"MECHANISMS OF ABERRATION PRODUCTION IN EUKARYOTE CHROMOSOMES"

Final Report

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PROJECT ABSTRACT

Hardware for a semi-automatic chromosome analysis device was constructed and tested, and software written for its application to chromosome measurement and karyotype analysis.

A comprehensive theory of eukaryote chromosomal aberration production by ionizing and non-ionizing radiation and by chemical agents was developed during the Contract period. Experiments were carried out to determine the patterns of aberration production by several specific agents, including 8-methoxypsoralin plus light, ozone, and ultraviolet light at 265 and 280 nm. "Double-labeling" experiments with tritiated and cold 5-bromodeoxyuridine, differential fluorescence and autoradiography were begun, in order to test whether induced sister chromatid exchange always involves all four DNA polynucleotide strands.

Experiments were carried out with cells from the human genetic diseases Hutchinson-Gilford progeria syndrome, Fanconi's anemia, Bloom's syndrome, ataxia telangiectasia and incontinentia pigmenti, in order to determine whether they are truly radiation sensitive, and to test whether they may involve specific DNA repair deficiencies. Both Fanconi's anemia and ataxia telangiectasia were found to show a characteristic G_2 phase chromosomal radiosensitivity; this was ruled out in one case of progeria originally reported to exhibit a DNA strand break repair deficiency.

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PROJECT SUMMARY AND REVIEW

A. Semi-automatic chromosome analysis device.

During the two year period of this Contract the hardware for this device was completed, installed and tested. Prototype "breadboard" interfaces had been designed under the previous Contract (At-(40-1)-4065) at Vanderbilt University. The machine, with these prototype interfaces, was shipped from Vanderbilt to the Johns Hopkins University during the first year of the present Contract. During the current year, the final interface hardware was built, installed in the device, and tested. The development of the required software for the Digital Equipment Corp. PDP-8/E minicomputer which is incorporated in the device has continued during the current year and may be considered reasonably complete.

PDP-8/E software development and testing was greatly facilitated by the acquisition of an extra 4K of core memory, giving the machine a total of 12K, and of a new 256 word read-only memory. The original 8K hardware configuration was supposed to allow the use of the Digital Equipment Corp. operating system (OS-8) for this minicomputer, but it proved incompatible after delivery. It was thus necessary to load the programs required for software development and testing (Symbolic Editor, Loaders, Program Assembly Language Compiler and Debugging program) from punched paper tape. Though possible in theory, this procedure proved impossibly tedious and time consuming in practice. Since the new hardware configuration has permitted use of the magnetic tape OS-8 system, program development has proceeded rapidly and efficiently.

With the current interfaces and the newly-developed software, the semi-automatic chromosome analysis device is complete, and chromosome arm measurements may be made and stored on magnetic tape very rapidly by relatively unskilled operators. These measurement lists serve as input for the more

extensive FORTRAN karyotype and aberration analysis programs developed earlier under the Vanderbilt Contract (Bender, Kastenbaum, Lever and Pelster, 1972). Some preliminary work has also been done on programming approaches to several other possible applications of the semiautomatic chromosome analysis device during the current Contract period. These applications include analysis of quinacrine fluorescence chromosome banding patterns and the analysis of the distribution of sister chromatid exchanges. It is anticipated that this work will be continued as part of the Principal Investigator's new program at the Brookhaven National Laboratory.

B. Mechanisms of aberration production in eukaryote chromosomes.

A major accomplishment during the Contract period was the development of a comprehensive theoretical model of chromosomal aberration production for virtually all known clastogenic agents, including ionizing and non-ionizing radiation and chemical agents (Bender, Griggs and Bedford, 1974a) during the first Contract year. Most of the experimental work accomplished during the Contract's two years has been closely related to this development.

1. Chemical clastogenesis. Preliminary experiments and observations were made for two specific agents: 8-methoxypsoralin and ozone. When psoralin treated cells are exposed to visible light, DNA lesions, believed to be mainly interstrand crosslinks, are produced, and chromosomal aberrations result. Since the crosslinking is light-dependant, the lesion can be induced at known stages in the cell cycle in synchronized cultures, and an analysis analogous to those we performed earlier for 5-bromodeoxyuridine-plus-light-induced polynucleotide strand breaks (Bender, Bedford and Mitchell, 1973) and for ultraviolet-induced pyrimidine cyclobutane dimers (Bender, Griggs and Walker, 1973) is feasible. The preliminary experiments conducted during the Contract period were designed to achieve the required degree of synchrony in Chinese hamster V-79 cells

(necessary because of the new culture conditions existing at the Johns Hopkins laboratory being used), to determine suitable levels of 8-methoxypsoralin and light exposures, and to determine the division delays which need to be allowed for in the design of the aberration experiments.

