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NOFAL, Mohamed Magd Eldin, 1929-
RADIOBIOLOGICAL EFFECTS OF RADIOIODINE
THERAPY; CHROMOSOMAL CHANGES.

The University of Michigan, Ph.D., 1967
Health Sciences, radiology

University Microfilms, Inc., Ann Arbor, Michigan

RADIOBIOLOGICAL EFFECTS OF RADIOIODINE THERAPY; CHROMOSOMAL CHANGES

by
Mohamed Magd Eldin Nofal

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in the
University of Michigan
1967

Doctoral Committee:

Professor Claire J. Shellabarger, Chairman
Professor William H. Beierwaltes
Professor Norman E. Kemp
Associate Professor Margery W. Shaw

Abstract

RADIOBIOLOGICAL EFFECTS OF
RADIOIODINE THERAPY;
CHROMOSOMAL CHANGES

By Mohamed Magd Eldin Nofal

Evaluation of the radiobiologic effects of irradiation from radioiodine therapy for thyrotoxicosis and thyroid carcinoma was performed through analysis of chromosomal changes in peripheral leukocytes.

After a short-term culture, lymphocytes were analyzed for chromosomal changes. Abnormalities in count and morphology were analyzed through direct microscopic visual scoring.

First Evaluation:

This was done on:

1. Sixty-five hyperthyroids treated 3 to 14 years previously with 6 to 54 mC ^{131}I. Their chromosomal analysis was compared to that of 27 controls matched for age and sex.

2. Fifteen treated hyperthyroids. Chromosomal analysis was done before and one-half hour after an oral dose of ^{131}I of radioiodine ranging from 7 to 13 mC.

3. Twelve thyroid cancer patients. Chromosomal studies performed before and at intervals up to 48 hours following a larger dose of ^{131}I ranging from 150 to 208 mC.

In all these groups, evaluation under circumstances described suggested a statistically higher incidence of abnormalities in the study groups than in the control groups. The incidence of abnormalities was highest shortly after irradiation (groups III and IV).

No hematologic phenomena seemed to accompany these abnormalities, either shortly after irradiation or as a late manifestation.

Re-evaluation:

Repeat study of the chromosomal changes was performed on the latter two groups (treated hyperthyroids and thyroid cancer patients) using a more accurate and detailed method of analysis, eliminating investigator bias. Results obtained under these circumstances suggested no statistically significant difference between the chromosomal picture before and after ^{131}I therapy, in both hyperthyroid and thyroid cancer patients. The explanation for the discrepancy and comparison with the literature are discussed.

Data also showed no significant difference in the chromosomal analysis between non-irradiated hyperthyroid patients and patients with thyroid carcinoma, suggesting no specific effect of either disease on the chromosomes.

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PREFACE

The study on which this dissertation reports was conducted during the years 1963-1967 in the University of Michigan Medical Center. It was supervised by Dr. William Beierwaltes, Coordinator of the Nuclear Medicine Unit and by Dr. Claire Shellabarger, Chairman of the Radiation Biology Program.

The idea for the study occurred to us in connection with an assignment from the U.S. Public Health Service, to do a follow-up evaluation on patients treated with ^{131}I for thyrotoxicosis at this center since 1948. The Public Health Service was interested to know whether any undesirable aftereffects of such treatment had meanwhile come to light. The results of that study have been written up and appeared in a recent issue of the J.A.M.A. (77).

My thanks must go in the first instance to all patients involved in the study, as well as to the women prisoners and other volunteers who contributed blood samples to serve as controls.

I also wish to take this opportunity to express my gratitude to the members of the Doctoral Committee for their invaluable help and constructive criticism; to Dr. Beierwaltes for his indispensable advice and generous support at every stage of the project, including the use

of his laboratory and hospital facilities; to Dr. Margery Shaw for all the time she spent in teaching me the procedure of analysis and in reviewing the results; to Dr. Shellabarger for his continuous guidance, and to Dr. Abdul-Amir Al-Saadi for his assistance in working out the cytogenetic technique.

Finally, I would like to thank all the staff of the Nuclear Medicine Unit, in particular Mrs. Nancy Nemacheck and Miss Natalie Rhodes for their technical assistance and Miss Catherine Lafkas for her help in typing and secretarial work.

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Chapter I

INTRODUCTION

The necessity of living with atomic energy and ionizing radiation is becoming increasingly evident and one can no longer remain aloof from the problems involved, as radiation is gradually becoming a part of everyone's daily life. The development of nuclear power, and the increasing use of radiation in medicine and industry cannot be reversed.

Essentially all kinds of radiation sources are currently being used with increasing frequency. These sources include: X-ray machines, ^{60}Co sources, radioisotopes used in diagnosis, therapy and medical research, nuclear reactors and their high-energy particle accelerators, and nuclear weapons with their associated fallout. The number of X-ray machines in use in the world is progressively rising; the number of cobalt units in use is constantly expanding. The application of radioactive isotopes to medical purposes is rapidly increasing in frequency and new isotopes are being put into use every year. The predicted accumulated total radioactivity resulting from nuclear power industry will increase by a factor of 10 over the next 10 to 15 years. The volume of accumulated radioactive waste material of high and intermediate level will increase proportionally (33). The use of atomic devices for

experimental detonations has increased markedly over the past several years. The increasing use of all of these sources has resulted in a steady though small increase in the amount of radiation exposure of the population over the inescapable background.

Thus, it is probable that man-made radiation will be a permanent feature of the human environment from now on. On the other hand, it is conceivable that the use of atomic energy will be merely a transient phase in the history of technology and that substitution of solar energy for atomic energy will make widespread use of the latter unnecessary some 5 or 10 generations from now (113).

As with all agents that constitute a potential hazard to the health of human beings, the radiobiologist is and must be a key figure. It becomes of increasing and extreme importance that radiation workers have some knowledge of this problem in order to be able to advise with regard to the potential hazard entailed. Of even greater importance than recognizing a potential hazard from radiation is the capacity to differentiate the true hazardous situation from a negligible to non-existent hazard.

The widespread use of isotopes and the by-products of nuclear reactors, imposes a whole set of additional problems in medical use of radiation. The hazards of radioisotopes were emphasized

especially when it was brought to light that the individuals who had ingested radium later developed bone sarcoma and many died (124). Another example is the increased frequency of stillbirths among parents who were atomic bomb survivors of Hiroshima and Nagasaki (126).

The question has thus become not "Does ionizing radiation cause biological damage?" but rather, "how much biological change can ionizing radiation produce?" For many years, workers concerned with radiobiological protection have been interested in the development of methods of estimating exposure to ionizing radiation based upon biological rather than physical techniques. Many possibilities have been investigated, including studies of the frequency of lymphocytes with bilobed or double nuclei, or of neutrophils carrying particles with specific staining properties; and studies of the excretion in urine of the products of protein breakdown (30). None of these methods has so far proved satisfactory.

We have chosen to study the chromosomal changes as a manifestation of the radiobiological effect of ionizing radiation. We were mainly concerned with chromosomal abnormalities that might be produced and probably persist many years after radioiodine therapy for thyrotoxicosis, as the most commonly used isotope in the field of

internal radiation therapy. Pochin in 1960 (85) estimated the number of thyrotoxic patients treated with radioiodine over the past 20 years to be at least 60,000.

The recent and rather rapid development in the field of cytogenetics enabled us to take advantage of its method and look beyond the level of the cell and nucleus to deeper structures: the chromosomes.

In the past few years, there have been a number of major advances in the field of cytogenetics in general and human cytogenetics in particular.

For about 30 years, the general belief was that normal human chromosomes numbered 48. This concept was only changed in 1956 when Tjio and Levan (106) proved beyond a doubt that the number is 46. The inaccurate estimate of chromosomes was due partly to technical difficulties, to the small size and large number of human chromosomes as compared to some plant and animal species, and to the relative difficulty of doing experimental work with human material. Subsequently, the development of methods for producing well-spread metaphase figures from human cells grown in tissue culture has led to a wide interest in human cytogenetics.

The potentialities of cytogenetic studies have been great and allowed for the use of chromosome analysis as a research tool on a variety

of human problems in different areas. To mention some of these, we may think of developmental syndromes, tumor studies, especially leukemias, and problems related to radiation. A good amount of information has been accumulated and investigations are being undertaken to determine whether radiation might induce demonstrable chromosomal changes. Studies have included patients with accidental, diagnostic and therapeutic exposure to radiation. Most of these studies, however, were concerned with a relatively small number of subjects, or followed for a short time.

Mammalian somatic cells in culture when applying these techniques are well suited to cytogenetic investigations. These cells are easily manipulated, and are readily accessible to chemical agents, such as colchicine. In addition, the use of human material is particularly appealing because of the wide interest in radiation damage to humans.

Chromosomal abnormalities have been demonstrated in peripheral white blood cells following external irradiation from X-rays, both therapeutic and diagnostic, and internal irradiation from isotopes from both therapeutic and accidental exposure.

Controlled radiation exposure has also been performed on a variety of plants and animals, and chromosomal abnormalities evaluated. Moreover, many experiments have been carried out in vitro, utilizing

tissue culture cell populations, on both mammalian and human cell lines.

Review of the Literature on Human Chromosome Damage by Irradiation

In 1959, Fliedner et al. (44) reported the presence of chromosome stickiness, clumping and anaphase bridge-formation in bone marrow preparations from eight persons accidentally exposed to mixed neutrons and gamma-rays.

Tough et al. in 1960 (107) noted the presence of chromosomal abnormalities in leukocytes of two patients given X-ray treatment for ankylosing spondylitis. Twenty-four hours following single exposure to 250 rads, the modal count dropped from 98% to 72%, and structural abnormalities had risen from 1% to 22%. By the fifth day, however, both non-modal count and structural abnormalities had dropped to near normal levels.

In 1961, Boyd et al. (17) found acute chromosomal changes in the leukocytes of four patients treated with 6-11 mC ^{131}I for thyrotoxicosis and two patients treated with 100-150 mC for thyroid cancer. They concluded that a 100 mC dose produced significant chromosomal abnormalities, and even smaller doses in the order

of 10 mC might produce recognizable changes.

Stewart et al. in 1961 (104), reported the occurrence of chromosomal aberrations in peripheral leukocytes from patients with Klinefelter's syndrome shortly after exposure to diagnostic X-ray. They assumed that the aberrations might have been induced by diagnostic irradiation in vivo or by background radiation, but admitted that they might either be related to the patient's abnormal karyotype or could have arisen in culture.

Buckton et al. in 1962 (19) showed that in leukocytes of patients with ankylosing spondylitis who received X-ray therapy, chromosomal abnormalities can persist for as long as 18 years after irradiation.

Goodlin in 1962 (49) reported the existence of a relationship between chromosome damage of peripheral leukocytes and radiation therapy in 17 patients with carcinoma of the cervix.

Macintyre et al. in 1962 (60) reported the occurrence of numerous abnormalities of number and structure of chromosomes in the leukocytes of one patient during 14 days after a therapeutic dose of 167 mC ^{131}I for metastatic thyroid carcinoma. They also concluded that the maximum frequency of anomalies occurred during the period of 3-12 hours after therapy and corresponded to the maximum

concentration of ^{131}I in blood.

Conen et al. in 1961 and 1963 (27) reported structural chromosomal abnormalities in the leukocytes of an infant with congenital anomalies probably produced by a diagnostic X-ray dose of 0.8 rad.

In 1962, Lindgren et al. (59) studied peripheral blood chromosomes in a 5-year old boy, one of a pair of identical twins, who was exposed to 1100 rads X-ray to the spine. Chromosomal analysis showed significant abnormalities in both count and structure, while no abnormality was observed in the unirradiated twin brother.

Bender and Gooch in 1962 and 1963 (7,8) demonstrated persistent chromosomal aberrations in both number and form in leukocytes of eight men two and one half years and to a lesser extent three and one half years after accidental whole-body exposure to mixed gamma ray and fission neutrons with dose calculated to vary from 22.8-365 rads.

Sasaki et al. in 1963 (95) found significant chromosomal changes in peripheral leukocytes of 15 radiologists and radiation workers exposed to a total dose of 2 to 88 rem as compared to a roughly comparable control group.

Bloom and Tjio in 1964 (16) reported the presence of chromosomal abnormalities in 20 patients 72 hours following diagnostic X-ray procedures, especially cardiac catheterization, which dissappeared after $2\frac{1}{2}$ months.

Norman et al. in 1964 (80) studied peripheral blood chromosomes of 33 radiation workers with a lifetime dose in excess of 10r and found the frequency of abnormalities in number and form to be higher than in the population at large.

Visfeldt (111) in Denmark in 1964 demonstrated the presence of chromosomal aberrations in cell cultures from skin biopsies taken at different intervals after local irradiation.

Moore et al. (73) in 1964 disclosed aberrant chromosome morphology in circulating lymphocytes in 10 patients with uterine cancer following radiation therapy (up to 9212 r).

Friedman et al. (48) in 1964 observed a higher incidence of endoreduplication in 11 patients with disseminated carcinoma after total body irradiation, than before radiation therapy.

Amarose and Baxter (1) in 1965 reported a higher proportion of cells with abnormal chromosome counts shortly after beginning ^{60}Co therapy in 6 patients with pelvic cancer. A bell-shaped curve of response was readily reached during the course of irradiation therapy. They also noted that increasing exposure to radiation did not alter the initial response, i.e. plateau.

Millard (71) in 1965 examined peripheral blood chromosomes in 6 men with testicular tumors at different intervals after radiation therapy.

By the end of radiotherapy, 32% of the cells showed structural aberrations which dropped to 8% after 17-19 months.

Doida et al. in Japan in 1965 (38), comparing chromosome changes in seven atomic bomb survivors (17 to 18 years after exposure), five patients with breast cancer who had received radiotherapy, and six radiation workers, noted increased aberrations, especially in the first two groups.

Dekaban in 1965 (36) reported a case of a woman who had shown two types of chromosomal abnormalities in her peripheral blood. One of them was an increased rate of chromosomal aberrations occurring at random, which he believed to be associated with previous exposure to X-irradiation.

Warren and Meisner in 1965 (115) studied chromosomal changes in patients receiving X-irradiation for cancer of the cervix. They concluded that the incidence of cells containing structural chromosomal aberrations tends to increase with dose throughout the course of therapy; while the incidence of aneuploidy observed 24 hours after a single X-ray therapy changed little in the course of further treatment, and tended to return to normal levels after the conclusion of therapy.

Dekaban et al. in 1966 (37), in a study of chromosomal changes on selected patients, observed that the rate of chromosome-type

aberrations induced by in vitro irradiation of peripheral blood was significantly higher in a woman who was previously exposed to irradiation.

Nasjleti et al. in 1966 (76) reported that the occurrence of polyploidy and endoreduplication was increased in leukocytes following irradiation in vitro and in vivo, with occurrence of other structural abnormalities. They also found that gamma radiation of ^{131}I and ^{60}Co and X-rays had similar qualitative effects as measured by leukocyte culture.

Norman et al. (79) in 1966 measured the frequency of chromosome aberrations in 36 women who had received radiation therapy for cervical carcinoma. They found two groups of aneuploid cells; one showed a stable frequency of about 5.8% with no structural aberrations; the other containing structural aberrations was eliminated at a rate of 530 ± 64 days.

Fischer et al. (43) in Vienna in 1966, examined 20 patients who were known to have received thorotrast 19 to 27 years previously. In 19 patients who received thorotrast for arteriography, there was evidence of chromosomal breakage at a rate of 0.03 to 0.7 breaks per cell. One patient who received thorotrast for retrograde pyelography showed no evidence of chromosomal damage. Polyploidy up

to 6% was present in 15 cases.

Bloom et al. (15) of the Atomic Bomb Casualty Commission, Japan, in 1966 evaluated chromosomal damage in 94 survivors of atomic bombings of Nagasaki and Hiroshima in 1945. Although there was no difference in the degree of aneuploidy between study and control groups, there was a statistically higher incidence of structural chromosomal abnormalities among the exposed group. However, there was no direct relation to exposure dose or clinical symptoms of radiation sickness at the time of exposure.

Cantolino et al. (22) in 1966 studied chromosomal abnormalities in 5 hyperthyroid patients before and at different intervals after ^{131}I treatment. Although there was no real difference in the frequency of aneuploidy between control and post-treatment series, they found significant chromosomal aberrations at all intervals after therapy with no peak. There was no correlation between the level of radioactivity in plasma and the incidence of aberrations.

Bender and Gooch (10) in 1966 found chromosome-type aberrations in the peripheral blood of 3 men who were accidentally exposed to acute whole-body mixed gamma ray and fission-neutron radiation. Physical dose estimates ranged from 12 to 47 rads. They reported a drop in the frequency of the aberrations after several weeks.

Chapter II

TREATMENT OF THYROTOXICOSIS WITH RADIOACTIVE IODINE

Two years after Fermi had produced radioactive iodine in 1934 by means of neutron bombardment of iodine, a colloquium was held on the subject, "What Physics Can Do for Biology and Medicine." The results of this conference led to the use of radioiodine in a cooperative research study in Boston. This and subsequent studies clearly opened the way to thyroid gland therapy and, in 1942, groups in Boston and California reported the use of radioactive iodine for control of thyrotoxicosis.

After the release of ^{131}I for national distribution by the Atomic Energy Commission in 1946, a flood of reports on both clinical and laboratory investigation of thyroid function and disease appeared in the literature. Now, after more than 20 years of experience, this isotope continues to hold its position as the most useful tool for the treatment of thyroid disease.

