CHROMOSOME ABERRATIONS IN LEUKEMIA

1973-1974

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INTRODUCTION and LITERATURE REVIEW

To attempt to identify one particular cause of leukemia by studying several patients over a period of one year is an impossible task. However, an appreciation for the large number of variables that exist in such an analysis may be gained.

Leukemia is a group of disorders in which "proliferation, maturation, and release of leukocytes is no longer kept within bounds by normal physiological mechanisms of control."¹ Leukemia, which may produce qualitative as well as quantitative changes in white blood cells, has received considerable attention as a killer of man in the last twenty years. Many theories have been proposed as to the causes of this disorder. Among suggested causes are environmental contaminants, viruses, and physiological contaminants such as medications. But in the last decade, because of the putative relationship between chromosomal anomalies and the malignant cells of cancer, interest in chromosomal aberrations as possible causes of leukemia has increased. Thus far, only chronic granulocytic leukemia has been associated with a particular chromosomal alteration—-that being the Philadelphia chromosome.² The remaining types of leukemia have yet to be linked consistently with a particular abnormality. Therefore, it was interesting and enlightening to compare chromosomal abnormalities of several leukemia patients at the Bell Memorial Hospital.

Cancer is an irreversible process, that is, cancer cells can not transform back to normal cells. Also division in cancer cells results in subsequent formation of more cancer cells. Therefore cancer might be a "molecular biological disease, i.e., one involving abnormal genetics and further that the process is continued as a kind of mutation."³ To account for the genetic origin of cancer cells, four concepts have been hypothesized.

1) All cells contain cancer genes, i.e., genes which when expressed cause cancer. In non-cancer cells these genes are repressed.
2) Either at an early stage or following some type of viral or other infection, lysogeny (complete or partial) occurs as a result of which "cancer genes" or growth genes that are normally portions of viral genomes become incorporated into the cancer cell genome and are expressed, regardless of whether other viral genes are functional.

3) Cancer results from one or more "somatic mutations".

4) Cancer results from epigenetic (non-gene) changes in cells.

A chromosomal abnormality existing in leukemia is probably caused by a mitotic malfunction. According to Peo C. Koller, "the significance of mitotic disorders lies in the fact that 1) they indicate the metabolic instability of tumor cells which are easily affected by environmental factors and 2) they represent the machinery by which cellular heterogeneity is introduced, maintained, and increased in tumor tissues." Several mitotic malfunctions have visible effects on the chromosomes of leukemia victims. Dicentric chromosomes, binucleate cells, and endoreduplication may be observed in some cells. Also abnormal cells are present due to fragmentation, stickiness of chromosomes, non-disjunction, and lagging.

Fragmentation is probably the most common chromosome anomaly observed in cancerous tissue. Chromosome fragments are scattered throughout the cell. The fragments usually disintegrate in the cytoplasm because they do not possess a centromere and consequently cannot move to opposite poles at anaphase, thus resulting in their not being incorporated in the daughter nuclei at telophase.

Dicentric chromosomes are the result of breaks in two chromosomes followed by the broken parts being joined in a new arrangement that results in a single chromosome with two centromeres. The remaining ends form acentric fragments. Koller states that "if the region between the two centromeres is very short, the dicentric chromosome can be incorporated into one nucleus, in which case it will appear again at the next mitosis."6

"Stickiness" is evidenced by the chromosomes clumping together at metaphase. Lagging of chromosomes sometimes occurs if chromosomes are on the outside of the mitotic spindle. Eventually the chromosomes will appear as fragments. Non-disjunction, which also may be caused by stick-
ness, results in one daughter cell containing more chromosomes than normal and another containing fewer.

The irregular distribution of chromosomes into several nuclei is brought about by a multipolar mitotic spindle. The nuclei tend to be different sizes and are often held together by bridges. Cells with two distinct nuclei have a complete cell membrane forming between daughter cells. But when the nuclei divide the chromosomes mix. Cells now contain the hap number of chromosomes. Repetition of the process results in "giant" cells with hundreds of chromosomes.

The above malfunctions in the mitotic process are possible causes of the chromosomal aberrations that exist in leukemia patients.

