortunately, the first autoradiographs exhibited no grains, presumably to the low specific activity of the sample. Taylor talked about his excitement upon seeing autoradiographic grains scattered over the chromatids of chromosomes in cells following the treatment of root-tips with another preparation of tritiated thymidine. Not only did the distribution of the grains demonstrate that DNA replicated semiconservatively at the chromosomal level but exchanges of material between sister chromatids could be detected by switches in the grain pattern in the autoradiographs. The subsequent studies on chromosomes in plant and mammalian cells using this autoradiographic technique by Taylor and others gave valuable insight into chromosome structure and function. Taylor discussed in detail the conclusions that were made from these studies during these years and the questions that remained unanswered.

Latt discussed the second major impetus to research involving SCEs, the introduction of biochemical techniques based on the incorporation of an analogue of thymidine carrying a heavy polarizable atom, e.g., bromine, such as would be the case for BrdUrd, into replicating DNA. Latt indicated that these experiments were designed from the very beginning to provide a new tool, more powerful than autoradiography, for the examination of DNA replication in eukaryote cells. Latt then went on to discuss the nature of the sister chromatid differential staining process involving Hoechst 33258 and subsequently, Hoechst 33258 plus Giemsa, the early findings on DNA replication and SCEs which were obtained from studies with BrdUrd, and concluded with a discussion of ongoing studies in which recombinant DNA techniques were being used in cells selectively exposed to BrdUrd to evaluate gene location and expression.

CONCLUDING GENERAL DISCUSSION

The symposium concluded with a general discussion of the relevance of SCE studies to public health, prevention and intervention, chaired by R. R. Tice (USA) and A. Hollaender (USA). The general discussion was preceeded by an introduction from T. A. Tsongas (USA) in which several key questions were raised, the answers to which are needed by regulatory agencies if SCE data is to be used correctly in an analysis of human health risks. These questions dealt with the proper rationale for using SCE studies for health hazard evaluation, the significance of an increase in SCEs in terms of individual future adverse health outcomes, the possible correlations between SCEs and other biological manifestations of genotoxic damage or ill health, the relevance of SCE data obtained from animal

studies to estimates of human health risks, the nature of the statistical requirements for biomonitoring studies involving SCEs, and the extent of the possible confounding factors which may influence the expression of SCEs. These concerns were reiterated by T-M Ong (USA) who also added questions about the possible need for a standard SCE protocol to be used in biomonitoring studies, the best approach for obtaining cooperation among the various parties involved in occupational studies, the mechanism and extent to which workers should be informed of their individual results, and the duration over which follow-up studies in occupational exposure situations would be needed and/or useful.

As would be expected, considerable discussion among the particpants was evoked by these questions, and it was obvious that the questions and their answers were not limited to SCE studies alone but included any biological approach for biomonitoring. While it is not feasible within the context of this summary to include every individual response or comment (a detailed summary of the General Discussion is available in the symposium proceedings), a selected number of consensus statements are possible.

1. The induction of SCEs is a manifestation of DNA damage and, as such, can be used to identify genotoxic agents posing a mutagenic and/or carcinogenic hazard.

2. Thus, SCEs provide a useful tool for biological effect monitoring studies, especially when used in conjunction with other biological effect indicators of genotoxic exposure, and especially where prior experimental data indicates a potential risk exists. In any monitoring study, potentially confounding variables, i.e., differences in age, gender, smoking history, drug usage, etc., must be controlled.

3. However, SCEs can also be induced by indirect mechanisms, including altered nucleotide pools, and awareness of these interactions should be included in any evaluation of SCE data.

4. As with any biological indicator of genotoxic damage, it is currently impossible to quantitate the health risk associated with an increased frequency of SCEs for a group of exposed individuals, and even more so, for the individual. Only longitudinal, prospective studies will enable this problem to ever be addressed. Such studies, involving the use of multiple biological indicators and worst case populations, need to be funded now.

5. SCEs can be correlated with mutations, cellular transformation, and cancer but the correlation is not constant and depends on the spectrum of lesions induced by a specific agent. Some of these lesions may elicit only one biological response while many other lesions will elicit multiple responses.

6. Statistically correct and robust approaches for analyzing SCE data are currently available. However, to determine the most sensitive method for the analysis of SCE data obtained under different experimental conditions, adequately large and accessible data bases must be made generally available. This goal is being actively sought by participants at the symposium.

7. More basic research is necessary to increase the understanding of how SCEs occur, whether their formation is error-free or error-prone, what they signify and the limitations involved in their applications.

8. It was hoped that in four or five years, a second International Symposium on SCEs would be held to address advances in SCE research.

ACKNOWLEDGEMENTS

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PROGRAM COMMITTEES

EUROPE: B. Lambert (Chairman), A. Brogger, B. Kihlman, A. T. Natarajan, G. Obe, E. Passarge, P. Perry, H. W. Rudiger, and M. Sorsa.

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USA: R. R. Tice and A. Hollaender (Chairmen), J. W. Allen, A. V. Carrano, S. A. Latt, and S. Wolff.

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DISCLAIMER

Figure 1. Keynote Speakers and Program Chairmen

Front Row (left to right) Richard Setlow, Kanehisa Morimoto, Pete Hughes, J. Herbert Taylor.

Back Row (left to right) Bo Lambert, Jane Setlow, Alexander Hollaender, Raymond Tice, Samuel Latt.