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Some rare autosomal recessive diseases of humans have been reported to have in common a high frequency of spontaneous chromosome aberrations, developmental abnormalities, and a predisposition to malignancy. The three diseases most frequently mentioned in the so called "chromosome breakage syndromes" are ataxia telangiectasia (AT) (also known as Louis Bar syndrome), Bloom's syndrome (BS), and Fanconi's anemia (FA). Some investigators have included xeroderma pigmentosum (XP) in that listing.

The initial purpose of this investigation was to find if there were ample evidence for classification of all four diseases in one category. Thus the primary goal was to determine the kinds of cytogenetic aberrations reported in each disease. The secondary goal was to establish from the literature whether or not the entire genome in these diseases was randomly affected. The third goal was to discover similarities and differences in them that might elucidate the nature of the repair defect and its consequences at the cellular, tissue, and clinical levels of organization.

A Medline search of the 2600 journals listed in Index Medicus was conducted. Clinical studies, case histories, and cytogenetic reports were analyzed concurrently with studies in molecular biology such as position effects, properties of constitutive heterochromatin, and phases of the cell cycle. Scattered information from all these

sources was arranged in tabular form for easier comparison. Finally, statistical analysis was made in order to determine likenesses and differences not evident on first examination. From data obtained from the literature it was found that in each of the diseases there were reports of hypogenitalism. Stunted growth in utero, post partum, or during both periods was reported for all four. Hypo and/or hyperpigmentation of the skin was found to occur in all these diseases. It was concluded that the majority of malignancies reported have been in tissues with high mitotic indices. Recent literature confirms previous reports of an increased level of spontaneous chromosome instability in AT, BS, and FA. There is also recent validation of an increased incidence of chromosome aberrations in XP. The data indicate that all the diseases have some defect in DNA repair mechanisms. In AT and FA there is a significantly higher percentage of excision type aberrations when compared to other types of abnormalities such as spindle defects and chromosomal rearrangements. Through chi square analysis in the present study it was found that group D chromosomes in AT are more involved in aberrations than if the damage were random. Groups E and F show more involvement in anomalies in BS patients than random damage would indicate. FA patients have more C group defective. Too few cytogenetic reports exist for XP to make a definitive statement about specific chromosome group involvement. All of these diseases were reported to have impaired response to PHA or to have cells that grew more slowly than controls, and each of them except FA have shown impaired cellular immunity. An increased incidence of infection was reported for all, although only AT and BS showed low immunoglobulin levels.

A COMPARISON OF THE CYTOGENETIC, CLINICAL, AND IMMUNOLOGIC ASPECTS OF FOUR "CHROMOSOME BREAKAGE SYNDROMES": ATAXIA TELANGIECTASIA, BLOOM'S SYNDROME, FANCONI'S ANEMIA AND XERODERMA PIGMENTOSUM

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Statement of the Problem

Some rare autosomal recessive diseases of humans have been reported to have in common a high frequency of spontaneous chromosome aberrations, developmental abnormalities, and a predisposition to malignancy (Bochkov et al., 1974; Tatte and Good, 1971; Passarge, 1972; Bloom, 1972). The three diseases most frequently mentioned in the so-called "chromosome breakage" syndromes are ataxia telangiectasia (AT) (also known as Louis Bar syndrome), Bloom's syndrome (BS), and Fanconi's anemia (FA). Some investigators have included xeroderma pigmentosum (XP) in that listing (German, 1972; Schroeder, 1974).

This study was undertaken to compare these four diseases clinically, cytogenetically, and immunologically. An effort was made to determine similarities and differences in the basic molecular defects causing the increased level of spontaneous chromosome breakage. In the case of xeroderma pigmentosum, the search included determining if, indeed, there was an increased incidence of chromosomal aberrations. Listed below are the specific questions asked at the beginning of this study.

1. Does recent literature verify the fact that these diseases have an increased level of chromosomal instability?

2. What types of aberrations occur? Are there correlations

among the four?

3. Does the literature reveal specific break loci? Are there correlations here? Is there any correlation between those breaks and heterochromatic areas?

4. Do any of the other three diseases show evidence of DNA repair defects as has been reported for XP?

5. Since all these diseases have been reported to have an increased incidence of malignancies, and since chromosomal changes and efficient immunological responses are both suspect in the origin of carcinogenesis, do any or all of them show defects in the immune mechanism?

6. Although these four diseases present a very different clinical picture on the surface, are there any underlying likenesses that might help to elucidate the basic defect?

7. Are there any abnormalities in the cell cycle or cell proliferation that might help explain the increased level of malignancies?

There are many reports available concerning the various aspects of these four rare genes. Some of these supply only cytogenetic data. Others are clinical case histories. Some authors have compared one facet between two of the diseases. Based on a recent Medlars search, this paper is the first attempt to compare the cytogenetic, clinical, and immunological aspects of all four of these diseases.

Significance of the Study

Genes which affect phenotype of chromosomes

James German (1972) termed the four rare genes to be compared in this paper "genes which affect the phenotype of the chromosome." He pointed out that there are several examples of the fact that unusual morphological features and unusual functioning of human chromosomes can be under genetic control. These four genes have in common severe clinical manifestations in addition to the unusual chromosome behavior they appear to determine. In 1972, German pointed out that for three (AT, BS, and FA) of the diseases to be discussed here, direct cytogenetic observation showed an unusual feature when the gene is in the homozygous state. Chromosomes of cells from patients afflicted with these diseases when in tissue culture exhibited a high degree of instability and tended to undergo disruption and rearrangements. At that time an increased tendency for instability and rearrangement had not been directly observed in cells from XP patients. But clones of fibroblasts with pseudodiploid complements had been seen growing in the midst of normal diploid fibroblasts. He postulated that at some earlier time either *in vitro* or *in vivo*, a tendency for chromosome rearrangements existed in those XP cells. This probably occurred when the cells were exposed to ultraviolet light. It is well-known that irradiation and certain chemicals can produce chromosome aberrations (German *et al*, 1974).

A 1974 paper (Harnden) noted that certain rare genes known in nonhuman species fall into two categories: (1) those that have

been demonstrated to exert an effect, directly or indirectly, on the behavior or function of the chromosome at some stage in the cell, and (2) those that predispose the affected animal to cancer. An example of the first type would be the sticky chromosome in maize. That gene results in chromosomal disruption and aberrant segregation of the chromosomes at cell division. The viable yellow gene in mouse shows the second type. When the gene is present, cancer usually develops whether the host is from a low or high cancer strain. The four genes compared in this paper could belong to one of these groups or both.

Number of patients with each disease

None of these four genes have been assigned to their autosome, and nothing is known about their linkage relation to other genes. There are no reliable estimates of their frequency, but a rough estimate would be around 1:100-1:350. Up to 1972, there had been 40 reported cases of Bloom's syndrome, 200 with Fanconi's anemia, 150 cases of AT, and 100 with XP (Passarge, 1972).

A 1972 paper (Bloom) noted that Bloom's syndrome, FA, and AT have in common the clinical characteristics of intrauterine growth retardation, short stature, immunologic deficiency, and an increased risk of malignancy. Higurashi and Conen reported in 1973 the same common features for the three diseases listed above. They also listed skin disorders as an additional common clinical manifestation.

A 1975 report (Bloom) also listed the same common clinical features for Bloom's, FA, and AT, but added the footnote that the

immunological, and cutaneous manifestations differ to some extent. This author's analysis of reports on the clinical symptoms of all four diseases, shown in Table 1, page 6 gives support to the possibility that XP could be added to this list.

Knudson et al. (1973) pointed out that whether the defect in the syndromes is in the mitotic apparatus, DNA synthesis, or repair is not known. The unusual phenomenon of chromosome breakage raises two questions: (1) What is the relationship of chromosome breakage to the disease, and (2) How is the chromosome breakage connected with the development of malignancy (Schroeder and Kurth, 1971)?

TABLE 1.

Comparison of Symptoms in "Chromosome Breakage" Syndromes				
Clinical Symptoms	AT	BS	FA	XP
Reproductive System				
Hypogenitalism	+	+	+	+
Skeletal Defects				
Stunted growth	+	+	+	+
Microcephaly			+	+
Thumb deformities		+	+	
Cutaneous Abnormalities				
Hypo and/or Hyperpigmentation	+	+	+	+
Telangiectasias	+	+		+
Malignancies	+	+	+	+
Lymphoid Tissue	+	+	+	+
Skin	+			+
Gastrointestinal tract	+	+		
Immune System				
Hypogammaglobulonemia	+	+		+
Increased infection	+	+	+	+
Impaired response to PHA, PWM, serum	+	+	+	+
CNS				
Purkinje cell loss	+			+
Ataxia	+			+
Mental retardation			+	+
Choreoathetosis	+			+
Chromosomes				
Increased breakage and/or rearrangements	+	+	+	+

Sources: 2, 8, 10, 15, 128, 134, 138, 161, 162, 174, 177, 178, 215.

CHAPTER II

REVIEW OF LITERATURE

Clinical Characteristics

In addition to the common clinical characteristics previously mentioned, there are also those unique to each of these four diseases. Some of them are so different they seem to create a wide disparity among these diseases.

Ataxia Telangiectasia

This is a unique syndrome because of its specific array of pathological systems of the body. Clinically there is evidence of involvement of the central nervous system, the conjunctiva and skin, the upper and lower respiratory tract, the lymphatic system, and immunological functions (Birth Defects Atlas ed. by Bergsma, 1973).

Children afflicted with this disease appear normal at birth. Their growth and development seems normal until about 18 months to two years. Then they begin to have difficulty in walking because of defective balance. This follows a variable progression which leads to complete incapacitation, usually around eight to ten years old. Only later do the "bloodshot" eyes become evident and confirm the diagnosis (Peterson and Good, 1968). These telangiectasias in the conjunctiva may delay diagnosis until between four and six years. Telangiectasias (dilatation of small blood vessels) are often present also around the ears, face, neck, hands, wrists, and knees and antecubital fossae (German 1972; German 1969). Reed et al. (1966)

noted that the pattern of telangiectasias suggests that the areas of greatest sun exposure are most affected and that the changes in the skin suggest premature aging. Louis Bar delineated the syndrome in 1941. In 1966 Hecht et al. first noted the high frequency of chromosome breakage. The sex ratio appears to be 1:1 (Birth Defects Atlas ed. by Bergsma, 1973).

Skin

Reed et al. (1966) have observed that the sun exposed skin of AT patients is very similar to that of young XP patients. Some persons have noted a mottled pattern of hypo and hyperpigmentation (Reed et al., 1966; German et al., 1974).

Gonadal abnormalities

Harden (1974) has reported abnormality of the ovaries, especially ovarian follicular agenesis. There have been reports of the absence or hypoplasia of gonads or gonadal dysgenesis (German et al., 1974). German et al. (1974) theorized that mesenchyme involvement could be associated with the abnormality in the development of germ cells.

Other clinical features

Other clinical features are such things as jerky eye movements, slow slurred speech, a "fixed" facies, and early graying of hair. Abnormalities in the immune system will be discussed in a separate section further in this paper.

Malignancies

One estimation is that at least 10 percent of these patients die with a malignancy (Gatti and Good, 1971; Kersey et al, 1973).

Data from the Immunodeficiency - Cancer Registry in 1973 showed tumors in 52 AT patients. Eleven percent of these were in epithelial tissues; 62 percent were in the lymphoreticular system; 21 percent were leukemia; 2 percent were mesenchymal; 4 percent were in the nervous system. This listing gave 12 families in which tumors of identical histologic type developed in two or more siblings with the same primary immunodeficiency disease. Seven percent of these families had AT (Kersey et al., 1973). A 1976 study by Swift et al., noted that for AT heterozygotes younger than age 45, the risk of dying from a malignant neoplasm was estimated to be greater than five times the risk for the general population. The frequent occurrence of malignancies include particularly reticulum cell sarcomas, lymphomas, and lymphosarcomas (Broumsell, 1975).

Bloom's Syndrome

The predominating clinical features of Bloom's syndrome are small body size, sun-sensitive telangiectatic skin lesions in a butterfly distribution over the face, and a defective immune response. The average full term weight is 4 pounds 6 ounces, and the average height of patients past 20 years old is 4 feet 9 inches (Schroeder and German, 1974). It has not been definitely proven which comes first, the telangiectasias or the sun-sensitivity. The lips may be affected as well as the eyelids. The skin lesions usually appear during the first year and tend to become less severe as the patient grows older. Some lose their eyelashes. Clinodactyly, undescended or small testes, a high pitched voice, and pilonidal cyst or dimple also are common manifestations (Passarge 1972; Landau et al., 1966).

Certain observations point to heterogeneity. There is a distortion of the sex ratio with some reports listing 4:1 M/F (Landau et al., 1966; German, 1969). But the most recent report of the sex ratio shows 1:.61 M/F (Harnden, 1974). In a majority of patients there is Ashkenazik Jewish ancestry. But there are several patients lacking that heritage. Several postulations have been put forth to explain the preponderance of male patients. The phenotypic manifestations could be milder in females or there could be reduced viability of females (German, 1969; Harnden, 1974). This disease was first recognized as a clinical syndrome by Bloom in 1954.

Skin

Many researchers have reported cafe-au-lait spots (German, 1974; Bloom, 1966; Bourgeois, 1975).

The immunologic deficiencies will be discussed in a later section of this paper.

Malignancies

There seems to be an increased tendency to leukemia among these patients (German, 1972; German et al., 1974). But there are other malignancies as well. Several have developed at least one cancer of the gastrointestinal tract (German, 1974). Out of 44 patients whom German had studied by 1974, eight had developed cancer. Four of these had acute leukemia and four had cancers in the alimentary canal. Of the heterozygotes connected with these patients, four parents have had cancer as have nine grandparents. Controls risk of leukemia is one in 2,900. Miller (1966) has estimated the risk for Bloom's

syndrome patients as one in eight.

Fanconi's Anemia

Fanconi's anemia is characterized by a hematological abnormality consisting of pancytopenia and bone-marrow hypoplasia. These symptoms usually appear between four and twelve years of age. It is a chronic and progressive disease which causes death in childhood frequently from hemorrhage or some other marrow failure. In addition, there are many anatomical defects. Among these are skeletal deformities, especially of the thumb and radius, renal anomalies, hypogenitalism, stunted growth, and defects of the ear and heart. There may also be a lack of radial pulse, transposition of the penis and scrotum and extra fingers (Fanconi, 1967; Guanti, et al. 1971). Two separate reports (Fanconi, 1967; Lisker and Cobo, 1974) have reported increased fetal hemoglobin. Fanconi (1967) reported defects in glucose metabolism and postulated that a mutation causes a defect in the mesenchyme. He noted that autopsies showing atrophy of the spleen speaks for this hypothesis. The variability of symptoms and malformations is difficult to explain by a single gene mutation.

Fanconi (1967) has listed 31 years as the average age for mothers of these patients. He also listed two-thirds of the patients as male, which he thought suggested external influence on a genetically determined constitution. Often there is mental retardation (Lisker and Cobo, 1974). This disease manifests an unusual combination of a functional defect in a relatively unstructured cellular system plus major anomalies of solid structures like the

heart and kidney (German, 1973).

The average life expectancy is two to four years after the first anemic symptoms. There seems to be no racial or geographic predilection (Caldwell, 1969). Fanconi first described this type of progressive pancytopenia in conjunction with congential abnormalities and peculiar brownish skin pigmentation in 1927. Some evidence is accumulating for genetic and clinical heterogeneity (Passarge, 1972). This disorder may present a wide spectrum of clinical and pathological change with varying age of onset and history (Beard et al., 1973).

Malignancies

In addition to the increased risk of malignancy among FA patients, there have been reports of an increase in malignancy among relatives (Higurashi and Conen, 1971). Swift (1971) has listed the risk for heterozygotes of FA of malignancy as about three times as great as normal. Swift (1971) has pointed out that if a neoplastic cell line is to emerge, it must do so before these patients die with hemorrhage or infection. Several reports note their increased risk of leukemia (German, 1973; German et al., 1974).

Xeroderma Pigmentosum

The skin of the XP patient is extremely sensitive to sunlight. The outstanding clinical feature is the very high incidence of actinic skin cancer on exposed regions of the face and arms. At an early age, these persons show some of the characteristics of severely sun-damaged or aged skin. There are two clinical forms. Both show the skin symptoms, but one has in addition very severe

neurological complications (Cleaver, 1970). About fourteen percent of cases have neurological involvement such as mental deficiency, ataxia, choreoathetosis and spasticity (Ramsay et al., 1974).

The skin appears normal at birth but usually before three years of age, changes due to sun exposure appear and progress relentlessly (German, 1972). The sex ratio is 1:1. Death usually occurs before the patient reaches his twentieth birthday (Birth Defects Atlas, ed. by Bergsma, 1973). Hebra and Kaposi first described the disease in 1874. The neurological disorders associated with it were recognized by de Sanctis and Cacchione in 1932. Other clinical characteristics include immature sexual development, telangiectasias and small areas of hypopigmentation (Robbins et al., 1974).

One study of the de Sanctis Cacchione type of XP (Reed et al., 1969) showed many clinical manifestations similar to those of AT. In autopsies, the cerebellum showed mild to moderate loss of Purkinje cells which is very characteristic of AT. The authors stated that the cerebral atrophy in XP differs from AT in severity by the lesser degree of cerebellar folial atrophy and by the lesser degree of spinal cord nerve cell damage. Cleaver and Bootsma (1975) noted that progressive loss of neurons could be age-related because base damage from physical and chemical agents in nondividing brain cells could cause an abnormal rate of cell death.