The ozone exposure work has involved observations of peripheral leukocyte chromosomal aberration levels in human subjects breathing air containing ozone at 0.5 ppm for 6 to 10 hours. Our observations are being reported in a paper in press in the journal MUTATION RESEARCH, a preprint of which is included with this Report as C00-2382-10. Briefly, we found significant increases in the frequencies of achromatic lesions in samples obtained immediately after exposure for either six or ten hours, and increases in both the achromatic lesion and the chromatid deletion classes two and six weeks later. We did not, however, find any increase in the frequencies of chromosome-type aberrations at any sampling time. The latter observation is important, because earlier reports of Zelac, Cromroy, Bolch, Dunavant and Bevis (1971a, 1971b) had indicated a significant increase in chromosome type aberration in Chinese hamster lymphocytes following their exposure to similar ozone levels, and human populations are currently being environmentally exposed to such levels from photochemical smog in some areas.

We have also begun experiments in which peripheral lymphocyte cultures are exposed to ozone in vitro at various stages in the cell cycle. Though these experiments are as yet incomplete, it is already evident that direct exposure to dissolved ozone during the G_0 or G_1 phases of the cell cycle does indeed induce achromatic lesions. By analogy with other chemical clastogens, this suggests the induction of a long-lived single polynucleotide strand lesion. A major objective of experiments being conducted as the Contract period ends, and which will be analyzed later, is to determine whether in

vitro exposure will produce an increase in chromatid deletions in second post-exposure mitoses, and derived chromosome type deletions in third post-exposure mitoses. The observation of such increases would provide an explanation of both our observation of delayed increases in chromatid deletions in our human subjects, and of Zelac et al.'s observation of chromosome-type aberrations in their Chinese hamster experiments.

2. Ultraviolet light. Earlier work under the predecessor of the present Contract had shown that the pyrimidine cyclobutane dimer accounted for virtually all chromosomal aberration production by exposure to ultraviolet light of 256 mμ wavelength (Griggs and Bender, 1973). However, chromosomal aberration production by exposure to 280 mμ ultraviolet, at the protein absorption peak, has also been reported (Chu, 1965), suggesting the possibility that some other lesion, possibly even involving chromosomal protein, may also result from dimer formation, since some dimers are induced at this wavelength. Preliminary experiments to test this possibility were undertaken in collaboration with Dr. H. G. Griggs during the Contract period. Populations of the A8W243 *Xenopus* cell line, which possesses the photoreactivating enzyme, and which we had used for our earlier ultraviolet experiments, were synchronized by mitotic shakeoff and exposed to either 265 mμ or 280 mμ light from the large quartz prism monochrometer in the Department of Biochemistry of the Johns Hopkins School of Hygiene and Public Health. Samples exposed to both wavelengths were either kept in the dark or exposed to photoreactivating light from daylight fluorescent tubes, and the cells were fixed for cytogenetic examination when the first mitoses began to appear. These preliminary experiments served largely to establish suitable ultraviolet doses and the division delays to be allowed for in later experiments. Nevertheless, some aberration analyses were done, and the results are so far consistent with the

production of only chromatid-type aberrations by 280 nm irradiation, and the photoreactivation of at least a large fraction of them. Should these preliminary results be confirmed by later experiments, it will be possible to conclude that the DNA lesion responsible for aberration production by 280 nm ultraviolet is indeed the pyrimidine cyclobutane dimer, and consequently that chromosomal protein lesions are not important contributors to aberration formation.

