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MASTER

MITOMYCIN C-INDUCED SISTER CHROMATID EXCHANGES AND CLASSICAL  
CHROMOSOME LESIONS IN CULTURED LYMPHOCYTES OF SAGUINUS  
OEDIPUS OEDIPUS AND SAGUINUS FUSCICOLLIS ILLIGERI

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*[Handwritten signature and date]*  
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#### ABSTRACT

Lymphocyte cultures from 10 tamarins of the "cancer prone" S. oedipus species and from 10 tamarins of the "cancer resistant" S. fuscicollis species were treated in vitro with the clastogen mitomycin C to determine whether there were differences in sensitivity to mutagen-induced cytogenetic lesions between the two species of tamarins or within S. oedipus animals. No differences in either spontaneous or mutagen-induced sister chromatid exchange were observed between S. oedipus and S. fuscicollis, but there was significant variability in induced sister chromatid exchange frequencies within S. oedipus. Further studies of animals related to the tamarin with the highest induced sister chromatid exchanges demonstrated that the same trait was present in lymphocytes of a sibling and the female parent. Induced classical chromosome lesions evaluated in cultures from these same animals showed the same increased response over other animals tested from either species. These data suggest that individual S. oedipus animals may have increased sensitivity to mutagen-induced cytogenetic lesions and that this sensitivity may be the expression of a genetic trait. Whether this sensitivity is related to the increased incidence of colon cancer in animals of the same species is not known, but chromosomal sensitivity has been demonstrated in cells from patients with several genetic diseases, also characterized by an increased risk of cancer.

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## INTRODUCTION

It has been established pathologically that within the same colony of tamarins at Oak Ridge Associated Universities (ORAU), in excess of 15% of the animals of the Saguinus oedipus species die with adenocarcinoma of the colon, whereas the disease is not observed in members of the closely related species, Saguinus fuscicollis (1). Because the high incidence of colon cancer appears to be restricted to S. oedipus, it is possible that these animals have a genetic trait that predisposes members of this species to this specific malignancy.

The possibility that colon cancer in the tamarin may be the manifestation of a genetic trait is strengthened by observations in man. It has been extensively documented that persons with several rare genetic diseases [i.e., ataxia telangiectasia (AT), Bloom's syndrome (BS), Fanconi's anemia (FA), Xeroderma pigmentosum (XP), and Gardner's syndrome (GS)] are at increased risk for developing several malignancies. Early cytogenetic studies of somatic cells from persons with BS, FA, and AT demonstrated that high frequencies of spontaneous chromosome breakages were a characteristic finding in these syndromes. This discovery in turn prompted the name "constitutional chromosome breakage syndromes" for these specific conditions. In addition, recent studies have also shown that cells from persons with BS exhibit high frequencies of spontaneous sister chromatid exchanges (SCEs), while cells from persons with XP only show increased SCE frequencies when subjected to mutagenic challenge.

The reasons for the aberrant spontaneous and induced cytogenetic findings in these persons are not known, but it has been speculated that these individuals may have a specific gene trait that predisposes both to the evolution of malignancy and the frequently associated chromosome instabilities. Should this be the case, then chromosome instability may be a visible manifestation (marker) of inherited predispositions to malignancy (2,3).

Because of the association between chromosome instability and increased susceptibility to malignancy in persons with several inherited diseases, it is possible that members of the S. oedipus species of tamarin which will develop colon cancer might have a genetic marker which could be identified by cytogenetic techniques. If a relationship between chromosome instability and colon cancer in the tamarin can be demonstrated, then we may be able to use cytogenetic methods to detect animals which will develop cancer in the future, (i.e., identify "cancer-prone" animals early in life). Therefore, using cytogenetic techniques, this study was designed to determine: (1) whether there were differences in the spontaneous SCE frequencies in tamarin lymphocytes [as seen in XP (Table 1)] between the "cancer-prone" S. oedipus animals and the "cancer resistant" S. fuscicollis, and/or (2) whether the differences mentioned above were demonstrable only within S. oedipus animals.

Table 1

## Cytogenetic and Genetic Findings in Chromosome Instability Syndromes

Syndrome	Classical Lesions	SCEs	Defects in DNA Repair
Bloom's Syndrome (BS)	Increased spontaneous nonrandom breaks and exchanges (symmetrical quadriradials) in lymphocytes, marrow cells and fibroblasts (2-12). Increased induction of breaks--not much over spontaneous (10).	Increased spontaneous SCEs in lymphocytes, marrow cells and fibroblasts (4-8, 10-12). Increased induction of SCEs when cells exposed to low concentrations of MMC (10).	Defect in rate of repair (19, 26, 27).
Fanconi's Anemia (FA)	Increased spontaneous nonsymmetric and random breakage and rearrangements in lymphocytes, marrow cells and fibroblasts, especially asymmetrical quadriradials (13-18). Increased induction of aberrations UV = 5x control MMC = 30x control (6).	No increase in spontaneous SCE frequency (6, 7, 10, 13, 15, 25). Difference in challenged response in various tissues (15). MMC causes decreased SCEs in lymphocytes (6, 7).	Decreased repair rate (25) after UV and alkylators (14, 15, 16, 28, 29-33).
Ataxia Telangiectasia (AT)	Increased spontaneous and variable induction of nonrandom chromatid and chromosomal breaks and rearrangements (triradials) in lymphocytes, fibroblasts and marrow cells (6-8, 19-22).	No increase in spontaneous SCE frequency (7, 8, 16). Induction of SCE frequency varies with tissue type (7).	Decreased repair with certain mutagens-- $\gamma$ , X-rays, and some chemicals (6, 7, 21, 34, 35).

Table 1 (continued)

Syndrome	Classical Lesions	SCEs	Defects in DNA Repair
Xeroderma pigmentosum (XP)	No increase in spontaneous breakage or rearrangements over normal cells (2, 3, 4, 19). Increased induced breakage (4, 23); in vivo (19).	No spontaneous increase in SCE frequency (16, 17, 25). Increased induction of SCE frequency in lymphocytes, fibroblasts and marrow cells (16, 17).	Repair--functionally normal (19, 33). UV induced dimers (caused by increased sun sensitivity). Decreased DNA excision repair except in one complementation group (6, 16, 30, 32, 36, 37).
Gardner's Syndrome (GS)	Increased spontaneous tetraploidy only in tissues which are or will become malignant (24). No increased breakage seen in fibroblasts (9).	Not done.	Not known.

## CHAPTER 1

### REVIEW OF LITERATURE

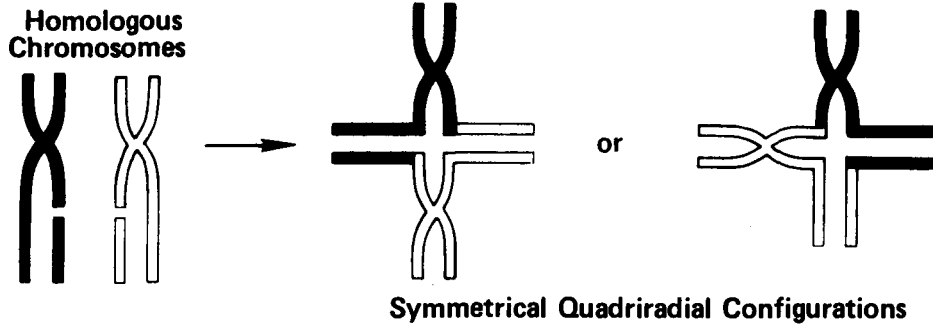
#### Classical Chromosome Lesions in Persons with Constitutional Chromosome Breakage Syndromes

In 1954 Bloom recognized and described the autosomal recessive condition (named after him) characterized by sun-sensitive telangectatic lesions on the face and growth retardation found in a group of Eastern European Jewish immigrants (2,4). Soon afterwards, German described an excessive amount of spontaneous chromosome breakage in cultured lymphocytes of individuals referred to him by Bloom. The chromosomal rearrangements or lesions found in the cultures of several Bloom's syndrome individuals were remarkably similar. The most prominent and striking lesions were symmetrical chromosomal configurations called quadriradials formed from broken and rejoined homologous chromosomes (2,4) (see Figure 1a).

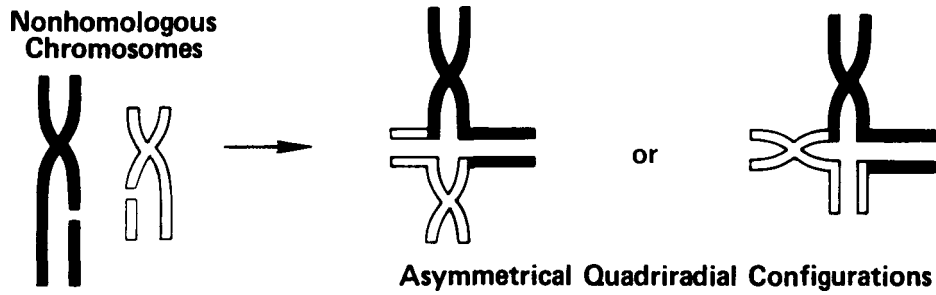
Since that time similar increased chromosome instabilities have been documented in three other autosomal recessive syndromes (i.e., FA, AT, and XP) and in the autosomal dominant condition, Gardner's syndrome (GS) (see Table 1). Other than the already mentioned quadriradial configurations found in BS, distinguishing cytogenetic characteristics are associated with the other four inherited syndromes. In FA, increased random and nonsymmetric spontaneous breakage and rearrangements formed from broken nonhomologous chromosomes have been found in three different body tissues, marrow cells, lymphocytes, and

Figure 1. Illustrations of characteristic cytogenetic configurations formed from broken chromosomes in three autosomal recessive syndromes. Notice the manner in which the broken chromatid rejoins--always realigning with its original sister chromatid.

(a) BLOOM'S SYNDROME



(b) FANCONI'S ANEMIA



(c) ATAXIA TELANGIECTASIA

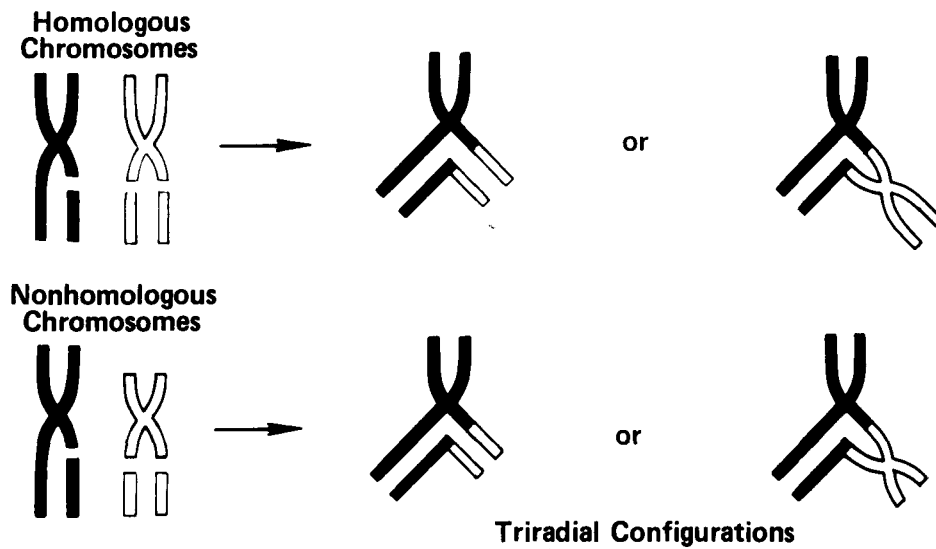


Figure 1

fibroblasts (see Figure 1b) (2-6, 13-16). Cells from persons with AT show increased spontaneous and nonrandom chromatid and chromosomal breaks (38) resulting in the very characteristic triradial configuration (see Figure 1c) (2, 6-8, 19-21). Of the four autosomal recessive conditions, XP is the only syndrome whose cells do not demonstrate increased spontaneous chromosome breakage above the normal rate (2,3,19); yet lymphocytes, fibroblasts, and marrow cells of these persons demonstrate a characteristically increased induced breakage following exposures to mutagenic chemicals and ultraviolet (UV) irradiation (16,23). Cells from persons with the dominantly inherited Gardner's syndrome show no increased spontaneous breakage in any of the tissue types but exhibit increased in vitro spontaneous tetraploidy in tissues which are or will become malignant (24).