MATERIALS and METHODS

The present study of chromosomes was conducted on peripheral blood and bone marrow samples of leukemia patients at Ball Memorial Hospital. Standard leukocyte and bone marrow procedures were used. After culturing, slides were prepared which in turn are analyzed to establish a modal chromosome count. Visual scanning for abnormalities was done using a light microscope and photographs of unusual cells were prepared.

Chromosome technique: Chromosome culture medium purchased in one hundred ml bottles from GIBCO was used. This medium is pipetted in 5 ml aliquots into sterile screw cap tubes and the tubes are taped shut. This is important to maintain pH. However, if the medium is pink upon thawing, a drop or two of .01 N HCl will return it to its original yellowish color, which indicates the proper pH. The medium is then frozen until required.

A sample of four or five ml of peripheral blood or bone marrow is drawn in a heparanized tube. Two culture tubes are set up from this sample by dropping four or five drops of whole blood into the medium.

After the blood is placed into the tubes of medium, the tubes are slanted in the incubator for seventy two
hours at thirty seven degrees Centigrade. Since it is difficult to maintain thirty seven degrees absolutely, it is better to fluctuate a few tenths of a degree below rather than above the optimum thirty seven degrees.

At the end of seventy two hours the cells are harvested following a modification of the Rhode Island Hospital Method.

1. Add 0.5 ml concentrated colchicine to each vial 1½ hour before harvesting, to prevent formation of the spindle fibers.

2. Add 5 ml of N saline by pipette and mix.

3. Spin in centrifuge at 300 rpm's for 10 minutes.

4. Evacuate the tube down almost to the bottom using an aspirator. Carefully mark each pipette to correspond with the different patients. Add 5 ml hypotonic KCl solution and suspend cells with the pipette. Leave for four minutes and then spin at 800 rpm's for 10 minutes.

5. Evacuate down to the bottom (it is important to do this as quickly as possible to all tubes so that the cells are not exposed to KCl too long). Add 1 ml of fixative (one part glacial acetic acid to three parts 200 proof ethanol) to the tube and mix the cells immediately, getting as fine a suspension as possible. Spin at 800 rpm's for 10 minutes and once again evacuate down to the bottom. Add 3 to 5 ml of fixative and centrifuge. Repeat two times.

6. Make one slide from each culture. Air dry. Stain eight minutes.

Colchicine is prepared by adding 1 ml concentrated colchicine to 100 ml Earle's Salt solution. This gives a dilution of 1 ml = .0005% colchicine.

KCl hypotonic solution is .075M and is kept at 37 degrees centigrade in the incubator.

For staining slides Giemsa stain is used. Commercial stain may be used or stock Giemsa stain may be prepared by placing one vial powdered Giemsa (0.5 gm) into an Erlenmeyer flask with 30 ml of glycerine. This is allowed to dissolve in a water bath (55-60 degrees for 1½ - 2 hours). Add 45 ml of acetone-free alcohol and store in amber bottle.

Before making slides dilute stock Giemsa as follows:
24 ml of water
3 ml phosphate buffer at pH 6.1.
3 ml stock Giemsa stain.
Filter through Whatman #1 filter paper.
Phosphate buffer (10%)
73.5 ml KH₂PO₄ (1M)
26.5 ml K₂HPO₄ (1M)
Adjust to pH 6.1 with 1N KOH.

Priest gives some additional advice on culturing chromosomes:

For direct cytogenetic examination of tissues, the cells to be studied must be dividing rapidly at the time of sampling. In some cases, cell dispersion creates a problem if the tissue is dense. A short term cell incubation may be employed 1) to increase the number of dividing cells and 2) to allow time for an in vitro colchicine effect. However, any incubation step inserted between obtaining the tissue and examining the chromosomes raises the criticism that artifacts may be introduced.

Chromosome medium 1A contains phytohemagglutinin.
This substance acts as a stimulant to cell proliferation. However, it does not intensify growth of malignant cells; sometimes, in fact, it may even retard growth. Dr. Palmer, geneticist at Indiana University Medical School, suggested to use medium without phytohemagglutinin to replicate the in vivo condition in vitro. Only two patients were cultured in this manner and the results were very poor.