3. Sister chromatid exchange and recombinational repair. During the Contract period a theoretical analysis was published of the consequences of a recombinational form of DNA repair in eukaryote cells and the explanation the existence of such a form of repair would provide for some of the puzzling observations that have been made regarding the phenomenon of sister chromatid exchange (Bender, Griggs and Bedford, 1974b). Such repair would, of necessity, involve exchanges of single polynucleotide chains, a somewhat controversial idea for eukaryotes, but one which has also been proposed by others (Gatti, Pimpinelli and Olivieri, 1973, 1974). The recent development of a new technique for visualization of sister chromatid exchanges (Latt, 1973) has provided a means of testing this hypothesis. The technique involves growth of cells for two consecutive S periods in the presence of 5-bromodeoxyuridine, resulting in chromosomes which at the following metaphase have one chromatid with a thymidine-bearing polynucleotide chain and one with only chains in which 5-bromodeoxyuridine has been substituted for thymidine. When such substituted chromosomes are stained with the dye Hoechst 33258 or with acridine orange (Kato, 1974), the thymidine-bearing chromatid fluoresces differentially under the ultraviolet microscope, thus allowing sister chromatid exchanges to be detected. Since the thymidine bearing chromatid may be detected without autoradiography (as was formerly necessary), a "double

labeling" technique is possible so that one may follow independently the segregation of both of the "old" polynucleotide chains present after the first S period. This may be done simply by using 5-bromodeoxyuridine labeled with tritium during the first S period, followed by cold 5-bromodeoxyuridine during the second. Thus the location of the thymidine bearing, unsubstituted strand may be detected by its differential fluorescence, while the substituted strand may be detected by autoradiography. If sister chromatid exchange always involves both subunits, then both "labels" should always switch from one chromatid to the other at each sister chromatid exchange point. If some induced sister chromatid exchanges involve only one polynucleotide chain, however, then there should be cases where the fluorescence exchanges but the radioisotope does not, and vice versa.

Such "double labeling" experiments have been carried out with both V79 Chinese hamster tissue culture cells and human peripheral lymphocyte cultures. In one series of experiments nothing was done to stimulate sister chromatid exchange, so that all exchanges seen were spontaneous. In another, the cultures were exposed to visible light curing the second S phase in order to induce exchanges (Ikushima and Wolff, 1974a). Analysis has so far been completed for only the V-79 spontaneous exchange group. Among 137 exchanges in 38 cells which could be analyzed by both fluorescence and autoradiography, no clearcut case was found of either label switching without the other, though there were several questionable cases of non-coincidence. Thus it is evident that at least the bulk of spontaneously-arising sister chromatid exchanges involve exchanges of whole double helices. Analysis of the induced exchanges has been begun, but an answer to the question must await the autoradiographic results.

4. Chromosomal mononemy. The general theory of aberration pro-

duction developed under this Contract (Bender, Griggs and Bedford, 1974a) assumes that the eukaryote chromosome is mononeme, with a single DNA double helix per chromosome or chromatid. There is much evidence to support this view. Recently, however, Ikushima and Wolff (1974b) published data from experiments on aberration production by ultraviolet light and by X-rays which they interpreted as being best explained by a polyneme chromosome structure. Experiments bearing on this conclusion were carried out during the Contract period, resulting in the publication of a paper (Bender, Bedford, Griggs and Merz, 1975) a reprint of which is submitted as Report C00-2382-11. Briefly, Ikushima and Wolff's polyneme interpretation rested largely on their observation of a few chromatid type aberrations in colcemide-induced tetraploid and octoploid cells following irradiation of (largely) diploid cultures. This was taken as evidence that ionizing radiation induces long-lived sub-chromatid lesions capable of giving rise to new aberrations for at least two post-irradiation cell cycles. The aberration frequencies they observed were very small, and it seemed likely they could be accounted for in any case by the facts that synchrony of the irradiated population was not perfect, and that there were some tetraploids already present. Nevertheless, we undertook experiments in which synchronous populations were gamma-irradiated in either G_1 or the G_2 phases of the cell cycle and then fixed in their first, second or third post-irradiation mitoses. That the cells were indeed in these mitoses was ensured by resynchronizing the populations at each division. In contrast to what was observed by Ikushima and Wolff, no evidence for any increase in chromatid aberrations in either the second or third post-irradiation metaphases was observed. It appears, then, that their results must reflect some technical artifact, and that all of the available evidence remains consistent with a mononeme model of eukaryote chromosome structure.