Malignancies

In a study of 15 XP patients, (Robbins et al., 1974) most tumors were basal cell carcinomas; some were squamous cell carcinomas. There was almost 50 percent prevalence of malignant

melanoma of the skin which is much higher than the one per 10,000 in the average population. The authors noted that whereas malignant melanomas usually develop in the average population between the fourth and sixth decade of life, these developed much earlier. This disease represents a unique conjunction of both genetic and environmental factors in cancer etiology. Because of that, the elucidation of its biochemical basis may provide understanding of the genetic changes involved in carcinogenesis from many chemical and physical agents (Cleaver and Bootsma, 1975).

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Cytogenetic Characteristics

Chromosome Abnormalities in General

Instability

In 1972, Passarge stated that chromosal instability had been demonstrated consistently only in Bloom's syndrome and Fanconi's anemia. He concluded that the evidence for XP was still rather preliminary and that it was rather inconsistent in AT. But in 1974, Schroeder reported that in all four of these diseases, clones with rearrangements had been found. In FA, the abnormal clones have been observed in bone marrow and lymphocyte cultures. Such clones have been seen in fibroblast cultures of BS and XP as well as in lymphocyte cultures of AT. A variety of structural chromosome abnormalities have also been observed in patients with megaloblastosis due to pernicious anemia, malabsorptive states, DiGuglielmo syndrome, and acute leukemia (Landau et al., 1966).

Breakage in bone marrow

The incidence of chromosome breakage in the bone marrow of healthy persons is unknown. The only available control value is 0.6 percent found in non-hematological diseases (Schroeder and Kurth, 1971).

Causes of chromosome breakage

Environmental situations which predispose to cancer and genes which predispose to it have chromosal instability and damage in common (German, 1972). That enzymatic digestion of DNA by DNA-ase may cause chromosome breakage has been suggested (Cohen and Hirschorn, 1971). They do not believe that lysosomal enzymes are

an integral part of the chromosome damage caused by viruses. Their investigation of the breakage of chromosomes by certain chemicals produced no direct evidence for lysosomal damage (Cohen and Hirschhorn, 1971). But there was an apparent decrease in chromosome damage when these drugs were combined with lysosomal stabilizers. These data suggested that selection against cells with greater chromosome damage occurs producing two results. There is a reduction in the mitotic rate and an apparent reduction of chromosome damage secondary to preferential survival of the "healthier" cell. Although vitamin A ruptures lysosomes, there was no suggestion of increased chromosome damage induced by vitamin A compounds.

Chromosome rearrangement - role in cancer

Chromosomal instability leading to an increased number of *de novo* chromosome rearrangements characterizes the cells of certain persons with an increased risk of cancer (German, 1972). He noted that many cancers in animals and most in humans do not have a normal karyotype. Therefore, he postulated, chromosome rearrangement must play some essential role in the conversion of a cell to the cancerous state. Otherwise cancer cells would not have any more markers than normal cells. One support for a relationship between specific marker chromosomes and oncogenesis is the consistent extra band on a number 14 chromosome in Burkitt lymphomas. A low percentage of patients do not show it (Manolov and Manolova, 1972). One working hypothesis that has been advanced is that in certain cases chromosomal aberrations may give rise to an increased sensitivity of the cell to carcinogenic agents (German *et al.*, 1974).

In searching for differences in the karyotypes of preinvasive and malignant tumors, Atkin and Baker (1969) noted that premalignant lesions frequently yield near diploid karyotypes and that those usually have fewer chromosome changes than do invasive tumors. They also confirmed a previous report that there was lower than expected representation of the D and G group chromosomes. In addition, they reported less representation of B group chromosomes than could be expected. They postulated that those deficiencies could be caused by a loss or involvement in structural rearrangements. Preinvasive lesions did not show the same deficiency in chromosomes of the D and G groups. They did show a moderate lack of B group chromosomes.

It has been pointed out (Knudson et al., 1973) that chromosome abnormality characterizes nearly all human cancers. They also noted there was impressive evidence that many tumors arise from a single cell. A conclusion of one study (Taylor et al., 1973) pointed out the seemingly overwhelming evidence that tissues with a significant increase in chromosome breakage are predisposed to malignancy. The authors point out two major lines of evidence that suggest chromosome abnormalities are deleterious in man. The first line is the indirect evidence shown by many epidemiologic studies that human populations exposed to chromosome breaking agents have an increased incidence of neoplastic disease. They also point out the direct evidence of the increased transformation of fibroblasts from FA and BS patients by SV40. These conclusions ended a study of skin biopsies from patients with porokeratosis of Mibelli, a rare inherited disease associated with malignancy. Clones of abnormal cells were found in

eight of sixteen lines of fibroblasts from affected areas of skin. All data pointed to the fact that cells with chromosome abnormalities were found mostly in affected skin areas (Passarge, 1972).

Schroeder and Kurth (1971) reported that a high percentage of chromosome breakage had been found in the diseases discussed in this report plus glutathione reductase deficiency, Kostmann's agranulocytosis, and pernicious anemia. They pointed out that in BS, FA, and AT the incidence of leukemia is increased above the general population. A conclusion of that paper stated that in order to connect the breakage phenomena with the development of malignancy, a relationship must be established between the in vitro and in vivo events. They suggested that might be shown with direct chromosome preparations from bone marrow and bone marrow smears. One report (Knudson et al., 1973) notes that in all forms of cancer, two or more changes are necessary; and the first change is apparently in the chromosome. Further changes could be mutational or not, they state.

Preferential breakage of certain regions

When chromosomes break, do they break randomly? And, if they do not, why? There are single cases and families known with increased chromosome breakage in specific sites of chromosome number 2, 9, and 16. The breakable sites are sometimes characterized by visible constrictions which show an elevated amount of very late-replicating constitutive heterochromatin. The consistency in incidence and distribution of these anomalies is difficult to explain with a virus infection. Although that possibility has not

been completely excluded, Brogger concluded that a virus is not responsible for the breaks reported in this study.

Brogger (1971), who viewed 1468 leukocyte cultures from fifty persons, concluded that the pattern of chromosome damage was clearly nonrandom. He found an excess of damage in chromosome number three and shortage of damage in the C, F, and G groups. His data also pointed to a clustering of damage in a specific site on chromosome three, the middle part of the short arm. In addition, he noted the same clustering in the distal portion of the long arm of number 16 and hints of it in the midportion of the short arm of number one and two. Damage was mainly in the long arms of the B and C groups. He determined that the distribution of damage was not related to ordinary secondary constrictions, late replicating regions, or the pattern of quinacrine mustard-induced flourescence.

Reeves and Lawler (1970) pointed out that a high level of breaks in particular chromosome regions was occasionally seen in humans. They also noted that time in culture could increase the number of breaks. They studied cells from persons with various diseases which showed preferential breakage of C group chromosomes. These involved the secondary constriction region of C9, and the distal one-third of the long arms of C9, C8, and C10.

Inhibitor of lymphocyte response to PHA

Most lymphocytes respond to PHA by dividing. But it has been demonstrated that there is an inhibitor of that response in the plasma of sixty percent of patients with various types of solid tumors (Gatti and Good, 1971). The same phenomenon occurs in lymphocytes

of patients with AT, Hodgkins disease, tuberculosis, syphilis, multiple sclerosis, and hepatitis. They pointed out that the role this lack of response may play in oncogenesis is unclear.

Sister chromatid exchange (SCE)

The discovery that alkylating agents induced a large number of SCEs in normal cells provided a new approach for studying these "chromosome breakage" syndromes (Kato, 1974; Latt, 1973; Latt, 1974). Since SCEs involve an interchange of DNA duplexes between chromatids followed by ligation, they may constitute a repair response (Latt et al., 1975).

Kato (1974) concluded that both chromatid aberrations and sister chromatid exchanges were caused by the same initial DNA damage which would not be repaired instantly. He noted that evidence was accumulating that, in post-replication repair in mammalian cells, such gaps are not filled by a recombinational mechanism but by a process involving de nova DNA synthesis. Their data implied that both SCEs and chromosomal anomalies might result from errors in a repair process which involves a caffeine-sensitive step(s). That theory still leaves unexplained how proflavin induces SCEs. The fact that methyl-nitro-nitrosoguanidine (MNNG) fails to induce SCEs in spite of its ability to produce chromosome aberrations is also not answered by that hypothesis. But Comings (1975) thinks there is no correlation between chromosome breakage and SCEs. Woff et al. (1975) noted no relationship between SCEs and DNA repair. Another article (Galloway, 1975) noted that SCEs seemed to be dependent on post replication repair.

DNA repair

Rasmussen and Painter (1964) first reported a phenomenon called "unscheduled DNA synthesis". That was an autoradiographic demonstration of the uptake of labeled thymidine into DNA by cells not in S phase. Most mammalian cells exhibit "unscheduled synthesis". Rasmussen and Painter (1966) and Cleaver and Painter (1968) showed that much of the synthesis in Hela cells after UV exposure was not of the semiconservative type but was similar to that of repairing strains of E. coli; that has been termed "repair replication".

Most researchers agree that there are four steps and four enzymes in the pyrimidine dimer excision repair system of UV damaged DNA (German, 1974). These are:

1. Breakage in the single strand next to the dimer - UV specific endonuclease
2. Excision of dimerized pyrimidine - exonuclease
3. Filling gap with new base - DNA polymerase
4. Closure of broken strand - ligase.

Post replication repair

The common conclusion from many experiments (Buhl et al., 1974; Lehmann, 1974) is that DNA made on a UV - damaged template is initially of lower molecular weight than DNA made in an unirradiated cell. It is thought that daughter strands might contain temporary gaps opposite dimers; these are filled in later.

Ataxia Telangiectasia

Types of anomalies and in which cells seen

Interest in information on chromosome instability in AT is subject to several investigatory factors: (1) study of the mechanisms of spontaneous mutation processes at the chromosome level, (2) the idea of a possible correlation between chromosome anomalies and the high risk of lymphoreticular neoplasia in these patients, and (3) elaboration of the techniques and treatment of such patients (Bochkov et al., 1974). There have been conflicting reports concerning whether or not there actually is an increased level of chromosome breakage in AT. Pheiffer (1970, 1972) reported no increase in the number of chromosome breaks. Hayashi and Schmid (1975) concluded that the negative results in one of the five patients they studied demonstrated once more that cytogenetic anomalies are not an obligate finding in AT.

But in many other studies, there have been reports of various chromosome anomalies. One report listed widespread aneuploidy in cells of the liver, adrenal, kidney, lungs, heart and the anterior pituitary. The most frequent type of abnormality in one (Bochkov et al., 1974) was acentric fragments. The ratio of chromatid to chromosome aberrations was sixty to forty. That same report noted high indices of endoreduplication and polyploidy in some patients. A study on lymphocytes and fibroblasts (Knudson et al., 1973) showed that fibroblasts were about five times as sensitive to breaks as lymphocytes. The authors concluded that gave support to the fact that chromosomal damage in AT is not limited to cells of lymphoid origin.

They reported that about fifty percent of these patients show chromosome breakage similar to that seen in FA and Bloom's syndrome. Hayashi and Schmid (1975) agreed with Harnden's 1974 study that the incidence of chromatid breaks and chromatid exchanges were either in the normal range or the upper limit for controls. That report listed chromosome type aberrations as more characteristic.

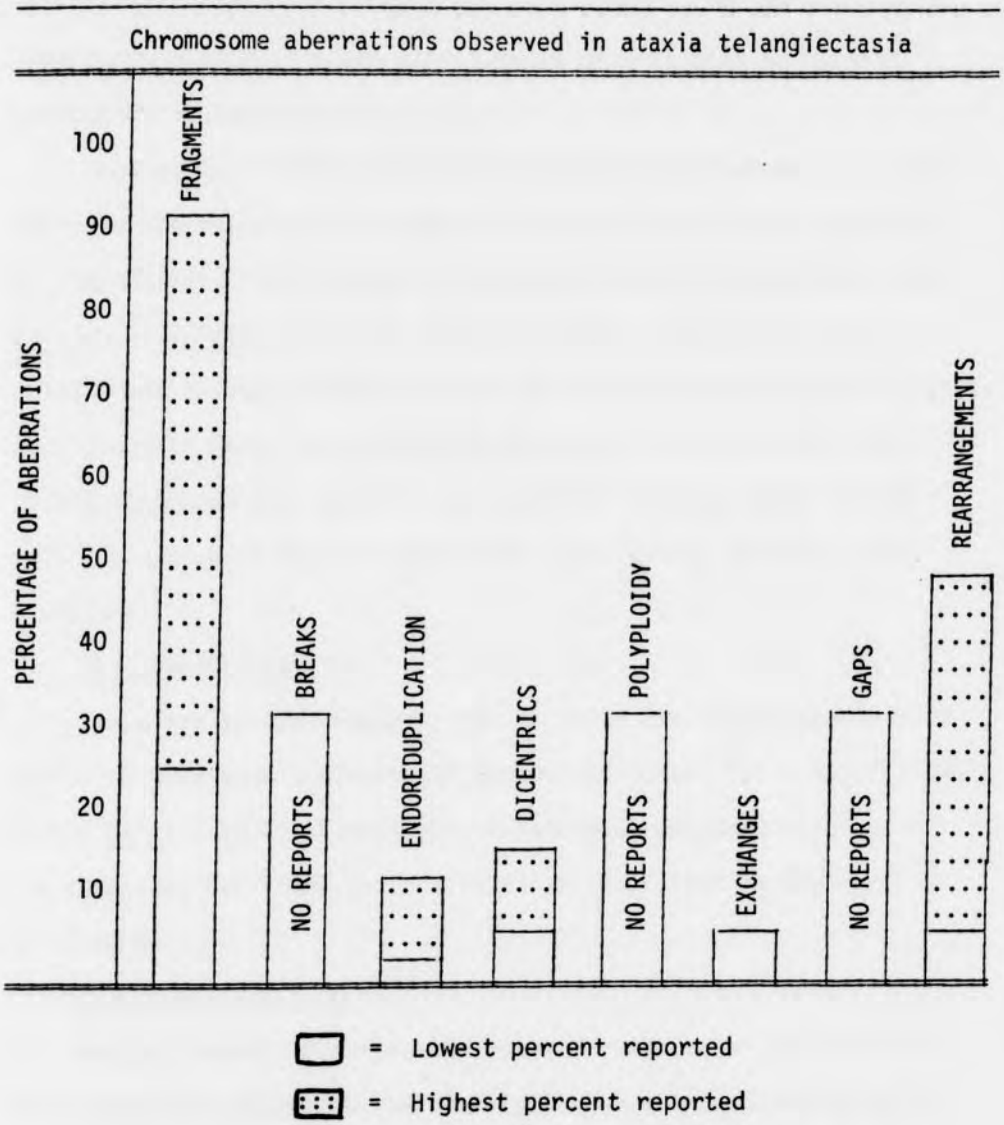
Harden (1974) noted that breakage was not always a consistent feature. He concluded that of much more significance was the increase in rearrangements leading to dicentrics or abnormal monocentrics. His postulation was that perhaps gaps and breaks were more common in younger patients and rearrangements in older ones. He further concluded that chromosomal instability in AT patients was not confined to lymphoid cells. (Refer to Figure 1., page 24).

Relationship with malignancy - clones

One study (Gatti and Good, 1971) listed six families in which more than one sibling with AT had developed the same type of cancer. Other siblings without AT did not develop any malignancies. That study also reported that chromosome abnormalities were present whether or not malignancy was involved.

Hecht et al. (1973) reported on a clone of lymphocytes marked by a translocation which was followed in an AT patient for fifty-two months until his death at the age of twenty-three. At the beginning, one to two percent of the lymphocytes had a translocation. Later, the main population of cells, fifty-six to seventy-eight percent, contained this translocation. These researchers concluded that this data in conjunction with other reports suggests that clones of

FIGURE 1.



In each report, the number of aberrations was totaled. Each figure listed here is a percentage of that total figure.

lymphocytes with a specific translocation sometimes arise and proliferate, perhaps representing a step toward lymphoid neoplasia. They postulated that those clones could be analogous to chronic myelocytic leukemia cells.

Hook et al. (1975) reported six adjacent metaphases, each with the same translocation observed in the microscopic field on a slide of lymphocytes. This seemed to indicate that the clone arose either in vivo or in vitro, and its members remained contiguous through culture and harvest perhaps because of some membrane abnormality that made daughter cells less likely to separate. These results were obtained through the use of a new method of placing drops of the cell suspension on a dry slide rather than letting them fall onto a wet one.

Radiosensitivity

There are several reports that AT cells are significantly more radiosensitive than controls (Higurashi and Cohen, 1973; Gotoff, 1967; Taylor et al., 1975). Incidence of simple chromosome deletions did not increase, but there was a significant elevation in the level of exchanges.

SV40 transformation

Another aspect of chromosome behavior studied in relation to the neoplastic process is how readily they are transformed by SV40 virus. Harden (1974) reported that AT lymphocytes are not usually susceptible to SV40 transformation.

Which chromosome(s) affected most

Bochkov et al. (1974) reported an excess of breaks in the A, C, and D chromosome groups and a deficit in the F and G groups. That does not happen in cytogenetic studies of cells from the average population. The abnormal marker that was seen frequently in one study (Cohen et al., 1973) was from the D group. In two cases banding helped to identify it as a rearranged number 14 chromosome.