At the University of Michigan, ^{131}I was first used in 1946 in the treatment of thyrotoxicosis and thyroid cancer.

The simplicity of radioiodine treatment is its chief recommendation. It can be accomplished with minimal disturbance to daily

activities. It is especially useful when coincident organic disease, especially heart disease, increases the hazards of surgery. Although not strictly a medical consideration, the total cost of ^{131}I therapy to the patient is probably less than for other forms of treatment.

The use of ^{131}I in the treatment of thyrotoxicosis was and still is limited to older ages. Such restriction is based on the fear that radiation exposure (5,000-20,000 rads to the thyroid gland and 5-15 rads total body) would eventually cause thyroid cancer, leukemia, or genetic change. This concept was based on the observation that irradiation of the thyroid of rats can induce thyroid carcinogenesis (47). Also, exposure of the thyroid of the infant or child to external radiation has been clearly implicated in the genesis of thyroid cancer (39).

There is now less concern that leukemia will follow ^{131}I therapy, since a survey by Pochin (85) revealed fewer than the anticipated number of cases of leukemia in over 10,000 treated patients.

To date, no case of cancer of the thyroid definitely attributable to ^{131}I administration to adults has been reported in the literature.

The third cause for restriction of the use of radioiodine to certain age limits is the fear that irradiation of the gonads during therapy will induce genetic damage that will be transmitted from parent to child. Radioiodine would be routinely used in the treatment of thyrotoxicosis

in the young, were it not for the fear that such radiation would cause damage to the germ cells or induce neoplastic changes in other tissues. However, the actual genetic hazard involved cannot be assessed with accuracy. The danger is probably slight, and the effect may even be imperceptible; gonadal irradiation from a single average ^{131}I treatment is only 5-10 times that occurring during a barium enema or intravenous pyelography (116). Starr (102) reported that 20 young females who were treated with ^{131}I later gave birth to 31 normal children.

Blomfield (14) reported the case of one patient who was treated 3 weeks after conception and another who conceived about one week after the administration of a therapeutic dose of ^{131}I. Both pregnancies were uneventful and the children developed normally during the subsequent 5 years.

McGirr (67) concluded that in 3 patients treated with radioiodine in the first trimester, no untoward events were noted in the offspring.

Both Werner (117) and Chapman (23) independently observed no ill effects from the treatment with ^{131}I during pregnancy.

Therefore, until recently, ^{131}I treatment of thyrotoxicosis at the University of Michigan Medical Center was limited to patients forty years of age or older unless they had:

1. Persistent or recurrent disease after a previous surgical thyroidectomy.

2. Refusal of operation by surgeon or patient.

3. Failure of adequate preoperative preparation with antithyroid drugs during a four to six-month trial period.

4. Progressive exophthalmos.

The average single dose considered necessary to free the patient of thyrotoxicosis was estimated as 10 mC ^{131}I and was decided on the basis of 3 factors: the results of the 24-hour thyroidal uptake test, the estimated weight of the thyroid gland, and the transit time of ^{131}I through the gland.

Figure 1 is a graph of the average number of millicuries given in the first dose each year from 1948-1963.

In a recent review of 848 thyrotoxic patients who were treated at this center with ^{131}I (77), we found that 50% of all patients had received a total dose between 10 and 20 mC of ^{131}I while 22% had received an initial dose of 10-11 mC. We also observed that 71% of patients had received only one dose of ^{131}I, while 15% required two doses. We also found that 66% of the patient population received antithyroid medication prior to ^{131}I therapy, usually propyl thiouracil and Lugol's iodine for variable periods.

When those patients were followed for a period of 10 years after ^{131}I therapy, we found that the cumulative incidence of hypothyroidism was higher in this group than in a comparison group treated only with surgery. Following an initial rise in the incidence of hypothyroidism after the first year, the cumulative increase was 2.8%/year in the ^{131}I-treated group as compared to 1.7%/year in the surgery-treated group.

The exact cause of this phenomenon is not definitely understood, but one of the many explanations put forward is that radiation may cause profound damage to the chromosomal apparatus, which might prevent replication of cells needed to replace those lost through normal attrition (101).

Radioiodine:

^{131}I is obtained from the reactor as a fission product. It has a physical half life of 8.07 ± 0.02 days, a biological half life of 138 days, and an effective half life of 7.5 days. It decays, by emitting both beta and gamma rays, to stable xenon-131. Its average beta energy is 0.187 MeV and average gamma energy is 0.364 MeV.

About 90% of the effect of therapeutic ^{131}I is produced by beta rays and the remainder by gamma rays. This fact is of great signifance, since beta rays travel only about 2 mm or less in tissues,

in contrast to gamma or X-rays which penetrate much farther. Since ^{131}I can become fixed in the thyroid in concentrations much greater than in other areas of the body, up to 10,000 times that in serum in thyrotoxic patients, and because the radiation is confined largely to the gland, ^{131}I therapy is considered superior to X-ray therapy. It is estimated that only about 3000 REP can be given to the thyroid gland with X-ray without risk of serious damage to surrounding structures, but many times this amount can be given with radioiodine.

Blood Radioactivity:

Figure 2 shows serum radioactivity concentration following administration of 8.0 mC of ^{131}I dose for hyperthyroidism. Within half an hour following oral administration of the dose, serum activity was about 0.25 μC/ml, which rapidly dropped to 0.06 μC/ml at 8 hours, 0.01 μC/ml at 24 hours, and 0.004 μC/ml at 48 hours.

The calculated average whole body radiation dose from such therapy, assuming that the effective half life is 6 days and weight of patient 70 kg., could be derived from this formula (51).

$$D_{body} = 1.2 \times 10^{-3} \times \text{dose in } \mu C$$
$$1.2 \times 10^{-3} \times 8 \times 10^{3} = 9.6 \text{ rads}$$

A therapy dose of ^{131}I was calculated to achieve a concentration in

the thyroid gland of 185 μC/gm for a total thyroid dose of 16,700 rads.

A smaller dose of 4 mC ^{131}I was calculated to produce a concentration of 63 μC/gm thyroid tissue with a total radiation to the gland of 5,600 rads (77).

Green et al. (50) found that mean blood irradiation measured in 191 thyrotoxic patients after a single dose of ^{131}I was 8 rads per therapeutic dose. The mean blood irradiation dose for complete treatment of the disease was estimated in 802 patients previously treated as 16.6 rads. Bone marrow dose was estimated as 80% of the blood dose.

Pochin (86) estimated that the use of radioiodine in thyroid disease contributes only about 0.6% of the total exposure from medical radiology. Radiation exposures of the gonads, the marrow and the body tissues, generally are much less than those of the thyroid by a factor of several hundreds. In radioiodine therapy of hyperthyroidism, the mean dose to blood was likely to be between 15-20 rads.

Rall et al. (89) in 1949 studied the tissue distribution of radioiodine 56 hours after oral ingestion of 63 mC in a patient with metastatic thyroid carcinoma. His estimates showed:

Organ	Percent of Dose	μC ^{131}I/gm Tissue	Tissue Dose(r)
Thyroid	20.0	1260.0	181,000
Blood	1.7	-	-
Red Marrow	3.0	1.26	182.0
Liver	3.9	1.9	274.0
Spleen	3.3	0.04	6.0

They concluded that except for the thyroid gland and one metastatic growth, the quantity of radiation appeared negligible.

Seidlin et al. (98) in determining the blood radioiodine concentration in 26 patients with metastatic thyroid carcinoma found that the generalized body radiation was about 50% of that received by blood. Mean specific radiation dose delivered to blood was calculated as 56 ± 34 REP per 100 mC.

For the sake of comparison, we performed the same study of determining serum radioactivity following administration of 180 mC ^{131}I for treatment of thyroid cancer (fig. 3). At 2 hours after oral administration of ^{131}I, serum radioactivity was about 4.0 μC/ml; 1.5 μC/ml after the first day, and 0.22 μC/ml at 3 days. The estimated average whole-body radiation dose was calculated as 64 rads, assuming an effective half life of 1 day.

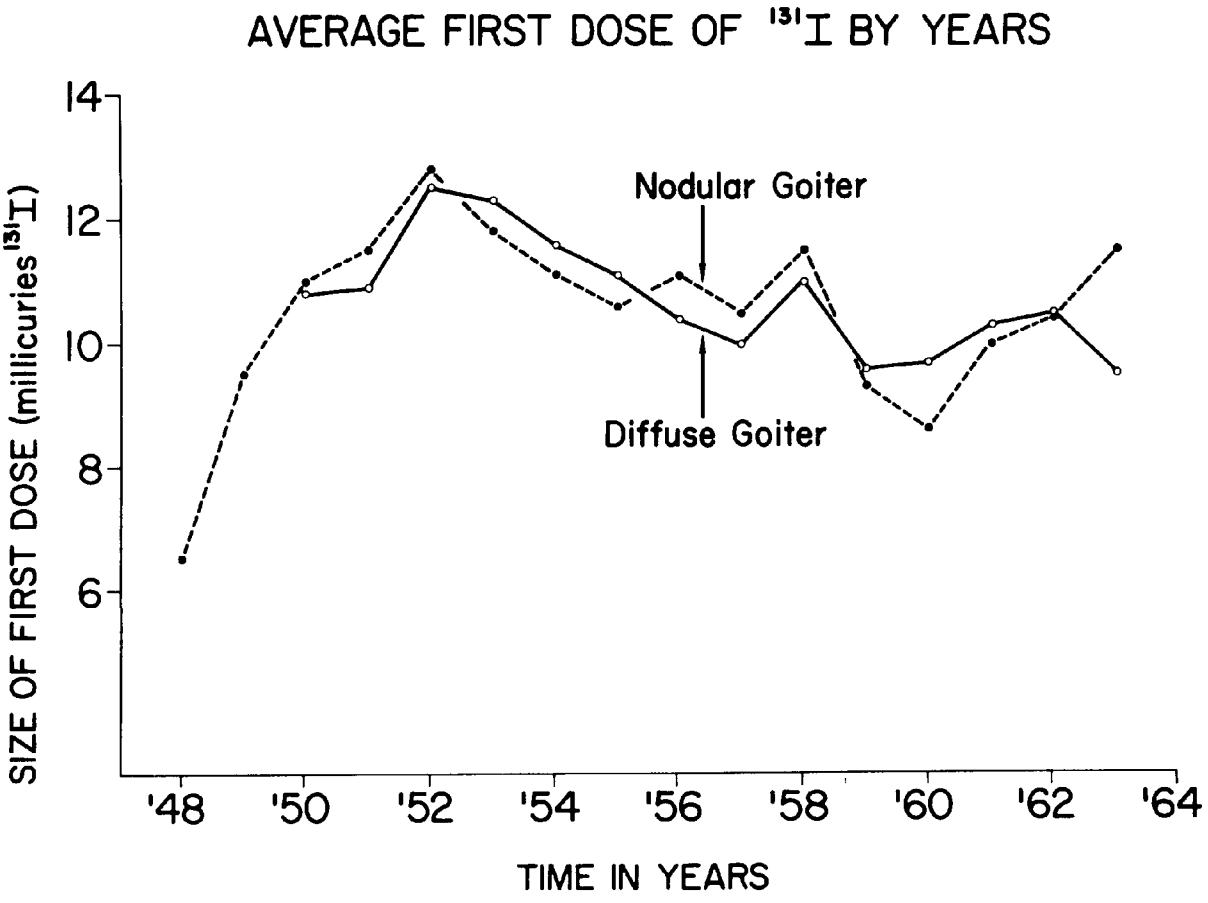

AVERAGE FIRST DOSE OF ^{131}I BY YEARS
SIZE OF FIRST DOSE (millicuries ^{131}I)
14
12
10
8
6
Nodular Goiter
Diffuse Goiter
'48
'50
'52
'54
'56
'58
'60
'62
'64
TIME IN YEARS

Figure 1

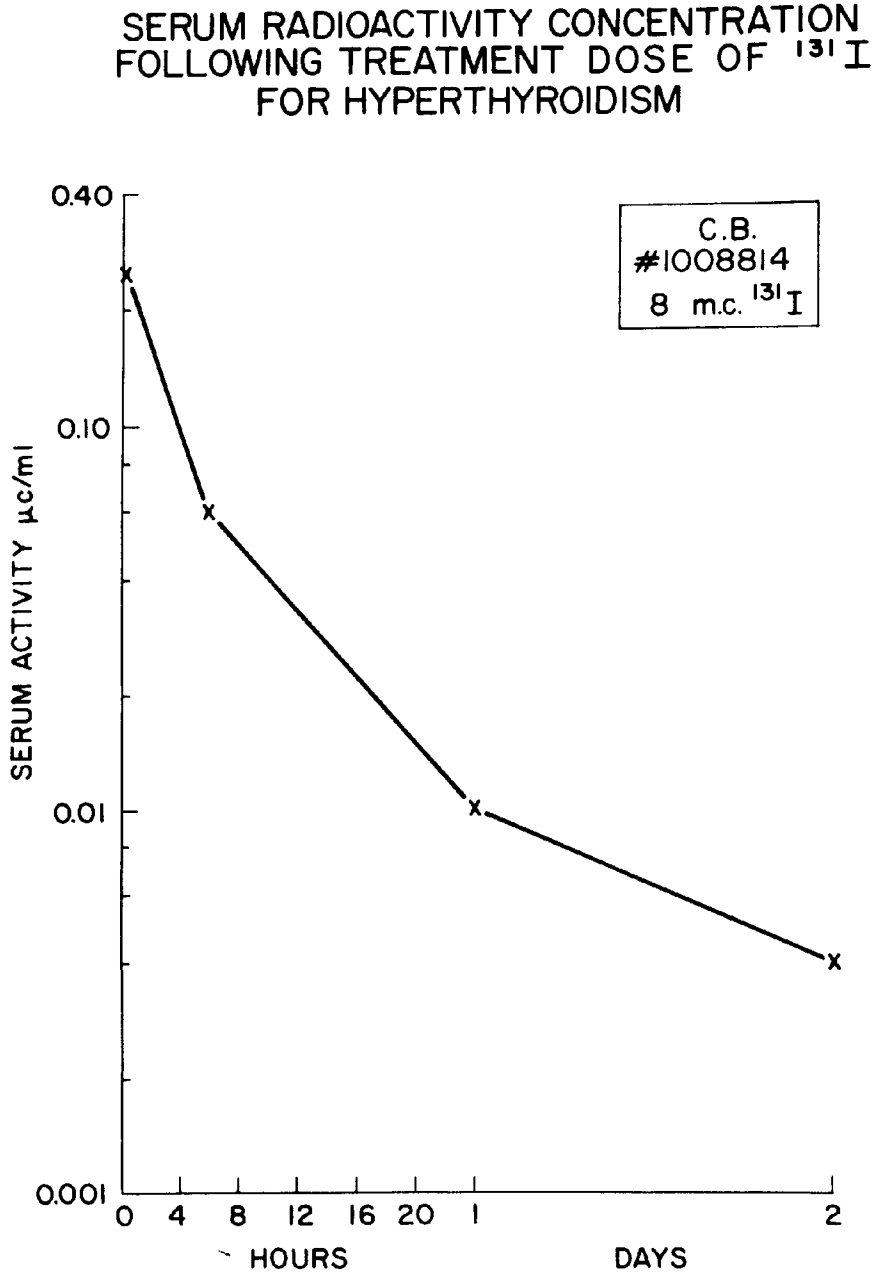

SERUM RADIOACTIVITY CONCENTRATION
FOLLOWING TREATMENT DOSE OF 131I
FOR HYPERTHYROIDISM
C.B.
#1008814
8 m.c. 131I
SERUM ACTIVITY µc/ml
0.40
0.10
0.01
0.001
0 4 8 12 16 20 1 2
HOURS
DAYS

Figure 2

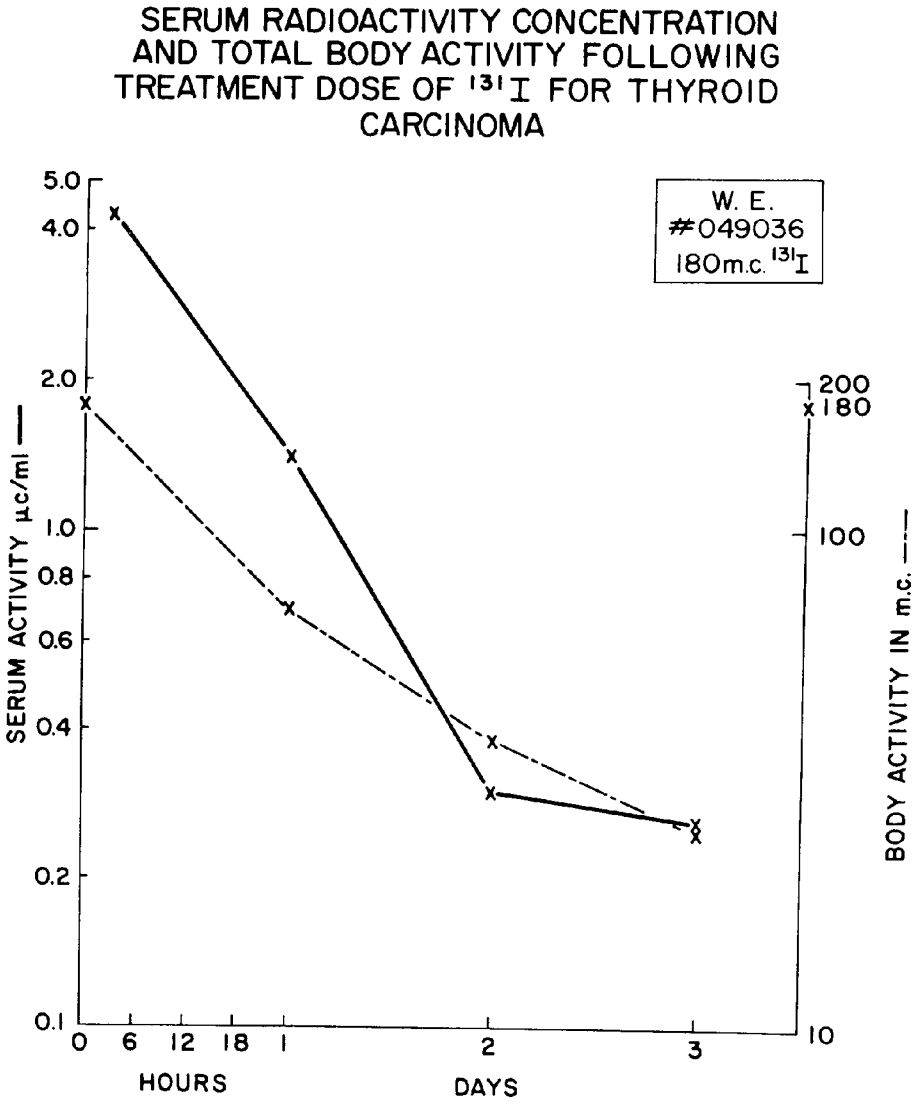

SERUM RADIOACTIVITY CONCENTRATION
AND TOTAL BODY ACTIVITY FOLLOWING
TREATMENT DOSE OF 131I FOR THYROID
CARCINOMA
W. E.
#049036
180m.c. 131I
SERUM ACTIVITY µc/ml
BODY ACTIVITY IN m.c.
5.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
200
180
100
10
0
6
12
18
1
2
3
HOURS
DAYS

Figure 3

Chapter III

MATERIALS AND METHODS

Materials

We studied a total of 119 subjects; (control and patients) their peripheral blood was cultured and their chromosomes analyzed for abnormalities in number and structure. These were grouped as follows:

Group I-Controls:

These were 27 subjects; 22 females and 5 males; their ages ranged from 22 to 78 years (Appendix I). They were especially selected from among medical students and other volunteers, and a thorough history was secured from each, making certain that they did not have a history of thyroid disease or exposure to therapeutic radiation. They were selected to match in age and sex with the follow up population (i.e. group II).