#### Increased Incidence of Cancer

The aberrant cytogenetic findings in persons with these conditions are of interest because individuals with each of these rare genetic diseases are at substantially increased risk for developing malignancies (Table 2).

Of the four autosomal recessive diseases, XP individuals are the most likely to develop multiple skin cancers due to the nature of the disease (6,19,39,40). However, because of their sun-sensitivity, BS and AT patients also are at increased risk for skin cancers. Bloom's, FA, and AT patients as a group have an increased susceptibility to leukemia, 367 times that found in the general population, and an increased risk of 32 times that of the population for development of all cancers (41).

Table 2

## Mode of Inheritance and Clinical Findings in Syndromes Manifesting Chromosome Instability

Syndrome	Mode of Inheritance	Cancer Risk	Immunological Deficiencies
Blooms Syndrome (BS)	Autosomal recessive (3, 5)	Increased: skin cancer due to increased sun sensitivity (2), leukemia (4, 19, 22, 32, 41). Other cancers increased (47).	Delayed hypersensitivities (T cells deficient). Immunoglobulin products (B cells deficient) (3, 4, 19). Immunologic deficiencies (22, 50).
Fanconi's Anemia (FA)	Autosomal recessive (19)	Increased: acute leukemia due to progressive marrow failure (2, 15, 22, 32, 41); squamous cell carcinoma (15, 26); other cancers (47).	Deficient due to leukopenia (progressive marrow failure) (2, 15, 22).
Ataxia Telangiectasia (AT)	Autosomal recessive (2, 22)	Increased: acute and chronic lymphocytic leukemia; Hodgkin's disease; lymphomas (22, 32, 41, 47, 48).	Hypoplastic lymphoid system. Decreased T cells. Impaired B cells (not responsive to mitogens) (6, 20, 21, 22). The above responsible for increased infectious diseases and neurological disorders (20, 21, 41).
Xeroderma pigmentosum (XP)	Autosomal recessive (39)	Increased: skin cancer due to increased and extreme sun sensitivity (6, 19, 39, 48). Pseudodiploid clones (No malignant characteristics) (19).	Cellular response of B and T cells normal (51).
Gardner's Syndrome (GS)	Autosomal dominant (9, 24, 42-44, 46)	Increased: colon cancer (42, 43, 44) whose primary lesions were colorectal polyps (9, 24, 42-44, 49); skin cancer (46).	Not known

Eighty percent of patients with Gardner's syndrome, the only dominantly inherited syndrome of the group, develop adenocarcinoma of the colon (42,43,44) by 30 years of age. Penetrance of the GS gene is essentially 100%, and because the trait is dominant, there is wide variability in expressivity (45). Colon malignancy is preceded by primary colorectal polyps (9,24,42-44), but there are many other manifestations associated with the syndrome such as epidermoid cysts, dental anomalies, osteomas (45) and an increased incidence in skin cancer (46). In addition to the cytogenetic findings and increased incidence of cancer, several other similarities, such as defects in DNA repair mechanisms and immunological deficiencies have been characterized and documented in these human syndromes (Tables 1 and 2, pages 3 and 9).

Sister Chromatid Exchanges in Persons with Constitutional Chromosome Breakage Syndromes

In 1956, Taylor (52) observed spontaneous sister chromatid exchanges (SCEs) while studying tritiated thymidine labeled chromosomes of the broad bean, Vicia faba. Then in 1972 Zakharov and Egolina (53) showed that tritiated bromodeoxyuridine ( $^3\text{H-BUdR}$ ) caused a delay in spiralization of late replicating chromosome regions. These regions corresponded to the chromatids with doubly substituted DNA in Chinese hamster ovary cells after CHO cells had undergone two rounds of DNA synthesis. In 1973, Latt described an SCE microfluorometric detection method using fluorescent dyes (54). Subsequently, in 1974 Korenberg and Freedlender (55) and Perry and Wolff (56) described a more usable and permanent fluorescent plus Giemsa (FPG) staining technique for the visualization of sister

chromatid exchange. Since that time an overwhelming amount of literature on the phenomenon has been published.

The new FPG technique has proven to be much more accurate in the assessment of SCEs than the autoradiographic method because staining improved the resolution of the exchanges (56). This technique is based on two assumptions: (1) that the thymidine analogue, 5-bromodeoxyuridine (BUdR) is substituted for thymidine during two periods of DNA replication, and (2) that the fluorescent dye, 33258 Hoechst, is bound more tightly to the singly substituted chromatid which, in turn, counter stains with Giemsa darker than the doubly substituted chromatid. A schematic diagram of BUdR incorporation into mammalian cells cultured *in vitro* is shown in Figure 2. Because BUdR is substituted for thymidine in only the newly replicating strands of DNA during interphase, chromatids which are stained, counter stained and viewed under the light microscope at Metaphase I after incorporation of the analogue are uniformly dark in appearance. However, after two rounds of replication followed by the FPG staining technique (described briefly in Figure 2), one of the Metaphase II chromatids microscopically appears more lightly stained than the other. If an exchange of DNA material has occurred between the two sister chromatids (SCEs), a differentiation between the light and dark points of exchange can be readily identified (See Figure 3). The actual exchange event has been shown to take place only in the S phase of the cell cycle (57).

Although the exact biochemical nature of the SCE has not yet been elucidated (58), SCE induction has been shown repeatedly to be a much more sensitive indicator of cellular exposure to many mutagenic and

Figure 2. Illustration of DNA replication cycle in stimulated lymphocytes cultured in the presence of 5-Bromodeoxyuridine (BUdR) and the microscopic appearance of the chromosomes after having been stained using the fluorescent plus Giemsa (FPG) technique.

- (1) 33258 Hoechst fluorescent dye
- (2) fluorescent light exposure, and
- (3) Giemsa stain (counter stain)

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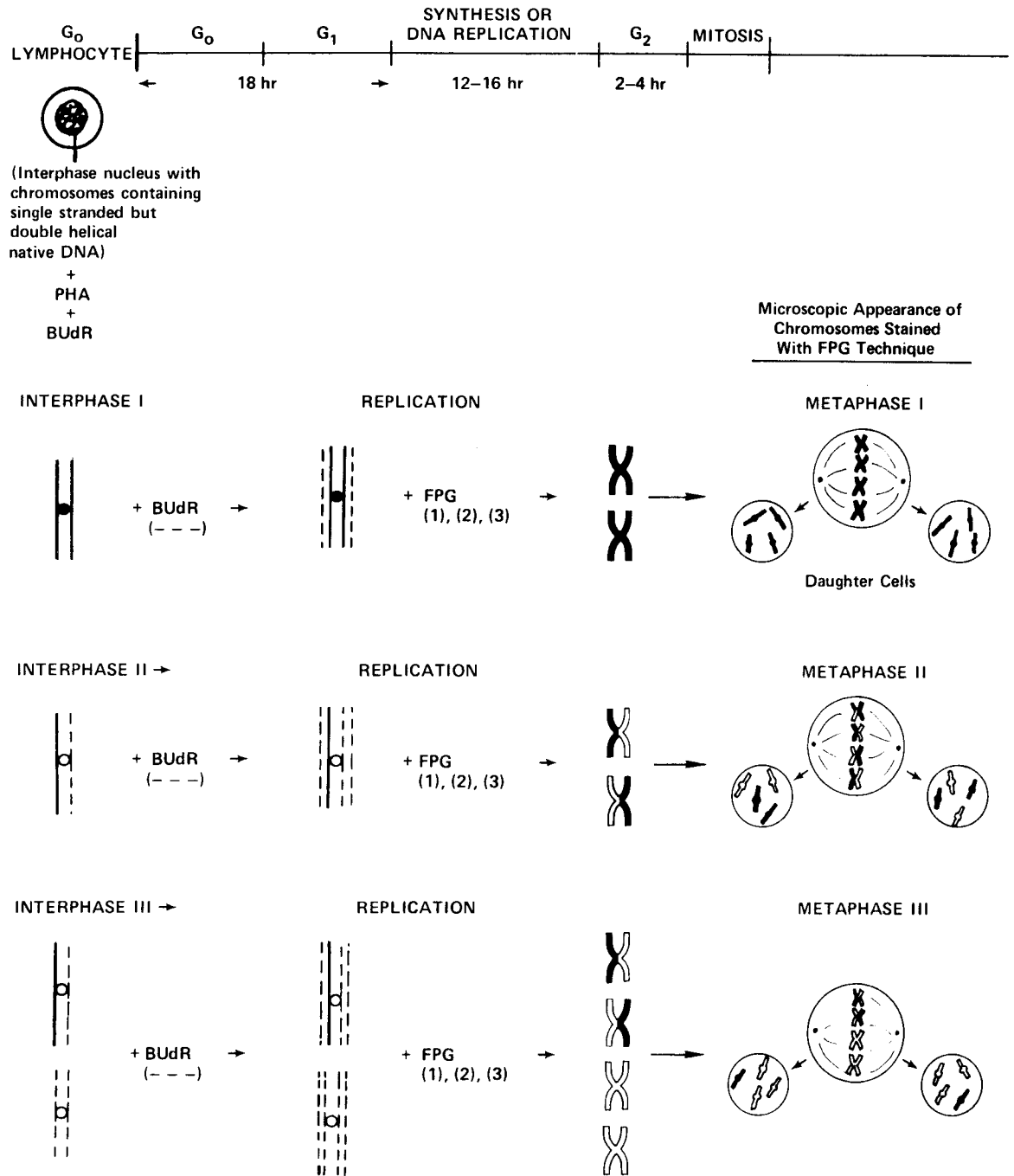
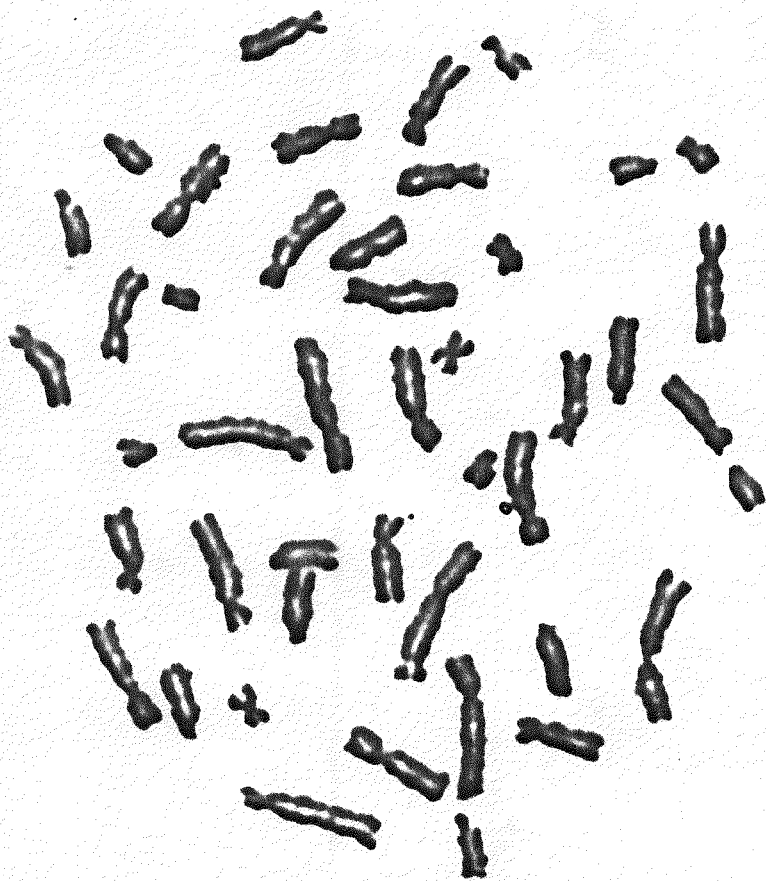
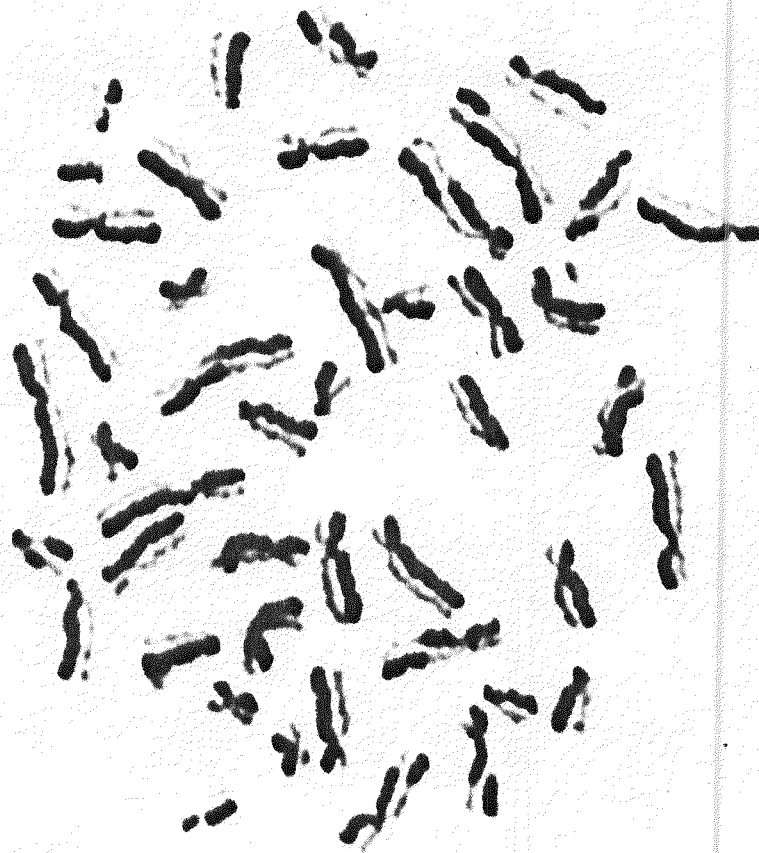