Photographic technique: Photos were taken through the microscope lens of a Leitz light microscope using a Leitz camera. Magnification was 1000x using the oil immersion objective. High contrast pan film was used along with a blue filter. The shutter speed was one half second and the light was maximum.

The negatives were processed onto single weight polycontrast P paper. The photographs were then used to prepare karyotypes, an arrangement of chromosome pairs in the metaphase stage in a standard array to study the comparative size, shape, and morphology of chromosomes.
### EXPERIMENTAL DATA

#### TABLE 1  CELL COUNTS

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<th>$\text{h}_6$</th>
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myeloproliferative syndrome, metastatic carcinoma - undifferentiated
DISCUSSION

The difference between acute leukemia and chronic leukemia is in the degree of maturation of the cells. In acute leukemia the new cells are dispersed from their zone of origin into the circulatory system so rapidly that there is not enough time for complete cell development. The onset of acute leukemia is rapid; the course is short and severe with such dramatic symptoms as fever, anemia, haemorrhage, tissue infiltration, buccal ulcerations and respiratory infections. Without treatment death results in three months.

In chronic leukemia the transition from a normal stem line to a malignant stem line is much slower. The disease progresses slowly, and remains mild. Symptoms include a pairless splenomegaly or lymphatic glandular enlargement. The patient can usually survive three to five years with this condition.

Chromosomal variation in leukemia really became of interest to geneticists when it was observed that acute leukemia is three to fifteen times more frequent in individuals with the chromosome aberration causing Down's Syndrome. Later it was found that patients in a "pre-leukemic" condition, who had in their bone marrow a clone of cells with chromosome abnormalities were very much more likely to develop leukemia than those without chromosomal aberrations. However, "if the disease does not manifest itself within a month of diagnosing the chromosome anomalies, the leukemia risk remains the same as in comparable patients without chromosomal changes."9

The present investigation allowed for the observation of chromosomal variation in four types of leukemia: acute granulocytic leukemia or chronic myeloid leukemia, acute lymphatic leukemia, chronic lymphatic leukemia, and chronic granulocytic leukemia.

The most consistent chromosome variations were observed in patients with acute leukemia.

Patients with acute leukemia can be separated into three groups: patients with apparently normal chromosomes, patients with a specific chromosome abnormality,
and patients in which there are a wide variety of chromosome abnormalities which do not apparently conform to a chromosomal pattern. Such changes include numerical aneuploidy and polyploidy, structural re-arrangements and the finding of abnormal marker chromosomes of unknown origin.

Three of the twelve patients studied were diagnosed as having acute granulocytic leukemia or chronic myeloid leukemia. Each of the three patients corresponded to one of the above categories in their karyotypic picture.

**Patient A** - wh: Negro male; 33 years of age; admitted because of anemia and a "preleukemic condition". He was diagnosed as having acute granulocytic leukemia within two weeks. After diagnosis patient A was placed on COAP treatment which is dosages of cytosan, cytoxan, oncocin, and prednisone. Some radiation therapy was also administered. No apparent remission was noticed. The patient succumbed within six months. Only one peripheral blood sample for chromosomal analysis was obtained. The cell growth of the patient was very limited; however, the metaphases that were present were excellent for analysis. The chromosomes were long, extended, and almost filamentous. A type of "threading" was visible (Figure 1) but was not pronounced enough to be classified as fragmentation. This threading occurred predominantly in the short arm portions of A-group chromosomes and the long arm portions of C-group chromosomes. Large satellites were visible in the D and C-group chromosomes. The presence of these large satellites led to the suspicion of the predisposition of cancer.

As the acrocentric chromosomes with satellites contain the special "molecular organizer" segment, translocations involving these chromosomes may lead to a progressive loss of such regions. Early cytochemical and cytological studies of cancer cells drew attention to the possible role of the nucleolus in the initiation of malignant cell behavior (Koller-1943).