Group II:

It consisted of 65 patients, 47 females and 18 males. Their ages ranged from 21 to 82 years (Appendix II). These patients had the

established diagnosis of thyrotoxicosis and were consequently treated with radioactive iodine during the period from 1950-1961. Some of these patients were placed on antithyroid medication for variable periods, averaging 3 months, prior to ^{131}I therapy. The total dose of radioactive iodine was between 6.0 and 54.0 mC.

Thus, peripheral blood chromosomes of this group were analyzed 3 to 14 years following exposure to ^{131}I irradiation.

Group III:

It consisted of 15 thyrotoxic patients, 9 females and 6 males (Appendix III). Their ages ranged from 27 to 66 years. Blood samples were obtained from these patients at the time the decision was made to give them radioiodine as the treatment of choice for their condition. Two blood samples were obtained from each patient; one before and one within $\frac{1}{2}$ hour after administration of radioiodine. Doses given ranged from 7.2 to 12.7 mC of ^{131}I. Again some of these patients were under antithyroid medication prior to ^{131}I therapy.

Group IV:

It consisted of 12 patients, 10 females and 2 males, who had thyroid carcinoma (Appendix IV). In this group, the presence of thyroid

cancer was suspected clinically and proved by biopsy. All of these patients had total thyroidectomies, and 6 weeks later they had a thyroidal ^{131}I scintiscan (done with 300 μC) which showed the presence of remnants of functioning thyroid tissue in the neck region. Depending on the presence and extent of this uptake as indicated on the scintiscan, the decision was made to ablate these remnants with ^{131}I and the patients were given a dose of radioiodine ranging from 150 to 208 mC. Three blood samples were obtained from most patients: one before administration of radioiodine, one 1/2-2 hours following administration, and the third 24 to 48 hours later.

METHODS

Peripheral Blood Culture Technique

The technique with its many different modifications is essentially a relatively simple method for obtaining samples of mammalian cells capable of division in vitro (75).

The blood culture technique has its advantages in application to humans because of the ease of biopsy, the short time of culture, and the simplicity of needed equipment and facilities.

The blood culture provides a dividing population of leukocytes which has not divided more than once or twice in culture and therefore

represents interphase cells in the body.

The culture itself consists of the following processes (74): 1. Separation of competent leukocytes. 2. Incubation in tissue culture medium. 3. Presence of phytohemagglutinin in culture. 4. Harvesting the cells during their mitotic peak. 5. Spreading of metaphases.

Leukocyte separation could be accomplished by any of these methods (74):

- simple gravity separation
- light centrifugation (at low speeds)
- strong centrifugation (to achieve a buffy coat)
- or use of dextran or similar compounds.

Autoradiographic studies with tritiated thymidine (62,11) have confirmed the fact that the cells undergoing eventual division after subjection to phytohemagglutinin were in the G-1 phase as circulating leukocytes. It was found that dividing cells were numerous at day 3, day 7, and even day 10, but diminished at day 14, and were absent at day 17. All chromatids showed labelling with tritiated thymidine in the first division, one chromatid of each chromosome was labelled at the second division, half of the chromosomes showed one labelled chromatid at the third division, and only about $\frac{1}{4}$ of all chromosomes showed labelling in one chromatid at day 5.

All quantitative studies indicated that the lymphocytes are the cells affected, and that these must first convert to a state capable of division.

I-Origin of Lymphocytes

The lymphocytic origin of dividing cells in cultures of peripheral blood was first postulated by Maximow in 1927 (66). In cultures of peripheral blood, mitotic indices at 1% were observed by the third day. Morphologic changes which occurred in the culture showed two types of mononuclear cells: small lymphocytes, and a larger type resembling monocytes which were believed to represent in part distorted lymphocytes. Granulocytes disintegrate during the first 48 hours of culture as shown by ^{32}P uptake (68). From the study of MacKinney et al. in 1962 (63) following the changes in cell counts, cell morphology and tritiated thymidine labelled cells at frequent intervals, it was shown that lymphocytes were responsible for the growth, and that the sequence of morphologic patterns of mononuclear cells represented developmental stages or transformation of small lymphocytes, as originally suggested by Maximow. In a subsequent paper (105) the same laboratory studied the detailed electron microscopic morphology of human peripheral blood cells in tissue

culture. They showed that small lymphocytes can transform into large pyroninophilic cells capable of division. Similar results were reported by other investigators (41). Nowell (81) showed that this transformation produced by phytohemagglutinin can be inhibited by prednisolone. It was also found that the delay of the addition of phytohemagglutinin to the culture for 1 or 2 days will delay the appearance of mitosis for the same period. Thus, the potential of the small lymphocyte for division appears to manifest itself only after specific stimulation accompanied by morphologic transformation.

In an article entitled "The Present Status of the Lymphocyte Problem," (122) Yoffey mentioned that there appear to be 3 major sites of lymphocytic activity. One is the bone marrow, where the role is hemopoietic. A second is in some of the lymphoid tissue, where it is concerned with immune responses. A third is in the connective tissue, where in addition to immune responses, transformation into monocytes and other connective tissue cells may occur. In the blood, it appears that the lymphocyte is merely en route from one region to another. The number of divisions between the parent reticular cell and the final small lymphocyte has been estimated at 6 in the mesenteric lymph nodes and 8 in the thymus. This means that one reticular cell would give rise to at least 64

small lymphocytes. But lymphoid tissue does not continually increase in size, because lymphocytes are continually leaving. Some pass directly through the walls of blood capillaries into the blood stream, while others reach the blood indirectly via the lymphatics.

Indirect evidence for a separate origin of lymphocytes and bone marrow cells came from observations on chronic myelocytic leukemia (118) in which dividing erythroid, granulocytic and probably megakaryocytic cells shared the Ph′ chromosome as a marker, while 3 or 4-day cultures of peripheral blood during remissions produced cells of normal karyotype predominantly, presumably because lymphocytes do not contain the marker.

Ottesen (84) in 1954 studied the age of human white cells in peripheral blood. Through 32P label, he was able to calculate the time the cells have spent in the organism, from the time of their labelling until they were sampled from the blood stream. The mean age of granulocytes was found to be 8.7 and 9.4 days. The main part of the granulocytes enter the blood stream at an age of 6 days. Less than 5% of the granulocytes in the blood stream were less than 5 days old and a negligible percentage was older than 3 weeks.

Lymphocytes formed two groups; one younger than 10 days with a mean age of about 3-4 days, the other having a mean age of about 100-

200 days. In one experiment he found that the short-living fraction represented 22%; the long-living 78% of the lymphocytes in blood. In another experiment, the short-living cells composed about 11%; the long-living 89% of the total population in circulation.

Recently it was shown that the average life of the lymphocytes obtained from women who had received radiation therapy for cervical carcinoma is about 18 months ± 2 (78). This deviated from another previous study, which estimated the average life of lymphocytes as 29 months (19).

II. Effect of phytohemagglutinin

When leukocytes of normal peripheral blood were cultured in vitro, few, if any, of the cells enlarge and undergo mitosis (91). Nowell (81) found that phytohemagglutinin was responsible for the presence of blastoid and dividing cells when added to cultures of normal human peripheral blood. This substance is derived from red beans (Phaseola vulgaris) as a mucoprotein which does not easily permit further purification.

Phytohemagglutinin has previously been used to agglutinate the erythrocytes (53), thereby facilitating their separation from the leukocytes which were to be cultured. This blastogenic effect was evidently not due simply to the removal of the agglutinated erythrocytes, for

phytohemagglutinin was found to induce blastogenesis even when erythrocytes remained in cultures of whole blood. Only after this "dedifferentiation" or conversion process, requiring 24 to 48 hours, do the cells proceed to divide. As explained previously, all studies pointed out that the small lymphocyte is the cell which undergoes blastogenesis. It seems that the monocyte does not undergo blastogenesis in the presence of phytohemagglutinin. It probably either remains unchanged or becomes a macrophage (97). This was further proved by the findings that none of the blastoid cells gave the cytochemical reactions characteristic of a monocyte (88).

On the third day of culture in cultures containing phytohemagglutinin, more than 70% of the small lymphocytes may be transformed into large cells with deeply basophilic non-granular cytoplasm surrounding a large rounded nucleus containing homogeneously staining chromatin and prominent nucleoli. The diameter of the largest cells may be 3 times that of a small lymphocyte. Small blastoid cells intermediate in size between these large cells and the small lymphocytes are also seen. Many of the blastoid cells are found in clusters and are actively ameboid. Some blastoid cells can be seen in mitotic division after the 48th hour of culture. Microdensitometric measurement of Feulgen-stained preparations indicated that DNA doubles in

amount as the nucleus enlarges during blastogenesis, in preparation for mitotic division (28).

It has also been suggested that phytohemagglutinin produces blastogenesis due to attachment of its blastogenic factor to some structures of the lymphocyte (110), possibly the cell membrane or in the nucleus area (69).

Sebasin (92) on the other hand reported that while culturing human peripheral lymphocytes in the absence of phytohemagglutinin, he observed a striking alteration in both size and cytological characteristics in some of the cells on the fifth day of culture. Between 5 and 40% of the cells were transformed, apparently spontaneously, into blast-like cells indistinguishable from those stimulated with phytohemagglutinin. Although the stimulus for transformation to blastoids is not clearly known, the immature cells that developed spontaneously or through phytohemagglutinin tend to appear in aggregates. As it has been suggested that the blastogenic properties of phytohemagglutinin may be related to cell membrane contacts caused by agglutination of leukocytes, he speculated that spontaneous transformation to blastoids is also related to lymphocytic aggregation that always occurs to some extent even when cells are cultured in its absence. He also raised the possibility that

transformation reflects a response by the small lymphocytes to antigens in culture media, especially those cell-bound resulting from cell degeneration.

III. Colchicine

Colchicine or its synthetic analog, colcemid, was added to the leukocyte culture about 4 hours before harvesting the cells, to disrupt the spindle formation in the dividing cells at the metaphase stage. It was noted that using an overdose of colcemid resulted in producing short, "curly" chromosomes, with a tendency for the sister chromatids to separate at the centromeric region (123).

Culture Method

The technique used in this study was derived from the method used by Moorhead (75), Shaw (99), and Bender (11).

A half ml of soluble sodium heparin (1000 units/ml) was drawn into a sterile disposable 10 ml syringe. Ten ml of venous blood were then drawn into the heparinized syringe, mixed and then transferred to a 15 ml vacuum tube. This was centrifuged at about 1200 rpm in an international clinical centrifuge for 10 minutes. The supernatant serum, the "buffy coat" containing leukocytes and the top

mm of erythrocytes were then drawn with a sterile Pasteur pipette and placed in a milk dilution bottle containing freshly prepared culture medium mixture.

The culture medium was composed of:

- 10 ml Difco Tissue Culture #199
- 1000 units penicillin G
- 1.0 mg streptomycin
- 0.3 ml bactophytohemagglutinin P (Difco)

This was thoroughly mixed with serum by pipetting to break up cell clumps; the bottles were tightly capped and placed in an incubator at 37°C in a horizontal position. At approximately 66-72 hours, 0.3 ml of colcemid solution was added to the culture bottle to make a final concentration of about 10^{-7} M/ml, keeping the bottle in a horizontal position in order not to disturb the growth.

Four hours later the bottles were removed from the incubator and their contents thoroughly mixed by pipetting to suspend the cells in the medium. The medium containing the cells was then transferred to a 15-ml-capacity centrifuge tube and centrifuged for 5 minutes at about 1000 rpm. The medium was then discarded, the remainder resuspended and 5.0 ml warm Hank's Balanced Salt Solution (PH7) added and thoroughly mixed with contents of the tube. This was

centrifuged for 5 minutes, followed by discarding the supernatant. The mixture was suspended and 5.0 ml of warm distilled water were added, contents resuspended and incubated at 37° for 10 minutes. The mixture was then centrifuged for 5 minutes at 1000 rpm and supernatant removed.

Without disturbing the "button of cells", 5.0 ml of freshly prepared Carnoy's fixative were added, drop by drop (3 parts absolute ethanol: 1 part glacial acetic acid) and the tube allowed to stand for 30 minutes at room temperature. Following, the cells were resuspended, centrifuged for 2-3 minutes and the supernatant replaced by fresh fixative. This was repeated 2-3 times, until the solution became clear. Finally, all supernatant was removed and the cells resuspended in about 0.5 ml of fixative (depending on the density of the cell suspension). Two drops were then placed on clean chilled slides which were first fan dried for 1 minute, then passed through a spirit flame and left to dry at room temperature overnight. Six to eight slides were obtained from each culture.

Staining was performed for 20 minutes in 2% aceto-orcein; then slides were rinsed in 2 changes of 70% alcohol and one change of 90% each for 1 minute. After they were completely dry, they were mounted in Euparal and covered.

Interpretation:

Slides were labelled according to a special code and were then scanned under the low magnification of the microscope for the presence of mitotic figures. Examination was performed on metaphase plates which appeared intact. Under the high power and using an oil immersion apochromat × 100 objective of a Zeiss photomicroscope, chromosome sets were analyzed for any abnormality in number or structure. Fifty countable metaphases were considered necessary for scanning, but in many cases 100 cells were analyzed.

In general, three criteria had to be satisfied for chromosome analysis: 1. The cell must not be obviously broken. 2. Chromosomes should be spread enough to allow no ambiguity in interpretation for purposes of counting. 3. There must be no stray chromosomes in the same optical field.

If any of these criteria was not satisfied, the cell was usually discarded.

Selected metaphases with apparent or suspected abnormalities were photographed, using an apochromatic-aplanatic condenser, a green interference filter and 35 mm high contrast copy film. From the negatives, 8″ × 10″ prints were prepared and chromosomes cut and rearranged in groups by the paste-up method. The Denver

Classification was used for preparing these karyotypes (52). The Denver Classification can be summarized as follows:

Chromosomes are arranged in 23 pairs, 22 somatic and one sex pair, according to their size and long-to-short arm ratio.

The 22 autosomes are classified into seven distinctive groups. Within these groups, further identification can (in many cases) be made among the individual chromosomes. The quantitative characteristics can be summarized as follows:

Group A: Pairs 1-3:

Large chromosomes with approximately median centromeres, distinguished from each other by the size and centromere position.

Group B: Pairs 4-5:

Large chromosomes with submedian centromeres. Although pair #4 is slightly longer, the two pairs are rather difficult to distinguish.

Group C: Pairs 6-12:

Medium-sized chromosomes with submedium centromeres. Individual pairs are usually difficult to identify. The X chromosome belongs to this group and resembles the longer pair (#6) from which it is difficult to distinguish.

Group D: Pairs 13-15:

Medium-sized acrocentric chromosomes. Pairs 13 and 14 each have a satellite on the short arm; pair 15 has no satellite.

Group E: Pairs 16-18:

Rather short chromosomes with approximately median (in pair 16) or submedian centromeres.