Figure 2



Before BUdR incorporation



After BUdR incorporation

Figure 3. Photographs of normal tamarin lymphocyte metaphases before and after incorporation of BUdR--showing the differentiation between sister chromatids.

carcinogenic chemicals than is chromosomal aberration induction (59-62).

Many studies indicate that the SCE may be the result of repair or misrepair of DNA (29,58,63-65).

Soon after publication of the new staining techniques, SCE frequencies were evaluated in persons with several of the chromosome instability syndromes (see Table 1, page 3). Lymphocytes, marrow cells, and fibroblasts from individuals with BS demonstrated an increased spontaneous (4-8,10-12) and induced (10) SCE frequency, whereas this did not occur in tissues of FA (6,7,10,13,15,25), AT (7,8,16), or XP (16,17,24). As can be seen from Table 1 (page 3), XP cells, which demonstrate no spontaneous SCE above baseline frequency, have the most consistent induced cellular response of the three syndromes; all three tissues show an increased response following mutagen challenge (17,19,23) or UV irradiation (66,67). In FA and AT various tissues show varied responses toward the induction of SCEs (7).

Considering the correlation between increased cancer risk (Table 2, page 9) and the aberrant cytogenetic findings (Table 1, page 3) in persons with several autosomal recessive and dominant syndromes, it is reasonable to hypothesize that should the increased incidence of colon cancer in S. oedipus be the manifestation of a genetic trait, these animals might also have an associated cytogenetic marker. This project, therefore, was undertaken to investigate this possibility through the use of cytogenetic techniques.

## CHAPTER II

### MATERIALS AND METHODS

#### Cell Culture, Harvest, and Slide Preparation

Blood from the femoral vein of 20 New World tamarins (10 of S. fuscicollis and 10 of S. oedipus) was drawn into sterile heparinized syringes. The ages of the animals, or length of time in the colony, ranged from one and one-half to nine years. For all cultures, lymphocytes from 0.5 ml whole, heparinized blood were cultured in the dark for 68 hr at 39°C in 70% RPMI 1640 medium (with HEPES buffer) supplemented with 29% fetal calf serum, 1% penicillin-streptomycin solution (100 unit penicillin G and 100 ug streptomycin), 0.3 ml reconstituted phytohemagglutinin (PHA-M) and 25 ug 5-bromodeoxyuridine (BUdR) in 10 ml medium. To determine whether SCE frequency in control cultures varied as a function of lymphocyte number, total white counts and differentials were completed on each blood sample. In addition, several cultures with varying concentrations of lymphocytes from three animals were initiated.

To assess the induction of SCEs in tamarin lymphocytes following challenge with a known mutagen, replicate cultures from each animal were exposed for the entire culture period to 0, 20, or 30 ng/ml Mitomycin C (MMC). This clastogenic antibiotic is a potent bifunctional alkylating agent which damages DNA (68,69) and causes a significant increase in SCEs over control values at very low concentrations (70) in normal human lymphocytes (60) as well as in cells from all four of the

autosomal recessive syndromes which have been mentioned [BS (10), FA (15), AT (7), and XP (17,30)].

In both the control and treated cultures metaphases were accumulated by addition of Colcemid (GIBCO) 4 hr before harvest, at a final concentration of 0.1  $\mu\text{g/ml}$ . Following a 20 min hypotonic treatment in 0.9% sodium citrate (a 10 ml washing and then placing the cells in 10 ml fresh hypotonic solution at 39°C) the cells were fixed in 6 ml of 3:1 methanol:acetic acid mixture, allowed to stand for 20 min, and washed two times in fresh fix before final dilution for test slide preparation. Test slides were made and examined for chromosome morphology and correct cell concentration to be used for further preparations and evaluations. After 24 hr at 4°C, the cells were carried through one change of fresh fixative, resuspended, and dropped onto clean chilled slides. The slides were flamed and allowed to dry overnight.

#### Staining Procedure

The slides were first stained for 30 min at room temperature in 10  $\mu\text{g/ml}$  33258 Hoechst in pH 7.0 buffer (0.01 M potassium phosphate). After rinsing with tap water, they were immersed face up and layered singly in the potassium phosphate buffer in covered petri dishes and placed on an x-ray viewbox. A lamp with cool-white fluorescent light bulbs was also placed above the dishes. (Slides received fluorescent lighting from above and below.) Two different times for light exposure were used. When chromatids did not differentiate within the "normal" 6 hr exposure, the slides were left overnight under fluorescent light for approximately 17-18 hr.

For differentiation of sister chromatids and SCE identification, the chromosomes were stained in 5% Giemsa (Gurr R66 in pH 7.0 0.01 M potassium phosphate buffer) for 10 min, rinsed in tap water, and allowed to air dry.

#### Microscopic Analyses

The preparations were scanned with a magnification of 100 × the length of the slide. From each culture, 20 differentially stained second division metaphases having a full complement of chromosomes were studied under oil with a magnification of 900 ×. At least two slides per culture, 10 cells per slide, were examined for the SCE determination. In several cultures treated with 30 ng/ml MMC, 25-100 first division metaphases were examined for assessment of classical chromosome lesions. For every metaphase examined the coordinates were recorded to preclude duplicate scoring and allow reexamination.

## CHAPTER III

### RESULTS

#### Cytogenetic Evaluations in Control Lymphocyte Cultures

Influence of lymphocyte concentration on SCE frequency. Because it has been noted in several animal species that SCE frequency in control cultures varies depending upon the number of lymphocytes inoculated into the culture medium (71), a preliminary study was conducted to evaluate SCE frequencies in second division metaphases in replicate control cultures containing different cell concentrations from three tamarins, one of S. fuscicollis and two of S. oedipus. The data in Table 3 indicate that there are no significant differences in the control SCE frequency between the replicate cultures of either species tested over a two- to three-fold range in cell number and concentration of BUdR per lymphocyte. Because of these findings, it was not considered necessary to adjust the lymphocyte cell number to a constant value in the remaining cultures tested for SCE frequency in this study. However, to reconfirm the initial observation, that SCE frequencies are not dependent upon lymphocyte concentration within the range tested, a visual comparison of SCE data versus cell number was made (Figure 4). Total white counts and differentials were completed on each of nine S. oedipus and nine S. fuscicollis animals for this final comparison (Table 4).

Table 3

Frequency of SCEs in Relation to Total Lymphocyte Count  
in Replicate Control Cultures from Three Tamarins

	Lymphocyte Count (Total Lymphocytes in Medium) ( $\times 10^6$ )	BUdR <sup>a</sup> ug/Cell ( $\times 10^{-6}$ )	SCEs per Metaphase
<u>S. oedipus</u>			
97-68 D	2.8	8.9	5.60
	5.5	4.5	5.40
	8.4	3.0	5.30
03-65 A	6.0	4.2	5.85
	9.0	2.8	5.40
<u>S. fuscicollis</u>			
3711	0.7	36.0	5.00
	1.5	17.0	5.25
	2.1	12.0	4.55

<sup>a</sup>Total BUdR concentration in 10 ml cultures: 0.025 mg.