**Patient B** - ca: Caucasian male; 77 years of age; admitted because of swelling and eruption of legs plus a skin rash on the lower extremities; complained of back pain. He was diagnosed as having a relapse of acute granulocytic leukemia.

Patient B was placed on the COAP chemotherapy program in January 1973. By April of 1973 the oncogenic portion of
COAP was dropped because of neuromuscular toxicity. White
blood cell count was 3300 with 72% granulocytes. The
platelet count ranged from 93,000-110,000. Prior to this
time, January 1973, patient B received external radiation
therapy for a chomatous mass at the nape of the neck.
This treatment induced leukopenia and anemia of a mild
degree.

Several peripheral blood samples were taken from
patient B for chromosomal analysis during the progression
of his disorder, but it was not until July, 1973, that a
sample was obtained which provided adequate growth. At
this time the patient was receiving dosages of cytoxan,
cytoxan, prednisone, and dalmane.

Chromosomal analysis revealed an 80 percent normal
modal count with 20 percent aneuploidy. It appeared that
most of the marker chromosomes were in the C group although
not consistently the same chromosome.

Approximately 50 percent of all patients with acute
granulocytic leukemia who have been studied have
had aneuploid stem lines in marrow preparations.
These aberrant clones may be hypodiploid, hyper-
diploid, or pseudodiploid. Specific stem lines are
generally quite consistent in leukemic cells from
one individual. Although specific karyotypic alter-
ation may disappear during remission, the
original stem line may reappear during successive
relapses.

Sandberg and his co-workers also support this viewpoint.

Sandberg and co-workers (1968) analysed the chromo-
some constitution in 219 cases of acute leukemia, and
found a very variable pattern: nearly 50 percent of
the cases had aneuploid modal chromosome numbers
ranging from hypodiploidy to hypertetraploidy, while
the others apparently had diploid modes. These in-
vestigators also found that in cases of acute leukemias
with the same modal chromosome number, the karyo-
types were different. Comparative karyotypic analy-
yses of many cases seem to indicate that in acute
leukemias certain autosomes of group C are more fre-
quently affected than other chromosomes.

Patient C - sw: This patient exhibits the third condition
which may exist in acute granulocytic leukemia which is
a specific chromosomal abnormality. In most cases this is
the Philadelphia chromosome.

Nowell and Hungerford (1960) demonstrated a specific
chromosome abnormality in chronic myeloid leukemia,
and this was rapidly confirmed. The abnormality found in blood and marrow cells in the great majority of CML patients is a small chromosome replacing one of the G-group chromosomes and resulting from a presumptive deletion of about one-half to two-thirds of the long arm (16,XX or XY, Ga-). This chromosome is also known as the Philadelphia or Ph' chromosome. 14

Hemerton further states that this Ph' chromosome is closely related to the etiology and progression of the disease. The Ph' chromosome is the result of a specific chromosome rearrangement in a myelogenous stem line. The rearrangement must be caused by a mutagenic agent in adult life as is shown by the case of monozygotic twins in which only one member of the pair had the Ph' chromosome. 15

Patient C was a Caucasian female in her early 30's. No bone marrow or peripheral blood samples were obtained for analysis until she had entered the blastic crisis stage. At this time she was also receiving radiotherapy and experimental chemotherapy. Consequently, growth was limited and distorted. Fifty percent of her blood cells exhibited the Ph' chromosome. In other studies the overall frequency is about 80 percent. 16

That further changes (other than just the Ph' chromosome) appear in blastic crisis may be substantiated.

The terminal stage of CML is usually associated or preceded by a blastic phase in which the chromosome pattern of leukemic cells with Ph' undergo further changes. The chromosomal changes are not necessarily identical in different patients but have a tendency to form a uniform pattern. The appearance of two or more Ph' chromosomes usually coincides with the onset of the blastic phase (Pedersen, 1968). Hyperdiploidy is the most common type of aneuploidy which appears in marrow cells during blastic crisis. 17

The chromosomes of patient C also exhibited considerable breakage and secondary constrictions (Figure 2). These variations also appear to be consistent with past research.

