The ability of normal diploid arterial smooth muscle cells to grow and propogate in a serum-free, hormone-supplemented medium extends the theory that the role of serum in cell culture is to provide hormones (*31*). The formation of polypoid nodules, possibly resembling atherosclerotic plaques (*28, 29*), by hormone-supplemented smooth muscle cells is consistent with the concept that serum presents an abnormal environment for cultured smooth muscle cells (*32*), since in vivo they are exposed to the full complement of serum hormones only after endothelial injury (*5, 33*). We believe that we have achieved a more integrated understanding of the influences of hormones on the medial smooth muscle cell (Fig. 3). This may lead to an appreciation of atherosclerosis as a disease mediated by a complex endocrine system.

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34. We obtained EGF and MSA from Collaborative Research, Inc.; DME, Ham's F-12, FBS, and trypsinated EDTA from Grand Island Biological Co.; Cohn fractions I and IV from Armour Pharmaceutical Co.; bovine insulin, human transferrin, ovalbumin, soybean trypsin inhibitor, and hydrocortisone from Sigma Chemical Co.; and cell culture dishes (Costar) from Bellco. We thank H. N. Antoniades for the generous gift of PDGF (specific activity, 5 U per nanogram of protein); R. Humbel for purified IGF-I; D. Bing for purified human α-thrombin (original source, J. Fenton II); and R. Wolfe for the trace elements. We are indebted to J. van der Spek for technical assistance and L. Sanford for preparing the manuscript. Supported in part by NIH grants HL 07374, AM 07026, HL 06197, HL 25066, HL 22602, and AG 00599.

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Normalization by Cell Fusion of Sister Chromatid Exchange in Bloom Syndrome Lymphocytes

Abstract. *Fusion of fresh lymphocytes from a Bloom syndrome (BS) patient with those of normal subjects or a BS heterozygote resulted in complete normalization of the frequency of sister chromatid exchanges in the chromosomes of BS cells. This normalization took place by the first mitosis in hybrid cells. In contrast, cultivation of BS lymphocytes with those of normal subjects or the BS heterozygote had no effect on sister chromatid exchanges. The cell fusion experiments suggest that the normalization of the sister chromatid exchange frequencies in BS cells can be achieved by factors conserved in the cells of various mammalian species. These findings are compatible with the concept that BS is a recessive genetic mutation at regulatory levels of the DNA repair function.*

Lymphocytes, fibroblasts, and marrow cells of Bloom syndrome (BS) patients exhibit high frequencies of chromosome breaks and rearrangements and spontaneous increases in the number of sister chromatid exchanges (SCE) (*1, 2*). Even though BS cells are competent in excision and postreplication repairs and single-strand break rejoining and have normal levels of DNA polymerases, the findings are compatible with a deficiency in some aspect of the DNA repair function (*3–5*). Bryant *et al.* (*6*) observed normalized SCE frequencies in hybrid cells of BS and euploid cells. Alhadeff *et al.* (*7*) reported normalization of SCE in somatic cell hybrids 15 to 20 days after fusion between Chinese hamster cells (CHO-YH21) and BS fibroblasts (GM 1492) that was independent of the type and number of human chromosomes retained in the hybrid cells. In these studies (*6, 7*), the SCE frequencies were examined 10 to 20 days after fusion and varied from one BS cell to another. The mechanism of normalization of SCE is still obscure, especially in relation to (i) the stage of the cell cycle in hybrids when normalization of the SCE frequencies takes place and (ii) the class of cell populations involved in fusion. Fusion experiments with cells cultured for a long time should be evaluated with caution, since cells with high SCE frequencies were able to correct the defect in BS cells (*8*). Furthermore, cultivation of BS and normal cells led to different results and conflicting conclusions (*9–11*).