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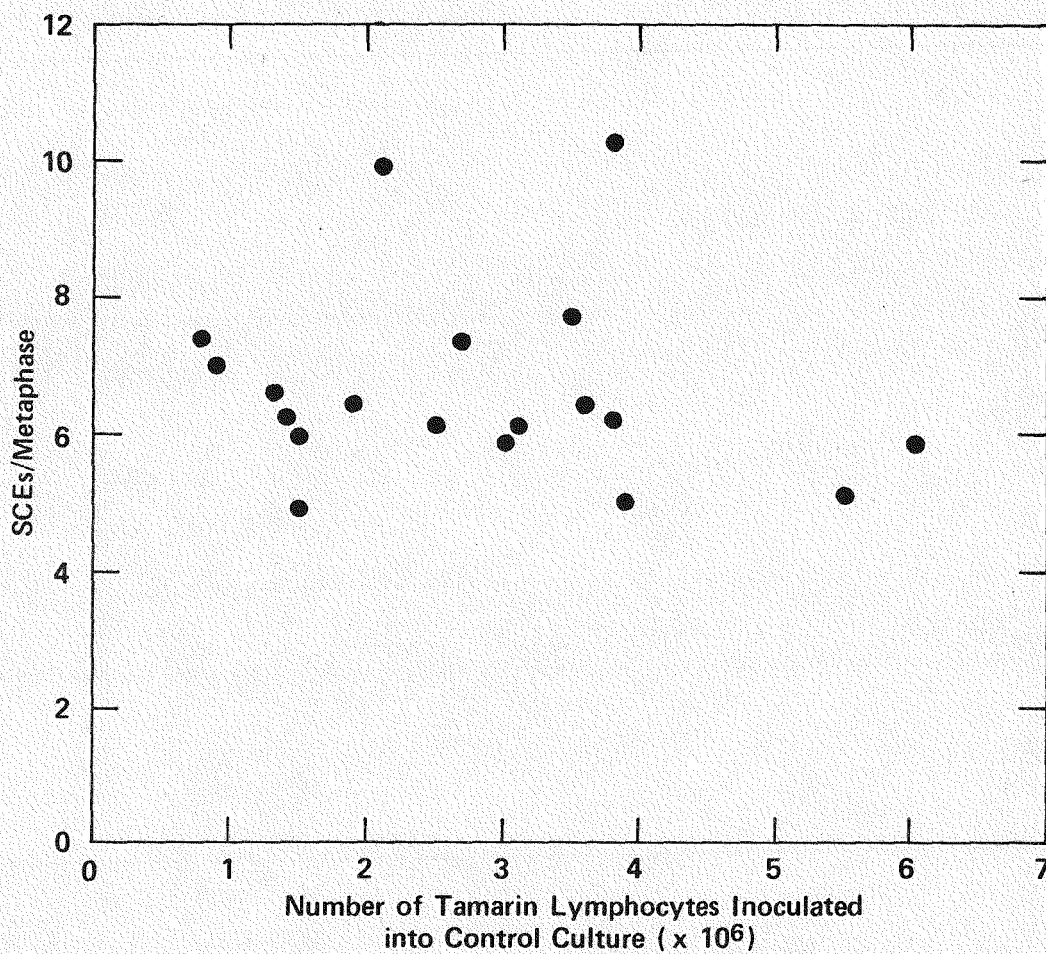


Figure 4. Plot showing independency of spontaneous SCE frequency upon lymphocyte concentration.

Table 4

Hematological Data from Eighteen S. oedipus and S. fuscicollis Tamarins

Animal	White Blood Count/mm <sup>3</sup>	Percent Lymphocytes	Absolute Number of Lymphocytes	Number Lymphocytes/ml of Blood Inoculated (x10 <sup>6</sup> )	Number Lymphocytes Inoculated in 0.5 ml Blood (x10 <sup>6</sup> )
<u>S. oedipus</u>					
03-65 A	17,500	68	11,900	12.0	6.0
03-65 A	12,050	63	7,592	7.6	3.8
97-68 D	56,400	19	10,716	11.0	5.5
01-33 B	20,700	37	7,659	7.7	3.9
97-106 D	10,500	58	6,090	6.0	3.0
3211	19,300	37	7,141	7.1	3.6
3152	32,300	9	2,907	2.9	1.5
3188	9,400	66	6,204	6.2	3.1
1715	21,250	13	2,763	2.8	1.4
1591	11,400	15	1,710	1.7	0.9
<u>S. fuscicollis</u>					
3711	9,350	31	2,898	2.9	1.5
18-31 D	9,200	40	3,680	3.7	1.9
52-14 D	9,750	78	7,605	7.6	3.8
3763	16,800	30	5,040	5.0	2.5
1914	8,700	48	4,176	4.2	2.1
2716	10,950	24	2,628	2.6	1.3
73-54 J	12,950	54	6,993	7.0	3.5
s-76-64 I	13,500	40	5,400	5.4	2.7
3741	6,400	28	1,792	1.8	0.9

Comparisons of spontaneous SCE frequencies in second division

lymphocytes of S. oedipus and S. fuscicollis. Cytogenetic studies were conducted on 20 different South American tamarins from two species [i.e., the "cancer prone" S. oedipus and "cancer resistant" S. fuscicollis as determined by Lushbaugh, et al. (1)]. For these evaluations 24 control cultures (eight with varying lymphocyte concentrations) were initiated, 13 from 10 S. oedipus and 11 from 10 S. fuscicollis for analysis of spontaneous SCE frequency in second division metaphases. All cultures were incubated for 72 hr in medium containing 10  $\mu$ M BUdR.

In S. oedipus tamarins, SCE frequencies varied little among cultures from 10 animals (i.e., from 4.75 to 7.40 SCEs/metaphase in individual cultures with a mean frequency of 5.93 SCEs/metaphase for the group). The range was also consistent among cultures; the highest number of SCEs observed in a single metaphase was 14 (Table 5). Within S. fuscicollis there was more variability between animals in control SCE frequencies (i.e., from 4.88 to 9.30 SCEs/metaphase in cultures from 10 animals with a mean of 6.32 SCEs/metaphase for the group). The range among different cultures within animals of this species was also consistent; the highest number of SCEs observed in a single metaphase was 19 (Table 6). In neither group does it appear that there is a correlation between the age of the animal and the control SCE frequency (Tables 5 and 6). The average frequency of SCEs in control cultures from the 10 S. oedipus animals was not significantly different from the average frequency of SCEs in the 10 S. fuscicollis (i.e., 5.93 versus 6.32, respectively). Examples of second division metaphases from control cultures of both

Table 5

Frequency of SCEs  $\pm$  S.E. (Range) in Control Lymphocyte  
Cultures from Ten S. oedipus Animals

Animal Number	Sex (Animal/Co-Twin)	Age <sup>a</sup>	Date of Culture	Number of Cells Scored	SCEs/Metaphase
03-65 A	♂/♀	2.0	11-17-78	20	5.85 $\pm$ 0.83 (1-14)
			12-15-78	20	6.20 $\pm$ 0.58 (1-9)
97-68 D	♂/♂	2.0	10-9-78	20	<sup>b</sup> 4.75 $\pm$ 0.56 (1-10)
				20	<sup>b</sup> 5.20 $\pm$ 0.54 (1-10)
			11-17-78	20	5.40 $\pm$ 0.49 (2-12)
99-203 B	♀/♂	1.5	9-26-78	20	6.55 $\pm$ 0.55 (3-12)
01-33 B	♀/♂	1.5	9-12-78	20	5.15 $\pm$ 0.57 (1-9)
97-106 D	♀/♂	1.5	9-12-78	20	5.85 $\pm$ 0.55 (1-10)
3211	♀/♀	1.5	2-5-79	20	6.45 $\pm$ 0.54 (2-12)
3152	♂/♂	2.0	2-5-79	20	5.95 $\pm$ 0.60 (3-14)
3188	♀/♀	2.0	2-5-79	20	6.15 $\pm$ 0.30 (4-8)
1715 <sup>c</sup>	♂/?	2.5	3-26-79	20	6.20 $\pm$ 0.34 (4-9)
1591 <sup>c</sup>	♀/?	3.5	3-26-79	20	7.40 $\pm$ 0.66 (3-14)
Mean					5.93 (1.14)

<sup>a</sup>Years in colony.

<sup>b</sup>Cultures with different hypotonic treatments.

<sup>c</sup>Animals with chronic colitis.

Table 6

Frequency of SCEs  $\pm$  S.E. (Range) in Control Lymphocyte Cultures from Ten S. fuscicollis Animals

Animal Number	Sex (Animal/Co-Twin)	Age <sup>a</sup>	Date of Culture	Number of Cells Scored	SCEs/Metaphase
48-21-B-11	♀/♀	1.5	10-9-78	16	<sup>b</sup> 4.88 $\pm$ 0.32 (3-7)
				20	5.00 $\pm$ 0.63 (1-10)
3711	♂/♂/♀	1.5	11-17-78	20	5.25 $\pm$ 0.88 (1-18)
13-31-0	♂/♀	9.0	2-20-79	20	6.40 $\pm$ 0.68 (2-13)
52-14 D	♂/♂	8.0	2-20-79	20	9.30 $\pm$ 0.77 (4-19)
3763	♂/♀	3.0	3-5-79	20	6.10 $\pm$ 0.69 (1-14)
1914	♂/♂	2.0	3-5-79	20	8.90 $\pm$ 0.53 (5-14)
2716	♀/♂	2.0	3-5-79	20	6.60 $\pm$ 0.55 (3-13)
73-54 J	♂/♂	4.0	3-26-79	20	5.75 $\pm$ 0.37 (2-9)
s-76-64 I	♀	3.0	3-26-79	20	7.40 $\pm$ 1.17 (2-15)
3741	♂/♀	2.0	4-24-79	20	7.00 $\pm$ 0.71 (2-15)
Mean					6.32 (1-19)

<sup>a</sup>Years in colony.

<sup>b</sup>Duplicate cultures.

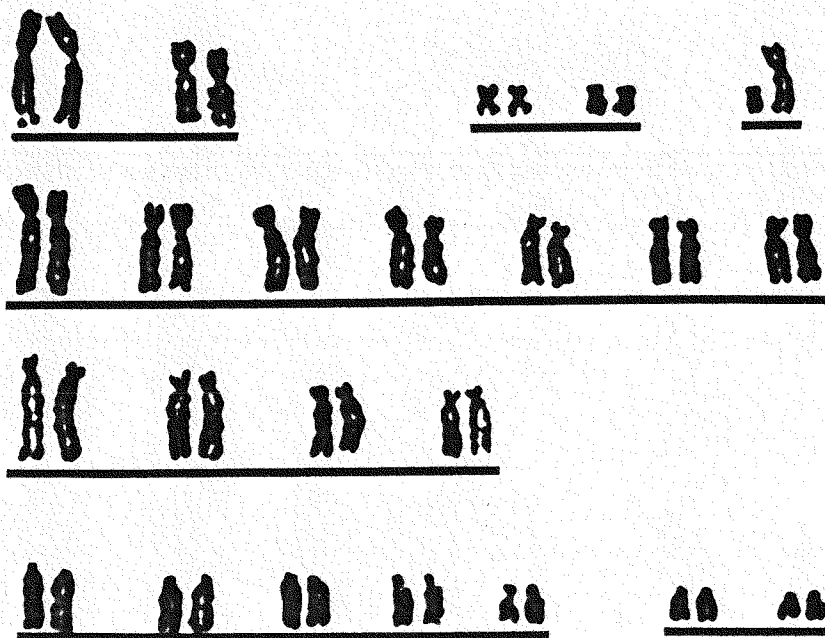
species are shown in Figure 5. A karyotype of each species is shown in Figure 6.

As a second method of comparing SCE frequencies in control cultures from these two species, the distribution of SCEs in 260 second division metaphases from S. oedipus animals and in 216 second division metaphases from S. fuscicollis is illustrated in Figure 7. The percentages of total metaphases scored having 1-19 SCEs are virtually identical in the two species.