Another report (Hecht and McCaw, 1974) listed eleven unrelated AT patients in whom there were lymphocyte clones marked by a translocation. In every clone the long arm of a number 14 chromosome had lost material so that the authors considered it a "donor" chromosome. They reported that the "receptor" chromosome was variable. Chromosomes six, seven, fourteen and X served as "receptors".

The translocation clone followed in the Hecht et al. (1973) report was listed as a 14/14. They cited additional evidence for the involvement of the number 14 chromosome. They listed one patient with a 14/7 translocation and another with a 14/1 translocation. The break point on number 14 appeared to be the same in all cases. Contrary to those reports, Gropp and Flatz (1967) concluded that breaks were not specific and were similar to those observed after intensive irradiation.

Perhaps the most supportive evidence for the involvement of chromosome 14 is that cited in the McCaw et al. (1975) study. Those findings suggest that the structural rearrangement of 14q is the initial change in lymphocyte clones of cells from AT patients. Seven out of eight patients exhibited a translocation involving 14q. The

eighth patient had a ring 14. One of the patient's cells was studied before and after he developed leukemia. Before that diagnosis, his lymphocytes showed a clone with a 14_q translocation. That clone appears to have given rise to the leukemic cells. The authors hypothesized that the structural rearrangement of 14_q was directly related to the abnormal growth of lymphocytes and might have been a step toward the development of malignancy. That study also cites other evidence for nonrandom involvement of 14_q in African-type Burkitt's lymphoma and other lymphoid neoplasms. These authors postulate that the 14_q may be the change that is the common denominator in many kinds of premalignant clones of lymphoid cells in humans.

All the breakpoints in 14_q are clustered in band 12. The other chromosome most often involved is the 14 homologue. Chromosomes 6, 7, and X were other participants. The breakpoint in each recipient chromosome is at or near the end. The increased incidence of breakage throughout the karyotype cannot by itself explain the nonrandom involvement of 14_q12. No one has reported this band to be a "hot spot" or inherently fragile. And no evidence has suggested affinity of some environmental agent, such as a virus, for that particular band.

Another study of 7 AT patients (Oxford et al., 1975) also pointed out the specificity of D group involvement in rearrangements. The authors hypothesize that G-banding shows that it was a number 14 that was frequently involved in clone cells and that band $_q12$ may be a highly specific exchange point. They pointed out the necessity for determining the mechanism which brought about the involvement of this

specific locus. Their postulation was that it could be due to a virus which causes specific breakages or that some loci have an increased level of activity causing predisposition to breakage. There is no evidence to support either theory.

An additional case of a lymphocyte clone with a marker chromosome in one of three sibs with AT was reported (Rary et al., 1975). Including that case, eleven cases have been reported with a lymphocyte clone containing a D group rearrangement. When banding has been used it has been identified as a number 14. Hecht et al. (1973) failed to find the 14/14 translocation clone in bone marrow cells that he had seen in lymphocytes. In that particular case, the translocation could not be found in fibroblasts. But Cohen et al. (1973) has reported finding it in fibroblasts; so perhaps the marker clones are not restricted to lymphocytes.

In a study of five AT patients (Hayashi and Schmid, 1975) four showed an increased level of chromosome type aberrations. There was clonal development in one where ninety-six percent of the metaphases had a tandem duplication of almost the entire long arm of chromosome 14. Two others had a small proportion of cells with an unidentified abnormally long D group chromosome. In 724 metaphases there were 31 dicentrics that had no lost material and seem to have arisen by end-to-end fusions. The authors noted the rearrangement in the patient with the large percentage of abnormal 14 leads to structural aneuploidy, an almost complete trisomy 14. It is not known if this is a stable arrangement or if they would separate at anaphase.

One study (Harnden, 1974) reported excess breakage in the B, D, and G groups. Here too, there was a clone with a rearranged number 14 which composed seventy-three percent of the lymphocytes examined. One case in this same study showed a translocation affecting chromosomes in the D and F groups which was present in all cells examined. It has already been pointed out that Atkin and Baken (1969) have found that chromosomes from the B, D, and G groups are under-represented in malignant cells. They noted that if it could be shown that the lack of representation was because of their more frequent involvement in rearrangements, then the association with AT would prove most interesting.

Nelson et al. (1975) reported a translocation between 14 and X in a female with AT where the break on 14 occurred at q13 and the entire long arm was attached to X which had broken at q28. That patient showed an increase in the number of cells containing that translocation over a period of time. (Refer to Table 2., page 30).

Slowed down cell cycle - response to PHA

Bochov et al. (1974) reported that there was evidence of suppression of the cell division process in AT. Their theory suggested that the doubling and separation of chromosomes occurs in cells in which no spindle formed and whose nuclear membrane did not disappear. It was very difficult to obtain from AT patients enough metaphases to study. Those cells had to be left longer than average before harvesting. Many other researchers have reported the same phenomenon (Passarge, 1972; Naspitz et al., 1968; Oppenheim et al., 1966).

TABLE 2.

Distribution of Aberrations Among Chromosome Groups				
Chromosome Group	AT	BS	FA	XP
A	15-71-64	21-28-25	17-11-10	14-1-1
B	9-42-76	5-6-11	8-5-9	29-2-4
C	21-101-62	27-36-22	42-27-16	NR
D	33-157-86	9-12-7	15-10-5	43-3-2
E	6-28-47	17-23-39	11-7-12	14-1-2
F	3-15-38	17-22-55	5-3-8	NR
G	11-52-88	4-5-8	3-2-3	NR
X	1-14-100	NR	NR	NR
Total Number of Aberrations	470	132	65	7

Key to reading table:
First Number = Percentage of total number of aberrations in each disease occurring in that group
Second Number = Observed number of aberrations
Third Number = Percentage of total number of aberrations in each chromosome group occurring in each disease

No aberrations in the Y chromosome were reported
NR = no reports

The observed number of chromosome aberrations is a compilation of reports from many sources. A group was given a score of 1 each time a chromosome in that category was involved in any type of aberration. A clone of cells with a specific aberration also scored 1. Therefore, the total number of observed aberrations would be slightly higher if each cell were counted. But in the large majority of cases 1 = 1 cell.

Oppenheim et al. (1966) reported that the in vitro response of lymphocytes to PHA could be partially corrected by culturing them in normal homologous plasma. A study of AT lymphocytes by Naspitz et al. (1968) did not show the same result. Findings from another study (McFarlin and Oppenheim, 1969) indicated that plasma from about fifty percent of AT patients contains factors which inhibit the transformation of both normal and AT lymphocytes to PHA and pokeweed mitogen. Increasing the concentration of PHA improved the response. The concentration which elicited the elevated response is somewhat toxic to normal cells. The response and growth of lymphocytes from AT patients was improved also by culturing them in homologous plasma. Responses to SLW were not improved with homologous plasma. Increasing PHA or PWM in addition to culturing in homologous plasma showed even more improvement.

The results showed that the response of AT lymphocytes to PHA and PWM fall into three groups: (1) eighteen percent were normal, (2) twenty-five percent were abnormal and did not improve with the alteration of stimulant concentration or with culture in autologous plasma, (3) in the remaining fifty-seven percent, response was reduced but could be increased by either changing stimulant concentration or using homologous plasma. They noted that since lymphocyte response varies from time-to-time in the same patient, such studies are complicated by many variables. And that group was definitely heterogenous for lymphocyte transformation. Another interesting point in that same study was that AT plasma had an inhibitory effect on the response of normal lymphocytes to PHA

and PWM. The effect was most prominent at low concentrations of stimulant and could be reversed by increasing stimulant concentration level. The authors thought that suggested competitive interaction between stimulants and inhibitor.

Sister chromatid exchanges

Contrary to the results observed in cells from Bloom's syndrome patients, there is a normal level of SCEs in AT cells (Chaganti, et al., 1974; Galloway and Evans, 1975; Hayashi and Schmid, 1975). (Refer to Table 3., page 33).

DNA repair

Results of the study by Vincent et al. (1975) suggested that the increased spontaneous and X-ray induced chromatid aberrations seen in AT cells were not caused by a repair defect of single strand breaks. Evans (1974) and Wolff (1969) theorized that chromosome-type damage (rings, dicentrics and fragments) only would be seen in the mitosis following G_0 irradiation. At that phase, the chromosome responds to X-ray irradiation as though it were a single linear entity. Chromatid-type damage would be seen after irradiation in late G_1, S, and G_2.

The Taylor et al. (1976) experiment showed AT cells with chromatid damage when irradiated in G_0. There were a large number of gaps and breaks but there was a greater number of chromatid interchanges, particularly those resulting in triradials. Damage was apparently repaired before S in normal cells since chromatid aberrations were rarely seen at mitosis. The authors of that paper suggested that much of the damage to single strands of DNA in AT

TABLE 3.

Comparison of Similarities in Chromosome Anomalies in "Chromosome Breakage Syndromes" and Certain Chemicals									
Chromosome Defects	AT	BS	FA	XP	Proflavin	Mitomycin C	Trenimon	Lead Acetate	MNNG
Increased SCEs	-	*	-	-	*	*	*	-	-
Increased chromosome breakage	*/-	*	*	*/-	0	*	*	*	*
Chromatid interchanges	*/-	*	*	0	0	*	*	*	0
Homologous chromosomes mainly in interchanges	-	*	-	0	0	*	-	-	0
Increased SV40 transformation	-	*/-	*	-	0	0	0	0	0
Increased radio-sensitivity	*	*	*	0	0	0	0	0	0

Note similarities and differences in the four diseases in the left-hand columes as compared to the five chemicals in the right-hand columes.

Key: - = no increase
0 = no reports
* = increase
*/- = reports conflict
MNNG = methyl-nitro-nitrosguanidine

Sources: 11, 24, 25, 55, 63, 80, 82, 83, 107, 110, 112, 143, 174, 179, 189, 213

cells was not repaired and persisted to manifest itself as chromatid damage at the next mitosis. They commented that a high level of deletions after G_0 irradiation and chromatid damage after irradiation in G_2 in AT cells implied either a high level of double strand breakage or failure to rejoin. Paterson et al. (1976) confirmed that AT cells showed normal single strand rejoining and were defective in excision repair of X-ray induced base damage. They proposed a model in which the incision step of DNA repair was carried out by different enzymes such as endonuclease or UV endonuclease depending on the type of damage; the remaining steps could be carried out by common enzymes. (Refer to Table 4., page 35).

Bloom's Syndrome

Types of anomalies and in which cells seen

The incidence of structural changes in metaphases from cultured lymphocytes and fibroblasts from Bloom's syndrome patients is in the range of fifteen to twenty-five percent. Chromosome instability in cells from Bloom's syndrome was first demonstrated in 1964 by Passarge. Chromosome breakage and rearrangements in lymphocytes cause chromatin bridges, lagging or loss of chromosomes at anaphase and telophase. Many cells in interphase have aneuploid nuclei, binucleus or one or more micronuclei (Bloom, 1972). German (1969, 1972) noted that such cells would not be capable of many successful subsequent divisions. He hypothesized that fact could explain the lack of growth in these patients.

The types of breaks observed in cells from Bloom's syndrome patients are of the chromosome type and suggest that the damage

TABLE 4.

Disease	Comparison of Repair Defects — Defect	Source
AT	Spontaneous and X-ray induced aberrations not caused by repair defect in single strand breaks	205
	Lacks fully functional gamma endonuclease -- defective in excision repair of gamma-ray induced base damage	146, 202
BS	DNA chain elongation during S proceeds at slower than normal rate -- decrease in DNA polymerase activity	80
	Enzyme defect involves post replication	37
FA	Step needed for repair of damage induced by bifunctional, but not monofunctional alkylating agents may be deficient	119
	Defect in repair of interstrand cross links	172, 173
	Increased susceptibility to breakage by cross linking agents indicated defect in repair -- probably deficient exonuclease	154
	Rate of DNA chain growth normal	80
XP	Two excision repair deficient lines show intermediate time for conversion of low to high weight and in inhibition by caffeine	40, 70, 111
	Impaired capacity to repair DNA alterations induced by UV and some chemicals	7, 3
XP Variant	Longer time to convert low molecular weight DNA to high; that conversion drastically inhibited by caffeine	53, 122

occurred mainly in G_1 (Schroeder and Kurth, 1971; Samad et al., 1973). Fibroblasts show the abnormal chromosome behavior similar to that described for lymphocytes. The most characteristic finding in both types of cells is a quadriradial configuration. The quadriradial seen most often in these cells is composed of two homologous chromosomes. The figure is strikingly symmetrical. Quadriradials are rarely encountered in normal cells (German, 1972). If the in vitro observations reflect what has occurred in vivo, these quadriradials suggest a high rate of somatic crossing over. Figures similar to these have been seen in cells from a boy treated with X-rays for tumor and in normal lymphocytes or fibroblasts treated with mitomycin C and certain other chromosome breaking chemicals (German, 1969, 1973; Hand and German, 1975; Sawitsky et al., 1966). This could be a useful clue toward finding the basic molecular defect.

Mitomycin C is thought to cause "cross-linking" between bases on different chains of the DNA double helix. The quadriradials of Bloom's syndrome could result from abnormal joining of strands of two different DNA double helices (German, 1973). Comings (1975) suggested that somatic recombination in Bloom's syndrome cells and in those treated with mitomycin C may be the result of selection for recombination events that can occur only between homologous segments of DNA rather than the result of somatic pairing in the nucleus.

There is a tendency to "beading" which extends to "pulverization". This is probably caused by a different mechanism from that resulting in rearrangements. Beading might be the result of micronucleus

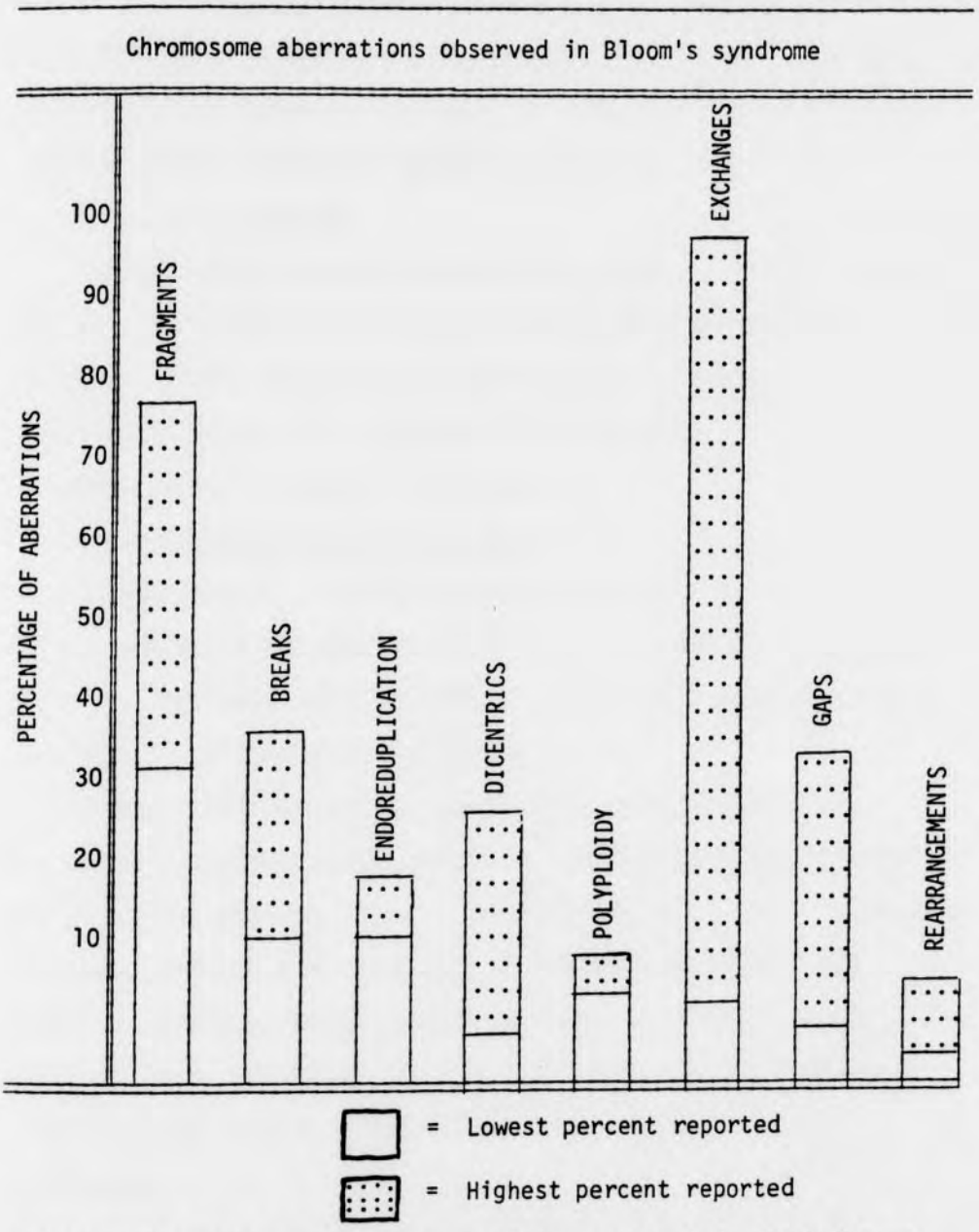
formation consequent to asynchrony of replication of chromosomes in the micronucleus in relation to those in the main nucleus (German, 1969). Nonsymmetrical quadriradials, dicentric chromosomes, triradial formations, rearranged monocentric chromosomes, chromatid breaks, acentric fragments, and telocentric chromosome fragments have also been seen in these cells (German, 1972). Regions of the chromosome most often affected in the chromatid exchange that results in quadriradial figures was at or near the centromere (German, 1972, 1974). German et al. (1974) suggested two possibilities to explain the increase in chromatid interchange in chromosomes of Bloom's syndrome patients: (1) increased "pairing" of certain regions, (2) predisposition to exchange between regions which pair normally. They also noted that the extent to which DNA replication in given segments is sychronized and the degree to which DNA sequences there are reiterated might influence whether a homologous interchange will occur.