5. Human "chromosome fragility" diseases. A number of human inherited diseases, including Fanconi's anemia, Bloom's syndrome and ataxia telangiectasia, are characterized by abnormally high spontaneous chromosomal aberration frequencies and by a significantly increased risk of neoplasia. It has been reported that cells from persons afflicted with the three diseases mentioned also exhibit abnormally high chromosome aberration rates when X-irradiated in the G_0 or G_1 phase of the cell cycle (Higurashi and Conen, 1971, 1973). Sasaki and Tonomura (1973) were unable to confirm the reported chromosomal radiosensitivity of Fanconi's anemia cells, though they did find a marked sensitivity to chemical crosslinking agents, a finding similar to the earlier report of Schuler, Kiss and Fabian (1969) of a chromosomal sensitivity to an alkylating agent for Fanconi's anemia.

Because these three diseases are all believed to be recessively inherited, and because of the reported chromosomal "fragility," all appear to be possible genetic DNA repair deficiencies, possibly analogous to the known genetically-irradiated repair deficiencies of prokaryotes, just as the one confirmed human DNA repair deficiency, xeroderma pigmentosum, appears analogous to exr⁻ in bacteria. In addition, cells from a patient believed to have the inherited Hutchinson-Gilford progeria syndrome have recently been reported to be deficient in the ability to repair radiation-induced DNA polynucleotide strand breaks (Epstein, Williams and Little, 1973), though whether such a deficiency is really characteristic for this disease has more recently been questioned (Regan and Setlow, 1974). A large fraction of the effort spent during the Contract's two year period has been spent in further study of the cytogenetics and radiosensitivity of several of these diseases, in collaboration with Dr. J. M. Rary of the Johns Hopkins School of Medicine, Department of Obstetrics and Gynecology and Dr. T. E. Kelly of the Department of Pediatrics.

a). Hutchinson-Gilford progeria syndrome. Because of the report of Epstein, Williams and Little (1973), and because of the expectation that a DNA strand break repair deficiency should result in a G_2 sensitivity to the induction of chromosomal aberrations by ionizing radiation, we obtained peripheral lymphocytes from the same patient studied by Epstein, Williams and Little, as well as from the patient's mother. Lymphocyte cultures from the patient, mother, and a normal control were X-irradiated and then fixed two hours later for examination of cells which had been in the G_2 phase of the cell cycle when irradiated. No elevation in the induced chromosomal aberration rate was seen, however. This is perhaps not surprising, in retrospect, in view of the later failure of Regan and Setlow (1974) to confirm the strand break repair deficiency. Our negative results were reported at the 5th International Congress of Radiation Research; the abstract was submitted as Report C00-2382-4.

b). Fanconi's anemia. During the Contract period a number of observations and experiments were conducted with cells from patients with Fanconi's anemia, in part as a research project of a graduate student in the School of Hygiene and Public Health, Miss Susan Brefach. So far, spontaneous chromosome aberration frequencies and the aberration frequencies in cells irradiated in G_2 have been investigated in leukocytes from four patients and in skin biopsy fibroblast cultures from three patients. Despite the numerous reports of increased spontaneous chromosomal aberration rates in Fanconi's anemia cells, we did not find a statistically significant increase over those in concurrent controls in any of these samples. Nevertheless, there was a consistent small elevation, particularly in the achromatic lesion class, even though it was not large enough to be significant in any individual case. When the results for the lymphocytes are pooled, however, the frequency in the

Fanconi's anemia cells is 0.47 ± 0.03 (285/611), while in the controls it was only 0.18 ± 0.02 (129/700), and the difference is highly significant, thus confirming the idea that Fanconi's anemia lymphocytes do, in general, show a higher spontaneous aberration frequency. In the fibroblasts, however, with a smaller number of cells scored (250 in each category), the difference is not significant. Whether this is simply due to the smaller numbers available at this point or to a failure of the fibroblasts to exhibit this characteristic cannot be decided as yet.

The G_2 X-irradiation experiments have shown a highly significant, consistently increased aberration frequency in both the Fanconi's anemia lymphocytes and the fibroblasts. Cells were irradiated with a dose of 50R (and other doses as well in one experiment), and mitoses collected for two (lymphocytes) or three hours (fibroblasts) to ensure a true G_2 sample. In each case an approximately two-fold greater aberration frequency was observed in the Fanconi's anemia cells than in the concurrent controls. When the data are pooled, the Fanconi's anemia lymphocyte rate at 50R, corrected for spontaneous rate, is 2.58 ± 0.06 (1807/700), as compared to 0.77 ± 0.03 (541/700) for the controls. In the fibroblast experiments the frequency is 2.29 ± 0.10 (573/250) for the Fanconi's anemia cells as compared to only 0.46 ± 0.04 (114/250) for the controls. Since the differences in 50R G_2 frequencies have in each individual case been highly significant, we believe that this may offer a relatively easy and reliable means of diagnosis; obviously the spontaneous aberration frequencies do not.