The acute blast transformation in patients with CML is usually accompanied by considerable increase in karyotypic instability. During the chronic phase, serial studies of blood or bone marrow usually show a fairly constant karyotype although aneuploid clones have been reported which often contain more than one Ph' chromosome. Treatment frequently reduces the proportion of Ph' positive cells. However with the onset of the acute phase of the disease, aneuploidy with loss or gain of chromosome unrelated to the Ph'
becomes frequent. Pedersen has suggested that the progression of the disease from the chronic to the acute stage is dependent to a large degree on the development of aneuploid clones. He also considered hyperdiploid Ph'-positive cells to be selected at the expense of other cell types and that chemotherapy might increase the selection of these more resistant cells.\textsuperscript{18}

It is evident however, that the Ph' chromosome which involves all marrow cells, i.e., normoblastic, granulocytic, and megakaryocytic, persists throughout the course of the disease regardless of the general response to therapy. However, the other chromosomal changes consequent to blastic crisis may disappear in response to therapy.

An interesting observation is the relation between therapy and whether the patient is Ph' positive or negative.

Patients with Ph' positive CML respond to therapy more readily and survive for a much longer period of time than Ph' negative patients with the same type of leukemia. It is possible that Ph' negative CML may be a different entity than Ph' positive CML and more akin to acute leukemia, particularly myeloblastic leukemia than to CML.\textsuperscript{19}

A second type of acute leukemia is acute lymphocytic leukemia. Two patients were studied with this disorder.

Patient D - rr: Male, Caucasian, six years of age. He was diagnosed as having acute lymphocytic leukemia (ALL) at the age of four. At that time he was placed on the therapy program outlined by Donald Pinkle. The program consists of three phases. I. Remission Induction Phase - Remissions are induced with Vincristine 1.5 mg per m\textsuperscript{2} of body surface area (BSA). One dosage weekly Iv. Also prednisone is administered 40 mg/BSA daily in three to four divided dosages. This is continued for four weeks. If complete remission of the marrow occurs at this time, the patient is entered into phase II. If not complete, phase I is continued for another two weeks. II. Intensive Chemotherapy is initiated after complete remission of the marrow. This consists of methotrexate 10 mg/BSA per day Iv x three days followed by mercaptopurine 1 gm/BSA/day Iv x three days followed by cyclophosphamide 600 mg/BSA Iv x 1 day. Simultaneously prednisone is tapered off and discontinued. III. Phase three is a continuation of
therapy. Upon completing the intensive phase period of therapy a two week rest period during which no medications are given is allowed. The continuation consists of mercaptopurine 50mg/BSA/day orally; one weekly injection of Vincristine 1 mg/BSA; methotrexate 20 mg/m²BSA; and cyclophosphamide 200 mg/m²BSA/week IV. 20

Patient D received twelve months of remission on therapy.

**Patient E - age:** Female, Caucasian, forty five years of age. She was diagnosed on May 22, 1973, on the basis of a bone marrow sample in the following manner: a) ALL in relapse; b) mild normocytic, normochronic anemia secondary to ALL and treatment; c) peripheral leukopenia and granulocytopenia secondary to ALL and treatment; d) peripheral thrombocytopenia and megakaryocytopenia secondary to ALL and treatment. By the end of May there was a worsening of the leukemic condition and an advanced degree of relapse. She was placed on COAP treatment but multiple efforts produced only partial remission.

On July 19, 1973 she was re-admitted to the hospital for a course of "AUR" chemotherapy which consists of 6MP 850 mg BID x 3 days; MTX 17.0 mg IV x 3 days; and cyclophosphamide 1000 mg IV x 1 day. Bone marrow studies indicated hypopcellularity. It was during this time that chromosome studies were performed.

The karyotypes of patient D were very varied. Many cells were hypodiploid with the missing chromosomes being in the G-group. One tetraploid cell and one hyperdiploid with 83 chromosomes were also observed. However several of the cells exhibited double minute chromosomes (DMS) (**Figure 3**). Double minute chromosome formation originates by the breakdown of existing chromosomes. No marker chromosomes characterizes cells containing DMS. Sandberg feels they are of neurogenic origin. The number of double minute chromosomes per cell is said to vary from one to more than fifty. The size and shape of the DMS are relatively constant. They are round and small with a diameter smaller than the width of a chromosome. 21 Eleven percent of the cells in
patient D exhibited DMS but the maximum number of double minute chromosomes per cell was five.