There are some reports of chromosome abnormalities in bone marrow cells of these patients. One analysis of fifty cells showed four breaks, one chromatid exchange, and one abnormal monocentric chromosome (Landau et al., 1966). One study (German, 1974) showed exchange figures and dicentrics in dividing bone marrow cells aspirated directly.

Another report listed (Schoen and Shearn, 1967) a high level of chromosome breakage when a patient was examined at four years, eight months old and normal chromosomes when he was studied at two years of age. They postulated that aging may play a role in the development

of chromosome defects in these patients. Bloom (1975) confirmed the fact that the proportion of cells with abnormal configurations is increased in the skin, peripheral blood, and bone marrow of Bloom's syndrome patients. His study listed the figures of one-to-two percent of normal skin fibroblasts with aberrations, ten-to-fifteen percent for Bloom's syndrome patients and five-to-seven percent of obligate heterozygotes for Bloom's syndrome. German (1973) postulated an interesting theory. He stated that the symmetrical quadriradials seen so frequently in Bloom's syndrome could give rise to daughter cells which have genomes different from one another and different from all other cells in the body. By a process of crossing over, each cell would have become homozygous for all genes on affected chromosomes distal to the point of exchange. Such a daughter cell and its progeny, though retaining a balanced and complete genome, would have lost by somatic recombination certain alleles represented in all other somatic cells. He felt that a search was in order for what should be the resulting antigenic and enzymatic mosaicism. He theorized further that the café-au-lait spots and depigmented spots often seen in Bloom's syndrome could be equal to twin spots in maize and Drosophila. The dark areas would represent a clone of cells homozygous for genes with darker pigmentation, and light spots would represent a clone homozygous for genes with lighter pigmentation. (Refer to Figure 2., page 39).

FIGURE 2.



In each report, the number of aberrations was totaled. Each figure listed here is a percentage of that total figure.

Radiosensitivity

One experiment (Higuarashi and Cohen, 1973) showed that lymphocytes from Bloom's syndrome patients were significantly more radiosensitive than controls. The level of exchanges increased but the level of simple chromosome deletions did not.

SV40 transformation

German (1969) reported that Bloom's syndrome fibroblasts were not any more susceptible to SV40 transformation than normal cells. But Bloom (1975) reported twenty-to-fifty fold increase in Bloom's syndrome fibroblasts over controls in susceptibility to transformation by SV40. (Refer to Table 3., page 33).

Which chromosome(s) affected most

As described in AT there have been reports of lymphocyte clones with a rearranged D group chromosome in cells from Bloom's syndrome patients. But there have not been nearly so many reported as there have been for AT (Bochkov et al.,1974).

German (1972) stated that there was a strong predilection for certain autosomes to be involved in the quadriradials characteristic of Bloom's syndrome cells. In his report, the number 1 chromosome and those in the F group were most often involved in exchanges. The number 2 chromosome was least often involved. Passarge (1972) reported also a non-random involvement of homologous chromosomes in reunion figures with an excess occurring in the E and F group chromosomes.

Another report (German et al., 1974) listed chromosomes 1, 17, 18 and those in the C, F, and D groups as being the ones most

frequently involved in interchanges. Schroeder and German (1974) reported a non-random distribution of breakage with chromosome number 16 and those in the F group more affected than any others. The Sawitsky et al. (1966) study showed that quadriradials most often involved the number 1 pair of chromosomes. (Refer to Table 2., page 30).

Slowed-down cell cycle - response to PHA

German (1972) reported that cells from Bloom's syndrome patients respond normally to PHA. But he also stated that the skin cells did not develop into vigorous tissue culture cell lines as readily as did normal cells. Keutel (1969) noted that bone marrow aspiration was accomplished with difficulty in three patients and suggested some degree of cellular hypoplasia.

Sister chromatid exchanges

As mentioned before, cells from Bloom's syndrome patients have been shown to exhibit a great tendency for exchanges between chromatids of homologous chromosomes at homologous sites. In a study by German (1974), the cytogenetic technique by which sister chromatids are stained differentially was used to demonstrate a striking and possibly specific, but not recognized before, increase in the frequency with which sister chromatids also exchange segments. Normal controls had a mean of 6.9 sister chromatid exchanges per metaphase; the mean for Bloom's syndrome cells was 89.0. The exchanges in cells from Bloom's syndrome patients was so obvious that examining only one or two cells was sufficient to determine whether or not the pattern was present.

Chaganti et al. (1974) pointed out that the question remains unsolved as to whether or not the increase of SCEs and of chromosome breakage in Bloom's syndrome is due to the same effect. Hand and German (1975) presumed that the defect either concerns directly semiconservative DNA replication or results in disturbed cellular metabolism which in turn affects replication. Bloom's syndrome cells may serve as an example of the complexity of events taking place in the living cell prior to chromosome analysis. (Refer to Table 3., page 33).

DNA repair

One experiment (Hand and German, 1975) showed that the rate of DNA chain growth in dermal fibroblasts from Bloom's syndrome patients was significantly slower than controls. The authors assumed that the explanation could be either that an enzyme concerned directly with semiconservative DNA replication is defective or that a defective enzyme not concerned directly with replication results in disturbed cellular metabolism, which in turn affects replication.

The chromatid interchange that leads to the characteristic metaphase configuration of Bloom's syndrome is probably best interpreted as having taken place between homologous regions of two chromosomes during or following the period in the cell cycle when they engaged in semiconservative DNA replication. The possibility of such an exchange would be increased if that process were abnormally retarded so that replicating regions would remain "open" for longer than normal. In that way the cytogenetic

aberration characteristic of this disease could be caused by unusually slow DNA chain propagation (Hand and German, 1975). Comings (1975) stated that the enzyme defect in Bloom's syndrome probably involves post replication repair. (Refer to Table 4., page 35).

Fanconi's Anemia

Types of anomalies and in which cells seen

Chromosome instability in lymphocytes from Fanconi's anemia patients was first demonstrated in 1964 by Passarge. Although chromatid and isochromatid gaps and breaks are increased in both Bloom's syndrome and Fanconi's anemia, this disease presents a very different cytogenetic picture. Whereas in Bloom's syndrome homologous chromosomes had formed most of the interchanges, it is the non-homologous chromosomes that are most involved in the rearrangements common to Fanconi's anemia cells (Schroeder and German, 1974). The types of breaks in cells from Fanconi's anemia patients suggest that the damage occurred mainly in the G_2 period of the cell the cell cycle. Swift and Hirschhorn (1966) have attributed these breaks to an increased susceptibility to chromosome breaking agents, especially viruses.

An experiment (Sasaki and Tonomura, 1973) which tested the lymphocytes for breakage with several known mutagens showed that the chromosomes of Fanconi's anemia patients were more susceptible to breakage by caffeine and chloramphenicol than were the controls. It also proved a definite abnormal response to nitrogen mustard and mitomycin C and after being exposed to ultraviolet light in the

presence of 8-methoxypsoralen. All those are bifunctional alkylating agents. When treated with monofunctionally reacting agents such as methylmethanesulfonate, the level of chromosome breakage was within normal limits. Chromatid aberrations have been shown in lymphocytes, fibroblasts, and bone marrow cells (Bloom, 1972; Poon et al., 1974; German, 1972, 1973). There has also been a report of a high rate of somatic crossing over (Bloom, 1972).

Quadriradials are formed from chromatid breaks in two chromosomes, homologous or not, followed by abnormal fusion of these broken arms and chromatid interchange. Quadriradials are not seen as frequently in these cells as they are in BS (German, 1972). Chromosomal abnormalities also include anaphase bridges, fragments and micronuclei. One report (Passarge, 1972) listed chromatid exchanges as the most characteristic aberration. Endoreduplications were also increased. He listed the incidence of structural changes in metaphase figures from lymphocytes and fibroblasts as fifteen-to-twenty-five percent. One paper (Lisker and Cobo, 1974) noted that the main type of abnormality in these cells is chromatid gaps and breaks. That study indicated that, in addition, more complex rearrangements may be common leading to pseudodiploid cell lines. A 1972 paper by de Grouchy showed that the parents of Fanconi's anemia patients have more chromosome abnormalities in lymphocytes than did controls. That study found that none had over ten percent.

Bone marrow chromosome preparations differ significantly from lymphocyte cultures in that the short incubation time in vitro (two hours) includes only a small fraction of a single cell cycle.

Therefore, these findings become very important since it is likely that the DNA of those cells was synthesized while still in the patient's system. Abnormal anaphase figures in direct bone marrow aspirations strongly favor chromosome rearrangement *in vivo*. In comparison, lymphocytes undergo one to two cell cycles *in vitro* (48-72 hours) before chromosome analysis. Wolman and Swift's (1972) report showed ten percent of bone marrow cells, twenty-six percent of the fibroblasts, and sixty percent of the lymphocytes with significant structural damage. The authors noted that the difference between aberration frequency *in vivo* and *in vitro* may provide a clue to the nature of the inherited matabolic defect underlying Fanconi's anemia. Swift and Hirschhorn (1966) and Shahid *et al*. (1972) reported patients with abnormalities in ten percent of their bone marrow cells. The latter investigators also saw dicentrics and ring chromosomes which to them implied that at least some breaks had occurred *in vivo*.

Guanti (1971) recorded abnormalities in twenty percent of bone marrow cells. But there have also been several reports of no chromosomal abnormalities in the bone marrow of Fanconi's anemia patients (Bloom 1966; Hoefnagel 1966; Schmid 1967; Dosik 1970). Shahid *et al*. (1972) postulated that whether *in vivo* breakage of marrow chromosomes is spontaneous or induced by extrinsic agents including viruses, it is possible that the genetic deficiencies produced, may lead to a progressive loss of cells and result in aplastic anemia. They also noted that the apparent lack of involvement of lymphoid tissues (except the spleen) in spite of the

high rate of breakage *in vitro* may be attributed to the remarkable length of intermitotic interval and life span of some small lympocytes *in vivo*.

Chromosome aberrations may give rise to micronuclei by means of lagging chromatin. Eventually a micronucleus may be slowed down in its cell cycle in respect to the main nucleus and therefore undergo premature chromosome condensation when the latter undergoes mitosis. Obe *et al*. (1975) presented some evidence that this mechanism may operate *in vivo* without treatment with a mutagen. The authors supposed that the "pulverized" and chromatin of "segmented, beaded appearance" seen in Bloom's syndrome cells may have, in their case, represented patches of prematurely condensed chromatin. Micronuclei were reported in those cases of Bloom's syndrome which also listed "pulverized" chromatin. Bear *et al*. (1973) noted that the chromosomal changes in Fanconi's anemia could be an acquired phenomenon representing the abnormal and persistent effect of a virus within the cells. Or the changes could develop only when the inherited cell defect interacts with some exogenous agent. Schmid (1967) noted that some of the aberrations in these cells can be explained by chromosome breakage; others can be explained by deficiencies in the mitotic apparatus. But he theorized it was possible to explain all the anomalies by a deficiency in the spindle apparatus. Therefore, it could be this malfunctioning apparatus and not broken chromosomes that is responsible for the findings that have been reported in glutathione reductase deficiency and pernicious anemia. He further postulated that it was unlikely that fragile chromosomes is the primary defect.

He stated that it could be assumed that the process which breaks the chromosome at the same time kills the cell or at least incapacitates it for further divisions. The almost complete absence of chromosome-type aberrations suggests that the breaks are secondary to another, still unknown process that seems to be lethal to the affected cells. Swift and Hirshhorn (1966) pointed out that the high rate of chromosome breakage listed in the three diseases discussed thus far, whether spontaneous or environmentally induced, could result from defective chromosome structure, increased liability, or lysosomal membranes with increased release of destructive enzymes, or other unknown mechanisms. The cytogenetic instability could provide an explanation for the various features of the clinical syndrome. (Refer to Figure 3., page 48).

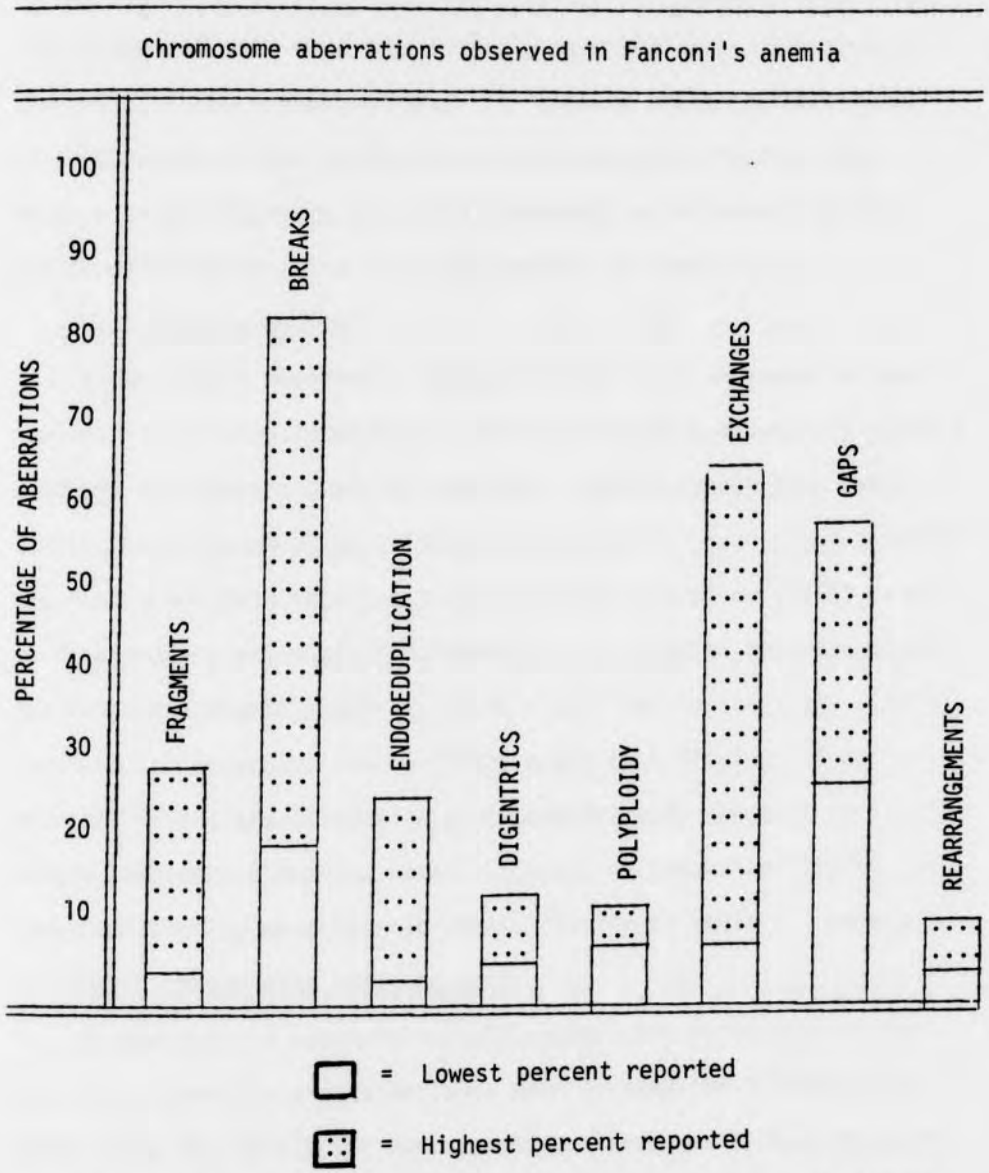
Relationship with malignancy - clones

Beard et al., (1973) reported that the spontaneous formation of abnormal clones suggests that these cells' sensitivity to transformation is a function of a cellular DNA replication or repair defect, rather than the abnormality of virus absorption or virus replication. They postulated that cells predisposed to developing abnormal karyotypes could probably be regarded as potentially malignant.

Radiosensitivity

An experiment by Higurashi and Cohen (1971) showed Fanconi's anemia lymphocytes to have four times the number of breaks per cell as did controls when they were irradiated with ten and one hundred rads. Fibroblasts showed two times controls in number of breaks per cell. The number of dicentrics and rings per cell also was

FIGURE 3.



In each report, the number of aberrations was totaled. Each figure listed is a percentage of that total figure.

significantly greater in lymphocytes from Fanconi's anemia patients. A later study (Higurashi and Cohen, 1973) showed that lymphocytes from these patients were significantly more radiosensitive than controls. Authors reported that the level of exchanges increased after irradiation but simple chromosome deletions did not. But another study (Beard et al., 1973) reported no difference in the effect of X-ray on these cells as compared to controls.

SV40 transformation

Bloom (1975) reported a twenty-to-fifty fold increase in susceptibility to transformation by SV40 in Fanconi's anemia and Bloom's syndrome fibroblasts over the controls. Other reports have been similar (Parrington et al., 1971; German, 1969). Bear et al. (1973) reported a ten fold increase. Torado (1966) and Bloom (1972) found an intermediate value of transformation for obligate heterozygotes for Fanconi's anemia. But the Young (1971) and Beard et al. (1973) studies were contrary. Bloom (1972) noted that findings of an increase in the transformation of cytogenetically abnormal cells raises questions about the genetic control of susceptibility to transformation by an oncogenic virus. (Refer to Table 3., page 33).