One possible, and in a sense trivial, mechanism that would produce an apparent G_2 chromosomal radiosensitivity such as we observed for Fanconi's anemia might be that the Fanconi's cells simply have a longer G_2 phase than normal cells, and indeed this has been reported as typical of cells from one

patient (Loughman, 1973). Since aberration frequencies drop rapidly with post-irradiation time (See Bender, Griggs and Bedford, 1974a), a lengthened G_2 might be argued to produce samples at a given fixation time composed of a greater proportion of late G_2 cells, and consequently of cells with more aberrations. Accordingly, "terminal labeling" experiments were carried out with lymphocyte cultures and fibroblast cultures from Fanconi's anemia (one patient for each culture type) and from normal controls to determine the length of the G_2 phase. Cultures were treated with tritiated thymidine and then fixed at hourly intervals, with two hour colcemide metaphase collections, for nine hours. Autoradiographs were scored for the percentage of labeled mitoses. No difference from the controls was seen for either the lymphocytes (50% PLM= 5 hr.) or the fibroblasts (50% PLM= 6.5 hr.). Furthermore, no differences were seen in the ultimate labeling frequencies attained by the end of the experiments. Thus it does not appear that a lengthened G_2 is characteristic of Fanconi's anemia, or consequently, that this can explain the observed G_2 chromosomal radiosensitivity.

Further experiments will be necessary to resolve the apparent conflicts between the reports of Higurashi and Connen, of Sasaki and Tonomura, and our own observations. We have attempted G_1 raditation experiments like those of Higurashi and Conen, but since lymphocytes from Fanconi's anemia patients do not grow as well as those from normal controls, it is technically difficult to assure that similar populations of cells in their first post-irradiation mitoses are being sampled. We are attempting to resolve this difficulty by adding 5-bromodeoxyuridine to the cultures after irradiation and scoring only first-mitosis metaphases as identified by the lack of differential chromatid fluorescence following acridine orange staining.

Differential fluorescence of 5-bromodeoxyuridine-substituted chromosomes

has also provided another intriguing preliminary observation which, if we are able to confirm it, may be pertinent to the mechanism involved in the chromosomal effects characterizing Fanconi's anemia. It has been reported that lymphocytes from persons with Bloom's syndrome exhibit a characteristic very high frequency of spontaneous sister chromatid exchanges (Chaganti, Schonberg and German, 1974). Our observations indicate that Fanconi's anemia lymphocytes may be characterized by just the opposite, an abnormally low spontaneous sister chromatid exchange frequency. Though the data are as yet incomplete, statistically significantly low frequencies have already been observed in cells from three patients.

c). Ataxia telangiectasia. We have been fortunate enough to gain access to a number of cases of ataxia telangiectasia from several different families (and which may then represent separate genes) during the two years of Contract support. Our approach has been similar to that for Fanconi's anemia. Lymphocytes from patients and from their parents, and in several cases skin biopsy fibroblast cultures from patients have been utilized to determine spontaneous and G_2 , X-ray-induced chromosomal aberration frequencies. Our first attempts to make a meaningful analysis of G_0 or G_1 chromosomal radiosensitivity to see whether we could confirm the report of Higurashi and Conen (1973) were confounded by the problem of differential growth rate of the ataxia cells in culture, but further experiments have been done in which 5-bromodeoxyuridine was added after irradiation so that we could identify first mitoses by differential fluorescence. Experiments to measure both spontaneous and induced sister chromatid aberration rates have been started, but probably will not be completed before the Contract expires.

Spontaneous chromosomal aberration frequencies have been determined for lymphocytes from three affected sibs, and their parents in one family, from

two affected and two unaffected sibs and their parents from another family, and for a single affected child and his parents from yet another family. In a number of cases several samples were obtained over a period of more than one year. The (heterozygous) parents show no suggestion of any increase in spontaneous aberration frequencies over the normal controls. The affected individuals tend to show an increased frequency over concurrent controls, but, as was the case for Fanconi's anemia, they are usually not statistically significant. The same is true for fibroblast cultures from four affected individuals.