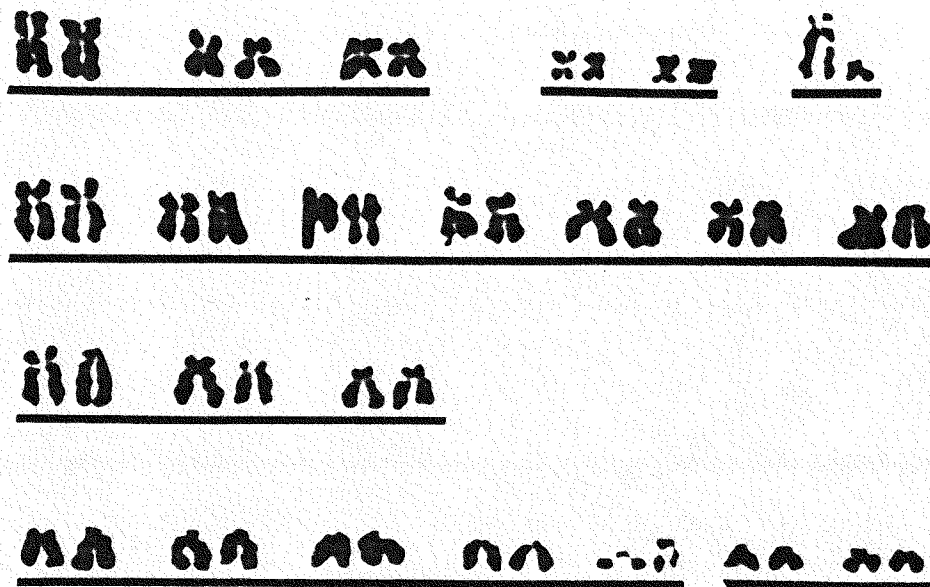
Although systematic scoring of first division metaphases for classical chromosome breakages was not routinely done on slides made from control cultures, note was made of any damage incidentally observed in cultures evaluated for SCEs. Generally, only an occasional chromatid gap and/or break was noted in metaphases from these control cultures; however, in preparations from one S. oedipus a first division metaphase with multiple G<sub>1</sub>-type aberrations (di and tricentric chromosomes and fragments) was noted. Veterinary history on this animal disclosed that the tamarin had had no drug or radiation exposure other than topical treatment of an open knee wound with Gentian violet, a triphenylmethane dye. It is likely that the animal ingested the dye while nursing its wound; and since several papers have cited the possibility that Gentian violet causes cytogenetic toxicity of mammalian cells cultured in vitro (72,73), it is possible that this cytogenetic damage occurred as a result of in vivo exposure to this clastogen.



Figure 5. Photographs of second division metaphases from control cultures of two tamarin species.



S. oedipus



S. fuscicollis

Figure 6. Karyotypes of two species of tamarins.

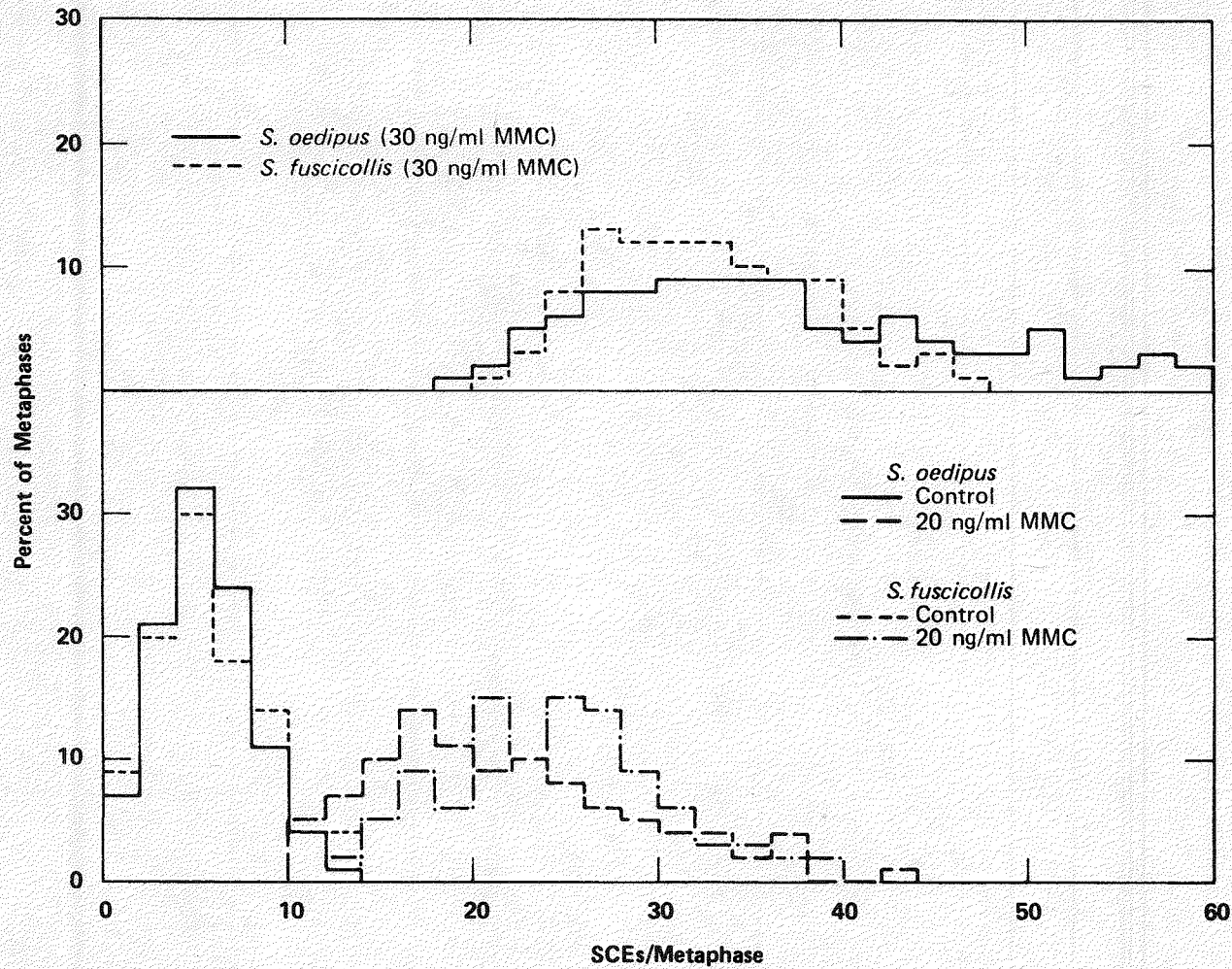


Figure 7. Distribution plot of SCE frequencies in control, 20, and 30 ng/ml mitomycin C-treated lymphocyte cultures from two species of tamarins.

Initial Comparisons of Mitomycin C-Induced SCE  
Frequencies in Second Division Lymphocytes  
of *S. oedipus* and *S. fuscicollis*

As has already been mentioned, MMC is a clastogenic antibiotic and a potent bifunctional alkylating agent which, among other actions, cross-links strands of DNA (32,75). Studies have shown that this compound is extremely effective in the induction of SCEs in mammalian chromosomes (17,70). Since MMC had not previously been evaluated for SCE-inducing ability in tamarin lymphocytes, a preliminary study was undertaken to determine the concentrations of this alkylator to be used for comparison of mutagen-induced SCEs in lymphocytes of *S. oedipus* and *S. fuscicollis*. Four cultures from each of two *S. oedipus* animals were initiated with varying doses of MMC (e.g., 0, 10, 20, and 30 ng/ml), harvested at the end of 72 hr, and scored for SCEs. It was determined that 20 and 30 ng/ml MMC induced high frequencies of SCEs in these cultures without excessively inhibiting culture growth. (See Figure 8 for an example of metaphases with SCEs induced in vitro by 30 ng/ml MMC.)

Based on these findings a total of 41 cultures from 10 *S. oedipus* and 10 *S. fuscicollis* animals were initiated with 20 or 30 ng/ml MMC and 10  $\mu$ M BUdR and held in the dark for 72 hr. The induced SCE frequencies in second division metaphases of these two animal species are shown in Table 7 and 8, respectively.

In *S. oedipus* tamarins the induced SCE frequencies shown in Table 7 were variable within both the 20 ng/ml dose level (mean values from 17.05 to 33.55 SCEs/metaphase with an overall mean frequency of 21.99 SCEs/metaphase for the 10 animals) and the 30 ng/ml MMC dose level (mean values from 22.75 to 48.20 SCEs/metaphase with an overall mean frequency

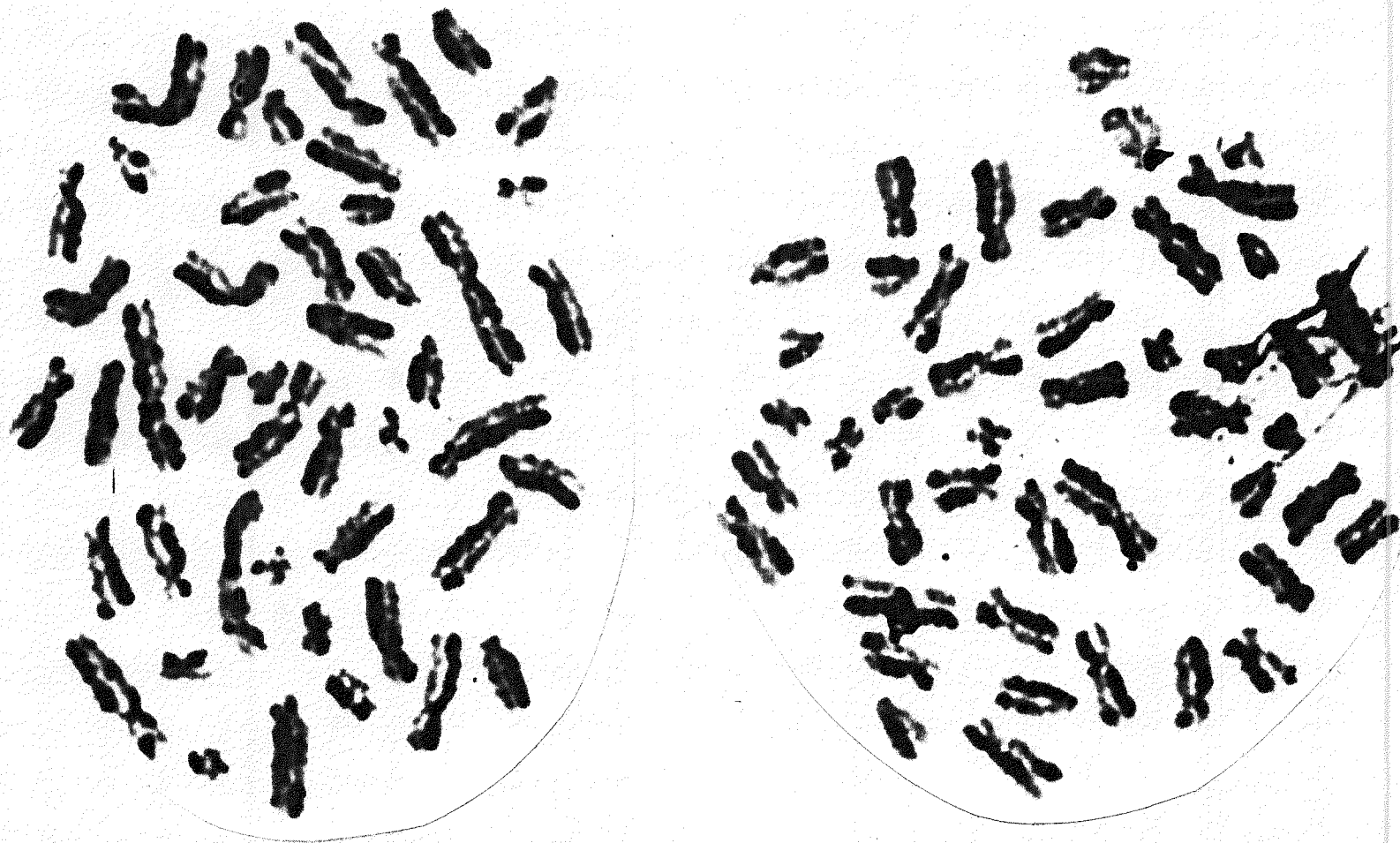


Figure 8. Photographs of second division lymphocyte metaphases from two different *S. oedipus* tamarins illustrating SCE frequencies observed after in vitro exposure to 30 ng/ml mitomycin C.