Group F: Pairs 19-20:

Short chromosomes with approximately median centromeres.

Group G: Pairs 21-22:

Very short acrocentric chromosomes; pair 21 has a satellite on its short arm. The Y chromosome belongs to this group.

Structural abnormalities in the chromosomes were visually scored as either chromatid-type or chromosome-type.

A chromatid gap was defined as a non-stained area or actual gap in a single chromatid with no displacement of the distal portion of the chromatid.

A chromatid break was visualized as a terminal deletion on a single chromatid or a complete break with displacement of the distal portion.

An isochromatid gap was scored as a non-staining area or gap on both sister chromatids at the same level, with no displacement of the distal portions.

A chromosome break was scored when a double acentric fragment was present: an apparent pair of linear or dot like portions of sister chromatids without a centromere was also considered to be due to a chromosome break.

An isochromatid break observed as a terminal deletion on both sister chromatids with displacement of the distal portion was scored as a chromosome break.

The latter two types are anatomically indistinguishable; the difference is only biological.

A dicentric was defined as a chromosome having 2 centromeres resulting from a rearrangement of chromosomal material.

A ring was scored if a double-stranded chromosome appeared to have its distal ends joined.

Re-evaluation:

Because of certain biases detected when the results were critically evaluated (discussion in chapter VI), re-analysis of the material of groups III and IV was carried out in a blind fashion using a different method of analysis.

From each blood culture five slides were randomly chosen; the two groups, including control pre-treatment samples, were pooled and slides recorded making a total of 277 "unknown slides." From each slide, a maximum of ten metaphase cells, selected for adequate chromosome spreading under low power of the microscope were counted for chromosome number using oil immersion phase contrast microscopy.

A separate form was made for each slide indicating the slide code number, the position of each scored metaphase on the microscope scale, the chromosomal landmark at two different positions, whether the cell is normal and what kind of abnormality if abnormal.

For each abnormal cell, whether in count or structure, a free-hand drawing karyotype was made using a different color for each chromosome group (Denver Classification). Through this, it was possible in many cases to indicate and report the missing or added chromosome as well as the one involved in any structural abnormality. Moreover the position of each abnormal cell was located on a "field finder slide" in case the cells were to be re-examined under a different microscope.

Each metaphase scored as abnormal was relocated and the abnormality scored independently by another cytologist.

Selected cells demonstrating typical abnormalities observed were photographed and paste-up karyotypes prepared from the prints, for illustrative purposes.

After all the slides were analyzed and reviewed by the advisor, they were decoded and data from slides from each blood sample combined.

Chapter IV

RESULTS

A. First Evaluation

(performed according to the method described on page 37)

I. Control Group:

The detailed analysis of 27 controls shown in appendix I is summarized in table 1. The modal number of chromosomes was observed in 95.2% of the counted cells, with a total aneuploid frequency of 4.8% (including 0.5% tetraploids). The frequency of chromatid gaps among 1275 counted metaphases was 0.4%. No other chromatid or chromosomal aberrations were noted in this group.

II. Follow-Up Group:

As shown in appendix II and table 1, of 5000 cells scored, the incidence of aneuploid cells was 6.5%, again including 1.1% tetraploid cells.

A chi-square test applied to these data comparing it to the control group showed a significant difference ($X_2 = 5.5$; d.f. = 1; P = 0.01 - 0.02).

According to this analysis (table 1) data showed the presence of both chromatid and chromosomal abnormalities at a higher frequency than in the control group.

III. Treated Hyperthyroid Group:

In this group, blood samples were obtained from 15 patients before and one-half hour after ingestion of ^{131}I. The therapeutic dose for each patient is given in appendix III.

As shown in table 2, of 720 cells analyzed before therapy, 3.6% were aneuploid (including 0.1% tetraploid). Post-therapy samples showed 6.7% aneuploid cells. When these data were subjected to a chi-square test; a significant difference was observed ($X_2 = 7.64$; d.f. = 1; P = 0.001 - 0.01). Structural aberrations were also higher in the post-therapy samples.

IV. Treated Thyroid Cancer Group:

Twelve patients were included in this group (appendix IV), most of them had 3 blood samples; one before ^{131}I therapy; second at two hours and third within 24 to 48 hours following treatment. Aneuploidy was observed in 4.4% of the counted cells before therapy and in 6.2% and 9.4% of the cells 2 hours and 24 to 48 hours after treatment respectively (table 3).

A chi-square test showed significant difference after treatment as compared to the pretreatment samples ($X_2 = 10.9$; d.f. = 2; P = 0.005 - 0.001).

Also structural abnormalities as shown in table 3, were higher

after than before treatment; the higher incidence was observed in the 2 hours samples.

B. Re-evaluation

Again a different method was used in getting the results of the re-evaluation of groups III and IV.

When scoring the metaphases in these groups; cells with chromosome counts below 43 were not reported; these cells were considered broken and thus discarded.

In computing the frequency of aneuploidy, tetraploid metaphases were not included and were considered separately.

The identity of the missing or added chromosome in aneuploid cells was always reported whenever a definite decision could be made.

In the case of metaphases with 4n, it was reported whether it was just tetraploid or endoreduplication (fig. 13 & 14).

Isochromatid gaps were reported as chromatid gaps but were specially noted under "Remarks" in the appendices.

Treated Hyperthyroid Group:

As shown in appendix V, chromosomal analysis was re-studied on 13 patients before and $\frac{1}{2}$ hour after receiving their therapy dose of ^{131}I.

Table 4 summarizes the findings in both pre- (451 cells) and post-therapy (349 cells) samples. Total aneuploidy was 7.76% in pre-therapy control samples as compared to 5.16% in the post-therapy samples under study. A chi-square test applied on these data showed no statistically significant difference ($X_2 = 2.06$; d.f. = 1; P = 0.1 - 0.2). Tetraploidy was slightly higher post-therapy (1.15% vs. 0.44%) but not statistically significant. Again there was more or less similar chromosome-type aberrations, in the form of one hit (breaks) or two hit lesions (dicentrics). However, there was a statistically higher incidence of the chromatid-type lesions after therapy (3.7%) as compared to before therapy (1.8%) ($X_2 = 3.11$; d.f. = 1; P = 0.05). No abnormal pattern was noted for the missing or added chromosomes in aneuploid cells (table 5). The blood picture on these patients showed no abnormality.

Treated Thyroid Cancer Group:

As shown in appendix VI, chromosomal analysis was restudied on 11 patients in this group, before treatment and at intervals up to 48 hours after receiving ^{131}I therapy dose. Here slides of different post-therapy blood samples were pooled together and 5 slides were randomly selected to represent the post-therapy blood cultures.

Table 6 summarizes the findings before and after treatment. Of 375

cells scored from control pre-therapy blood, 5.8% showed aneuploidy and 1.87% were tetraploids. After therapy, the frequency of aneuploidy in 376 cells analyzed was 6.65% with 2.93% tetraploid cells. Statistical analysis of these data showed no significant difference (X_2 = 0.215; d.f. = 1; P = 0.4 - 0.7).

While chromosome-type aberrations occurred at a more or less similar frequency before and after treatment, there was a higher incidence of chromatid type lesions in the control pre-therapy blood as compared to the post-therapy study blood.

No definite pattern different from the expected was found in relation to the missing or added chromosome in the aneuploid cells (table 7).

One patient (J vH) showed a constant incidence of tetraploidy in the form of 3 cells with endoreduplication which were present both in the pre- and the post-therapy blood cultures (appendix VI).

The complete blood count performed on these patients at the time of the study was normal.

Table (1)

Comparison, Control vs. Follow Up Groups (I & II)

(a) Chromosome Count Distribution

Group		<44	44	45	46	47	48	±92	Total	Aneuploid
Control (I)	# of Cells	11	17	18	1214	5	4	6	1275	61
	Percent	0.9	1.3	1.4	95.2	0.4	0.3	0.5	99.9	4.8
Follow-up (II)	# of Cells	18	62	140	4651	34	16	54	4975	342
	Percent	0.4	1.2	2.8	93.5	0.7	0.3	1.1	100	6.5

(b) Structural Abnormalities

Group	Chromatid-type				Chromosome-type			
	Gaps		Breaks		Dicentrics		Breaks	
Control (I)	5/1275:	0.4%	0		0		0	
Follow-up (II)	67/4975:	1.3%	6/4975:	0.1%	11/4975:	0.2%	5/4975:	0.1%

Table (2)

Treated Hyperthyroid Group (III)

(a) Chromosome Count Distribution

Group		<44	44	45	46	47	48	±92	Total	Aneuploid
Pre-therapy (III-a)	# of Cells	1	8	13	694	1	1	2	720	26
	Percent	0.1	1.1	1.8	96.4	0.1	0.1	0.1	99.9	3.6
Post-therapy (III-b)	# of Cells	5	5	29	722	5	2	7	775	53
	Percent	0.6	0.6	3.7	93.2	0.6	0.3	0.9	99.9	6.7

(b) Structural Abnormalities

Group	Chromatid-type		Chromosome-type	
	Gaps	Breaks	Dicentrics	Breaks
Pre-therapy (III-a)	9/720: 1.3%	0	0	1/720: 0.1%
Post-therapy (III-b)	19/775: 2.4%	1/775: 0.1%	2/775: 0.3%	5/775: 0.7%

Table (3)

Treated Thyroid Cancer Group (IV)

(a) Chromosome Count Distribution

Group		<44	44	45	46	47	48	±92	Total	Aneuploid
Pre-therapy	# of Cells	2	4	14	502	0	0	3	525	23
(IV-a)	Percent	0.4	0.8	2.7	95.6	0	0	0.6	100.1	4.4
Post-therapy	# of Cells	5	7	17	563	0	1	7	600	37
2 hrs. (IV-b)	Percent	0.8	1.2	2.8	93.8	0	0.2	1.2	100.1	6.2
Post-therapy 24-48 hours	# of Cells	0	9	20	453	8	0	10	500	47
(IV-c)	Percent	0	1.8	4.0	90.6	1.6	0	2.0	100.1	9.4

(b) Structural Abnormalities

Group	Chromatid-type		Chromosome-type	
	Gaps	Breaks	Dicentrics	Breaks
Pre-therapy (IV-a)	8/525: 1.5%	0	0	0
Post-therapy within 2 hrs. (IV-b)	41/600: 6.8%	9/600: 1.5%	14/600: 2.3%	13/600: 2.2%
Post-therapy 24-48 hours (IV-c)	9/500: 1.8%	4/500: 0.8%	9/500: 1.8%	3/500: 0.6%

Table (4)

Re-evaluation

Treated Hyperthyroid Group

Chromosome Count Distribution (%)

Group	43	44	45	46	47	48	±92	Hypodip.	Hyperdip.	Aneuploid
Pre-therapy	0.44	0.66	5.54	92.24	0.66	0.44	0.44	6.65	1.11	7.76
Post-therapy	0.29	0.86	3.44	94.84	0.29	0.29	1.15	4.58	0.57	5.16

Group	Chromatid Lesions (%)			Chromosome Lesions (%)				Total Structural Lesions (%)	Total # of Cells
	Gap	Break	Total	Break	Dicent.	Ring	Total		
Pre-therapy	1.11	0.66	1.77	2.22	0.44	0.22	2.88	4.65	451
Post-therapy	2.58	1.15	3.72	2.58	-	-	2.58	6.02	349

Table (5)

Pattern of Missing or Added Chromosomes
In Aneuploid Cells

(a) Treated Hyperthyroids. Pre-Therapy

	A	B	C	D	E	F	G	Total
Observed	0	1	9	5	5	3	2	25
Expected	3.5	2.2	8.7	3.5	3.5	2.2	2.2	

$X_2 = 2.25$

d.f. = 6

P = 0.9

(b) Treated Hyperthyroids. Post-therapy

	A	B	C	D	E	F	G	Total
Observed	0	0	4	3	1	0	3	11
Expected	1.4	0.96	3.8	1.4	1.4	0.96	0.96	

$X_2 = 6.33$

d.f. = 6

P = 0.3 - 0.5

Table (6)

Re-evaluation

Treated Thyroid Cancer Group

Chromosome Count Distribution (%)

Group	43	44	45	46	47	48	±92	Hypodip.	Hyperdip.	Aneuploid
Pre-therapy	-	1.33	3.47	94.13	1.07	-	1.87	4.80	1.07	5.87
Post-therapy	0.27	1.06	3.99	93.35	1.06	0.27	2.93	5.32	1.33	6.65

Group	Chromatid Lesions (%)			Chromosome Lesions (%)				Total Structural Lesions (%)	Total # of Cells
	Gap	Break	Total	Break	Dicent.	Ring	Total		
Pre-therapy	1.87	-	1.87	1.33	0.53	-	1.87	3.73	375
Post-therapy	-	0.27	0.27	0.80	0.27	-	1.06	1.33	376

Table (7)

Pattern of Missing or Added Chromosomes
In Aneuploid Cells

(a) Treated Thyroid Cancer. Pre-Therapy

	A	B	C	D	E	F	G	Total
Observed	1	1	5	0	3	1	5	16
Expected	2.1	1.4	5.6	2.1	2.1	1.4	1.4	

$X_2 = 9.99$

d.f. = 6

P = 0.1

(b) Treated Thyroid Cancer. Post-Therapy

	A	B	C	D	E	F	G	Total
Observed	0	0	2	1	3	2	3	11
Expected	1.4	0.96	3.8	1.4	1.4	0.96	0.96	

$X_2 = 8.25$

d.f. = 6

P = 0.2

Chapter V

MECHANISM OF RADIATION INJURY TO CHROMOSOMES

Since Muller's original discovery in 1927 (125), that X-rays can produce gene mutations, much study has been devoted to the effect of radiation on genetic systems. One area in particular is the production of chromosomal aberrations by different kinds of radiations.

When a chromosome is broken by radiation, the broken ends are usually left in joinable condition (58). They may rejoin restituting the original chromosome, or if a chromosome is broken in two places, the broken ends may rejoin in new ways. Such "abnormal" union will clearly lead either to a single chromosome in which the portion between the breakage points has been taken out and reinserted in reverse order (inversion), or two chromosome bodies. One may be a ring formed by joining of the ends of the segment between the breakage points, and the other formed by the joining of the two remaining segments. One of these two chromosome bodies lacks a centromere and is likely to be lost at cell division. If the cell is able to survive the loss, one will be left with a cell in which one of the chromosomes is deficient of a certain number of genes, i.e. deletion. If two different chromosomes are broken, abnormal union between the broken ends

leads to reciprocal translocation with the production of two chromosome bodies, each having a portion of one of the two original chromosomes. If one new chromosome body has both centromeres (dicentric), and the other has no centromere (acentric), this latter fragment is likely to be lost at cell division.

Any change in the chromosomes has to be gross to be visible at all. If there is a structural abnormality of a chromosome, we may be able to recognize it, but only if it is relatively massive. For example, subchromatid aberrations, involving subdivisions of the chromosomes that are smaller than the chromatid, are not usually visualized.

Although chromosomes are only seen during cell division, and observations of structural change are made by examination of metaphase or anaphase figures, irradiation during these phases does not cause immediate chromosome breakage, perhaps because of the existence of a matrix of protein and nucleic acid which binds together to produce the coiled chromosome. Even though, at these stages, the chromosome thread may be broken, the chromosome does not separate into two pieces.

For the production of structural changes, it is necessary to irradiate during interphase, or early prophase (58). Aberrations observable in metaphase are then due to irradiation which has occurred at a

stage prior to metaphase corresponding to the time interval which elapsed between irradiation and fixation.

As a direct effect of radiation, chromosome breakage is caused by an ionizing particle passing through or in the immediate vicinity of a chromosome at the point at which breakage occurs. Breakage and the resultant rearrangements are not the only change; there may be alteration of the surface properties of the chromosomes so that they tend to stick together, producing bridges at anaphase stage. In severe cases, the chromosome may remain clumped at metaphase so that no further division stages ensue; or the bridges at anaphase may fail to break so that separate daughter nuclei cannot be formed. This "physiological effect" as described by Lea (58), is probably produced by a general change in the surface properties covering the whole surface of the chromosome. It was explained that the matrix of nucleic acid deposited on the chromosome takes the form of a fluid unpolymerized state instead of the normal polymerized non-sticky form. Again, this effect is exhibited in cells which are already in division at the time of irradiation. By the time cells enter division, recovery from the physiological effect will occur, as the chromosomes in metaphase do not exhibit the surface stickiness, i.e. reversible process. Lea concluded, therefore, that the physiological effect, but not the structural changes,

are exhibited by the cells already in division at the time of irradiation; and that the structural changes, but not the physiological effects, are exhibited by the cells which enter division.

If at metaphase, all aberrations are seen to involve both chromatids in the same way, this is evidence that at the times when structural rearrangement took place, the chromosome was single. If, on the other hand, it is observed that one chromatid takes part in a structural rearrangement in which the other does not take part, this is evidence that the chromosome was already split into chromatids at the time when the structural rearrangement occurred.

I. Chromatid Breaks:

Sometimes, the broken fragment remains close to the unbroken chromatid fragment in metaphase, possibly being held by its matrix, and appears as a gap or achromatic lesion. In some materials, constrictions are seen in the chromatids at anaphase, and these may be due to breaks in only one of the two threads of the chromatids; this is rather difficult to score. If the broken fragment becomes separated from the chromatid, it might be lost in the matrix and we get a shorter chromatid, i.e. deletion. It is believed that chromatid breaks observed at metaphase constitute only a minority of those

initially produced; the majority restituting, or uniting with other breakage ends to give the more complex structural changes.