Karyotype analyses have been done with quinacrine fluorescent and/or Giemsa banding techniques. No case of a karyotypic abnormality characteristic of the affected individuals was found. However, one patient displayed a clone of lymphocytes which had the constitution 45,XY,-14-14+t(14q;14q). The breakpoints of the translocation were identified as 14q12 and 14q31. In the first sample the clone was observed with a frequency of only 6%, but its frequency was 46% about a year later, and was about 25% after two more months. No translocation-bearing cells were seen in a sample of 50 fibroblast culture cells. This case has been described in a paper (Rary, Bender and Kelly, 1974) which is submitted as Report C00-2382-12. Such clones of chromosomally abnormal lymphocytes have been reported in other cases of ataxia telangiectasia, and frequently, if not always, contain a rearranged chromosome 14 (see recent review by Harnden, 1974); often, possibly always, the 14q12 region is involved. The clinical significance of this clonal tendency in ataxia telangiectasia is as yet unknown. We plan to follow both this and our other patients to attempt to correlate the appearance and relative frequency of rearrangement clones with the clinical status of the patients.

As was the case for Fanconi's anemia, both lymphocyte and fibroblast

cultures have displayed a consistent, statistically significant G_2 chromosomal radiosensitivity. Though scoring of aberration frequencies in all experiments has not yet been completed, the pooled results from single samples from each of six patients and five normal controls are illustrative: the rate was 3.67 ± 0.08 aberrations per cell (2201/600) for the ataxia telangiectasia patients, as compared with only 1.61 ± 0.06 for the controls (the somewhat higher rates for the ataxia experiments than for the Fanconi's anemia experiments are the result, at least in part, of selecting a somewhat shorter colcemide collection time for experiments with ataxia). A similarly increased frequency over that in normal controls is seen in experiments with fibroblast cultures as well: the induced aberration frequencies were 3.80 ± 0.28 and 1.51 ± 0.19 per cell in the one experiment for which scoring has been completed to date. The induced aberration frequencies in lymphocyte cultures from the parents of the affected individuals are the same as in those from the normal controls. Our preliminary results from these experiments were reported at the 1974 Annual Meeting of the American Society of Human Genetics; the abstract of this paper is submitted as Report No. C00-2382-13.

One possible explanation of the apparent G_2 chromosomal radiosensitivity of cells from persons with this disease, as suggested for Fanconi's anemia, is that they might have a longer than normal G_2 phase. To test this possibility a "terminal labeling" experiment was done like that already mentioned for Fanconi's anemia. Lymphocyte cultures from four of the patients, two parents, and two normal controls were used. A "terminal label" of tritiated thymidine was given, and cultures fixed at hourly intervals for eight hours. Autoradiographs were made, and the percentage of labeled mitoses determined from a sample of approximately 200 mitoses for each time point. The duration of G_2 , as measured by the time to reach 50% labeled metaphases, did not differ between

the patients, parents or controls; the average time was 4.8 hr.

Erratic aberration frequencies in our first experiments attempting to confirm the G_0 or G_1 chromosomal radiosensitivity reported by Higurashi and Conen for ataxia telangiectasia lymphocytes in vitro have led us to employ the technique of differential chromatid fluorescence following incorporation of 5-bromodeoxyuridine to determine the rates at which ataxia and normal lymphocytes progress through successive cell cycles following culture initiation. This may easily be done following staining with acridine orange, since all first mitoses will have both chromatids of each chromosome equally bright, only one chromatid of each chromosome in second mitoses will fluoresce brightly, only one-quarter of the total chromatid length will be bright in third mitosis, and so forth. Somewhat to our surprise, we found that in spite of the low yield of mitoses characteristic of early fixation times for ataxia lymphocytes, second and third mitoses begin to appear at about the same time as in normal control lymphocyte cultures. Thus for fixations early enough to ensure that only first mitoses are present there simply are not enough mitoses to make a G_1 experiment feasible. For this reason we have done experiments in which G_0 cells are X-irradiated in vitro and then cultured in medium containing 5-bromodeoxyuridine for scoring with the fluorescence microscope following acridine orange staining so as to allow identification of the first post irradiation mitoses, as already mentioned.