Patient D exhibited aneuploid and tetraploid cells with no specific variation. This is also consistent with past findings.

Since June 1972, we have been studying the chromosomal constitution of cells in the bone marrow and peripheral blood in children with acute leukemia at diagnosis, and throughout the course of the disease. Recently developed techniques for more precise identification of chromosomes' structure are being used in a search for minimal deviation karyotypes. In acute lymphoblastic leukemia we classify the patients into four classes according to the cytogenetic analysis at diagnosis: 1) predominantly hyperdiploid (eight cases); 2) normal cells together with an occasional hyperdiploid or pseudodiploid cell (ten cases); 3) hypodiploid (one case); and 4) normal (one case).

During the course of treatment in ALL, the marrow does not revert immediately to cytogenetic normality. It is essential to examine the patient before any treatment is given, in order to know the composition of the original cell population. At present we cannot say whether hematological relapse is associated with the reappearance of a population of cells chromosomally related to those found at diagnosis.22 Very high chromosomal numbers (above 80) occur more frequently in ALL than in acute lymphoblastic leukemia. The appearance of aneuploid cells can be used for diagnostic purposes.

The aneuploid cells in acute leukemia may disappear completely, particularly in acute lymphoblastic leukemia, when the disease responds to therapy. When relapse occurs, the leukemic cells reappear with the same karyotypic picture that characterized them prior to relapse and this cycle may repeat itself several times. In fact, the appearance of occasional metaphases with an aneuploid chromosome constitution in an otherwise normal marrow may herald the relapse of acute leukemia and this may prove to be a valuable therapeutic index in chemotherapy.23

The third classification of leukemia studies consisted of patients with chronic lymphocytic leukemia.

Patient E - hz: Caucasian male, 64 years of age. He was admitted on July 31, 1973. Diagnosis of CML, which he had had for approximately ten weeks was made on the basis of bone marrow analysis. The bone marrow was quite hyperplastic with sheets of cells. For chemotherapy he was placed on chlorambucil and prednisone.
FIGURE 1: threading
patient A

FIGURE 2: secondary constrictions
patient C

FIGURE 3: double minute chromosomes
patient D

FIGURE 4: regular chromosome
patient F

chromatid that
did not separate
A peripheral blood sample for chromosome analysis was obtained from patient F on August 8, 1972. The modal chromosome count was 46XY with an occasional random cell with 45 chromosomes. Several cells with a 46 count were noted in which the A1 chromosome seemed to be involved in either a translocation or an inversion. Another possibility would be incomplete separation of the chromatid as illustrated in Figure 1.

Many of the cells of patient F also revealed a chromosome in the G-group that was much smaller—often referred to as the Christchurch chromosome. Scientists disagree as to the significance of this marker chromosome.

Gunz et al. (1962) found an abnormal G group chromosome segregating in a family in which two members were affected with chronic lymphocytic leukemia; several other members also carried the abnormal chromosome but were otherwise unaffected. They suggested that this chromosome predisposed to the development of chronic lymphocytic leukemia. This, however, has never been confirmed and it seems likely that the abnormal chromosome which was termed the Christchurch chromosome, Ch', and appeared telocentric (Gp-), was a normal variant of the karyotype with little clinical significance.24

Patient G - sex: Caucasian male, 69 years of age. He was diagnosed as having chronic granulocytic leukemia in acute blastic crisis. Drug therapy, COAP, induced remission. There was a decrease in the number of red blood cells with mild anisocytosis, poikilocytosis, and polychromasia. Abnormal cell forms include racquet cells, burr cells, helmet cells, tear drop forms and ovalocytes. There was an increase in the number of white blood cells.