Which chromosomes affected most

In von Koskull and Aula's (1973) experiment where lymphocytes from five Fanconi's anemia patients were stained for G bands, all breaks were in lightly stained segments. There was a clustering of breaks at 3_q and 13_q and the distal region of A group chromosomes. Fewer breaks occurred near the centromere. All chromatid exchanges involved two lightly stained regions. Statistical calculations showed

significant nonrandom distribution with an excess of breaks in chromosomes 1, 2, 3, 6, and 13. The authors pointed out that since the DNA and Q at G bands is late replicating which indicates a higher adenine: thymine ratio at those loci, those breaks occurred at guanine:cytosine rich segments. They theorized the intrigue of comparing break points by a virus and a chemical in Fanconi's anemia cells. Caldwell (1969) reported that most of the breaks in the Fanconi's anemia cells he studied were in chromosome groups A, B, and C.

A study by Lisker and Cobo (1974) showed a patient who had sixty percent of bone marrow cells that had a D group chromosome with a larger than normal long arm. Another report of bone marrow cells by Shahid et al. (1972) showed that chromatid breaks involved primarily B and C group chromosomes. Wolman and Swift (1972) stated that group A had slightly more breaks in their study. Swift and Hirschhorn (1966) reported that there appeared to be a predominance of breaks in the distal portion of one arm chromosome number 1 and on one arm of chromosome number 1. Beard et al. (1973) reported on the apparent spontaneous production of two clones of abnormal karyotype in the fibroblasts of a Fanconi's anemia patient. One clone showed forty-six chromosomes with two abnormal chromosomes in group B and one missing number 3 chromosome. The second clone had forty-seven chromosomes with an extra F. (Refer to Table 2., page 30).

Slowed cell cycle - response to PHA

When Young (1971) was investigating the transformation rate of Fanconi's anemia fibroblasts by SV40 he noted that these cultures grew

more slowly than controls and were difficult to maintain.

Sister chromated exchanges (SCEs)

Results of Chaganti (1974) and Sperling (1975) indicated that the process leading to breaks does not act by simultaneously increasing SCE rates. Sperling et al. (1975) pointed out that the involvement of homologous chromosomes in interchanges in BS indicated a different biochemical pathway was affected than that in FA. There non-homologous interchanges predominate. Perhaps different repair processes are affected too. The fact that it has been found (Poon et al., 1974) that the DNA repair deficiency in Fanconi's anemia is an exonuclease deficiency is not contradictory to the findings of that study. For it was shown in an ultraviolet light experiment that SCE formation seems to be dependent on post-replication repair but not on replication repair. (Refer to Table 3., page 33).

DNA repair

The fact that Fanconi's anemia lymphocytes showed an increased susceptibility to chromosome breakage by DNA cross linking agents indicated to Poon et al. (1974) that there might be some defect in the DNA repair mechanism. From their experiment they concluded that there was a defect and that it was in the excision of DNA lesions. They postulated that this was probably a result of deficiency of a specific exonuclease. They further theorized that these cells could produce single strand breaks after being exposed to ultraviolet irradiation but could not complete the process. Fanconi's anemia fibroblasts also showed unscheduled DNA synthesis after exposure to 4-nitroquinoline-N-oxide. This is a carcinogen which has proven to

induce unscheduled synthesis in human cells (Stitch et al., 1973). The fact that it induced unscheduled synthesis in those patients' cells suggested they could make single-strand scissions after exposure to it as well as ultraviolet light. Those cells also seemed capable of rejoining X-ray induced breaks suggesting that a ligase was present.

The results of that experiment that FA cells are unable to remove a thymine dimer is at odds with Setlow and Regan's (1972) conclusion. The reason for the disagreement is unclear, but Regan could have used lower doses of ultraviolet light. Perhaps these cells are not as sensitive to UV as are xeroderma pigmentosum cells, but are more sensitive than normal. Poon et al. (1974) pointed out that there may not be a complete absence of the exonuclease, which would explain the lack of UV induced skin cancers in these patients. They noted that differences in results might also be caused by the use of different cell lines. They concluded that it can not be said that an exonuclease defect exists in all Fanconi's anemia patients. Hand and German (1975) reported that the rate of DNA chain growth in fibroblasts is normal. Another study (Saski, 1975) found that Fanconi's anemia cells had a specific defect in the repair of preaberration lesions induced by bifunctional mitomycin. They theorized that the lesions were possibly DNA cross links of the interstrand type. They also noted that the results strongly suggested that those cells were defective in a biological system which constitutes an essential step in the repair of DNA interstrand cross links. One study noted (Latt et al., 1975) that the reduced ability of FA cells to form additional

SCEs after exposure to alkylating agents suggested that a defect related to chromosome repair existed. They suggested that a step needed for repair of the damage induced by bifunctional, but not monofunctional, alkylating agents might be deficient in those cells. (Refer to Table 4., page 35).

Xeroderma Pigmentosum

Type of anomalies and in which cells seen

Reports indicate a different kind of cytogenetic disturbance from the other three diseases considered in this paper. There seems to be a tendency toward pseudodiploid clones. This is very rare in the laboratory studies of normal human cells (German, 1972). Pseudodiploid clones in xeroderma pigmentosum cells were first reported by Passarge (1972). Cytogenetic data for these cells is very scarce. Reed et al. (1969) reported normal karyotypes in lymphocyte cultures from four patients. German detected a low incidence of chromosome rearrangement in three of six fibroblastic cultures. Bloch-Shtacher et al. (1972) studied chromosomes in fibroblasts from different areas of one patient. They found eighty-four percent of the cells from unexposed areas with normal diploid karyotype. Only fifty-seven percent from exposed areas had normal karyotypes. And only ten percent of the cells from skin that had developed squamous cell carcinoma had normal karyotypes.

Huang et al. (1975) listed dicentrics as the prevailing aberration. They also noted that chromosome and chromatid breaks were seen frequently. Rings and pulverization were seen, but rarely. They concluded that XP cells were less stable than the controls at

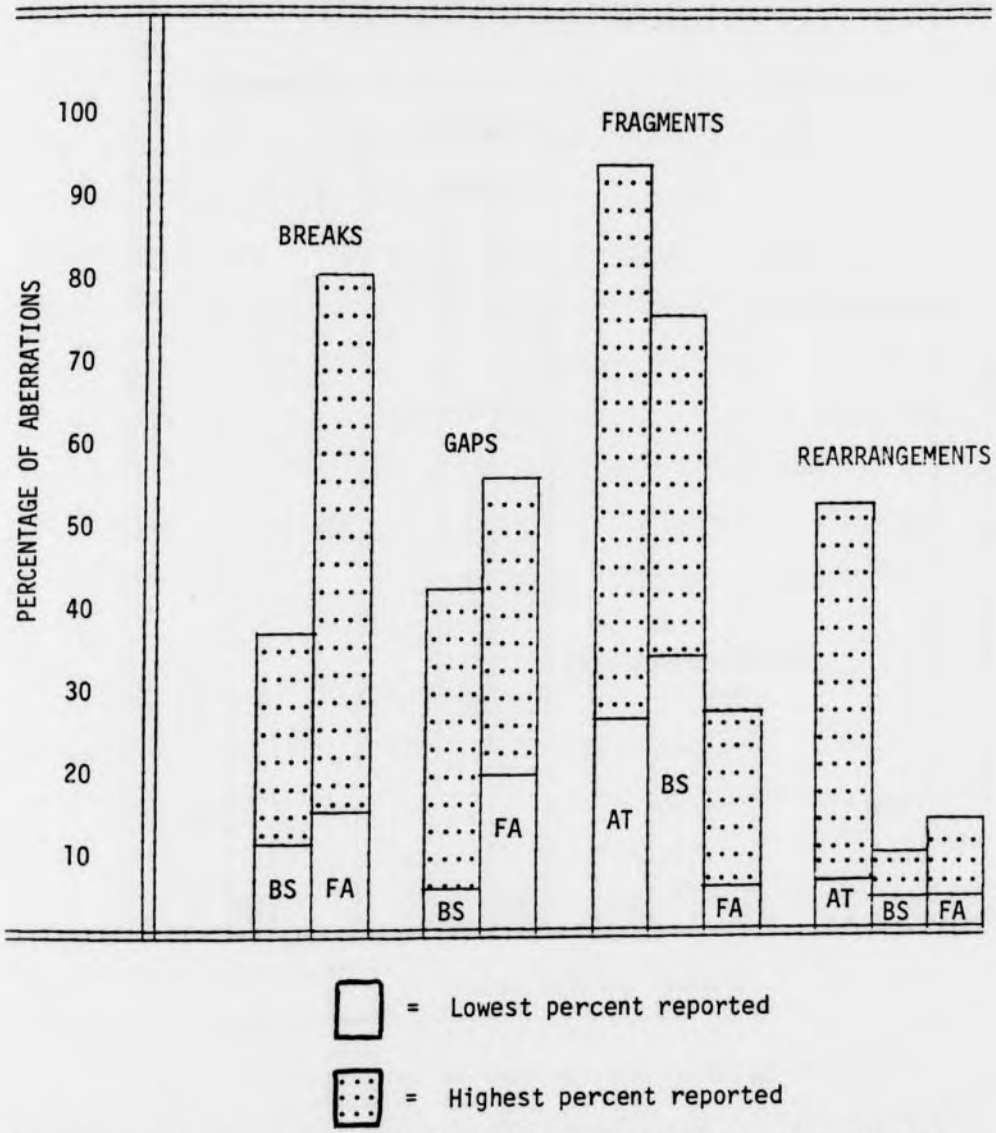
late passage, but the degree of chromosome instability varied greatly among cell lines. At early passage, XP cells had no more aberrations than controls. At late passage, two of four XP lines had up to fifty percent polyploidy and up to seventy-nine percent aberrations. The other two lines had a slightly higher incidence of aberration than controls. Parrington et al. (1971) reported that XP cells have a higher rate of aberrations than do controls after exposure to ultraviolet light but that the degree there also varied among cell lines. They noted that since there seems to be varying degrees of repair levels, there could also be different levels of chromosome instability. Cleaver and Bootsma (1975) reported normal karyotypes with no increase in breakage or rearrangement. Harnden had reported in 1974 that there was no excess of breaks or rearrangements in XP cells under ordinary tissue culture conditions but there was an increase over controls after ultra-violet light exposure (Refer to Figure 4., page 55).

Relationship with malignancy - response to carcinogens and mutagens

Impaired DNA repair capacity of XP cells seems to result in their greater sensitivity towards lethal and chromosome damaging action of those physical and chemical carcinogens which induce irreparable DNA alterations. But they do not differ from controls when treated with any of several potent alkylating mutagens and carcinogens. Stitch et al., (1974) reported that the sensitivity of XP cells varied towards different physical, chemical, and viral carcinogens. Fanconi's anemia cells also vary in their response,

FIGURE 4.

Comparison of chromosome aberrations observed in "chromosome breakage" syndromes



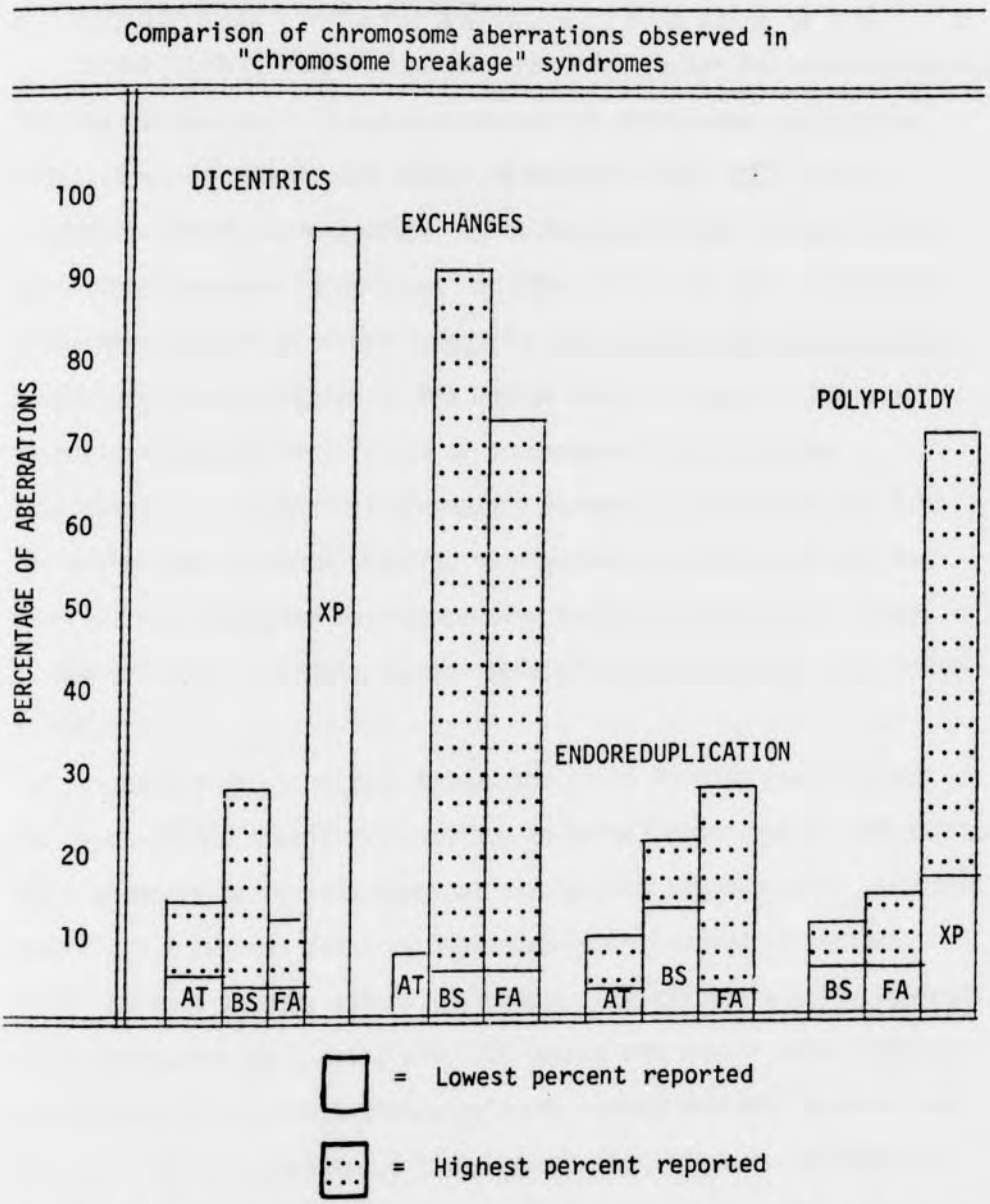
= Lowest percent reported

= Highest percent reported

In each report the number of aberrations was totaled. Each figure listed here is a percentage of that total figure.

SOURCES: 16, 84, 100, 116, 139, 187, 196, 207, 214.

FIGURE 4. (continued)



In each report the number of aberrations was totaled. Each figure listed here is a percentage of that total figure.

SOURCES: 16, 84, 100, 116, 139, 187, 196, 207, 214.

but cells from patients with these two diseases differ in their response toward the same carcinogen.

Sasaki (1973) compared normal and XP cells for DNA repair capacity and its consequences in the development of chromosome aberrations elicited by treatment with methyl methanesulfonate (MMS) and 4-nitroquinoline-1-oxide (4NQO). XP cells were highly susceptible to chromosome breakage by 4NQO but not MMS. Since XP cells had already been shown to have a severely reduced capacity to repair 4NQO-induced damage and normal repair for MMS damage (Stitch et al., 1973), he strongly suggested that faulty or incomplete DNA repair was responsible for structural chromosome changes. He pointed out that the mechanisms by which specific agents damage chromosomes may be diverse, but indicated results pointed to the importance of damage to the DNA and its repair in the production of chromosome aberrations by 4NQO.

In Stitch et al. (1973) study, the clone forming capacity and the level of DNA repair was examined in normal human and XP fibroblasts after exposure to various chemical carcinogens and mutagens. XP cells showed (1) a reduced level of DNA repair when exposed to various carcinogenic N-oxides, (2) no unscheduled DNA synthesis after exposure to 6-nitroquinoline 1-oxide and, (3) normal DNA repair after exposure to N-methyl-N^1-nitro-N-Nitrosoquanidine. Those authors hypothesized that the results suggested a link between the extent of DNA damage, the level of repair, and the degree of sensitivity in cells exposed to these chemical carcinogens and mutagens. They raised the question of whether the observed or inferred links between DNA damage, DNA repair,

elevated cell sensitivity, and increased mutation rate are relevant to neoplastic transformation induced by chemical agents or whether they represent mere epiphenomena. They theorized that a deficient DNA repair system could: (1) elevate the mutation rate thus creating a genetically heterogenous cell population from which a neoplastic cell arises, (2) increase the frequency of chromosome aberrations which would contribute to genetic variation among cells exposed to carcinogens, or (3) lead to frequent cell death followed by repeated compensatory proliferation. It could also facilitate the incorporation of viral genomes.