d). Bloom's syndrome. In spite of repeated attempts, it has proven possible to obtain leukocyte samples from only one case of Bloom's syndrome during the Contract period. This was used for a G_1 X-irradiation experiment in an attempt to confirm the chromosomal radiosensitivity reported by Higurashi and Conen (1973). Like our first G_1 experiment with ataxia telangiectasia, the results were erratic, presumably because of the inclusion

of second or even third post-irradiation mitoses, and we have not yet had the opportunity of repeating it using the 5-bromodeoxyuridine substitution technique.

e). Incontinentia pigmenti. This additional disease, believed to be inherited as an X-linked dominant, has been reported to be characterized by a high spontaneous aberration frequency in peripheral lymphocyte mitoses (de Crouchy et al., 1973; Cantu et al., 1973). Toward the close of the Contract period, we obtained peripheral blood samples from two girls afflicted with this disease and from their unaffected mother. Scoring is incomplete, but it is already apparent that we not only can confirm the earlier reports of high spontaneous aberration frequencies, but it appears that this may be a more striking and consistent feature in this disease than it is in Fanconi's anemia and in ataxia telangiectasia. A G_2 X-irradiation experiment has been done with lymphocyte cultures from these patients, but we have not yet had the opportunity to score the slides for aberrations.

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PUBLICATIONS

Two papers; "Mechanisms of chromosomal aberration production III. Chemical and ionizing radiation," by M. A Bender, H. G. Griggs and J. S. Bedford, and "Recombinational DNA repair and sister chromatid exchanges," by M. A Bender, H. G. Griggs and J. S. Bedford, submitted as preprints last year (Reports C00-2382-2 and C00-2382-3, respectively) have now appeared in print. Reprints are submitted with this Report.

Two papers were submitted, accepted and published this year. Reprints of "X-ray and UV-induced chromatid aberrations: evidence for polynemic chromosomes?," by M. A Bender, J. S. Bedford, H. G. Griggs and T. Merz are submitted as Report C00-2382-11. Reprints of "A 14/14 marker chromosome lymphocyte clone in ataxia telangiectasia," by J. M. Rary, M. A Bender and T. E. Kelly are submitted as Report C00-2382-12.

A contributed paper entitled "Cytogenetic studies of ataxia telangiectasia," by J. M. Rary, M. A Bender and T. E. Kelly was presented at the Annual Meeting of the American Society of Human Genetics in Portland, Oregon on October 16-19, 1974. The abstract is submitted as Report C00-2382-13.

An invited symposium paper entitled "A unifying theory of radiation and chemical clastogenesis" was presented at the Annual Meeting of the Radiation Research Society in Miami Beach, Florida on May 11-15, 1975 by the Principal Investigator. The abstract is submitted as Report C00-2382-14.

Finally, one manuscript is in press in MUTATION RESEARCH as the Contract Period ends. Preprints of "Observations of aberrations in chromosomes of lymphocytes from human subjects exposed to ozone at a concentration of 0.5 PPM for 6 and 10 hours," by T. Merz, M. A Bender, H. D. Kerr and T. J. Kulle are submitted as Report C00-2382-10.

In addition to the publications listed above for the final Contract year,

the following publications were produced during the Contract's initial year:

1. Uniformity of radiation-induced mutation rates among different species," by S. Abrahamson, M. A. Bender, A. D. Conger and S. Wolff. (Nature 245: 460-462, 1973) - Report C00-2382-6.
2. "Mechanisms of chromosomal aberration production I. Aberration induction by ultraviolet light," by M. A. Bender, H. G. Griggs and P. L. Walker (Mutation Research 20:387-402, 1973) - Report C00-2382-7.
3. "Mechanisms of chromosomal aberration production II. Aberrations induced by 5-bromodeoxyuridine and visible light," by M. A. Bender, J. S. Bedford and J. B. Mitchell (Mutation Research 20:403-416, 1973) - Report C00-2382-8.
4. "Spontaneous and X-ray-induced chromosomal aberrations in progeria," by M. A. Bender and J. M. Rary (Abstr. 5th International Congress of Radiation Research, pg. 181, 1974) - Report C00-2382-4.
5. "Chromosome aberrations and cell death in V79 Chinese hamster cells," by J. S. Bedford, H. G. Griggs, J. B. Mitchell and M. A. Bender (Abstr. 5th International Congress of Radiation Research, pg. 204, 1974) - Report C00-2382-5.