In our study, we examined the mechanism of SCE in BS cells by simultaneously performing cocultivation and hybridization experiments, using fresh lymphocytes from normal subjects, a BS heterozygote (HBS), and a BS patient. Fresh lymphocytes were used in order to exclude a possible metabolic shift in hybrids during long-term culture. We report that fusion of BS cells with either normal or HBS cells results in complete correction of the SCE incidence in the BS chromosomes by the first mitosis in hybrid cells, probably occurring during the preceding DNA synthesis period.

Heparinized peripheral blood was collected from two healthy females, a 20-year-old male with BS (*2*), and the mother of another BS patient, (HBS). The lymphocytes were purified by the Ficoll-Hypaque density sedimentation method (*12*).

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The technique of Davidson *et al.* (*13*) was used for cell fusion. Approximately 48 hours after stimulation with phytohemagglutinin, BS lymphocytes were exposed to bromodeoxyuridine (BrdU) (5.0 μg/ml) for 20 hours (a period covering one round of DNA replication for most of the cells). Equal numbers (2×10^7) of labeled BS lymphocytes and unlabeled normal or HBS lymphocytes cultured for 68 hours were mixed and then rinsed with 0.25 percent trypsin for 3 minutes. After centrifugation, a solution of 50 percent polyethylene glycol and 10 percent dimethyl sulfoxide (0.5 ml) was placed over the cells for 1 minute and then removed with a Pasteur pipette. The cells were washed three times with RPMI 1640 medium, once with medium containing 10 percent fetal calf serum, and then cultured without BrdU at 37°C in a CO_2 incubator and harvested every 24 hours. Cocultivation experiments were carried out as follows: normal and HBS cells labeled with BrdU during the first cell cycle were cultivated in the absence of BrdU with nonlabeled BS cells during the second cell cycle, and the effect of BS cells on SCE in the normal and HBS cells was evaluated. To test the effects of the latter types of cells on the SCE frequencies in BS cells, the BS cells were exposed to BrdU and cultivated in the absence of BrdU with nonlabeled normal or HBS cells. Chromosome preparations and the differential staining of sister chromatids with the FPG (fluorescence plus Giemsa) method were accomplished with techniques described in (*2*).

Before cocultivation or hybridization, the average SCE frequencies per cell were 4.9 ± 0.42, 6.1 ± 1.86, and 66 ± 1.81 in normal, HBS, and BS cells, respectively. The frequency of SCE in BS cells was more than ten times that in the normal or HBS cells. The SCE frequency did not differ significantly between normal and HBS cells.

Table 1 summarizes the effects of cocultivation and cell fusion on the incidence of SCE. The BS lymphocytes that had incorporated BrdU for one round of DNA replication were either cultivated or fused with normal or HBS cells; thereafter, these cells were cultivated in the absence of BrdU through another round of DNA replication. Thus when the next mitoses were encountered, only the chromosomes of BS cells had differentially labeled chromatids (*2*).

The results show that normal or HBS cells cultivated with BS cells (1:1 cell ratio) maintained their original low SCE frequencies, whereas the BS cells retained their high SCE frequencies. Thus, cocultivation did not seem to alter the frequency of SCE.

In the hybridization experiments, only the BS cells had incorporated BrdU for one round of DNA replication. After fusion with unlabeled normal or HBS cells, the mixture of unfused and fused cells was cultured in the absence of BrdU and harvested every 24 hours. After 24 hours, all mitoses showed a diploid chromosome number, indicating that only unfused cells underwent mitosis in the first 24 hours. The unfused cells maintained their original SCE frequencies. Regardless of fusion combinations, no hybrid cells underwent mitosis until 48 hours after fusion, indicating that their cell cycle time had been expanded beyond the normal range. On the basis of the mitotic index measured at 24, 48, and

Table 1. Evaluation of SCE frequencies in cell fusion and cocultivation between Bloom syndrome (BS) lymphocytes and normal (N) lymphocytes and between BS and heterozygote Bloom syndrome (HBS) lymphocytes. In experiments 2 through 4, cultured lymphocytes were labeled with BrdU (5 μg/ml) for 20 hours and then cultured for 24 hours without BrdU.