SV40 transformation

Some other genetically determined diseases with a high incidence of neoplasia show increased susceptibility to *in vitro* transformation by SV40. Fibroblasts from XP patients do not exhibit this characteristic (Parrington *et al.*, 1971; Cleaver and Bootsma, 1975; Key and Torado, 1974). Key and Torado (1974) also studied the effect of SV40 on XP cells after exposure to ultraviolet light to see if the biologic defect in relation to carcinogenesis in XP could enhance the susceptibility to triggering by sunlight of viral oncogenesis. No increase in transformation was observed. They concluded that the question still remains whether ultraviolet light in conjunction with other oncogenic agents such as chemicals, or RNA tumor viruses might serve better to explore the role of sunlight in the genesis of skin cancer. (Refer to Table 3., page 33).

Which chromosome(s) affected most

Bochkov et al. (1974) reported observing lymphocyte clones with a rearranged D in XP cells. Stitch et al. (1974) observed the effect of UV AD12 on these cells and controls. In both cases over fifty percent of all chromosome aberrations were in chromosomes 1 and 17. Most chromatid aberrations were near the end of chromosome 1 and in the region of the secondary constriction on number 17. This agrees with previous observations on normal and trisomic cells.

In the Huang et al. (1973) experiments it was previously mentioned that chromosomal changes occurred in two of four XP lines. In both cases there was a high incidence of a longer chromosome in the B group. Waltimo et al. (1967) reported on two sisters with XP. One had forty-five chromosomes in which the karyotype showed a D/D group translocation. The other sister plus their parents had normal karyotypes.

Cell cycle

Chopa and Forbes (1974) noted that results indicated that UV light-induced epidermal production in hairless mice was associated with progressive shortening of the cell cycle and reduced cell death. The duration of the S phase decreased significantly in the hyperplastic epidermis and continued to decrease in nonmalignant and malignant stages of tumor growth. They also indicated a proportion of cells above the basal layer which under normal circumstances would have keratinized still retained the ability to proliferate and therefore contribute to tumor growth. They concluded that whether the production of UV-incuded tumors involves changes at the chromatin

level is more difficult to answer. But divergencies in cell proliferative behavior from normal may be attributed to some modification at this level.

Huang et al. (1975) noted that these cells grew much slower than controls; consequently mitosis was rare. He questioned it as a sign of aging. He also reported that the incidence of cells with forty-four or forty-five chromosomes was much higher than controls and concluded that XP cells may be more sensitive to osmotic shock. Reed et al. (1969) and Schroeder (1974) also reported that XP and AT cells grew poorly in vitro.

Sister chromatid exchanges

All reports thus far have indicated that there is no increase in sister chromatid exchanges in XP cells (Cleaver and Bootsma, 1975; Kato and Stitch, (1975). That holds true in both excision-repair-deficient and post-replication-repair-defective cells (Wolff et al., 1975). This is contrary to results for chromosome aberrations which increase in excision-repair-defective cells after exposure to UV or 4-nitroquinoline-1-oxide. Wolff et al. (1975) suggests that SCEs are unrelated to DNA repair. They report that neither the amount of excision repair in various XP lines nor the presence of defective post-replication repair as in the variant form seems to have any effect on SCE levels. Chromosome aberrations, on the other hand, are higher in excision-repair-defective cells after UV exposure (Parrington et al., 1971) or 4N1 oxide (Sasaki, 1973). (Refer to Table 3., page 33).

DNA repair

The DNA repair of single-strand breaks (produced by ionizing radiation) and of base damage (produced by UV light) (Rasmussen and Painter, 1964) are two repair mechanisms most mammalian cells possess. Genetic defects in the former mechanism are found in cells from the human premature-aging disease, progeria, which fail to rejoin single strand breaks. Some XP cells exemplify the other defect. They are incapable of repairing UV damage (Epstein et al., 1973).

Solar radiation contains UV equivalent to about 1-2 ergs/mm^2/min. of 254 nm. wavelength. The sensitivity of normal cells is about 30 ergs/mm^2. Therefore, most unprotected fibroblasts would be killed after thirty-to-sixty minutes in direct sunlight (Cleaver, 1973).

XP cells repair chemically induced DNA damage less efficiently than normal. Therefore, the enzyme missing is required not only for UV damage but for chemical damage as well (Huang et al., 1975; Robbins et al., 1974). 4-nitroquinoline-N-oxide induced no unscheduled synthesis in XP cells (Cleaver and Bootsma, 1975; Stitch et al., 1973). Poon et al. (1974) theorized that this agent does not produce single strand breaks directly. Since these cells exhibit normal repair after ionizing radiation, Cleaver and Bootsma (1975) suggested that the damage caused by this agent may be similar to UV damage and damage caused by N-acetoxy-2-acetylaminofluorene. Benz (a) anthacene epoxide causes unscheduled synthesis in controls, but very reduced repair levels in XP cells. These results resemble the increased susceptibility of these cells to UV light and the oncogenic 4NQO derivatives (Stich and San, 1973). XP cells and controls respond

about equally to MNNG damage. Stich et al. (1973) noted that it appears there is a different capacity for repair of 4NQO and MNNG induced DNA alterations.

Heterogeneity

Robbins et al. (1974) reported that cell fusion studies had shown that genetic complementation can occur between fibroblasts from certain pairs of patients overcoming the DNA repair defect in each member and demonstrating heterogeneity of the genetic lesion. Their paper reported four distinct complementation groups indicating that at least four mutations can cause defective repair. Each had its characteristic rate of unscheduled synthesis. Cleaver and Bootsma (1975) and Kramer et al. (1975) listed five complementation groups with excision-repair-defective XP syndrome. They stated this indicates intergenic rather than intragenic complementation. The existence of numerous complementation groups probably implies multiple gene loci specifying subunits and cofactors of UV endonuclease that initiates repair in XP cells.

A study of complementation in heterokaryons suggests that the 'XP-enzyme' is probably not a monomer and that its function may be dependent upon binding to an acceptor and the formation of a stable complex which turns over slowly (Giannelli and Pawsey, 1974). Patients with classical XP may carry defective enzymes which do or do not bind to the acceptor. Giannelli and Pawsey's (1974) findings suggested to them that the enzymes in this repair system may assemble to form a 'repair organelle'.

XP variant cells are defective in a stage of postreplication repair indicated by the drastic inhibition of gap filling by caffeine and slightly reduced in host cell reactivation of UV-damaged adenovirus. Therefore, Cleaver and Bootsma (1975) concluded, one could say that postreplication repair is less important than excision repair for the survival of human cells, though it is of obvious importance to clinical symptoms. The time taken to convert initially low molecular weight DNA similar to that in unirradiated cells is much greater in XP variants than in normal cells. In addition, that conversion is drastically inhibited by caffeine which has no effect on normal cells (Lehmann et al., 1975; Lehmann, 1974). Two cell lines of excision repair deficient XP classes show intermediate effects in time for conversion of low-to-high molecular weight DNA and in inhibition of the process by caffeine.

Despite low levels of excision repair, XP cells can tolerate many dimers without being killed because they have another mechanism for coping. One such mechanism is postreplication repair in which DNA synthesis is temporarily delayed by a dimer, then continues beyond the dimer, leaving a gap that is eventually filled. Howard-Flanders (1973) found that in bacteria, gap filling is achieved by a process involving sister-strand recombinational exchanges of large amounts of DNA. It is not definitely known that the process is the same in mammals. Lehmann et al. (1975) offers two explanations for this: (1) one or more enzymes are common to both excision and postreplication repair and one of these is defective in classical or de Sanctis Cacchione cells, (2) in normal cells some dimers are

excised in the first six hours after irradiation; consequently, fewer dimers have to be replicated than in excision deficient cells. It seems likely that deficiency in post replication repair is at least partly responsible for the clinical symptoms of XP in variants. This would seem to indicate that postreplication repair is a process of biological importance in human cells. Lehmann (1974) noted the interesting observation that a deficiency in post replication repair and various defects in excision repair seem to give similar clinical symptoms in man.

There is another form of XP termed de Sanctis Cacchione syndrome which has the same clinical symptoms and defective excision repair plus involvement of the central nervous system (de Sanctis and Cacchione, 1932). After cell fusion studies deWeerd-Kastelein et al. (1972) and Der Kaloustian et al. (1974) stated that the simplest interpretation of their data is that two different genes are involved in the basic defect causing de Sanctis Cacchione syndrome and classical XP. This could be termed intergenic or interallelic complementation. Kleizer et al. (1973) has reported that the parents of some de Sanctis Cacchione patients showed significant reduction in the level of repair synthesis.

One of the latest developments in the study of DNA repair is a new technique of DNA alkaline elution which helps in measuring single strand breaks in eukarotic cells. Fornace et al. (1972) and Haddow (1974) have reported an XP variant in which the clinical symptoms are the same as classic XP but has normal repair synthesis and normal UV sensitivity. The deficiency is in the rejoining rate.

Huang et al. (1975) noted a clear correlation of chromosome aberrations and DNA repair capacity in the various XP lines has been demonstrated after treatment with UV irradiation, chemical carcinogens, and infection with adenovirus type 12.

Host cell reactivation

The two phenotypic characteristics of XP cells in culture that correspond to the clinical symptoms of high UV sensitivity are colony-forming ability after irradiation and reduced ability to support the growth of UV damaged viruses. Huang et al. (1975) noted that this indicated host cell genetic defects are important in viral reproduction, a phenomenon known as host cell reactivation. They pointed out that observations implied the genetic defects in XP variant lines are much less important for the survival of radiation damaged cells and viruses than are the defects in other forms of XP. But since all forms show similar skin symptoms, genetic deficiencies appear equally important for carcinogenesis.

Takebe et al. (1974) reported host-cell reactivation high in the African green monkey, intermediate in normal human fibroblasts and human FL cells, and low in both XP and mouse L cells. Colony forming ability after exposure to UV light was high for FL cells, normal for human fibroblasts and L cells, slightly low for monkey, and extremely low for XP cells. They concluded that if survival of UV-irradiated Herpes simplex virus on a test line of human or other mammalian cells is as low as that on excisionless XP cells, then it is very probable that the test cell line is deficient in excision repair.

Using a sensitive host-cell reactivation technique, Day (1975) found that the fibroblasts from patients with all five known variants of classical XP do have deficiencies in their DNA repair rate after UV irradiation. Non-irradiated and UV-irradiated suspensions of adenovirus 2 (a nuclear-replicating, double stranded DNA virus) were assayed for their ability to form plaques on monolayers of human fibroblasts. The non-irradiated viruses or viruses repaired by the fibroblasts form plaques on such monolayers. Below are listed the characteristics of the five known XP variant lines:

	UV Induced Unscheduled Synthesis	UV Sensitivity of Colony Forming Ability	Reactivation of Adenovirus 2 (% Normal)
XP 4BE	Normal	Normal	64
XP 13BE	Normal	Normal	60
HG 85 9	Not Done	Not Done	63
XP 7TA	Normal	Not Done	57
XP 30RO	Normal	Not Done	57

These studies do not show which repair process is defective. It has been reported that fibroblasts from three variants have abnormal post replication repair (Lehmann, 1974; Lehmann et al., 1975). Refer to Table 4., Page 35).

Prenatal diagnosis

Ramsay et al. (1974) reported the first successful case of prenatal diagnosis of XP. Although the characteristic pigmentation does not show until the child is two to three years old, the DNA repair defect can be detected before then. The mother involved in this case

chose termination of the pregnancy at 22 weeks. The aborted fetus showed a 70 percent deficit confirming the prenatal test suspicions.

Immunologic Characteristics

General

Kersey et al. (1973) stated that clinical and laboratory evidence indicating a relationship between immunologic deficiency and cancer had accumulated rapidly in recent years. Gatti and Good (1971) noted that the frequence in malignancy in patients with primary immunodeficiency was roughly 10,000 times greater than that of a general age matched population. The question still remains whether increased incidence of malignancy in patients with immunodeficiency diseases is related to a genetic propensity to develop malignancy or is truly a function of their immunologic deficiency.

So far, immunoglobulin deficiency plus an associated increase of leukemia has been found in several diseases. These include Bloom's syndrome, agammaglobulinemia, AT, and Wiskott-Aldrich syndrome (Landau et al., 1966). You will note two "chromosome breakage syndromes" in this list. Of eight immune deficiency diseases listed by Knudson et al. (1973) seven have increased incidence of cancer. In the other, survival is too short to ascertain.

Ataxia Telangiectasia

From several case histories it appears that certain immunologic abnormalities antedate the telangiectasias and neurological symptoms of AT (German, 1972). Bloom (1975) noted that most cancers developed by these patients were lymphoid in origin.

Low lymphocyte levels

The depressed response of AT lymphocytes to PHA has already been discussed. It should be noted here that the small lymphocytes of these patients' peripheral blood are frequently decreased. Bochkov et al. (1975) noted that the markedly low reaction of blast-transformation in lymphocyte cultures in vitro may be explained by the reduced number of leucocytes and the low proportion of minor lymphocytes. Peterson (1968) reported that 35 percent of all AT patients had blood lymphocyte levels of less than 1,000 cells per mm. That was not simply a consequence of infection. Naspitz et al. (1968) thought the transformation of lymphocytes induced by PHA in vitro was a measure of the ability of the small lymphocytes to undergo transformation in vivo. They pointed out that it is still puzzling why the response to PHA is not constant from case to case.

I_gE and I_gA

Some cases have normal I_gA levels, but many have decreased to absent serum IgA. Sixty percent of the patients have decreased salivary IgA. The level of I_gE is also deficient in sixty percent of persons with AT (Birth Defects Atlas, ed. by Bergsma, 1973). In one study of 20 patients, (Reed et al., 1966) over one-half had depressed I_gA levels. Their parents' levels were normal, but siblings tended to have depressed levels. The IgA level was very low in ten of the patients, seven of twenty-one siblings, and two of twenty-seven parents. German (1972) noted various abnormalities in serum immunoglobulin levels, the most common being low I_gA.

Harnden (1974) also reported a low level or complete absence of I_gA as the most common defect in these patients.

A study of sixteen patients (Amman et al., 1969) showed eleven of them to be deficient in I_gE. The authors theorized that this abnormality could reflect a genetic association of this disease with a gene required for the synthesis of normal I_gE or IgE subclasses. Nine of these sixteen patients were deficient in both I_gA and I_gE. One was deficient in all immunoglobulins. They interpreted their results to indicate that the production of I_gA and IgE are linked both with one another and with the production of cell-mediated immune responses and dissociable from the production of I_gG and I_gM. Bloom (1975) listed both I_gE and I_gA levels reduced. A summary table (Peterson and Good, 1968) listed 25 percent of these patients with normal I_gA, three percent with high levels, and 72 percent with low levels.

I_gG and I_gM

McKusick and Cross (1966) reported normal or elevated levels of I_gG and I_gM in AT patients. Reed et al. (1966) listed these levels as nearly normal in his study. Harnden (1974) stated that these immunoglobulins are most often normal in AT patients but that there were reports of abnormalities. A summary table (Person and Good, 1968) lists 50 percent of these patients with normal I_gG, 11 percent with high I_gG, and 31 percent with low I_gG. The same summary shows 81 percent with normal I_gM levels, 18 percent with high levels, and one percent with low I_gM. (Refer to Table 5, page 71).

TABLE 5.

Comparison of Immunoglobulins					
IMMUNOGLOBULINS	AT	BS	FA	XP	SOURCES
A	60% decreased s. 25% normal	decreased		normal	60, 116, 78, 52, 46, 12
	50% depressed				162
	low		NO REPORTS		60, 82, 12
	72% low p.				152
	59% deficient p.				7
G	normal	low		normal	161, 82, 177, 46, 102
	50% normal 19% high 3% low				152
E	60% deficient	NO REPORTS		NO REPORTS	
	68% deficient				7
	low				12
M	normal	low		normal	161, 82, 60, 116, 12, 177, 46
	81% normal 18% high < 1% low				152

s = salivary

p = peripheral

Sinopulmonary infections

Recurrent viral or bacterial sinopulmonary infections become apparent in most of these patients by about six months of age, though this varies. Even those who have normal I_gA levels have many of these infections. Pneumonia is reported to be the most common cause of death for AT patients (German, 1972). (Refer to Table 1., page 6).

Defects in cellular immunity

AT patients exhibit defects in cellular immunity by prolonged survival of skin homografts and abnormal delayed hypersensitivity (German, 1972; Bloom, 1975). A study of 20 patients, 23 siblings and 27 parents, (Reed et al., 1966) showed family members had intermediate sensitivity rates. They stated that on a statistical basis, parents and siblings would be classed as nonreactors in a general population. (Refer to Table 6., page 73).

Defective thymus and lymph nodes

Naspitz et al. (1968) suggested a defect in the stage of conversion from an epithelial to a lymphoid thymus. Consequently, AT patients with this embryonic thymus would have underdeveloped and malfunctional peripheral systems. They theorized that an intrinsic defect of lymphoctyes is perhaps secondary to failure in thymic development. If so, that failure might best explain the PHA response and the immune abnormalities in AT.

Broumsell et al. (1975) hypothesized that a high percentage of the lymphoreticular malignancy is due to a reduction of thymic suppressor function which normally acts to retard continued lymphoid proliferation. The continued unregulated lymphoid proliferation

TABLE 6.

Comparison of Cellular Immunity		
Disease	Defect	Source
AT	Prolonged survival of skin homografts	60, 12
BS	Week delayed hypersensitivity and humoral immune response	102
FA	No Reports	
XP	Negative delayed hypersensitivity	46

would produce the increasing probability of malignant transformation. They hypothesized that the underlying defect in many diseases which predisposes to lymphoreticular malignancy is the loss of either T-cell suppression or B-cell suppression or both. German (1972) noted that both the thymus and lymph nodes were deficient in lymphoid elements in persons with AT. Harden (1974) reported complete or partial absence of thymus tissue in addition to abnormalities of other lymphoid tissues such as tonsils and lymph nodes found at autopsies of some AT patients.