II. Isochromatid Breaks:

In addition to cases where a single chromatid is broken, there are instances where both chromatids are broken at the same level. This type of break is believed to be produced by a single ionizing particle which passes through and breaks both chromatids of the already split chromosome.

The ratio of isochromatid to chromatid breaks due to X-rays varies with the stage of interphase at which irradiation occurs, being higher in early prophase where the two chromatids are closely associated. An ionizing event which passes through one has, therefore, a high probability of also passing through the other.

As prophase advances, chromatids spiralize in separate spirals and their distance apart increases. Thus, the probability of an ionizing event passing through the two chromatids diminishes.

Four types of isochromatid breaks could be identified:

1. NUPD, non-union proximal and distal.
2. NUD, non-union distal.
3. NUP, non-union proximal.

4. UPD, union proximal and distal.

In general, isochromatid-type breaks could be distinguished from chromosome-type in that the former always occur in cells in S and G2 stages. Also, almost all of them exhibit complete union. Occasionally we may get NUP and NUD, but hardly ever NUPD. This is in contrast to chromosome breaks, which always show the NUPD pattern.

III. Chromosome Breaks:

The simplest type of aberration produced in an unsplit chromosome is a simple chromosome break or terminal deletion. At anaphase, the chromatid fragments, formed by the splitting of the deleted portion, lag behind the centric chromosomes which are migrating to the poles. Lagging is due to the absence of the centromere and usually leads to failure of the fragments to be incorporated in the daughter nuclei. Consequently if many divisions elapse between irradiation and analysis, acentric fragments will not be observed. The two new ends formed by the breakage of an unsplit chromosome usually join either with each other, restituting the original chromosome or with other breakage ends giving various structural rearrangements. Thus, the terminal deletions observed

at metaphase are only a small fraction of those initially produced. In Tradescantia microspores, this fraction was estimated to be 5% of the original breaks. In Drosophila sperm, it was found to be 25% (58).

IV. Chromosome Interchanges:

If each of the two split chromosomes is broken simultaneously and they are in close proximity, rejoining of the four breakage ends can give rise, either to a pair of centric chromosomes (symmetrical), or a dicentric chromosome and an acentric fragment (asymmetrical).

The first type does not lead to any mechanical difficulty at anaphase, and should, therefore, be viable. However, they are not readily distinguished in mitosis from unchanged chromosomes. Asymmetrical interchanges result in the formation of acentric fragments which usually fail to be included in either daughter nucleus. At anaphase, the dicentric chromatids formed when the dicentric chromosome splits may either go to separate poles, or form a criss-cross bridge, or interlock. The relative frequencies of these events in onion root tip anaphases were 2:3:1. The proportion of cells carrying acentric fragments or dicentric chromosomes gradually diminishes in successive divisions following

irradiation. The death of the cell under these circumstances is due not only to genic imbalance resulting from losses of the chromosomes affected, but sometimes also to other causes, perhaps the mechanical difficulties experienced by dividing cells in which bridges are formed at anaphase. Similarly, if the two breaks occur in the same chromosome, a ring can be formed (plus a fragment).

If, however, the total dosage of radiation is divided into two fractions, only part of the breaks are produced at a given time. When a long enough period elapses before the second fraction of the dose is given, the first group of breaks will restitute before the second one is produced. Therefore, the two groups of breaks will not be able to rejoin with one another (121).

Radiobiologic Considerations:

According to the target theory, each break "hit" is caused by the passage of a single ionizing event through the breakage point, which provides the necessary energy for molecular transformation. A low dose produces a low number of ionizations with a low probability of hitting the target, and vice versa. According to Bacq and Alexander (2), this theory could be fitted to experimental data by postulating:

1. That the majority of breaks can rejoin by restitution or exchange for several minutes after the break has occurred and are not, therefore, detected;

2. That the number of ionizations which must be produced within a chromosome to produce a break is of the order of 15 to 20.

It was found that one-break aberrations increase linearly with dose, while two-break aberrations increase linearly with the square of the dose (42). If "P" is the probability that a hit is produced and is proportional to "D" dose, then the chance of two independently produced breaks occurring is "P × P" or P^2, which would be proportional to "D^2". However, it was found that asymmetrical exchanges in the plant Tradescantia increase linearly as 1.5 power of the dose. This was explained on the basis that the resultant yield represents a combination of one-hit and two-hit exchanges.

In case of neutron and alpha particle irradiation, all aberrations are linearly proportional to dose due to simultaneous production by the same ionizing particle of two breaks taking part in the exchange. Some breaks may be produced by ionization in the vicinity of, but not within, the chromosome by alpha particles.

The production of two-hit aberrations with sparsely ionizing radiation is influenced by two factors (42):

1. Distance: This was revealed in the non-randomness of rejoining due to the occurrence of limited numbers of sites within the nucleus where chromosomes, by chance, come close enough to one another. If there is a large number of such places, then a random distribution of exchanges would be expected.

As explained, three types of rejoining can occur:

a. Ends restitute - no exchange seen: ends are in the original position.

b. Reunite giving rise to translocations detected genetically but not cytologically, (detected only in first metaphase).

c. Rejoin asymmetrically, producing observable exchanges.

Thus, only one-third of the time would an observable change be formed.

2. Time: This factor was explained by dose fractionation experiments. Two-hit aberrations will decrease with a low dose rate with a rest period in between, as breaks restitute in the interval before other breaks are produced within the rejoining distances. Therefore, although equal numbers of breaks are produced with the same dose, the breaks will not be open concurrently and the chance for exchange formation is decreased.

Wolff(121) estimated that in the spermatocytes of the grasshopper, the distance over which broken chromosomes can unite is less than

0.3 μ. It was also observed that the shortest time between irradiation and observation of permanent chromosome alteration was two minutes.

Randomness:

As a general rule, radiation injury to the cell follows a random nature, producing different extents of damage. While all breaks are produced at random within the nuclei of the cell, chromosome exchanges might not be. As explained before, there seems to be a limited small number of sites within the nucleus where the two chromosome threads approach one another close enough to yield an exchange (58).

In vitro, Chu et al. (24) found that the frequency of spontaneous chromosomal aberrations is about 0.1/cell, (human somatic cell lines) while Bender (6) observed that the frequency in vivo is much lower; 0.0023 break/cell (peripheral leukocytes).

Chromosome Number:

When leukocytes are prepared by the short-term culture technique, a few percent of the cells are always found normally to have a chromosome number other than 2n = 46. Most of these cells contain fewer than 46 chromosomes, suggesting that they may result primarily from

breakage of cells during cytological preparation. With irradiation, there may be a higher frequency of aneuploidy. According to Bender (8) many may represent cells which have become aneuploid owing to abnormal segregation of aberrant chromosomes, in particular those with hyperdiploid counts. He tends to believe that the leukocytes of irradiated subjects may be somewhat hypersensitive to osmotic and mechanical stresses which cause broken cells in control preparations. In general, the mechanism involved is not entirely clear.

Age might be an important factor responsible for aneuploidy. Jacobs et al. (55) found higher frequency of non-modal count in older age groups among normal population. Similar results were observed on a subsequent study by the same group (54). They believe that a possible mechanism for aneuploid count is somatic non-disjunction in a normal diploid cell. Hypodiploid cells may also be produced by anaphase lagging (table 10).

Several biologic mechanisms could be postulated in this respect. One is that there is an increase of abnormal divisions with age. Another mechanism could be that the rate of abnormal division is a constant, but that some of the abnormal daughter cells are viable and maintain themselves in equilibrium with normal cells. Also, it may be that the cells were normal when taken from the body, and the

abnormal division is related to the condition of the culture. Some attributed this increase to the more frequent loss of sex chromosome (56).

Polyploidy:

In this condition, the cell comes into mitosis apparently normally, but, usually due to failure of the kinetic apparatus (spindle), the nucleus fails to divide, and consequently, the two sets of chromosomes remain within. This is not a great obstacle to the function of the cell; balance between chromosomes is what matters most. Apparently, it may be far more damaging to have one extra chromosome than to have a whole extra set (fig. 14).

Endoreduplication is a form of polyploidy characterized by typically paired diplochromosomes; a type of intranuclear doubling of chromosomes. Here, extra division of the chromosomes takes place during the resting phase without any mitosis-like manifestations. So when mitosis occurs, the chromosomes emerge already doubled (fig. 13).

Both phenomena were described in relation to malignancy, irradiation and certain anti-mitotic agents, especially in vitro (48).

In a recent report, Powsner (87) observed an incidence of endoreduplication much higher than previously reported in peripheral blood cultures of randomly selected controls.

Chapter VI

DISCUSSION AND INTERPRETATION

First Evaluation

Generally speaking, the data from this evaluation demonstrated higher incidence of chromosomal abnormalities both in count and morphology in all the study groups; this was mostly observed in patients studied shortly following treatment.

In the follow-up group, an attempt was made to find any correlation between the degree of aneuploidy or chromosomal aberrations and the duration after radioiodine therapy; no special pattern was detected.

The findings in the follow-up group as compared to the control group suggested that some abnormalities can persist for long periods of time after exposure to irradiation. This is in agreement with the impression given by Boyd (17) and Warren (115) who found that structural aberrations tend to persist. Bender (8) showed that in the 8 men accidentally irradiated, the frequency of abnormalities appeared to have dropped somewhat in most cases during a one-year period; while Cantolino (22) observed no noticeable loss or diminution of aberration frequency over a similar period.

The type of cell under consideration differs in the follow-up group from that in other treated groups. Shortly after irradiation, we were studying mostly the circulating lymphocytes which were irradiated by the therapy dose of ^{131}I. In the follow-up group we were dealing with lymphocytes that were the daughter cells that had risen from lymphopoietic stem cells which were irradiated years before. Thus in comparing the two groups, such factors should be considered; difference in radiosensitivity, degree of cell maturation, delivered radiation dose and the metabolic status of each tissue.

In the treated groups, especially group IV, to avoid irradiation during the culture period from the administered therapy dose of ^{131}I, only the buffy coat and the top mm of erythrocytes were drawn from the serum and used for culture; human A-B positive serum was then added to replace their "hot" serum.

To estimate the effect of the diagnostic tracer doses of ^{131}I which some of these patients had received before the chromosomal study, blood samples were obtained on 10 patients before and at different intervals after a tracer dose of 2-10 μC ^{131}I; no significant difference was noted.

Re-evaluation

As shown in tables 4 and 6 in Chapter IV, re-studying

chromosomal changes in these two groups showed no significant difference before and after ^{131}I irradiation. Despite the fact that this is not entirely in agreement with the previous results obtained in the first evaluation, it is not very different if one considers the results of post-therapy analysis only, without the controls.

It was interesting to find out from table 8 that the data were more or less in agreement in the two evaluations. In the first evaluation, aneuploidy among study groups ranged from 5.4% to 6.1% with a mean of 5.8%. In re-evaluation the range was from 5.2% to 6.7% with a mean of 5.9%; this is very close to the mean value in the first evaluation.

Also the difference in the two evaluations between the chromatid-type aberrations was not very striking, although the incidence was relatively high in the first evaluation of treated cancer group.

As for chromosome-type lesions, if we exclude the data on the long follow-up group, since this type of aberration tends to drop in subsequent division, we find that in the first evaluation the incidence ranged from 1.0% to 3.5% with a mean of 2.2%. In the re-evaluation, the range was 1.1% to 2.6% with a mean value of 1.9%.

Since the results from the two evaluations did not differ significantly in the post-therapy analysis, then the major discrepancy

should have been due to the results of the pre-therapy control samples as shown in table (9). The frequency of both aneuploidy and structural abnormalities was found to be different in the control group of the two evaluations.

There is generally a significantly lower incidence of chromosomal changes in the first evaluation especially in non-modal counts and chromosome-type aberrations. This might show certain bias in the first evaluation of the normal controls and control pre-therapy analysis.

1. In the first evaluation scoring of a blood sample was performed from any number of slides available depending on the yield of metaphases on these slides. Thus, the reported number of scored cells were obtained sometimes from as low as one slide and other times from as high as 8 slides. In re-evaluation, 5 slides from each sample were randomly selected and a maximum of 10 cells were always scored from each slide to avoid analyzing more cells in the better preparations with positive findings.

2. Identification of the origin of the slides in the re-evaluation analysis was entirely impossible, thus avoiding the possibility of any bias. Decoding of the slides was performed after all analyses were completed.

3. The technique of drawing a diagrammatic karyotype for each abnormal cell was applied only in the re-evaluation study. Besides checking for the chromosome count, it also allowed for identifying the group in which the chromosomes were missing, added or abnormal. In general, this method was very valuable for the accuracy of the scoring.

4. In the re-evaluation study, all abnormalities before being finally recorded, were passed upon by another observer; the advisor; a practice which was not followed in the first evaluation.

5. In the first evaluation, some cells with chromosome count less than 43 were scored and included in the reports. In re-evaluation, cells with counts below 43 were considered broken cells and were not reported.

6. When collecting the data for the first evaluation, tetraploid cells were included under aneuploidy; these were considered separately in re-evaluation.

In reviewing the literature for available data on leukocyte chromosomal changes from control non-irradiated subjects, it was interesting to find a wide variation from one laboratory to another (table 10). The degree of aneuploidy was reported as low as 0.2% in non-irradiated hyperthyroids (Cantolino) and as high as 29.7% in

non-irradiated patients with advanced cancer (Amarose).

Also the incidence of chromosomal aberrations ranged from 0 to 4.1% in the old age group (Jacobs, 1964).

However, if we consider, from this table, data obtained from a statistically significant number of cells, we find the range becomes narrower. Thus the degree of aneuploidy would become 6.0% to 9.6% as shown in the most recent study of Court Brown et al. (29), which also covers the range of other significant data. The same is observed with chromosome-type aberrations; the frequency then would be around 3.0%.

Considering this, we find that in the re-evaluation study of control pre-therapy blood, the range of aneuploidy was 5.9% to 7.8% and of chromosome type aberrations was 1.9% to 2.9%. Both fall very close to the values obtained from the most "reliable" data of the literature.

Thus, from the previous discussion, we may conclude that the results of the re-evaluation study are more reliable than that of the first evaluation.

We tend to think that this bias in the analysis of the controls might be as well responsible for the many reports done by others and briefly summarized in Chapter I which show chromosomal

abnormalities following exposure to irradiation.

The presence of non-modal count or a chromosomal abnormality in a control cell might represent either a spontaneous abnormality or an event related to natural background radiation.

Chromatid-type aberrations are not very significant since they are essentially an in vitro occurrence produced during the short-term leukocyte culture and so were considered separately. In general, they tend to be either lost completely or later may involve the whole chromosome after the first division (7).

Migeon et al. (70) showed that in both infant and adult controls, the frequency of chromatid-type aberrations ranged from 1.6% to 8.0%. They considered their presence is probably due to background radiation and that they may be a constant threat to the interpretation of the results. They stated that they might be initiated by technical procedures and that their presence in the absence of adequate controls should not be considered sufficient evidence of a response to previous insult by any agent.

Chromosome-type aberrations and many of the post-first chromatid aberration derivatives are lost some time after the first division. Many of the deletions are probably cell lethals and are thus expected to be lost quite rapidly from the cell population. In addition,

cell selection may well operate to increase or decrease the frequencies of various aberration-bearing clones of cells. Curtis et al. (35) found that chromosomal aberrations induced by X-rays in the regenerating mouse liver cells were eliminated after four or more cell divisions, possibly due to healing of broken chromosomes in the interphase nuclei.

The fact that we could not observe any specific pattern regarding the lost or added chromosome or the abnormal ones in both control and study groups supports the impression that the process is rather random.

Powsner (87) reported that endoreduplication may be found in approximately 10% of routine cultures of random subjects (non-irradiated). This is much higher than the frequency observed in our material either before or after treatment.

It was of interest to notice that there was no significant difference in the results of pre-therapy control samples from hyperthyroids and thyroid cancer patients despite the differences in the clinical picture. Before treatment, hyperthyroid patients were in a hyperactive metabolic state while all thyroid cancer patients were definitely myxedematous, being subjected to a total thyroidectomy before the treatment. This may lead us to assume that the thyroid

status of the patient does not affect the chromosomal apparatus; since neither the hyperthyroid nor the hypothyroid nor thyroid malignancy showed a chromosomal picture different from one another.

Examples of the above-mentioned chromosomal abnormalities are shown on pages 102 through 115.

Figures 4-11 show abnormalities detected in control pre-therapy blood samples. Figures 12-17 illustrate abnormalities observed in post-therapy blood samples.

Further Investigation:

It may be of interest to evaluate chromosomal changes after radioiodine therapy in the bone marrow.

Sandberg et al., in a study of chromosomal abnormalities in acute leukemia (94) reported that the chromosome pattern in freshly aspirated, non-cultured marrow in 6 patients, differed consistently from the findings in cultured blood of the same individuals. They concluded that future chromosome studies of acute leukemia will have to be based on examination of freshly fixed marrow aspirates, because of the following "advantages":

1. Marrow study exposed the cytogenic profile of the leukemia cell population in the patient's marrow and avoided false negative results from peripheral blood;

2. In vitro artifacts of peripheral blood are ruled out and mitotic arrest by colchicine is not required;

3. Most marrow aspirates of patients contain abundant mitoses, whereas cultures of leukemic tissue may grow poorly or not at all.