Table 7

Frequency of SCEs/Metaphase<sup>a</sup> ± S.E. (Range) in Control and Mitomycin C-Treated Lymphocyte Cultures from Ten S. oedipus Animals

Animal Number	Mitomycin C (ng/ml)		
	0	20	30
03-65 A	5.85 ± 0.83 (1-14)	--	45.90 ± 2.29 (30-67)
	<sup>b</sup> 6.20 ± 0.58 (1-9)	33.55 ± 1.24 (22-44)	48.20 ± 1.58 (36-63)
97-68 D	4.75 ± 0.56 (1-10)	17.15 ± 0.82 (12-25)	26.10 ± 1.03 (21-32) <sup>c</sup>
	5.20 ± 0.54 (1-10)	17.65 ± 0.96 (11-26)	
	<sup>b</sup> 5.40 ± 0.49 (2-12)	17.05 ± 0.69 (11-23)	
99-203 B	6.55 ± 0.55 (3-12)	21.20 ± 1.29 (12-35)	--
01-33 B	5.15 ± 0.57 (1-9)	17.53 ± 0.86 (11-27)	22.75 ± 1.02 (10-30)
97-106 D	5.85 ± 0.55 (1-10)	18.00 ± 1.42 (11-37)	27.87 ± 1.54 (18-42) <sup>d</sup>
3211	6.45 ± 0.54 (2-12)	21.35 ± 0.77 (16-24)	27.75 ± 1.02 (19-38)
3152	5.95 ± 0.60 (3-14)	25.10 ± 0.98 (19-32)	34.50 ± 1.89 (23-41)
3188	6.15 ± 0.30 (4-8)	23.65 ± 1.55 (13-44)	31.65 ± 1.24 (24-42)
1715 <sup>e</sup>	6.20 ± 0.34 (4-9)	28.05 ± 1.01 (20-36)	34.25 ± 1.13 (26-45)
1591 <sup>e</sup>	7.40 ± 0.66 (3-14)	23.55 ± 0.71 (17-29)	31.40 ± 1.09 (24-43)
Mean	5.93 (1-14)	21.99 (11-44)	33.04 (10-67)

<sup>a</sup>20 cells scored/culture unless otherwise indicated.

<sup>b</sup>Repeat culture.

<sup>c</sup>18 cells scored.

<sup>d</sup>15 cells scored.

<sup>e</sup>Animals with chronic colitis.

Table 8

Frequency of SCEs/Metaphase<sup>a</sup> ± S.E. (Range) in Control and Mitomycin C-Treated Lymphocyte Cultures from Ten S. fuscicollis Animals

Animal Number	Mitomycin C (ng/ml)		
	0	20	30
48-21-B-11	<sup>b</sup> 4.88 ± 0.32 (3-7)	21.40 ± 1.12 (12-29)	--
	5.00 ± 0.63 (1-10)	20.75 ± 0.96 (13-27)	--
3711	5.25 ± 0.88 (1-18)	--	32.07 ± 1.26 (25-41)
18-31-0	6.40 ± 0.68 (2-13)	22.75 ± 1.25 (14-38)	31.70 ± 1.99 (22-40)
52-14 D	9.30 ± 0.77 (4-19)	27.25 ± 1.20 (16-34)	35.55 ± 1.25 (25-44)
3763	6.10 ± 0.69 (1-14)	27.75 ± 1.27 (17-41)	34.35 ± 1.37 (25-47)
1914	8.90 ± 0.53 (5-14)	31.50 ± 1.52 (25-50)	39.55 ± 1.44 (28-54)
2716	6.60 ± 0.55 (3-13)	26.15 ± 1.10 (18-37)	34.85 ± 1.02 (27-45)
73-54 J	5.75 ± 0.37 (2-9)	22.95 ± 1.08 (15-33)	31.35 ± 1.75 (25-41)
s-76-64 I	7.40 ± 1.17 (2-15)	28.60 ± 1.23 (21-40)	33.20 ± 1.21 (24-42)
3741	7.00 ± 0.71 (2-15)	21.85 ± 0.95 (15-30)	28.10 ± 0.89 (23-37)
Mean	6.32 (1-19)	25.10 (12-41)	33.28 (22-54)

<sup>a</sup>20 cells scored.

<sup>b</sup>16 cells scored.

of 33.04 SCEs/metaphase for the group). The ranges in SCEs/metaphase also were variable among cultures from different S. oedipus, and as might be expected, as the dose of MMC increased, the ranges increased (with a difference of 33 SCEs/metaphase at 20 ng/ml and one of 57 SCEs/metaphase at 30 ng/ml MMC). In S. fuscicollis animals the induced SCE frequencies shown in Table 8 were quite consistent within both the 20 ng/ml MMC dose level (mean values from 20.75 to 31.50 SCEs/metaphase with an overall mean frequency of 25.10 SCEs/metaphase for the 10 animals) and the 30 ng/ml MMC dose level (mean values from 28.10 to 39.55 SCEs/metaphase with a mean frequency of 33.28 SCEs/metaphase). As in the S. oedipus animals, the distribution of ranges was increased in the 30 ng/ml MMC level (with a difference of 32 SCEs/metaphase) compared with the 20 ng/ml MMC treatment (with a difference of 29 SCEs/metaphase). Although comparisons of the mean frequencies of SCEs in MMC-treated cultures from the 10 S. oedipus and the 10 S. fuscicollis animals suggest no difference between the species (as seen in Table 9),  $t = -1.29$  and  $t = 0.30$ ,

Table 9

Mean SCE Frequencies (Range) in Mitomycin C-Treated  
Lymphocyte Cultures from Two Species of Tamarins

	20 ng/ml	30 ng/ml
<u>S. oedipus</u>	21.99 (11-44)	33.04 (10-67)
<u>S. fuscicollis</u>	25.10 (12-41)	33.28 (22-54)

the range of individual values within S. fuscicollis was not as great as within S. oedipus, particularly at the 30 ng/ml dose.

Three other comparisons of data demonstrated consistent response among animals of S. fuscicollis, but considerable variability in response among animals of S. oedipus. This variability is readily apparent when frequency distributions of SCEs in MMC-treated cultures from the 10 S. oedipus and 10 S. fuscicollis are plotted. As is illustrated in Figure 7, page 29, the distributions in cultures exposed to 20 ng/ml MMC from the two species show greater similarity than those plotted from cultures exposed to 30 ng/ml MMC. At both the 20 ng/ml and 30 ng/ml MMC dose levels, the range for S. oedipus animals is greater than that of S. fuscicollis but is more apparent at the 30 ng/ml MMC treatment.

When MMC-induced SCEs from cultures of the 10 S. oedipus tamarins alone are considered, SCE frequencies in one of the animals (i.e., 03-65A) are increased one and one-half fold compared with the overall mean frequency of the other nine animals (Table 10).

Table 10

Mean SCE Frequencies within S. oedipus at Two Concentrations of Mitomycin C (ng/ml)

<u>S. oedipus</u>	20	30
1 animal (03-65A)	33.55	47.05
9 animals	20.93	29.53

When data from the 30 ng/ml MMC treatment of lymphocytes from this animal was separated from that of the other nine tamarins, the resulting distributions plotted (Figure 9) and compared with the distribution for the entire species in Figure 7 (page 29), it is quite evident that the mean from that animal shifts the distribution for all S. oedipus animals. In addition, when the mean SCE frequency of this animal, 03-65A, was compared with the means of the other nine tamarins of the group (Tables 7 and 10), a significant variability among animals within S. oedipus was observed ( $F_s = 29.31$ ;  $p < 0.01$ ). This variability was not seen within the S. fuscicollis species ( $F_s = 1.61$ ;  $p < 0.20$ ).

Because a straight line appeared to adequately describe the SCE response of tamarin lymphocytes to 0, 20, and 30 ng/ml MMC, linear regression coefficients were calculated from the dose response of each animal for which these three points had been determined (Table 11). The linear regression method of Steel and Torrie (76) was used for these calculations. The regression coefficients for the nine S. oedipus animals varied by a factor of greater than 2.4, ranging from 0.58 to 1.40. Only the animal whose second division lymphocytes demonstrated increased sensitivity and SCE induction frequency to 20 and 30 ng/ml MMC had a coefficient of greater than one. On the other hand, the regression coefficients for the S. fuscicollis animals varied only by a factor of 1.4 ranging from 0.71 to 1.03.

Thus, within S. oedipus there is a third demonstration of variability between animals as to the lymphocyte response to MMC. This variability between animals is not as great within S. fuscicollis.

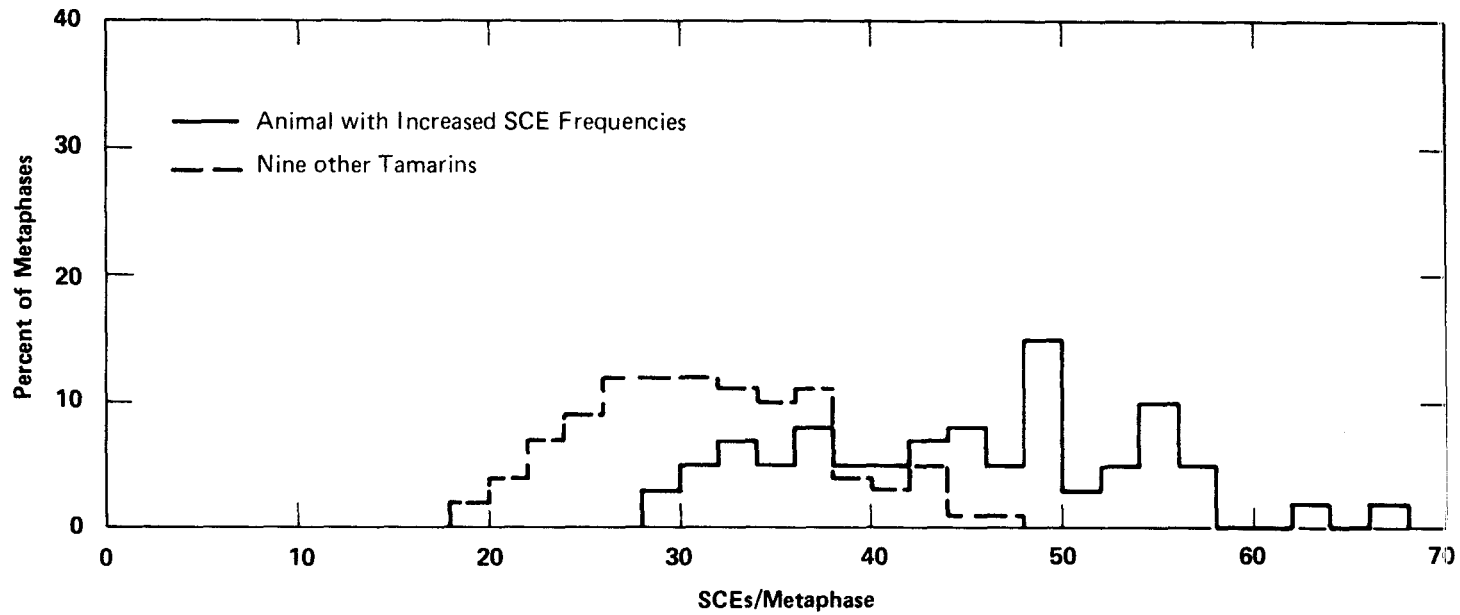


Figure 9. Distribution plot of SCE frequencies in 30 ng/ml mitomycin C-treated lymphocyte cultures from tamarin 03-65A and nine other animals from S. oedipus.