Defect in embryonic mesenchyme

German (1972) has theorized that the abnormalities of the lymphoid system and the immunological defect may be a result of a developmental defect of the embryonic mesenchyme. He hypothesized that would also explain the vascular anomalies.

Bloom's Syndrome

There have now been many reports of the increased incidence of infections and a disturbance of immune functions in Bloom's syndrome patients (Chaganti et al., 1974; Hutteroth et al., 1975; Schoen and Shearn, 1967). Hutteroth et al. (1975) stated that the immunologic abnormality found among persons with Bloom's syndrome in their study was not the same as that for AT or XP. But they theorized that one could still identify any biological links between "chromosome breakage", impaired immune responses and cancer. They also reported that the severity and frequency of these infections decreases with age. They noted that eight of fifty known cases who have survived infancy have developed one or more malignant tumors.

Low lymphocyte levels - PHA response - cellular proliferation

Hutteroth et al. (1975) studied four homozygotes and five heterozygotes. They found that their cells had impaired proliferative response and synthesized less immunoglobulins in five days than controls when cultured in the presence of pokeweed mitogen. They reported normal PHA response for these cells. They hypothesized that the defective immune function plus the small body size of these persons were a manifestation of a defect in some general activity such as cellular proliferation which would not be restricted just to the lymph and immune system. It could be a more specific defect of B- or T-lymphocyte function or abnormal processing or recognition of an antigen. The disparity between a normal response to PHA which suggests adequate T-lymphocyte function, and impaired response to PWM, which indicates deficient T-lymphocyte function, may show mixed lymphocyte culture to be a more sensitive indicator.

Hutteroth et al. (1975) also reported that the tissue cultures of Bloom's syndrome fibroblasts require longer periods of time between subculturing than normal and that the cultures tend to have a short life span. They concluded that the immune abnormalities of AT, BS and XP are secondary to a disturbance in cellular proliferation with a specific pattern of immune defects dependent on the nature and severity of that disturbance and the class of cells affected.

I_gA and I_gE

Several researchers have reported finding decreased levels of I_gA in these persons (Schoen and Shearn, 1967; Landau et al., 1966;

Bloom, 1975). I have found no reports on levels of I_gE for them.

I_gG and I_gM

Many papers list low levels of I_gM for Bloom's syndrome patients (Schoen and Shearn, 1967; Landau, et al., 1966; Bloom, 1975). Schoen and Shearn (1967) and Hutteroth et al. (1975) also reported low I_gG levels. An immunoflourescence study of the skin of a Bloom's syndrome patient (Conant and Crain, 1975) showed skin in the places exhibiting extreme sun sensitivity to have I_gG and I_gM deposited in subepidermal clumps. Uninvolved skin gave a negative response. (Refer to Table 5., page 71).

Cellular immunity

The four homozygotes studied by Hutteroth et al. (1975) did have delayed hypersensitivity and humoral immune response following tetanus and influenza vaccinations but they were weak. (Refer to Table 6., page 73).

Defective thymus and lymph nodes

So far there have been no reports of a defective thymus or lymph nodes in these patients as there have been for persons with AT.

Fanconi's Anemia

There is less information concerning the presence or absence of any immunologic abnormalities in FA than for any of the other three diseases discussed in this paper. Young (1971) reported that FA cultures grew more slowly than controls and were difficult to maintain.

Xeroderma Pigmentosum

Cellular proliferation

There have been several reports indicating that XP cells grew more slowly than controls (Schroeder, 1974; Haerer et al., 1969; Huang et al., 1975). Hutteroth et al. (1975) concluded that the immune abnormalities in XP patients was secondary to a disturbance in cellular proliferation.

DuPuy and Lafforet (1974) have made the most extensive report on immunological deficiencies in XP. The lymphocytes of four patients showed impairment of cell mediated immune function with negative delayed hypersensitivity, failure in dinitrochlorobenzene sensitization, and low PHA response. The authors noted that this immune defect may contribute to the high risk of malignancy among persons with XP. I_gG, I_gM, and I_gA levels were all normal. They postulated two possible reasons for the immune defect: (1) a diminished number of T lymphocytes, (2) inhibition of immunologic processes resulting from action of a blocking factor(s). They pointed out that abnormal immune surveillance could contribute to the high rate of malignancy. This theory suggests that a major function of cell mediated immunity is to eliminate malignant cells carrying "nonself" antigens. Impaired intracellular surveillance could fail to prevent malignant transformation by oncogenic agents. They concluded that the cell mediated immunity either by diminished T cells or blocking factors could also add to malignancy risk by lack of intercellular control over malignant cells. (Refer to Table 5, page 71 and Table 6., page 73).

CHAPTER III

SUMMARY OF LITERATURE REVIEWED AND DISCUSSION

Ataxia Telangiectasia

Embryonic mesenchyme

German (1972) concluded that the abnormalities of the lymphoid system and the immunological defect characteristic of AT may be the result of a developmental defect of the embryonic mesenchyme. He concluded that would also explain the vascular anomalies. Peterson and Good (1968) expressed the view that a mesenchymal defect may underlie the whole syndrome. They based that conclusion on the known fact that mesenchyme is necessary for the induction of lymphocytes; perhaps it is also necessary for the development of germ cells.

Degeneration rather than developmental failure

Contrary to the conclusions about a developmental defect are those opinions favoring the view that some type of degeneration causes the clinical symptoms of AT. German (1972) has pointed out that the degeneration theory is supported by the fact that cerebellar symptoms do not show in some patients until between four and six years.

Thymic development - immunoglobulins

It has been mentioned that many autopsies have shown abnormal thymus development in AT patients. Rosenthal et al. (1965) theorized that as a result of genetic abnormality, lymphocytes could be produced that lack normal immunologic competence caused by thymic

incompetence at a critical period of development. They further postulated that normal immunologic mechanisms apparently require some factor in addition to adequate amounts of immunoglobulins and lymphocytes that appear normal.

Telangiectasias - vascular

Peterson and Good (1968) noted there could be a vascular basis for the neuronal degeneration seen in these persons. These venous dilations must be connected to whatever basic abnormality is responsible for this whole syndrome. They also pointed out the close association between the development of the vascular endothelium and the cells of the hematopoietic system. Therefore, they concluded that, since the thymus is related to the hematopoietic system, a relationship might be assumed between that system and the vascular system.

Risk of neoplasia

The increase of clones with chromosomal anomalies observed in many of these patients so far is seen by some as a step toward neoplasia. Hecht et al. (1973) are convinced that property plus the tendency toward a high rate of chromosome breakage is intimately connected to the neoplastic risk.

Position effect

Much has been written about position effects in plants and lower animals. Some researchers believe that phenomenon may play an important role in the events that occur in the history of AT patients. McCaw et al. (1975) noted the possibility that the rearrangement which occurs in the 14_q12 band may give selective advantage to lymphocytes in disorders like AT. That advantage could be an increased rate of

cell division or a prolonged cell lifespan. Cell function or behavior could be changed through loss of genes or a duplication of genes. But changes could occur without loss or addition of genes, just through their rearrangement. One might suppose these happenings if genetic information concerning lymphoid cell growth were located on chromosome 14.

Bloom's Syndrome

Chromosome metabolism

The biochemical defect involved in Bloom's syndrome is still unknown, but some researchers suggest that it must be an enzyme concerned with chromosome metabolism. German (1972) postulated that it might be an enzyme that acts slower than normal. Such a defect might increase the frequency of openings in the DNA or could decrease their repair speed.

Schroeder and German (1974) used the cytogenetic comparison of BS and FA plus the fact that the basic defect in neither has been found to hypothesize about chromosome metabolism. They suggested that in each, the defect will be in an enzyme affecting the metabolism of the chromosome itself, but in quite different ways. Since chromosome instability exists in each of these diseases and since they both show a predisposition to cancer, they indicate, that gives adequate reason for considering the two together. Since those two characteristics are shared also by at least a good proportion of AT and XP patients, I would add them in that lumped consideration. Schroeder (1974) has noted that the cytogenetic differences in FA and Bloom's syndrome could indicate that the damage due to the genetic

defect simply occurs at different times of the cell cycle.

Cell growth

BS patients have stunted growth before and after birth. This could mean fewer or smaller cells. German (1969) has said that the evidence favors fewer. He postulated that chromosome rearrangements could result in cells incapable of many subsequent divisions.

Sister chromatid exchanges

The phenomenon of the high rate of SCEs in the cells of BS patients has been pointed out previously in this paper. Chaganti et al. (1974) noted that the study of this phenomenon in relation to the clinical features may prove helpful in understanding the biological significance of chromatid exchange in somatic cells.

DNA repair

Hand and German (1975) have pointed out that a possible explanation for the demonstrated disturbance in semiconservative DNA replication in BS patients is a decrease in DNA polymerase activity. Total absence of that enzyme would be lethal to a developing embryo; reduction, even to a mild degree, could have serious consequences. Of course, there might be a backup enzyme system if one is defective. They also postulated an alternative; polymerization could be reduced indirectly as a result of some other step in DNA synthesis, maybe one not immediately involved in chain propagation. They noted that a direct assay of the various DNA polymerases found in BS cells is needed.

German (1974) has postulated on the sequence of events in the abnormalities resulting in BS. He cites first a mutation of genetic material in all proliferating tissues. He suggests this probably occurs

repeatedly from embryonic life into adulthood mostly in tissues with the highest mitotic indices. This would include lymphoid tissues, bone marrow, and the mucosa of the alimentary tract and skin. Secondly, he theorizes a lag period of years before the third stage, clinical cancer. This seems to me to adequately sum up the other diseases being considered here as well. Of course, there could be interplay of these mutated cells with viruses.

Fanconi's Anemia

Defect occurring in embryogenesis

Althoff (1966) has suggested that since the organ systems most often found defective in FA patients undergo embryonic differentiation at a similar time (25-34 days), a single early defect could induce all the later manifestations. Such a defect would have to be quite widespread in gonadal tissues because the variation of symptoms is almost impossible to explain with a single gene mutation. He indicated that external influences are suggested by the excess of males affected.

As has been concluded about others of these "chromosome breakage" diseases, Caldwell (1969) noted that alterations in the genome by structural changes could derange metabolic processes so that cells would not survive. If enough were damaged, that could lead to organ damage; if the damage were to the stem cells, that could cause the anemia seen in this disease. He also pointed out that a delay in the appearance of symptoms could be caused by the necessity of passing through enough mitotic cycles. A long intermitotic interval occurs in lymphocytes.

Swift and Hirschhorn (1966) have also pointed out possible defects occurring in embryogenesis which could lead to all the later manifestations of FA. They pointed out that areas of rapid cell division such as the radial portion of forelimb buds might be more vulnerable to irregularities. They theorized, too, that the failure of endometrial proliferation and lack of response to gonadal hormones could result from depletion of stem cells in a highly proliferative area.

Biochemical error

In two papers, German (1969a; 1969b) reported that there is a disturbance in the hexokinase metabolism involved in FA. Others say they found normal hexokinase activity (Schroeder and Kurth; 1971; Sasaki and Tonomura, 1973). German noted that since hexokinase is the rate limiting glycolytic enzyme in blood cells, a lack of it caused a lowering of the cells' ATP level. He thinks that defect is directly related to the anemia and the increased level of spontaneous chromosome aberrations. It should be pointed out that in all the above experiments a higher than normal amount of chromosome abnormalities existed. Wolff (1969) has noted that ATP is necessary for adequate functioning of the chromosome's reunion system.

Chromosome breakage - DNA repair

Sasaki and Tonomura (1973) have found that FA lymphocytes have a specifically increased susceptibility to chromosome breakage by compounds that can introduce interstrand cross-links, such as nitrogen mustard, mitomycin C, and UV light. They interpreted that to mean that these lymphocytes are defective in a repair mechanism to tolerate cross links. They feel this defect may be implicated in these patients'

increased risk to develop malignancies. Though the mechanism is not identified yet, most mammalian cells must have an efficient repair system which tolerates DNA cross-links. FA cells are genetically handicapped. These chromosomes may be repaired by a DNA synthesis-mediated repair mechanism, but this may be error prone and be amplified into the structural abnormalities seen in cytogenetic observations.

Beard et al. (1973) have put forth another postulation concerning the chromosomes in FA cells. They feel that maybe only the cytogenetically normal lymphocytes can act as stem cells. That might explain the normal karyotypes in many bone marrow cells. This failure to supply enough functional stem cells may explain the delayed onset of critical bone marrow failure.

Xeroderma Pigmentosum

Developmental defect

Robbins et al. (1974) have noted that the finding in autopsies of de Sanctis Cacchione victims of loss of neurons may not be through atrophy but could be the result of a developmental arrest which correlates with the microcephaly often seen in these persons.

DNA repair

In 1970, Cleaver stated that XP, where malignancy had been correlated with low DNA repair ability, might illustrate a restricted mechanism of carcinogenesis possibly not to be found in other hereditary diseases with a high level of malignancies. But since then some defect has been found in the DNA repair ability of the other three diseases being compared here. It would be enlightening to know to what extent DNA repair mechanisms are involved in preventing malignancies in

persons without these diseases.

Cleaver and Bootsma (1975) have noted that DNA repair does find a logical place in the theories of carcinogenesis induced by physical and chemical agents. Unrepaired DNA might change its function and render surviving cells malignant. Another possibility is tumors developing because of overcompensating by surviving cells proliferating to replace nonviable cells. One definite point made by Reed et al. (1969) is that the connection here is not simply a lack of DNA repair ability which makes cells malignant. They noted this is proven by the fact that Hela cells do have adequate repair methods.

If damage to DNA bases accumulated during embryogenesis, those lesions would be unrepaired in classic XP. But if in de Sanctis Cacchione syndrome the UV endonuclease were functional and the exonuclease defective, there could result an accumulation of single-strand breaks. Cleaver (1974) has postulated this could lead to a loss of electrical activity and consequently play a role in development of neurological symptoms. He pointed for proof to the fact that this is what happens in rabbit retinal cells after irradiation that produces unrepairable single strand breaks (Wheeler and Latt, 1973; Wheeler et al., 1973). He concluded that two paths are open. The high level of carcinogenesis in XP could result from reduced repair because of increased genetic instability after irradiation or because of some reaction between the radiation damage and oncogenic viruses. Whether these possibilities are completely separate or part of a common process is not now known.

Lehmann (1974) has suggested that XP variant cells may not possess the ability to bypass defects on the template strand during replication. Fornace et al. (1976) concluded that a similar mechanism may account for their finding of retarded joining in the repair of pre-existing DNA. They pointed out that this defect would be expressed when two dimers occur near each other on opposite strands. According to their theory, one of those dimers would be excised normally by XP variants; but problems might arise if there were a dimer on the template. If all that is true, one might assume a mechanism for repair syntheses which could handle a lesion on the template. In XP variants, that mechanism would be defective and also caffeine-sensitive.

Lehmann (1974) pointed out that postreplication repair could well be an error-prone process which could lead to mutations and cancer. The discovery that XP variants have normal excision repair and defective postreplication repair lends support to some relationship between that repair and carcinogenesis. In addition, a connection is also suggested by the fact that caffeine affects both the post replication repair mechanism and the mutation rate. It certainly would seem that XP is a fertile field for study of radiation and chemically induced carcinogenesis.

German (1969) has listed three possibilities for the significance of genetically determined chromosome rearrangement. He noted that a tendency to rearrangement could be of primary importance if that happens in vivo. The range of clinical symptoms could depend on which cells die or are changed chromosomally and at what time in embryogenesis that occurs. For instance, if chromosomal breakage is generalized

before organogenesis in BS, that could be the cause of the total process of development resulting in fewer cells. Another possibility is that the breakage could occur in cells of one tissue type or certain organs. Then more specific defects probably would result. The pancytopenia seen in FA could be caused by breakage in hematogenic tissue. The relationship between the chromosome aberrations and immunoglobulin deficiencies seen in all these diseases remains undefined.

A second possibility pointed out by German is that a tendency to chromosome rearrangement could be the common denominator that predisposes these patients to malignancy. Or a tendency to chromosome instability could contribute to the development of aneuploid cells which seem to be more susceptible to transformation.

German lastly put forth the idea that this breakage phenomenon could happen only in vitro and have no role in vivo. Some more recent observations of chromosomal abnormalities in direct bone marrow preparations seem to be dimming that possibility though, since the chromosomes in bone marrow preparations probably went through their last cell cycle in vivo.

Chromosome Breakage

Chromosome breakage is known to be caused by irradiation, chemical carcinogens, UV light, and viruses. These four autosomal recessive diseases have spontaneous chromosome breakage similar to the physical and chemical agents listed above. Bloom (1975) noted that if there was too much chromosomal damage, the cell suffering the abnormalities probably dies. But if the damage is not excessive, the cell might survive and proliferate.