In another communication (93), in a study of chronic myelocytic leukemia, the same authors found that the frequency of the Ph′ chromosome in freshly aspirated marrow cells of 14 patients was significantly higher than that observed in cultured blood cells of the same subjects. They assumed that since myeloblasts, promyelocytes and myelocytes probably contribute most of the metaphases with the Ph′ chromosome observed in marrow, then dichotomy may be due to the larger number of these younger cells observed in marrow compared to blood. They also assumed that the abnormal autosome is present in normoblasts and other cells of the marrow, especially if the anomaly is present in the stem cells which may serve as the precursors not only of the leukemic cells but also of normoblasts and other cells. Another explanation they introduced was related to the mitotic capacity in vitro. Even though these karyotypically abnormal cells continue to divide, it appeared that they lag behind normal leukocytes in the incubated blood.

Reisman et al. (90) in a similar study of leukemia in children,

reported that in contrast to the findings in bone marrow, aneuploidy was seldom detectable in peripheral blood cultures because of the mitotic advantage of the normal diploid cells. Subsequently, they only considered findings obtained from the bone marrow.

Comparing chromosomal changes in the hemopoietic cells with those of peripheral lymphocytes after radioiodine therapy may be a worthwhile project.

Table (8)

Comparison of the Post-Therapy Results
of the Two Evaluations

Group	PERCENT		
	*Aneuploid	Chromatid	Chromosome
FIRST EVALUATION:			
Follow-up	5.4	1.4	0.3
Treated Hyperthyroids	5.8	2.5	1.0
Treated Thyroid Cancer	6.1	5.7	3.5
RE-EVALUATION:			
Treated Hyperthyroids	5.2	3.7	2.6
Treated Thyroid Cancer	6.7	0.3	1.1

*Not including tetraploids

Table (9)

Comparison of the "Control" Results
of the Two Evaluations

Group	PERCENT		
	*Aneuploid	Chromatid	Chromosome
FIRST EVALUATION:			
Normal Controls	4.3	0.4	0
Hyperthyroids	3.5	1.3	0.1
Thyroid Cancer	3.8	1.5	0
RE-EVALUATION:			
Hyperthyroids	7.8	1.8	2.9
Thyroid Cancer	5.9	1.9	1.9

*Not including tetraploids

Table (10)

Aneuploidy and Chromosome Aberration in Controls

Reference	# of Subjects	# of Cells	% Aneuploid	% Chromosome Aberrations	Remarks
Tough. Lancet II:849, 1960	6	205	7.3	-	Normals
Jacobs. Nature 191:1178, 1961	97	3528	6.5	-	5.7-9.5%, age 0-65 y.
Buckton. Lancet II:676, 1962	58	1250	3.7	1.5	Ankylosing Spondylitis
Buckton. Nature 193:591, 1962	38	1281	8.2	-	Cancer
Macintyre. J. Clin. End. & Met. 22:1117, 1962	174	2885	4.5	"rare"	Normals
Bender. Proc. Nat. Acad. Sci. 48:522, 1962	4	430	3.5	1.6	Normals
Bender. Rad. Res. 16:44, 1962	5	458	1.7	0	Normals
Bender. Rad. Res. 18:389, 1963	5	931	4.1	0	Normals
Cohen. Proc. Nat. Acad. Sci. 50:16, 1963	2	150	8.0	2.7	Normals
Sasaki. Radiol. 81:652, 1963	10	214	5.6	0	Normals

Table (10)
con't.
-2-

Reference	# of Subjects	# of Cells	% Aneuploid	% Chromosome Aberrations	Remarks
Jacobs. Nature 197:1080, 1963	124F	3909	6.8	-	4.0-10.2%, age 0-75y.
	123M	4471	6.5	-	
Jacobs. Ann. Hum. Genet. 27:353, 1964	102F	2669	12.8	3.4	Over 65 years.
	87M	2393	7.2	4.1	
Cohen. J. Cell. Biol. 23:386, 1964	5	626	-	3.0	Normals
Norman. Rad. Res. 23:282, 1964	23	464	4.5	0	Normal and hospital patients
Moore. Am. J. Obst. & Gyn. 88:985, 1964	10	250	12.8	0	Normals
	10	350	16.0	0	Cancer
Millard. Cytogenet. 4:277, 1965	6	298	15.0	2.0	Cancer
Warren. J.A.M.A. 193:351, 1965	-	-	10.0	-	Normals
			10.0	-	Hospital patients
			12.0	-	Cancer
Doida. Rad. Res. 26:69, 1965	3	262	3.6	0	Normal Japanese
	3	190	8.7	0	Cancer Japanese

Table (10)
con't.
-3-

Reference	# of Subjects	# of Cells	% Aneuploid	% Chromosome Aberrations	Remarks
Amarose. Obst. & Gyn. 25:825, 1965	5	350	29.7	2.9	Cancer (advanced)
Court Brown. Lancet II:1239, 1965	57	1524	-	2.0	Normals
Bender. Rad. Res. 29:568, 1966	-	1642	4.9	0.5	Normals
Court Brown. Chromosome Studies In Adults, Cambridge Univ. Press, London, 1966	231F 207M	6466 5954	9.6 6.0	3.1 2.6	Normals
Nasjleti. J. Nucl. Med. 7:159, 1966	10	1000	4.0	-	Cancer
Bloom. Lancet II:672, 1966	94	2813	4.9	0.03	Normal Japanese
Cantolino. New Eng. J. Med. 275:739, 1966	12 5	1569 482	0.5 0.2	0.1 0	Normals Hyperthyroids
Schmickel. Am. J. Hum. Genet. 19:1, 1967	12	1569	5.2	0.12	Normals
Present Study	12 11	451 375	7.8 5.9	2.9 1.9	Hyperthyroids Cancer Thyroid

Chapter VII

RELATION TO LEUKEMIA AND GENETIC RISK

The first clue that ionizing radiation is leukemogenic came soon after the discovery of X-rays, when individual cases of leukemia were reported in radiation workers (72). By 1942, there were at least 20 cases among radiologists and 4 among radioisotope workers (40). These data were consistent with the findings in laboratory animals. However, differences in medical care and diagnosis could as well have accounted for the reported higher leukemia rates in physicians, whose access to sophisticated medical care is much greater than that of the general population (72).

In 1950, March (65) showed that 4.7% of deaths among radiologists were attributed to leukemia as compared to 0.5% of deaths among other physicians. However, it was explained that this high incidence might be to some extent due to the relative youth of radiologists who were then in a young specialty (leukemia accounts for more deaths in middle than in later years.) Recently, Warren (114) observed a decreased incidence of leukemia among radiologists during the latter years, which may indicate that current occupational maximal permissible dose levels are providing adequate protection.

There was a definite increase in the incidence of leukemia among survivors of the atomic bomb in Hiroshima and Nagasaki (45). In contrast to the radiation exposure of radiologists, which is a daily low dose over a period of years, survivors of atomic bombing experienced a single instantaneous exposure over a wide range of doses. Mortality from leukemia among survivors reached a peak 6 to 7 years after the atomic explosion and has since declined to near normal levels (18).

In a recent study by Bizzozero et al. (12,13) on the radiation-related leukemia in Hiroshima and Nagasaki 1946-64, it was concluded that the maximal incidence rates occurred during 1950 to 1954, especially for chronic granulocytic and acute lymphocytic leukemias, in persons up to 29 years of age at the time of bombing and who were exposed within 1500 meters of the hypocenter. This initial period was followed by a transitional period of radiation effect during 1955 to 1959, characterized by elevated rates of acute granulocytic leukemia in those persons over 30 years of age at the time of bombings and exposed within 1500 meters. There was no significant clinical or symptomatological difference separating those cases of leukemia exposed to 0-1,500 meters from those exposed to 1,501-10,000 meters.

The findings of elevated incidence rates for both acute lymphocytic and chronic granulocytic leukemias in the young group and acute

granulocytic leukemia or chronic in the older age group suggested that age and possibly dose at the time of exposure are just two of the several factors that may determine the type of specific leukemia response in the human. Bizzezero also noted that experimental investigation in the mouse and other experience with humans tended to support these observations.

Court Brown et al. (31), in an evaluation of malignancy among over 13,000 patients with ankylosing spondylitis who were treated with X-rays, reported an excess of deaths from leukemia and aplastic anemia. Moreover, deaths due to other types of cancer originating in heavily irradiated tissues were increased approximately twofold 6 or more years after the first treatment. Fifteen years after first treatment, the excess showed no sign of diminishing. In contrast, the number of deaths from cancers originating in lightly irradiated tissues was not increased significantly. Neither ankylosing spondylitis nor the drugs used in its treatment were believed to have been important in the induction of leukemia.

Stewart et al. (103) concluded that about 8% of leukemias in children (other than lymphatic leukemias) were caused by maternal diagnostic X-rays.

MacMahon (64) estimated that mortality rates in children from

leukemia and other cancers were each about 40% higher when there was prenatal X-ray exposure than when there was not.

In 1960, Cronkite et al. (34) reported their observations as:

1. For high level, single-dose exposure in man, the incidence of leukemia is approximately linear with dose. At dose levels of perhaps 100r equivalent or greater, the incidence was approximately 1 to 2 per million persons at risk per year per rad, at least from approximately the second to fifteenth year following exposure.

2. Below these dose levels, and for other than single acute dose, data available were inadequate for prediction.

3. Although there was evidence that large dose radiation of sizable portions of the marrow may be leukemogenic, there were no adequate grounds for assuming that the highly localized radiation from internal emitters such as ^{90}Sr and radium were or were not leukemogenic.

4. They were not able to determine whether or not a threshold dose for the induction of leukemia does or does not exist.

In an article entitled "Is there Incriminating Evidence for ... Irradiation Leukemogenesis," Lawrence (57) summarizes his findings as:

1. The incidence of leukemia can be increased by exposure to irradiation.

2. No positive statement can be made as to whether there is a threshold or linear relationship between irradiation and leukemia.

3. There is no evidence that chronic lymphatic leukemia is produced by irradiation.

4. Naturally occurring radioactive substances and cosmic rays may cause approximately 130 mrem exposure a year.

5. Medical irradiation results in about one-half as much bone-marrow irradiation exposure as from natural sources.

6. Variations in radiographic techniques result in great differences in radiation exposure.

7. Fluoroscopy requires very careful monitoring.

8. Increased radiation exposure secondary to nuclear weapon testing is minimal, but it could become an important source of trouble.

Radioiodine and Leukemia:

Among a recently reported number of patients estimated at 60,000 treated for carcinoma of the thyroid with doses of ^{131}I ranging from 50 to several thousand millicuries, at least 10 cases of leukemia or aplastic anemia have occurred (112). In all but one case, the leukemia appeared between 2 and 4 years after the initial therapy.

As mentioned previously (Chapter II), Pochin (85) estimated that an expected number of 21 cases of leukemia would arise by chance in his thyrotoxic population, treated with radioiodine, with a range of 14 to 28 cases. He collected the 8 published cases and ten others, as well as one case of lymphosarcoma.

In the 8 cases collected, leukemia was of an acute type and the interval between administration of ^{131}I and the diagnosis of leukemia was 14 to 26 months (50).

Solomon and Rubenfeld (100) reported one case of leukemia and another of lymphoblastoma following radioiodine therapy for hyperthyroidism. Burns et al. (21) reported two cases of acute leukemia following ^{131}I, which makes a total of 20 cases.

In this respect, radioiodine is different from X-ray therapy used in the treatment of ankylosing spondylitis. In the latter case, irradiation was delivered to limited sections of the body in a series of isolated doses and the spinal bone marrow was particularly exposed. In case of radioiodine, the whole body is exposed to less intense internal irradiation by beta and gamma rays. Therefore, there may be a considerable difference in the leukemogenic effect of the two methods of irradiation. Green et al. (50) estimated that the average radiation dose delivered to bone marrow from a single dose of ^{131}I for

thyrotoxicosis is 10 rads. With multiple treatments, the marrow dose may be much larger. However, they concluded that, generally, the risk of leukemogenesis is slight and does not contraindicate ^{131}I therapy for thyrotoxicosis.

Pochin (86) reported that the use of radioactive isotopes made a small contribution only to genetically significant risks of the population as a whole. He considered that even after the high doses given for thyroid cancer, the risk of leukemia did not appear to be so high as to contraindicate the use of a form of therapy which is commonly valuable in the control of disseminated malignancy.

In view of the low doses of radiation to the blood forming organs resulting from treatment with therapeutic doses of ^{131}I, the lack of an increase over the expected incidence of leukemia is not surprising. There are certain atypical features about the small leukemia group as studied by Wald (112). Of the 19 cases in which the type of leukemia is known, 15 were diagnosed as acute. This proportion is higher than would be anticipated in a normal population of the same age and sex distribution. Also it is estimated that only about 20% of the hyperthyroid patients treated with ^{131}I were males. In contrast, over 70% of the cases of leukemia in these groups occurred in males. The atomic bomb exposure data also suggest a heightened sensitivity of

males to radiation-related leukemia. The mean age of leukemia patients is above that of hyperthyroid patients in general. This probably reflects the conservative use of ^{131}I in the younger population.

These facts would suggest either that these cases of leukemia occurred by chance, or that under these clinical circumstances the latent period of radiation-related leukemia was shortened.

A cooperative study of the late effects of radioiodine therapy in about 35,000 patients treated since 1946 is under completion now by the Division of Radiological Health, Public Health Service, which is expected to shed further light on the problem of radiation-induced leukemogenesis.

Chromosomes in Radiation-Induced Leukemia:

Studies in humans and mice have revealed demonstrable chromosome abnormalities in most cases of radiation-induced leukemia (49, 3).

Although stem lines characteristic of individual tumors have been noted, no consistent chromosome change has been described in a series of cases. This variability of demonstrable chromosome changes is typical of most types of malignancy studied, whether radiation-induced or not (with the exception of chronic granulocytic leukemia).

The failure to find such a specific change (Ph′ chromosome) in most types of malignancies suggests that the chromosome changes which are demonstrated in these tumors are not casual (82). This is probably due to their variability and the presence of similar changes without leukemia. It seems more likely that each type of malignancy may be characterized by a specific genetic change, but with the exception of chronic granulocytic leukemia, this primary change is too small to be demonstrated by present methods.

These chromosomal changes could be due to radiation. Although they do not cause the malignant state, they could occur coincidentally and be perpetuated simply because they confer no selective disadvantage on the neoplastic cell. Alternatively, these chromosome changes could be unrelated to radiation. Conditions associated with the neoplastic state itself appear to create a greater tendency for chromosomal rearrangements with resulting opportunity for selection of abnormal stem lines. Thus demonstrable chromosome changes could arise during the life of a tumor superimposed on the initial invisible radiation-induced changes, and the cells bearing these secondary changes could come to predominate, because of selective advantage over the original tumor cell population (82).

Genetic Risk Estimates

A recent United Nations report (109) considered that genetic risk estimates from radiation must, ideally, be comprehensive, therefore taking into account all major genetic effects of social rather than merely biological import.

The over-all risk of induction of all point mutations which are all assumed to have some degree of dominance and to be eliminated predominantly in heterozygotes, according to the report, includes the risk of induction of dominant mutations. One major practical limitation of the over-all risk estimate is due to the fact that the damage that is then assessed is expressed in term of loss of mutants through generations. This loss has a clear biological meaning and an undesirable character for the individual and for society. However, it is not known how many of the harmful mutations induced by radiation will at some point be eliminated through loss of a zygote before implantation, an event usually not detectable, rather than through drastic reduction in fertility, miscarriages, or serious genetic defects. This genetic risk is only part of chromosome anomalies. At present, there is no way of estimating the over-all risk of induction of chromosome anomalies. Their high frequency in human populations makes it likely that such a risk may not be negligible.

The present knowledge is inadequate even to guess its possible magnitude while the estimates available are based on assumptions that make conclusions conjectural or at best very tentative indeed.

In considering the significance of radiation damage to the genetic material, the report compares it with the rate of naturally-occurring genetic changes. It was estimated that on the average, a total of 140 point mutations arose spontaneously in 1000 gametes in each generation. Under conditions of acute irradiation at high doses, one rad induced a total of two mutations per 1000 gametes. Thus a dose of one rad per generation would add about one-seventieth to the total number of mutations arising spontaneously per generation. To this point mutational damage must be added that due to chromosomal abnormalities which occur spontaneously in 1% of live-born children.

The report concluded by stating that it is not possible at present to estimate the over-all rate of induction of anomalies by radiation, but the rate is expected to be very low at low doses.

Macintyre (60) raised the question that if all cell lines stemming from somatic cells with chromosomal abnormalities can remain viable, the same may be true in similarly affected reproductive cells in the gonads. He further explained that there is no assurance that spermatozoa or ova with an abnormal number of chromosomes are

infertile; Mongolism and Turner's syndrome probably arise from a zygote to which either sperm or ovum has contributed an abnormal number of chromosomes.

Later he studied chromosomal changes on cord blood from 23 newborn infants exposed to X-rays through maternal diagnostic procedures (61). He found no significant difference between the irradiated group and a group of 14 control babies born to non-irradiated mothers. However, he suspected a trend towards more breaks and abnormal counts in the irradiated series than in the control series.

Whelton et al. (119) reported the study of 3 children whose mothers were exposed to high-level gonadal irradiation. They concluded that neither the clinical nor the cytogenic investigations of these offspring gave any detectable evidence of genetic damage. However, they also added that the failure to demonstrate genetic damage in the first generation does not rule out the possibility of recessive mutations which will become apparent in a subsequent generation.