This type of leukemia exhibited a modal chromosome count of 46. However, many of the chromosomes were fragmented, or had distinct breaks. These breaks would permit loss of chromatid material or perhaps indicate an incomplete translocation. The presence of abnormally large chromosomes is a malfunction in mitosis—the chromosomes doubling in size but failing to separate. Such cells usually have giant nucleoli.
CONCLUSION

After completing this study it would be much easier to divide the material and construct a series of new research proposals.

To adequately trace a chromosomal variation in leukemia, it would be necessary to take samples for analysis from the time of diagnosis of the disorder, through chemotherapy, radiation therapy, remission, and release. Of course, this might take several years, but at least a comparison could be made between patients with the same disease at the same stage of development. The manner in which this study was conducted allowed for no control of variables. Whenever it was convenient to draw an extra blood sample, then chromosome studies were done.

Radiation therapy may produce temporary and permanent alterations. Temporary physiological effects include stickiness, abnormal spindle formation, and suppression of mitosis. Permanent effects, which are visible several hours after treatment, consist largely of injuries to chromosomes, such as a break in a chromosome strand. The distal segment usually appears as anacentric fragment.

Injuries to chromosomes by ionizing radiations are usually lethal to the cell. The damage consists of breaks in the chromosomes and the loss of acrocentric chromosome fragments during subsequent mitosis. In a diploid cell the loss of chromosome parts results in gene deficiencies which seriously impair cellular metabolism and leads to eventual cell death. Theoretically, it may be assumed that polyploid cells should be less vulnerable to radiation since chromosome losses could be compensated for by multiplicity of homologous chromosomes.

A similar viewpoint is shared by Busch.

Ionizing radiations of whatever source share with a number of other physical, chemical and biological agents the ability to produce transient and possibly permanent damage to the visible integrity of human chromosomes. The immediate effect of ionizing radiation is to produce damage to the chromosomes; this damage consists of deletions, translocations, acrocentric fragments, multicentric and ring chromosomes, and others which are probably deleterious to further
cellular division. Apparently such changes may be
totally present in human cells for as many as twenty
years.26
Chemotherapy may produce similar results.
The chromosome injuries produced by alkylating agents
are very similar to those produced by ionizing radiation,
whose these substances are referred to as "radiomimetic"
egents. Besides the strictly radiomimetic effects,
another kind of cytological disturbance has also been
observed in cells after the administration of alkyl-
ating agents. Amongst these effects superfragmenta-
tion of chromosomes and clumping of the injured
chromosomes into several cytometric bodies are the
most common; these effects may be referred to as
cytotoxic injuries; they occur about 72 hours after

treatment.27

Another handicap in studying chromosomal variations
is the difference in response of cell growth to different
types of media: i.e., with or without phytohemagglutinin,
or the length of incubation. Observations suggest that
the lower response to phytohemagglutinin by leukemic
lymphocytes in standard three day cultures may result
from a slowing down process of DNA and RNA synthesis.

The objectives of this research project were to:
1. Learn chromosome culturing techniques.
2. Ascertain frequency of chromosomal aberrations in
leukemic patients.
3. Determine types of chromosome aberrations in leukemia
patients.
4. Verify abnormalities in both bone marrow and peripheral
blood samples.
5. Consider prognostic significance of findings.
6. Correlate findings with other lab data on that point
or other research reports.
7. Study effect of therapy on chromosomal patterns.
It would be erroneous to say that any valid or new conclu-
sions were made in any of these areas. But at least each
point was considered and an awareness of the complications
that might arise was created. It was interesting to observe
on a first hand basis some of the same observations made
by other researchers.

In summary, it may be suggested that there are definitely
chromosome variations associated with leukemia, with the type
and frequency of the aberration being somewhat dependent
on the type of leukemia, progression of the disorder, and effectiveness of therapy.

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FOOTNOTES


3. Ibid., p. 11.

4. Ibid., p. 99.


6. Ibid., p. 19.


11. Koller, Peo C. op. cit., p. 82.


17. Koller, Peo C. op. cit., p. 66.


25. Koller, Peo C. op. cit., p. 32.
26. Busch, Harris. op. cit., p. 103-10h.
27. Koller, Peo C. op. cit., p. 10h.