Bloom, in another report, (1972) has pointed out that single chromatid breaks tend to rejoin in time so that later cytogenetic effects of viruses might be expected to be of the chromosome type in the same way as they are after irradiation. Even so, chromatid aberrations usually seem to be more characteristic of virus effects than are the chromosome type. One argument put forth is that inhibition of DNA or protein synthesis by some agent determines whether simple breaks or complex rearrangements are produced. It has been shown that in the presence of a chemical inhibitor of DNA synthesis, open breaks are produced; but chromosomal rearrangements occur if that inhibitor is removed. Bloom (1972) has postulated that a virus could inhibit DNA synthesis. If so, later effects could be chromosomal rearrangements that took place with a chromatid reunion after the release of an initial inhibition of DNA synthesis. Inability to repair breaks could lead to their proliferation, or it might cause cell death. Some of these genes that elevate the mutation rate could code for proteins important to the chromosome's morphology directly. Or they may code for proteins which only indirectly cause a metabolic imbalance in certain cells and predispose them to chromosomal instability.

It has been shown that chromosomal breakage actually occurs <u>in vivo</u> when pernicious anemia goes untreated (Schroeder and Kurth, 1971). B_{12} and/or folic acid treatment appears to "cure" that breakage. In glutathione reductase deficiency anemia, chromosome breakage has been demonstrated <u>in vitro</u>. In that case the extent of the damage seems to be related to the stage of the disease. That has not been the case

in experiments using cells from patients with either of the four diseases compared here. Ryan et al. (1965) investigated patients with psoriasis who had been treated with folic acid antagonists. The same defect in purine and pyrimidine synthesis as occurs in pernicious anemia took place in vitro and in vivo. Two of 171 patients treated in that manner developed leukemia. Hampel et al. (1969) has shown that chloramphenicol, an anemia inducing medication used in the treatment of glutathione reductase deficiency increases chromosome breakage in vitro.

Connection to neoplasia

German (1969) pointed out that the fact that the chromosomal aberrations and an increased level of malignancy is common to FA, AT, and BS suggested that the chromosomal changes, regardless of the cause, could be of fundamental importance in the pathogenesis of neoplasia. It would seem that the literature reviewed in this paper would indicate the addition of XP to that list. Schroeder and Kurth (1971) noted that as a consequence of chromosomal breakage, a change in the genetic material itself may create primary yet unknown conditions for malignant growth. They hypothesized that that might partially explain the correlation between spontaneous chromosome breakage and leukemia. They also pointed out that even though the chromosomal abnormalities often lead to a shortened life span for a cell, some do survive. Perhaps cells with invisible structural anomalies like point mutations, gene deletions and duplications which result from breakage-reunion continue to divide unrestrained. As a result that tissue which has an increased spontaneous mutation rate

plus aberrant cell lines could carry the seed of malignancy.

Every liver carcinogen studied thus far, with the possible exception of ethionine, induces in a matter of hours measurable single-strand damage to liver DNA in vivo as monitored by centrifugation in an alkaline sucrose gradient.

Hirschhorn et al. (1963) have demonstrated that the presence of extra genetic material may upset the genetic balance and create a situation which, due to instability, is susceptible to neoplastic transformation by viral or other agents. More evidences of genetic imbalance in patients and relatives in diseases in which the chromosome material may be damaged may help to clarify any possible relationship between genetic alteration and neoplasia.

One question continues to persist: In those tumors with chromosomal abnormalities, did the chromosomal change result from the neoplastic process or did the chromosomal abnormalities cause the development of the tumor? German (1972) strongly favors the view that chromosomal abnormalities occur in a cell that had originally converted to cancer and that the mutation is an integral part of the conversion. Perhaps one point that adds insight to this problem is the fact that an increased tendence to leukemia is found also in Down's syndrome, Klinefelter's syndrome, and other trisomies and translocation.

Schroeder (1974) surmised that the oncogenic implications of X-ray induced chromosome breakage, of UV-light induced damage in the DNA of XP patients, and of the spontaneous chromosomal instability in these hereditary diseases is one and the same. Chromosomal instability seems

to be characteristic of cell damage, from whatever cause, from which neoplasia may result. The incidence of cancer is about ten percent in FA and BS, perhaps less in AT and one hundred percent in XP.

Bloom (1975) noted that the exposure of these essentially mutant, chromosome damaged cells to oncogenic viruses causes a high rate of malignancy in the skin as in porokeratosis of Mebelli and XP or in lymphoid and other tissues in BS, FA, and AT. The question still remains -- what is the molecular basis of transformation?

Theory of carcinogenesis

One theory of carcinogenesis put forth by Comings (1973) is that cancer acts like a recessive trait. R genes are normally suppressed except during embryogenesis. Mutation of a regulatory gene releases the suppression and transforms the cell. Perhaps the inherent tendency towards chromosomal breakage and rearrangement makes these cells more susceptible to this transformation.

Lysosomes

One theoretically attractive hypotheses given to explain the primary mechanism of the action of physical or chemical agents on chromosomes involved enzyme release (Bloom, 1972). Later it was extended to involve a release of lysosomal nucleases by chromosomolytic agents followed by digestion of chromosomal nucleoproteins by those nucleases. Alison and Paton (1965) suggested that DNAase released by the lysosomes could enter the nucleus to produce chromosomal damage. They interpreted their experiment as representing the action of lysosomal DNAase released by exposure of photosensitized lysosomes to light. Later DNAase penetrated the nucleus and caused scission of two strands

of DNA. They pointed out that viruses, UV light, X-ray, and chemical carcinogens also bring about the release of enzymes from lysosomes. Other reports are contrary to those ideas. For viral produced aberrations, that hypothesis does not seem feasible. Agents which rupture lysosomes, like vitamin A and alcohol, did not produce a chromosomal damage. Much controversy over the lysosome theory still continues.

Cell Cycle

In each of these four diseases, there have been reports of difficulty in getting enough metaphases to study and the requirement of a longer length of time before harvesting. These facts seem to point to a slowed cell cycle.

Heterochromatin

It would be of interest to determine why certain chromosomes and certain areas of chromosomes seem to be preferentially damaged. One intriguing speculation involves the areas of heterochromatin on human chromosomes. Chromosomes in man that have nucleolar organizers are all of the D group chromosomes, numbers 13, 14, and 15 and the G group, 21 and 22. In mitosis these are frequently near the chromosomes with secondary constrictions which are numbers 1, 2, 18, and 1 C group.

Interesting observations were pointed out in a paper by Yunis and Yasmineh (1971) concerning the phenotypic effects of defects of autosomal chromosomes which involve heterochromatic chromatin. They noted that symptoms were largely nonspecific and showed a wide variation and overlap among patients with the same aberration. These symptoms usually involved physical and mental retardation and gross

malformations of the extremities and internal organs. They mentioned that the most distinct defect seemed to be interference in early development of many organs, plus a decrease in the number of cells per organ, and general disarrangement of cell metabolism. They concluded that it might be possible for some oncogenic viruses to insert themselves in the heterochromatic regions and cause them to replicate early and upset the chromosome's function. Many of the abnormalities in chromosome structure may involve heterochromatin due to its vulnerability to breakage by mutagens, its repetitive nature, and its tendency to form aggregates during the cell cycle. Likenesses between these statements to comments made previously in this paper concerning the symptoms of these four diseases are obvious. There is evidence in rats for non-random distribution of chromosome aberrations, especially in heterochromatic areas (Natarajan et al., 1973; Sugiyama, F., 1971).

Amniocentesis

One advance that may prove important to the elucidation of the basis for these "chromosome breakage syndromes" is amniocentesis. It should be possible with that process to determine precisely how soon in pregnancy the abnormalities appear and in which tissues (Bloom, 1972).

German (1969) said that he did not see much similarity among FA, BS, and AT except growth retardation after birth and a tendency to malignancy. Since then, as has been noted previously in this paper, more careful cytogenetic analyses have been made. It definitely seems that a consideration of the similarities among these four diseases, the chromosome abnormalities, immunoglobulin disturbances, growth

retardation, malignancy, hypogonadism, slowed cell cycle, and DNA repair defects may easily provide starting points for investigations of cell biology and oncology. It has been pointed out that the gene causing XP is an abnormal allele at a locus coding for one of the most important enzymes involved in the chromosome's metabolism (German, 1972). Perhaps the other diseases could be caused by abnormal alleles coding for equally important products that are necessary for maintenance of the genetic apparatus.

CHAPTER IV

CONCLUSIONS

Clinical

1. Reports indicate that all four diseases have in common the following clinical characteristics:
    a. Hypogenitalism
    b. Stunted growth (either intrauterine, post partum or both)
    c. Hypo and/or hyperpigmentation of the skin
    d. An increase in the incidence of malignancies, especially leukemia. The majority of these have been reported to occur in tissues with high mitotic indices.
2. Telangiectasias have been reported in the various areas of the body in AT, BS, and XP, but not in FA.

Cytogenetic

1. There seems to be ample evidence of chromosome aberrations in XP cells to include it in the category of "chromosome breakage syndromes".
2. There is proof of the existence *in vitro* of abnormal clones in all four diseases.
3. The increased level of chromosome instability apparent in karyotype analysis has become a part of the diagnosis

in BS and FA, but is not always a constant finding in AT and XP.

4. For all four diseases there is no apparent correlation in sister chromatid exchanges, incidence of chromosomal aberrations, and level of DNA repair.
5. The chromosome abnormalities are non-random. There is instead a very significant difference between the observed value and those that would be expected if all the chromosomes were involved in aberrations randomly.
    a. In AT, group D chromosomes are affected much more than would be expected, and where banding has been done there appears to be non-random involvement of a specific site, namely 14_q12.
    b. In BS, chromosome groups E and F are more frequently affected, and group D is involved much less than would occur randomly.
    c. In FA, chromosomes in the C group are more frequently affected.
    d. In XP, there are so few reports that it is not possible to make a definitive statement concerning the specific chromosomes affected by abnormalities. (Refer to Table 15, page 102).

TABLE 7.

Chi Square Analysis			
Chromosome Group	Observed	Expected	Difference
AT			
A	71	77.404	-6.404
B	42	38.353	3.647
C	101	114.362	-13.362
D	157	126.914	30.086
E	28	41.142	-13.142
F	15	27.893	-12.893
G	52	41.142	10.858
X	4	2.789	1.211
BS			
A	28	21.739	6.261
B	6	10.772	-4.772
C	36	32.119	3.881
D	12	35.644	-23.644
E	23	11.555	11.445
F	22	7.834	14.166
G	5	11.555	-6.555
X	0	0.783	-0.783
FA			
A	11	10.705	0.295
B	5	5.304	-0.304
C	27	15.816	11.184
D	10	17.552	-7.552
E	7	5.690	1.31
F	3	3.858	-0.858
G	2	5.690	-3.690
X	0	0.386	-0.386
XP			
A	1	1.153	-0.153
B	2	0.571	1.429
C	0	1.703	-1.703
D	3	1.890	1.11
E	1	0.613	0.387
F	0	0.415	-0.415
G	0	0.613	-0.613
X	0	0.042	-0.042

6. All four appear to have some defect in DNA repair mechanisms.
7. Analysis of variance of types of chromosome aberrations grouped according to excisions, repairs, and spindle defects showed the F value was significant at the .05 level for AT and FA. There were significantly more excision type aberrations than repair or spindle defects. For BS and XP that value was not statistically significant. It is suggested that the F value for XP was not significant because of the few reports available. (Refer to Table 8-15, pages 99-102).

Immunologic

1. All four diseases have been reported to have either impaired response to PHA or slower cell growth than controls.
2. All of them except FA have been reported to have impaired cellular immunity.
3. An increased incidence of infection has been reported for all the diseases, but only AT and BS have shown low levels of certain immunoglobulins.

TABLE 8.

Mean Percent of Chromosome Aberrations in Ataxia Telangiectasia		
Aberration	Number of Reports	Percent
Excisions	5	0.54
Repairs	6	0.13
Spindle Defects	4	0.04

TABLE 9.

Analysis of Percent of Aberrations in Ataxia Telangiectasia				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Aberrations	2	.69	.34	6.38*
Error	12	.65	.05	
Total	14	1.34		

* Significant at the .05 level

TABLE 10.

Mean Percent of Chromosome Aberrations in Bloom's Syndrome		
Aberration	Number of Reports	Percent
Excisions	13	0.25
Repairs	19	0.16
Spindle Defects	12	0.11

Table 11.

Analysis of Percent of Aberrations in Bloom's Syndrome				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Aberrations	2	.14	.07	1.53
Error	41	1.84	.04	
Total	43	1.98	.05	

Table 12.

Mean Percent of Chromosome Aberrations in Fanconi's Anemia		
Aberration	Number of Reports	Percent
Excisions	23	0.37
Repairs	13	0.17
Spindle Defects	7	0.13

Table 13.

Analysis of Percent of Aberrations in Fanconi's Anemia				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Aberrations	2	.48	.24	3.3*
Error	40	1.80	.45	
Total	42	2.29	.54	

* Significant at the .05 level

Table 14.

Mean Percent of Chromosome Aberrations in Xeroderma Pigmentosum		
Aberration	Number of Reports	Percent
Excisions	0	
Repairs	1	0.91
Spindle Defects	6	0.31

Table 15.

Analysis of Percent of Aberrations in Xeroderma Pigmentosum				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Aberrations	1	.31	.31	3.3
Error	5	.48	.10	
Total	6	.79		

CHAPTER V

SUMMARY

There is the definite possibility that some defect occuring during the development of the embryonic mesenchyme may underlie the array of symptoms in all of these diseases. Reports brought together in this paper that show hypogenitalism in all of them lends support to that theory. The fact that each of them show increased spontaneous chromosome aberrations and have a higher than normal level of malignancies seems to support the fact that chromosomal instability is an integral part of the development of neoplasia in these diseases.

The combination of a statistically significant increase in excision type chromosomal defects in addition to direct reports of DNA repair defects indicates support in the evaluation of this author for the theory that there is some problem in that mechanism in all four of these diseases. For even if some metabolic imbalance causes these chromosomes to break more easily than normal, the situation might be rectified if the repair mechanisms were working effectively.

Impaired cellular proliferation is certainly indicated as a common denominator for the problems in all these diseases. This seems to indicate some similar basic metabolic defect underlying each of them. Perhaps it is in the metabolism of the chromosomes themselves. Or perhaps whatever causes the increased spontaneous chromosome instability also contributes to the defect in cellular division. The

stunted growth for all four pointed out in this paper could be the clinical manifestation of the proliferation defect.

After reviewing the literature and making detailed comparisons of these four "chromosome breakage syndromes", several avenues for future research become apparent. It appears that one particularly enlightening area would be to investigate why there is decreased cellular proliferation in these diseases and if there is any involvement of a membrane abnormality. It should also prove helpful to compare specific spontaneous chromosome break points in the four diseases to the exact breaks caused by chemicals or the attachment points of certain viruses. Cytogenetic research using chromosome banding techniques is recommended in order to identify the specific loci on specific chromosomes where the breakage occurs with greatest frequency. When more precise determination of breakage points is carried out, it may well be found that a correlation exists between them and heterochromatic areas.

There seems to be ample evidence for grouping these four diseases together. First, there are reports noted in this paper of an increased level of chromosome aberrations in XP, already known to be deficient in DNA repair, in addition to DNA repair defects reported here in AT, BS, and FA, already listed as "chromosome breakage syndromes". Second there are the several common clinical symptoms and immunologic defects pointed out in this report.

Even though AT, BS, FA, and XP are quite different in many ways that have been pointed out in this paper, there are also many likenesses. And there seem to be enough of these similarities to

warrant further investigations into the molecular processes responsible and their connection to the development of malignancy. "Chromosome breakage syndromes" would seem to offer fertile fields for additional knowledge of chromosome behavior, immunologic deficiencies, DNA repair mechanisms, and their relation to oncogenesis.

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Appendix I. Percentage of Types of Chromosome Aberrations

Breaks	Gaps	Fragments	Endo-reduplications	Polyploidy	Dicentrics	Exchanges	Rearrangements
AT							
13		21	1		13	5	49
78		90	9		5	3	5
70							
BS							
16	7	2	13	5	1	4	3
37	3	76	18	9	24	89	6
9	37	34	9	10	8		
34		3			1		
		37			6		
		32			10		
FA							
70	21	3	0	8	9	13	9
29	48	2	26	11	2	5	2
42	42	26	19		7	67	8
34	34	9	16		11	20	63
64	18	11	14			7	
14	51	41					
78	55						
79							
59							
20							

Continued

Appendix I. Percentage of Types of Chromosome Aberrations

Breaks	Gaps	Fragments	Endo-reduplications	Polyploidy	Dicentrics	Exchanges	Rearrangements
XP							
				1	91		
				31			
				15			
				1			
				72			
				63			

In each report, the number of aberrations was totaled. Each figure listed here is a percentage of that total figure.

Appendix II. Distribution of Aberrations Among Chromosomes

Chromosome Group and Number	AT	BS	FA	XP
A			5-3-100	
1	4-20-44	16-21-47	5-3-7	14-1-2
2	5-24-89	1-1-4	3-2-7	
3	6-27-75	5-6-17	5-3-8	
B	9-42-76	5-6-11	8-5-9	29-2-4
C	20-93-60	27-36-23	40-26-17	
6	4-2-67		2-1-33	
7	1-5-100			
11	2-1-100			
D	24-111-82	9-12-9	14-9-7	43-3-2
13			2-1-100	
14	10-46-100			
E	4-20-54	11-14-10	5-3-20	
16	2-8-40	7-9-45	5-3-15	
17			2-1-50	14-1-50
F	3-15-38	17-22-55	5-3-8	
G	11-52-88	4-5-8	3-2-3	
X	1-4-100			

This appendix is a more detailed picture of Table 2, p. 30. When a report simply listed the chromosome group that contained an abnormality, it is listed that way. When a specific chromosome was mentioned, it is designated. Chromosome numbers not listed here were not specifically noted in any report.