Dekaban (37) reported a case of a 33-year-old mother who had previously received a therapeutic dose of 1550 rads to various parts of the body and gave birth to a malformed child. Her blood culture showed a high incidence of chromosomal breaks which mounted to 24% of the mitoses scored.

Chapter VIII

COMPLEXITY OF ENVIRONMENT

A. Radiation or Zero G

The question has often been raised whether there is a radiation or radiation-like hazard associated with space flight. Such an effect--if one actually exists--might be due, in part, to environmental features of space, such as prolonged weightlessness, which might interact synergistically with radiation to produce unexpected effects.

Bender in 1964 (4) planned a practical experiment to irradiate thoroughly studied biological material with a known quality and quantity of radiation during the zero gravity phase of an orbital space flight. The types and frequencies of effects were to be compared with those observed in suitable "in-flight" and ground-based controls. The parameters set up to be measured were the frequencies of various types of chromosome aberrations.

The experiment consisted of irradiation of a series of whole human blood samples with ^{32}P beta rays simultaneously in the spacecraft and on the ground during the orbital phase of the mission. After completion of the mission, a cytogenetic analysis was made of each blood sample and the yields of both single and multiple-break aberrations determined

for both in-flight and ground control samples.

Comparison of the results (9) showed that no significant difference in the yields of multiple-break aberrations existed between in-flight and ground samples. The frequency of deletions, however, was significantly higher for the in-flight samples.

Pre- and post-flight blood samples obtained from the astronauts showed no significant chromosomal abnormalities. Flight GT-III took place in March, 1965, and the duration of weightlessness was 50 minutes. Other factors which might be considered in this respect were acceleration and vibration of lift-off.

Bender concluded that synergism between radiation and some space-flight parameter thus appeared to exist for human chromosome aberration production. Though this effect is of interest, the experiment failed to confirm the existence of any large effect which might constitute a special obstacle to prolonged manned space flight.

However, in a further experiment during Gemini XI flight (5), he observed no synergetic effect for either single or multiple break aberrations.

His final conclusion was that no synergism appeared to exist between ionizing radiation and many of the factors associated with orbital flight; at least for gene mutations, production of chromosome aberrations, or cell killing.

It is still too early to obtain a complete picture of the interaction of irradiation with space flight factors, and so far there is no common opinion as to whether these factors by themselves are able to induce some kinds of genetic damage. The current findings, though, lead us to realize that the magnitude of the genetic and somatic risks encountered in space flights is not only determined by dose, dose rate and quality of the radiation received in those circumstances, but also by parameters that are particular to space flight situations.

B. Benzene and Caffeine

In a preliminary communication, Tough and Court Brown (108) reported that men exposed to ambient benzene may show structural chromosome aberrations in lymphocytes cultured from peripheral blood. They analyzed the chromosomes of 22 men working in a factory where benzene was being used as a solvent. The duration of exposure varied from 7 to 20 years. The frequency of unstable chromosomal abnormalities was higher among workers as compared to a control group ($P = <0.05>0.02$).

Ostertag et al. (83) treated HeLa cells for one hour with varying concentrations of caffeine, incubated the cells for 24 hours, then studied chromosomal abnormalities. They found that caffeine was effective in

breaking chromosomes of human cells in culture. The amount of breakage was almost linear with dose and extrapolates to control values at 0 dose. They concluded that it is possible that the much lower doses received by man in drinking coffee or tea are mutagenic as well. Rough calculations placed the amount of breakage in the neighborhood of the "natural mutation rate."

Thus we see that other factors besides radiation, including perhaps some which have not been identified as yet, exist in our environment and may also be responsible for chromosomal changes. There are many possibilities here offering scope to the investigators of the future.

Chapter IX

SUMMARY AND CONCLUSIONS

Evaluation of the radiobiologic effects of irradiation from radioiodine therapy for thyrotoxicosis and thyroid carcinoma was performed through analysis of chromosomal changes in peripheral leukocytes.

After a short-term culture, lymphocytes were analyzed for chromosomal changes. Abnormalities in count and morphology were analyzed through direct microscopic visual scoring.

First Evaluation:

This was done on:

1. Sixty-five hyperthyroids treated 3 to 14 years previously with 6 to 54 mC ^{131}I. Their chromosomal analysis was compared to that of 27 controls matched for age and sex.

2. Fifteen treated hyperthyroids. Chromosomal analysis was done before and one-half hour after an oral dose of ^{131}I of radioiodine ranging from 7 to 13 mC.

3. Twelve thyroid cancer patients. Chromosomal studies performed before and at intervals up to 48 hours following a larger dose of ^{131}I ranging from 150 to 208 mC.

In all these groups, evaluation under circumstances described suggested a statistically higher incidence of abnormalities in the study groups than in the control groups. The incidence of abnormalities was highest shortly after irradiation (groups III and IV).

No hematologic phenomena seemed to accompany these abnormalities, either shortly after irradiation or as a late manifestation.

Re-evaluation:

Repeat study of the chromosomal changes was performed on the latter two groups (treated hyperthyroids and thyroid cancer patients) using a more accurate and detailed method of analysis, eliminating investigator bias. Results obtained under these circumstances suggested no statistically significant difference between the chromosomal picture before and after ^{131}I therapy, in both hyperthyroid and thyroid cancer patients. The explanation for the discrepancy and comparison with the literature are discussed in Chapter VI.

Data also showed no significant difference in the chromosomal analysis between non-irradiated hyperthyroid patients and patients with thyroid carcinoma, suggesting no specific effect of either disease on the chromosomes.

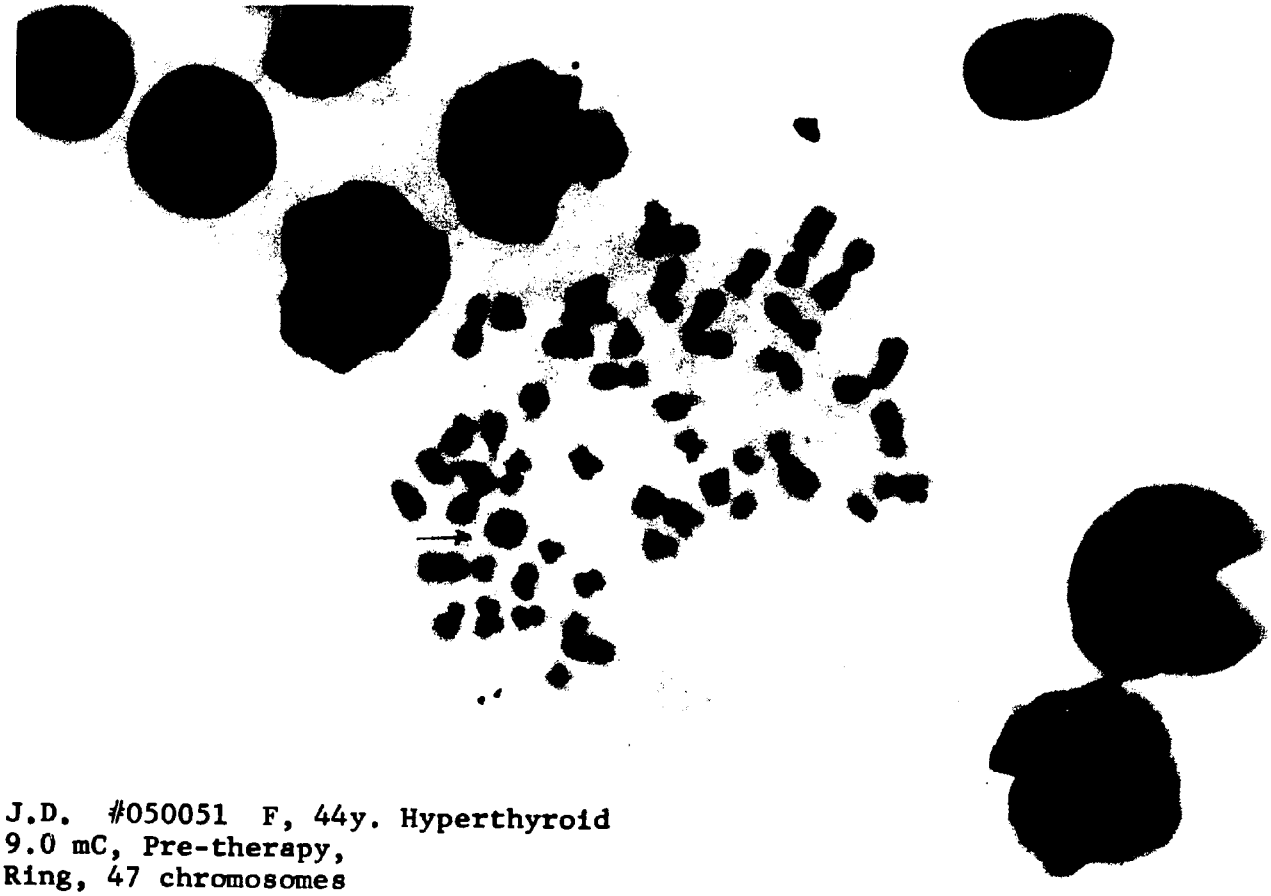
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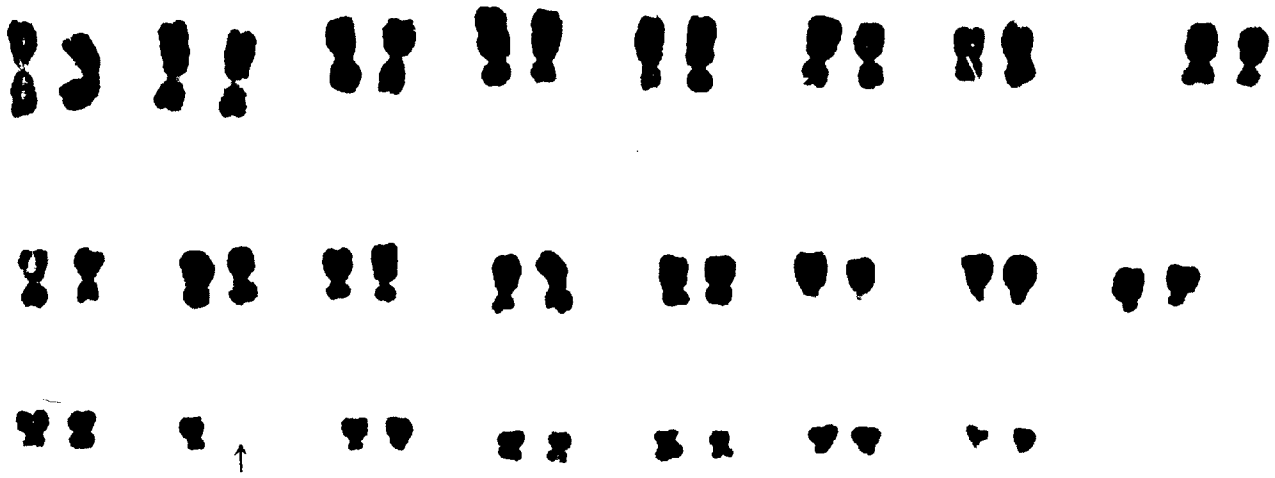
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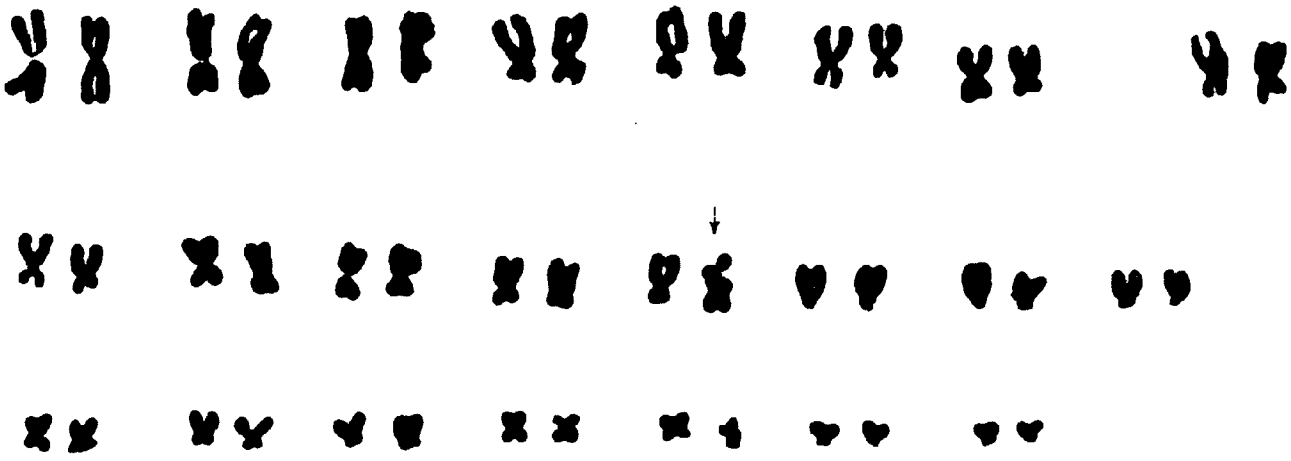
J.D. #050051 F, 44y. Hyperthyroid
9.0 mC, Pre-therapy,
Ring, 47 chromosomes

Figure 4



J.D. #055861 F, 31y. Hyperthyroid
9.5 mC, Pre-therapy,
45 chromosomes (-E)

Figure 5



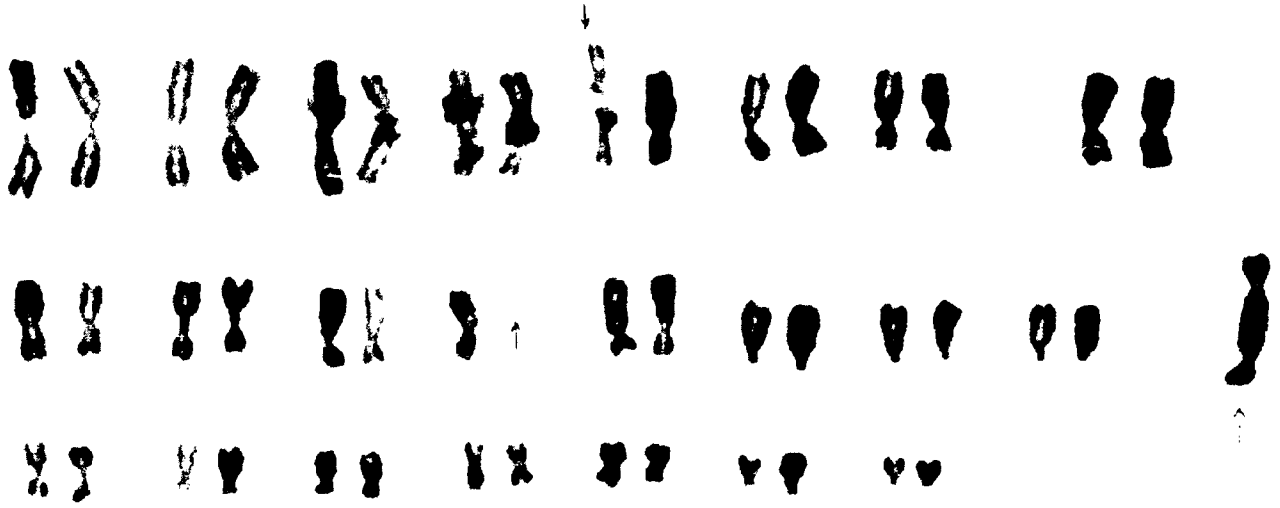
J.D. #055861 F, 31y. Hyperthyroid
9.5 mC, Pre-therapy,
Double acentric fragment

Figure 6



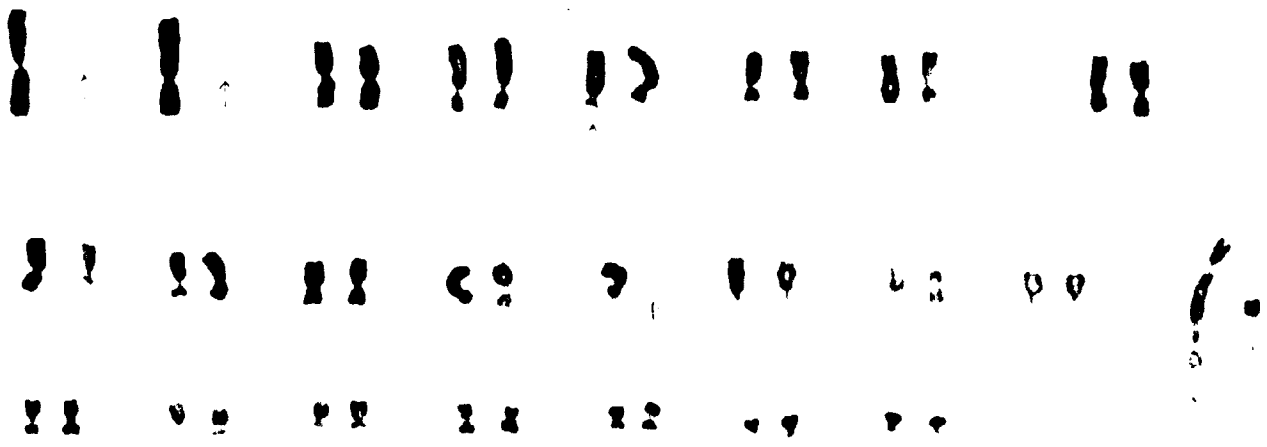
B.W. #748446 F, 61y. Hyperthyroid
8.8 mC, Pre-therapy,
Double long acentric fragment