Table 11  
 Regression Coefficients Calculated from Dose Response  
 Data from Two Species of Tamarins

<u>S. oedipus</u>		<u>S. fuscicollis</u>	
<u>Animal</u>	<u>Regression Coefficients</u>	<u>Animal</u>	<u>Regression Coefficients</u>
03-65 A	1.40	18-31-0	0.84
97-68 D	0.62	52-14 D	0.90
	0.69	3763	0.96
	0.58		
01-33-B	0.59	1914	1.03
97-106 D	0.72	2716	0.94
3211	0.72	73-54 J	0.85
3152	0.95	s-76-64 I	0.89
3188	0.85	3741	0.71
1715	0.96		
1591	0.80		

Additional Studies in Two *S. oedipus* Siblings Having Increased Lymphocyte Sensitivity to Mitomycin C; and in Parents of These Two Animals

To verify that the unusual response to MMC noted in animal 03-65A was a reproducible observation, repeat cultures containing 0, 20, and 30 ng/ml MMC were initiated. As can be seen from Table 7, page 32, the results of increased SCEs found in the first observation were duplicated.

In scoring for SCE analyses in this animal, a striking proportion of first division metaphases with classical chromatid-type lesions was observed on slides made from cultures treated with 30 ng/ml MMC. [Chromatid exchanges have been noted to occur in response to MMC by several authors (74,75,77).] To determine whether MMC-induced chromatid breakage was higher in lymphocytes of this animal, 50 to 100 first division metaphases were evaluated for damage and compared with analyses in 25 to 100 first division metaphases in two other *S. oedipus* and in four *S. fuscicollis* (see Table 12). The total amount of chromatid damage seen in lymphocytes of this tamarin was five to six times that observed in the first division cells of two other *S. oedipus* animals. It is also of interest to note that in this animal when a high amount of total damage occurred, one-half of it was extensive, precluding exact enumeration of the lesions but listed in Table 12 as "number" and "percent of cells scored with greater than 10 breaks/cell." An example of extensive chromatid damage is illustrated in Figure 10. No more than 12% first division chromatid damage (including breaks, isochromatid breaks and exchanges and a rare cell with multiple lesions) was seen in any of the *S. fuscicollis* animals. Thus, in this one *S. oedipus*

Table 12

Comparison of Classical Lesions in First Division Cells and SCE Frequencies in Second Division Metaphases in S. oedipus and S. fuscicollis Animals Following in Vitro Exposure to 30 ng/ml Mitomycin C

Animal	Number of First Division Cells Scored	Number of First Division Cells <sup>a</sup> with Damage	Percent of Cells Scored with Classical Damage	Number of First Division Cells with > 10 Breaks/Cell <sup>b</sup>	Percent of Cells Scored with > 10 Breaks/Cell	Mean SCE Frequency <sup>c</sup> in Second Division Metaphases
<u>S. oedipus</u>						
03-65 A <sub>d</sub>	100	37	37	16	16	45.90
03-65 A <sup>d</sup>	50	20	40	10	20	48.20
97-106 D	100	4	4	0	0	27.80
01-33 B	100	7	7	0	0	22.75
<u>S. fuscicollis</u>						
2716	50	4	8	1	2	34.85
18-31-0	25	1	4	0	0	31.70
1914	25	3	12	0	0	39.55
52-14 D	50	4	8	1	2	35.55

<sup>a</sup>Any cell noted with chromatid breaks or exchanges.

<sup>b</sup>Any cell with > 10 chromatid breaks or exchanges/cell (Large numbers of breaks precluded exact counting.).

<sup>c</sup>20 cells scored.

<sup>d</sup>Repeat culture.



Figure 10. Photographs of first division metaphases from S. oedipus tamarins whose chromosomes have been extensively damaged in vitro by 30 ng/ml mitomycin C.

first division chromatid sensitivity to 30 ng/ml MMC is increased as is the second division SCE response (Table 12).

To determine whether the increased MMC sensitivity in lymphocytes of the tamarin (03-65A) might reflect a familial condition, SCE evaluations were completed on lymphocytes from both parents and a sibling. The in vitro cytogenetic response to 30 ng/ml MMC demonstrated in the lymphocytes from the sibling (03-65C) from a consecutive litter and from the female parent (1651) were amazingly similar to the response observed in 03-65A. Both increased induced frequencies of SCEs in second division metaphases and increased chromatid sensitivity in first division metaphases were noted in three members of this particular S. oedipus family. These findings were not seen in any of the other animals of either species tested including the male parent (1650). A summary of the SCE frequencies of these family members (when their lymphocytes were challenged with 0, 20, and 30 ng/ml MMC for 72 hr in media containing 10  $\mu$ M BUdR) is shown in Table 13. A comparison of the first division chromatid sensitivity and second division SCEs in lymphocytes from these three related animals treated with 30 ng/ml MMC is shown in Table 14. Because of these interesting cytogenetic findings within one particular S. oedipus family of tamarins, a "family tree" was constructed to better illustrate the relationship of these animals with the results of the data (see Figure 11).

Another interesting observation noted about the three animals with the higher induced SCE frequency was of a more technical nature. The amount of time needed for fluorescent light exposure for good

Table 13

Frequency of SCEs/Metaphase<sup>a</sup> ± S.E. (Range) in Control and Mitomycin C-Treated Lymphocyte Cultures from Four S. oedipus Family Members

Animal	Mitomycin C (ng/ml)		
	0	20	30
03-65 A	5.85 ± 0.83 (1-14)	--	45.90 ± 2.29 (30-67)
	<sup>b</sup> 6.20 ± 0.58 (1-9)	33.55 ± 1.24 (22-44)	48.20 ± 1.58 (36-63)
03-65 C (sibling)	6.80 ± 0.53 (3-11)	29.10 ± 1.12 (19-38)	45.10 ± 1.30 (33-57)
1651 (female parent)	6.45 ± 0.49 (4-11)	No growth	42.75 ± 1.01 (36-54)
1650 (male parent)	6.05 ± 0.53 (4-11)	19.80 ± 1.02 (13-30)	25.00 ± 0.97 (19-34)

<sup>a</sup>20 cells scored/culture.

<sup>b</sup>Repeat culture.

Table 14

Comparison of Classical Lesions in First Division Cells and SCE Frequencies in Second Division Metaphases in Two S. oedipus Siblings and Their Parents Following in Vitro Exposure to 30 ng/ml Mitomycin C

Animal	Sex	Number of First Division Cells Scored	Number of First Division Cells <sup>a</sup> with Damage	Percent of Cells Scored with Classical Lesions	Number of First Division Cells with > 10 Breaks/Cell <sup>b</sup>	Percent of Cells Scored with > 10 Breaks/Cell	Mean SCE Frequency <sup>c</sup> in Second Division Metaphases
<u>Siblings</u>							
03-65A	♂/♀	150	57	38	26	17	47.05
03-65C <sup>e</sup>	♀/♀/♀	50	28	56	17	34	45.10
<u>Parents</u>							
1651	♀	25	12	48	7	28	42.75
1650	♂	50	3	6	1	2	25.00

<sup>a</sup>Any cell noted with chromatid breaks or exchanges.

<sup>b</sup>Any cell with > 10 chromatid breaks or exchanges/cell (Large numbers precluded exact counting of breaks.).

<sup>c</sup>20 cells scored.

<sup>d</sup>Repeat culture.

<sup>e</sup>Sibling of No. 03-65A and from different but consecutive litter.

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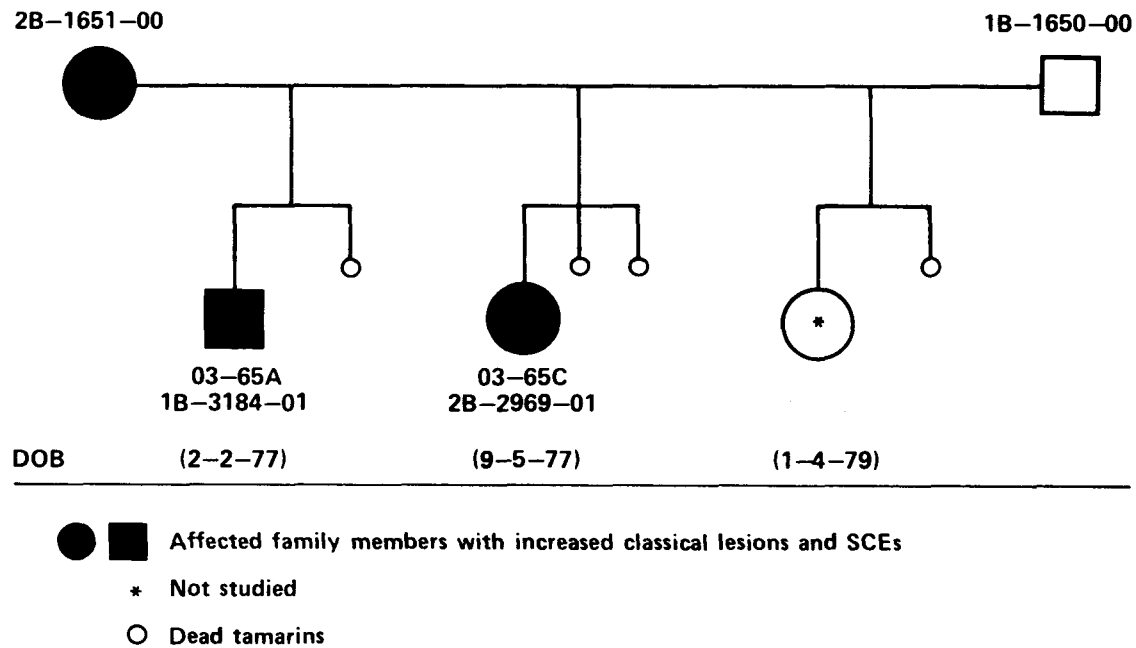


Figure 11. "Family Tree" illustrating the relationship between three related tamarins and the increased sensitivity of their lymphocytes to 30 ng/ml mitomycin C.

differentiation of the chromatids was two to three times that needed for the lymphocytes of the other animals from either species.

## CHAPTER IV

### DISCUSSION

Although colonic adenocarcinomas are among the most prevalent human cancers in the United States, they are infrequently seen as spontaneous cancers in animals (78). Therefore, when Lushbaugh et al. (1) reported a colon cancer incidence of 15% in the New World nonhuman primate, Saguinus oedipus, and not in the related species Saguinus fuscicollis residing in the same ORAU Marmoset Research Colony, much interest was shown in the possibility of establishing S. oedipus as a primary experimental animal model for colon cancer research. A cytogenetic study to determine whether there might be increased chromosomal sensitivity within the "cancer prone" species of tamarins was initiated to determine if this particular animal would be a useful model. The rationale for this cytogenetic evaluation stems from studies of patients with several genetic diseases in which increased frequencies of many types of cancer are associated with spontaneous or mutagen-induced chromosomal instabilities. This association between "chromosome instability" and increased risk of malignancy is clearly demonstrated in four human syndromes that have an autosomal recessive mode of inheritance (Tables 1 and 2, pages 3 and 9). Persons with these conditions, BS, FA, AT, and XP have an excessive risk for developing malignancies, increased frequencies of either spontaneous or induced chromosomal instabilities, frequent immune defects (4), and defective DNA repair in many cell types (6,16,32,47,79). Several authors have

also described an increased susceptibility of the fibroblasts of such patients to in vitro malignant transformation with the SV<sub>40</sub> virus (50,80-82), a phenomenon that has been cited as involving DNA repair processes (83,84). One or several of these defective features may be associated directly or indirectly with the evolution of malignancy in these persons (18). Regarding the aberrant cytogenetic findings that are characteristic of these inherited diseases, it is of interest that chromosomal instability can be demonstrated in lymphocytes and fibroblasts of patients with the recessively inherited FA years before malignant tumors develop (27,85), and that cells from the dominantly inherited Gardner's syndrome patients (in which multiple colon tumors occur) demonstrate increased in vitro tetraploidy if they are (or are destined to become) malignant (24). The objectives of this study, i.e., to determine if there were cytogenetic differences either in spontaneous or mutagen-induced SCE frequencies between two tamarin species (S. oedipus and S. fuscicollis), and if there were differences between animals within the species, was based on the rationale that if the increased frequency of colon cancer in S. oedipus were genetically controlled, there might be a cytogenetic marker which could be demonstrated by lymphocyte culture and chromosome evaluation.