Experiment	Cells	Label in first cell cycle	Procedure	SCE (number per metaphase*)
1	N			4.9 ± 0.42 (49)†
2	N	BrdU		65 ± 1.81 (43)
3	BS	BrdU		5.3 ± 0.49 (56)
4	HBS	BrdU		
5	N		Cocultivation	62 ± 2.02 (51)
	BS	BrdU		
6	N	BrdU	Cocultivation	5.2 ± 0.49 (66)
	BS			
7	HBS		Cocultivation	63 ± 1.96 (50)
	BS	BrdU		
8	HBS	BrdU	Cocultivation	5.7 ± 0.61 (48)
	BS			
9	N		Cell fusion	4.5 ± 0.31 (25)
	N	BrdU		
10	BS		Cell fusion	66 ± 1.96 (16)
	BS	BrdU		
11	HBS		Cell fusion	5.2 ± 0.29 (24)
	HBS	BrdU		
12	N		Cell fusion	6.2 ± 1.86 (38)
	BS	BrdU		
13	HBS		Cell fusion	7.1 ± 2.02 (18)
	BS	BrdU		

*Only the chromosomes of cells which had previously incorporated BrdU were used for SCE scoring. †Numbers in parentheses represent the number of cells examined.

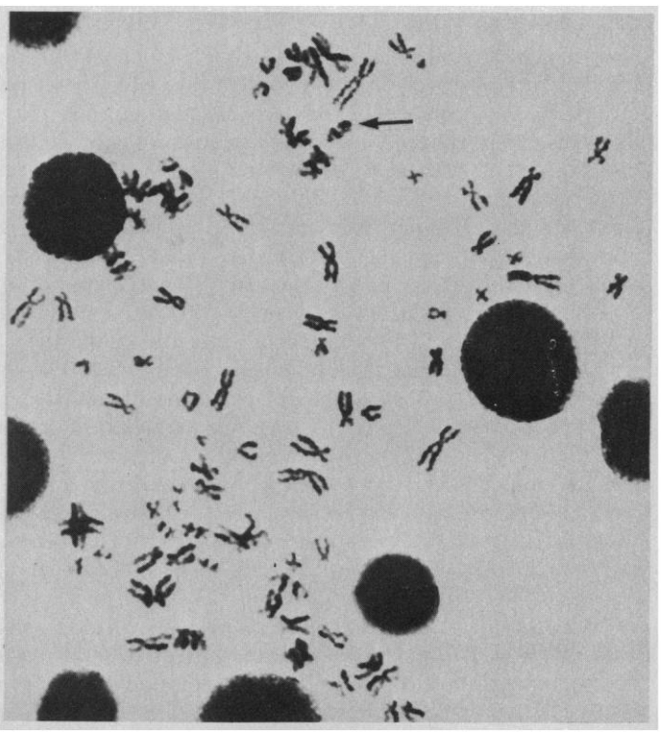

Fig. 1. First metaphase after fusion of a BrdU-labeled BS cell and a nonlabeled normal female cell. Arrow points to Y chromosome of the BS patient. The SCE incidence has been normalized in the BS chromosomes.

72 hours, the length of the cell cycle in hybrid cells between BS and normal or between BS and HBS cells appeared to be similarly expanded.

Metaphases of hybrid cells contained one diploid set each of BrdU-labeled and unlabeled chromosomes, indicating that hybrid cells between BS and normal or HBS lymphocytes reached mitosis after one complete DNA replication cycle subsequent to fusion. Further evidence of a genome of BS origin in the hybrids was established by the presence of a Y chromosome (Fig. 1). All the hybrids between BS and normal or HBS cells had a normal SCE incidence (Table 1 and Fig. 1). As shown in Table 1 (experiments 9, 10, and 11), no significant change occurred in the SCE frequencies when hybrids were produced between cells of the same origin. Thus, the process leading to normalization of SCE frequencies of BS chromosomes in hybrids with normal or HBS cells is not fusion per se or fusion-associated expansion of the cell cycle.