The results of this cytogenetic study indicate that lymphocytes from an S. oedipus female and two of her offspring exhibit increased sensitivity to SCE induction after in vitro treatment with low concentrations of MMC (68,69). As is illustrated in Table 12, page 40, the first division lymphocytes of these three animals also were shown to have increased chromosome breakage. This latter finding was not

demonstrated in any of the other S. oedipus animals tested nor was it seen in any of the preparations from the other species, S. fuscicollis. The heterogeneity (as demonstrated by SCE distribution frequencies, regression coefficients, and analysis of variance) among S. oedipus animals toward SCE induction in second division lymphocytes and chromosome damage in first division lymphocytes when exposed to 30 ng/ml MMC is analogous to the "chromosome instability" observed in persons with various genetic diseases. These findings suggest that possible genetic factors influencing DNA repair mechanisms may be responsible for the results obtained in these animals. Similar increased sensitivity to UV induced chromosome damage has recently been observed in fetal fibroblasts from New Zealand black mice which have increased risk for lymphoreticular malignancies of the spleen and other organs (86). Whether the chromatid sensitivity in S. oedipus is related to the high incidence of colon cancer is not known, but it is a hypothesis that should continue to be tested in this primate species.

Although chromosome instability has been noted in several inherited diseases, the cytogenetic findings in S. oedipus lymphocytes more closely mimic observations in XP. Cells from three family members within S. oedipus and from XP patients (17) demonstrate significant increased induction of SCE frequencies and increased chromatid sensitivity when challenged with mutagenic agents, yet spontaneous or control levels of sensitivity appear normal (see Table 15).

In addition to the similarities in chromosomal instabilities in S. oedipus and in various inherited human diseases, there appear to be corresponding similarities in several immunological findings between

Table 15

Summary of Clinical and Genetic Findings that May Be Related to Cancer Risk in S. oedipus Tamarins and in the Inherited Human Condition, Xeroderma Pigmentosum

Cytogenetic Findings	Immunological Deficiencies	Primary DNA Lesion	Cancer Risk	Mode of Inheritance
<u>S. oedipus (cotton-topped tamarin)</u>				
No difference in control SCEs between 13 animals tested. <sup>a</sup> Increased induction of SCEs by MMC in 3 related animals of 13 tested. Increased induction of classical aberrations in cells of same 3 animals in response to MMC (see Table 14). <sup>a</sup>	Less immunocompetence demonstrated than in <u>S. fuscicollis</u> (87).	Not known.	Increased spontaneous colon cancer (1).	Not known (Basis for thesis project).
<u>Xeroderma pigmentosum (XP)</u>				
Control and spontaneous SCEs within normal ranges for human, as are classical aberrations (32). Induced SCEs and classical aberrations are much increased over normal human cells and control values (30).	Response of B and T cells appear to be normal (51).	UV-induced pyrimidine dimers due to lack of UV endonuclease DNA repair enzyme in all but one group (22).	Increased skin cancer-- basal and epitheloid cell carcinoma (2).	Autosomal recessive.

<sup>a</sup>See Results section.

S. oedipus and the human syndromes BS, AT, and FA (Table 2, page 9).

It is of interest to note that Genogozian (87) and several other investigators (88) have also indicated that the South American tamarin, S. oedipus may be less immunocompetent than are other related nonhuman primates or man. In fact, one author contends that S. oedipus animals appeared to be more susceptible to Epstein Barr virus (EBV) infections, and they also developed a more pronounced clinical illness than did the S. fuscicollis (89). Several theories regarding the induction of colon cancer in the primate have been proposed. One of the most relevant to that seen in S. oedipus has been suggested by Scott (90). Considering the possible immunodeficiency of this primate, he proposes that the function of the animal's immunological system may be deficient due to a genetic predisposition, thus allowing the entrance of destructive organisms which cause ulcerative colitis, a chronic problem in these animals. This theory is supported by other authors (50). It is not known whether ulcerative colitis precedes the evolution of malignancy in the tamarin, but the two animals with this problem (i.e., S. oedipus 1715 and 1591) (see Table 7, page 32) and evaluated in the present study did not show the cytogenetic instability, nor has it been shown that there was any correlation between colitis and colon cancer in animals diagnosed as having the malignancy (1).

That the abnormal cytogenetic findings in S. oedipus may be related in some manner to the high incidence of malignancy is supported by several theories. First, the correlation between chromosomal abnormalities and a variety of tumors or malignancies has been known for some time. Sixty-five years ago Boveri, as discussed by Koller (91), postulated

that chromosomal aberrations were important in the etiology of malignancies. Since that time chromosomal abnormalities have been described in a variety of tumors including colonic tumors (92). More recently Knudson has proposed a "Two Stage Theory of Cancer" regarding the induction of malignancy. He proposes that it takes at least two mutational events for cancer expression and that every human cancer occurs in a genetic and a nongenetic form (37,93). His theory is illustrated in Figure 12 and was derived from his work based on in-depth studies of the time of expression of cancers in persons with dominantly inherited predispositions to retinoblastoma and those that do not have the inherited predisposition (94). Knudson found, for example, that the development of childhood retinoblastoma [an uncommon eye tumor which is dominantly inherited (37,95)] in the normal individual is one in 30,000, whereas the carrier of the gene will develop an average of three to four tumors early in life (39). Interestingly, retinoblastoma gene-carrying individuals also develop more bilateral tumors than unilateral (94). Knudson speculates that because some people are born with a dominant mutation that makes them susceptible to cancer (and yet only a rare cell even becomes a cancer cell), then two steps must be required for malignant transformation: (1) The first event may be either an inherited mutation or a subsequent mutation in a somatic cell (37). (2) In the case of persons genetically predisposed to develop cancer, a second mutational event must occur at a later time (48). This theory is supported by the observation that if the cancer is of the genetic form, it is likely to be expressed early in life (39,96), whereas if the cancer is not genetic the malignancy tends

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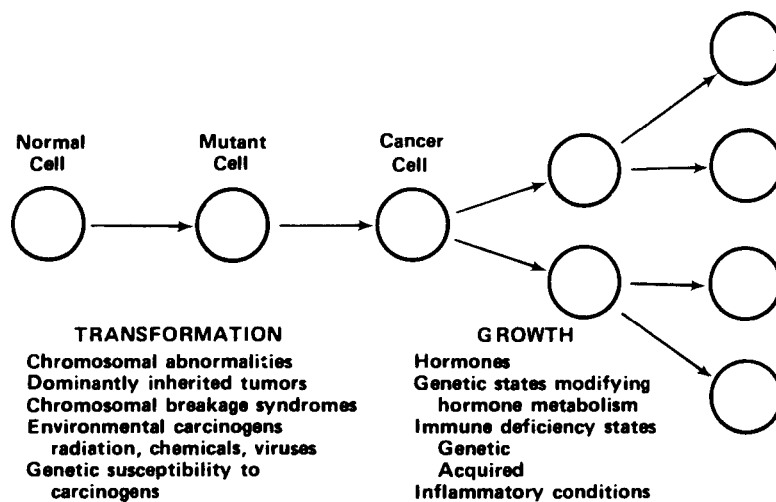


Figure 12. A model for carcinogenesis. A normal cell is converted to a cancer cell in two mutational steps in a transformation phase which can be accelerated by the means listed thereunder. The cancer cell then proliferates to form a tumor in a growth phase which can be accelerated by other means.

Source: Alfred G. Knudson, 1975.

to be age-associated and fundamentally a manifestation of accumulating somatic mutations (97).

Within the framework of Knudson's theory of tumor induction (98), an interesting comparison between the S. oedipus tamarins and XP autosomal recessive disorder may be considered. Assuming, as Knudson proposes, that it takes two mutations for the formation of the malignancy, it may be speculated that since persons with XP have an inherited gene mutation (i.e., the first event), only one additional mutation (i.e., the second event) is required for the manifestation of the malignancy. This may explain the high incidence of skin cancers in persons with this disorder. The "induced" and increased chromosomal breakage demonstrated in lymphocytes and fibroblasts of these patients may be a visible manifestation of this gene defect. In addition, it is known that these persons are extremely sensitive to UV radiation (sunlight sensitive) due to a lack of the UV endonuclease repair enzyme. This sensitivity could lead to an early accumulation of the "second" mutational event required for malignant cell transformation. The same rationale might be applied to explain the high incidence of colon cancer in S. oedipus. If the increased sensitivity of somatic cells from certain S. oedipus animals is a manifestation of an inherited trait, then it may be presumed that these animals have the "first mutational event" at birth. Thus, a "second event" might be induced at an early age in the susceptible animals following exposures to carcinogens. (For a summary comparing S. oedipus with XP, see Table 15.)

For inherited colon cancer as in retinoblastoma, discussed by Knudson, it has been theorized that there is a single gene defect in

individuals who develop these particular tumors that predisposes them to cancer (95,98) and that also predisposes their cells to produce chromosomal abnormalities (91), possibly as a result of faulty DNA repair (6). It is also known that the age-specific risk for human colon cancer is more than 1000 times as great for polyposis patients (those with multiple polyp formations) as for individuals who carry no predisposing gene (37,99). Ashley (99) tends to verify Knudson's theory by noting that in not all cases of intestinal carcinoma is there a stage of benign (somatic event) polyp formation leading to larger malignant ones.

In conclusion, because increased SCE induction is demonstrated in lymphocytes of two siblings of different and consecutive S. oedipus litters as well as the female parent, and because the finding of increased chromatid sensitivity occurs within the "cancer prone" species (1), additional experiments should be designed to further elucidate what appears to be a genetically predetermined susceptibility to a malignant disease in this primate. Since the neoplasms that develop in subjects with an innate predisposition to tumorigenesis might be highly expected to have multicellular origins (100), other tissues of these suspect animals should be evaluated to determine the cytogenetic response when challenged with MMC. Also, at the present time there is no simple method for a clinical diagnostic evaluation to determine if an S. oedipus animal has the cancer presently or may develop a colon malignancy in the future. The results of this study suggest that those animals expressing increased chromosome sensitivity should be closely monitored for possible development of colon cancer. Should colon cancer

develop in either of the two siblings, 03-65A and 03-65C, or the female parent, 1651, the evidence of increased chromatid sensitivity found during cytogenetic testing prior to malignant development would highly substantiate the hypothesis that the colon cancer in these animals is correlated with the increased chromatid sensitivity found in some of the animals within the species. This would provide validation for using cytogenetic screening as a method of identifying at risk (39, 101) animals within the S. oedipus colony.

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