Although chromosomal instability and high SCE frequencies in BS cells (*1, 2*) may reflect a deficiency in DNA repair function (*3–5*), no such defect has been demonstrated. Furthermore, findings incompatible with the concept of recessive mutation in BS, that is, that a substance produced by BS cells in culture can increase SCE in normal lymphocytes, have been reported (*11*). Other investigators have reported that cultivation of BS with normal cells leads only to decreased SCE in BS cells (*9, 10*).

Cell fusion experiments have been used by some workers to study the mechanism of SCE genesis in BS cells. Even though normalization of SCE frequencies was observed in the hybrids between BS and normal cells, the possibility that segregation of a particular population of hybrid cells during the long-term culture (10 to 20 days) might have taken place was not ruled out. The issue was further complicated by the finding (*8*) that the SCE in BS cells can be normalized by fusing BS cells with mouse tumor cells (L-A9) that show a high SCE incidence; thus, a certain metabolic shift might well have taken place and suppressed SCE.

We used fresh normal and BS lymphocytes and the cells of a BS patient who showed no bimodality with respect to SCE frequencies in his lymphocytes and analyzed the SCE incidence in mitotic cells immediately after fusion to circumvent some problems of other fusion experiments (*6–8*). The results demonstrate that the normalization of SCE in BS cells occurred in euploid cell hybrids after only one round of DNA replication subsequent to fusion. Since the HBS cells also corrected the BS defect as effectively as did normal cells, it appears that BS is, as expected, a result of a recessive homologous mutation.

Since correction of the SCE defect in BS cells can be achieved by fusion with cells of several species (*6–8*), our results suggest the presence of a factor in the cells of several species that is capable of correcting the defect responsible for the high SCE frequencies in BS cells, rather than the production of a DNA-damaging substance by the BS cells (*14*). The absence of such a factor in the BS cells leads to the high SCE frequencies.

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Increased Reproductive Effort with Age in the California Gull (*Larus californicus*)

Abstract. *Comparisons of reproductive behaviors of three age classes of California gulls demonstrate that reproductive effort increases with age in this seabird. These findings contradict the assumption that increased reproductive success with age results from increased experience and social status and demonstrate that selection for increased reproductive effort can occur in long-lived species.*

Several theoretical considerations have both supported (*1, 2*) and refuted (*3–6*) Williams's (*7*) hypothesis that long-lived organisms increase their reproductive efforts with age. However, there is a dearth of behavioral data on the topic. I report here that the reproductive efforts (risks taken and energy expended) of the California gull increase with the age of the parents.

The breeding site under study is on an island 24 km west of Laramie, Wyoming, in the Bamforth Lake Wildlife Preserve. Gulls in the colony have been banded yearly since 1959 (*8*); there are now more than 1000 banded gulls in a population of over 4000. To permit accurate mapping of the individual nests, the site was divided into a grid system, with metal stakes as reference points. The wings of 39 banded gulls were specially marked, and others were identified by the numbers on their leg bands. A sample of 21 pairs of young gulls (3 to 5 years old), 20 pairs of middle-aged gulls (7 to 9 years old), and 18 pairs of old gulls (12 to 18 years old) was obtained from previous banding records. The nests of these birds were marked with numbered stakes. Gull behavior was observed from a 15-foot observation tower.

Reproductive success was determined by counting the number of surviving chicks fledged by each pair throughout the breeding season. Data were also collected on the number of times offspring were fed, the number of acts of territorial defense, and the amount of time each parent spent on the nest during 437 1-hour observation periods (*9*) standardized for the time of day and the age of chicks when measurements were taken. The data were compared between age classes and on the basis of whether the nest was located in the center of the colony or the periphery. In comparing locations, equal numbers of parents from each age class were analyzed for each location. The behavior of parents while off the nest was also observed, with daily checks for marked birds on the beaches adjacent to the breeding colony. Finally, 420 observations of the foraging behavior of gulls were made at five lakes and

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