Figure 7



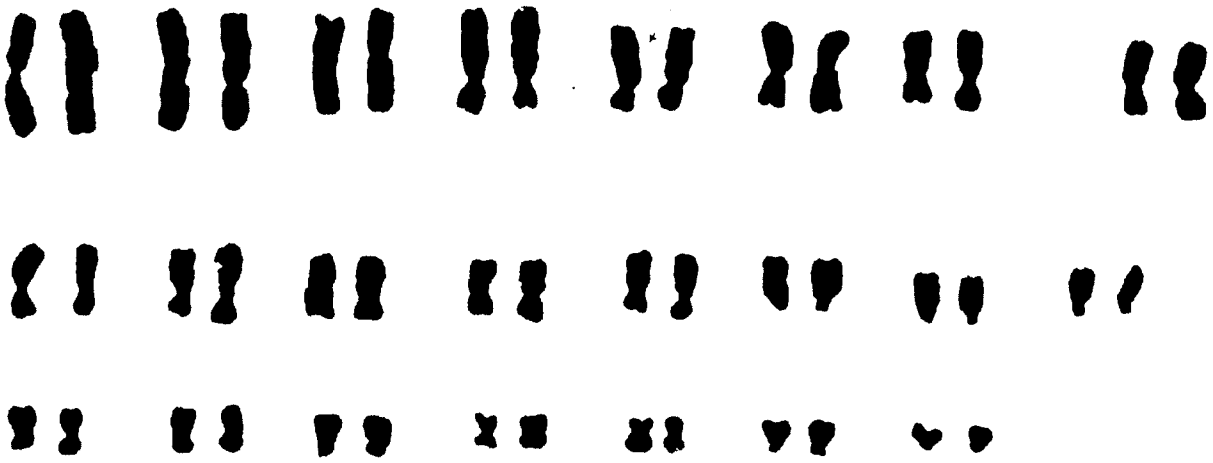
C. B. #052013 F, 40y. Hyperthyroid
10.0 mC, Pre-therapy,
47 centromeres, dicentric, -C, broken B

Figure 8



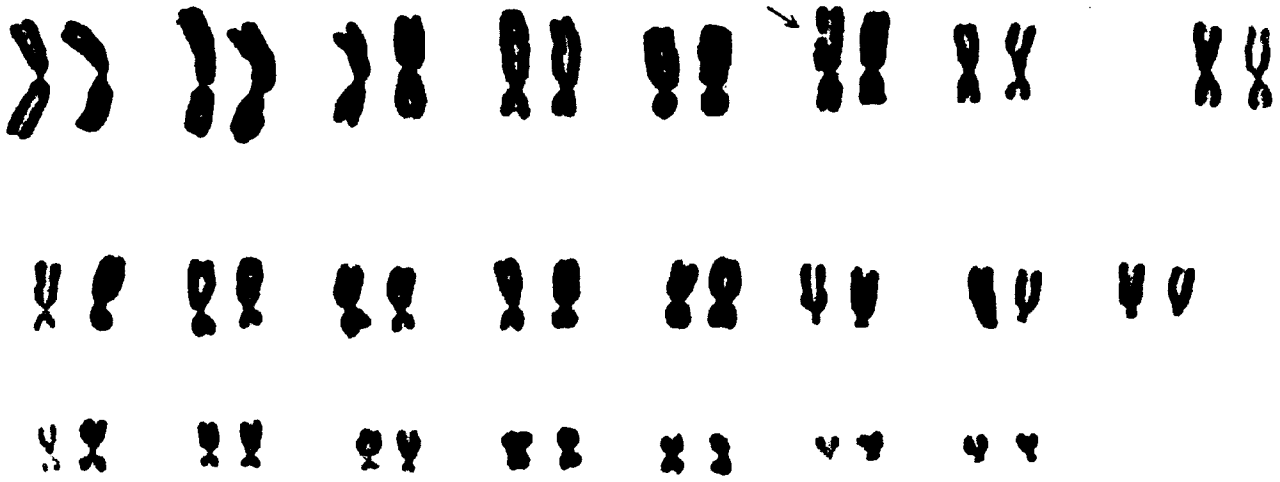
J.T. #039458 F, 37y. Thyroid Ca.
208 mC, Pre-therapy,
Dicentric, double acentric fragment, Cri du Chat "B", 44 chromosomes

Figure 9



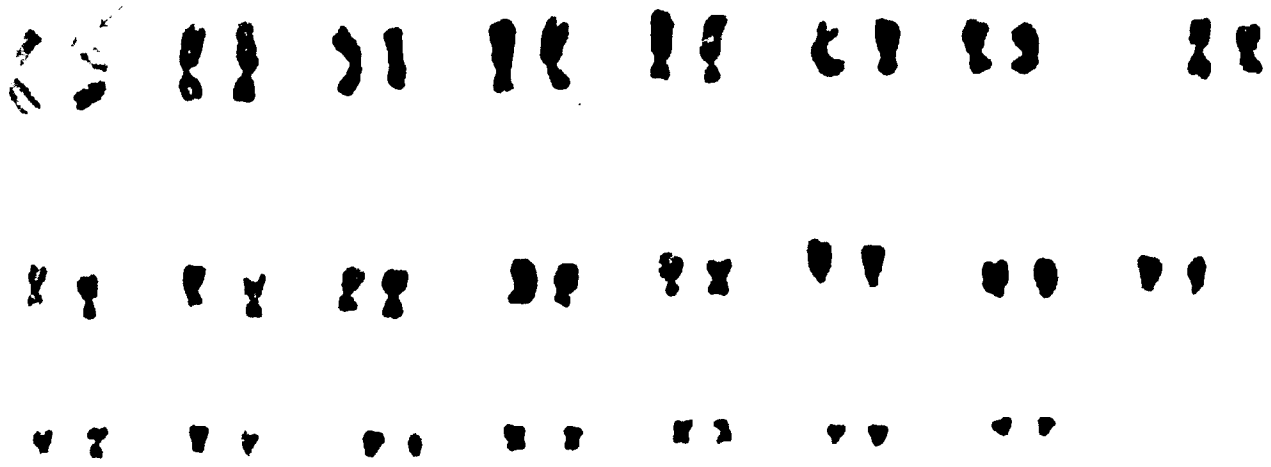
E.W. #049036 F, 49y. Thyroid Ca.
175 mC, Pre-therapy,
Chromatid gap in "B"

Figure 10



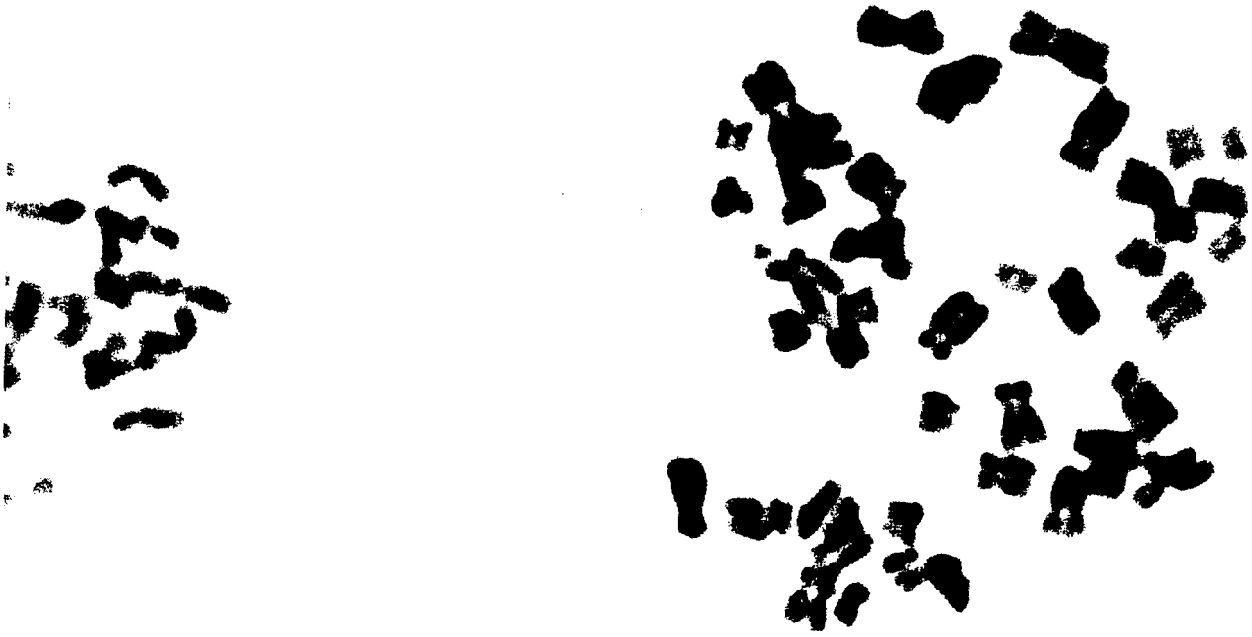
M.H. #033622 F, 31y. Thyroid Ca.
151 mC, Pre-therapy,
Chromatid gap in "C"

Figure 11



J.D. #050051 F, 44y. Hyperthyroid
9.0 mC, Post-therapy,
Double chromatid gap in "A"

Figure 12



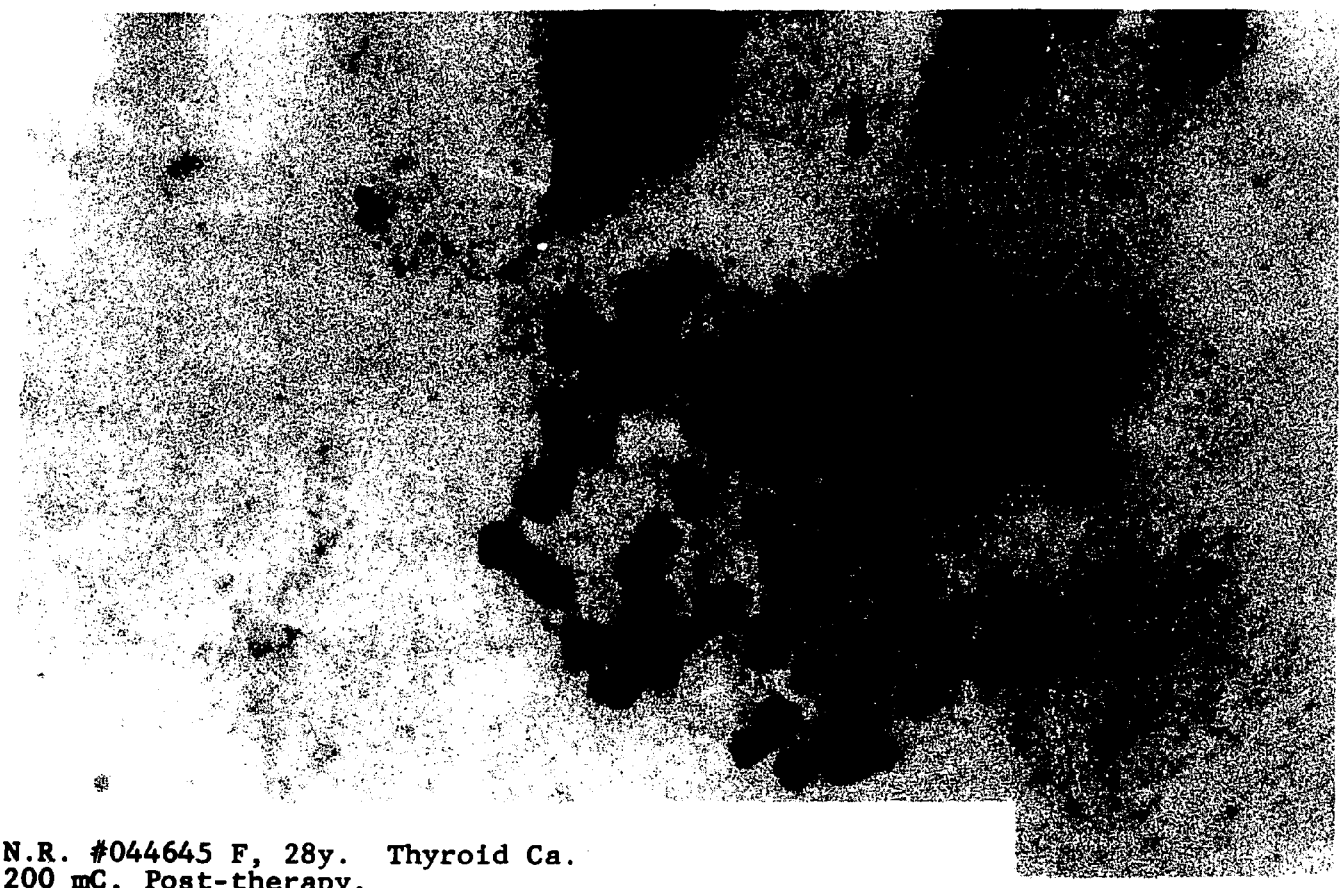
J.vH. #040606 M, 28y. Thyroid Ca.
196 mC, Post-therapy,
Endoreduplication

Figure 13



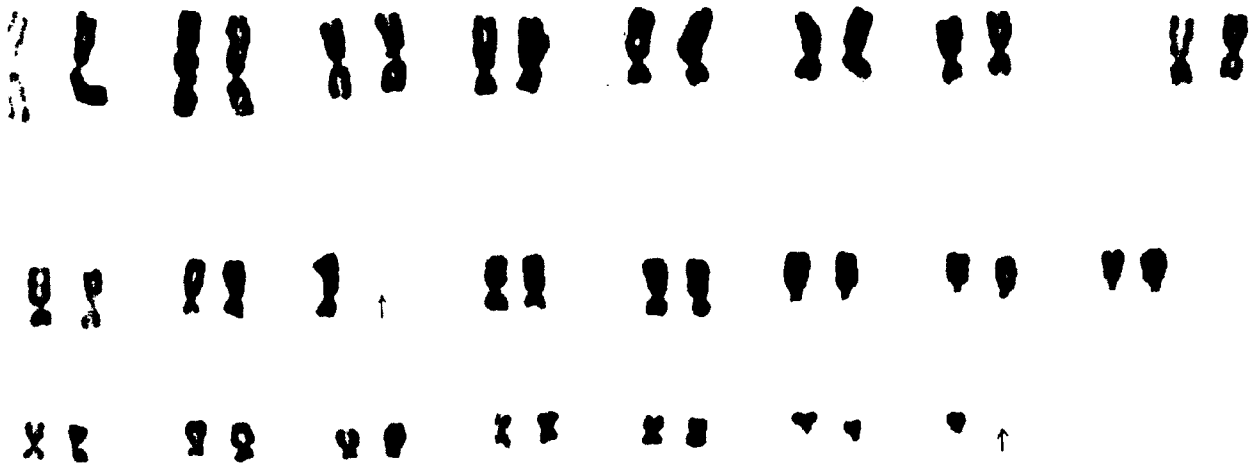
N.R. #044645 F, 28y. Thyroid Ca.
200 mC, Post-therapy,
Tetraploid

Figure 14



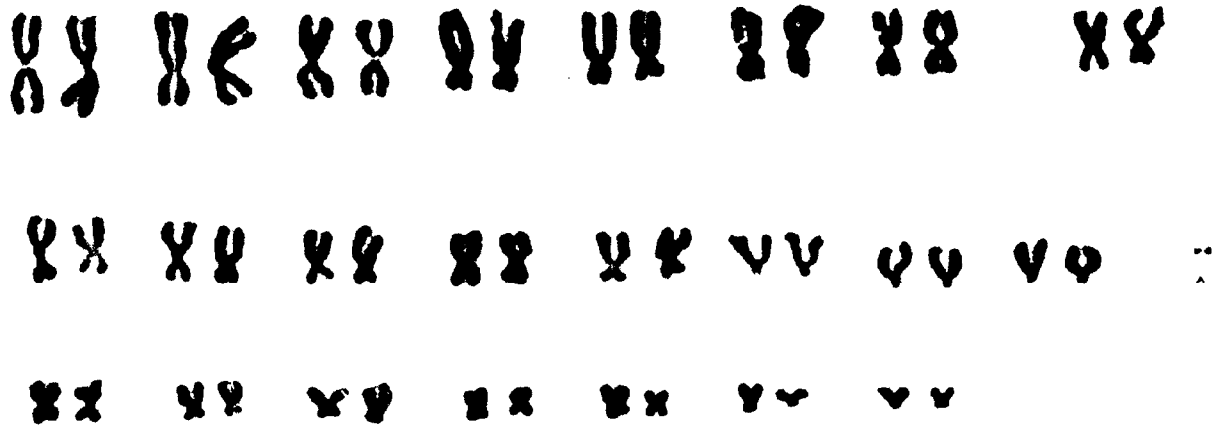
N.R. #044645 F, 28y. Thyroid Ca.
200 mC, Post-therapy,
Dicentric, double acentric fragment, 45 chromosomes

Figure 15



N.R. #044645 F, 28y. Thyroid Ca.
200 mC, Post-therapy,
44 chromosomes (-C, -G)

Figure 16



N.R. #044645 F, 28y. Thyroid Ca.
200 mC, Post-therapy,
Double dot-like acentric fragment

Figure 17

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Appendix I

Control Group

Initials	Age (years)	Sex	Chromosome Count (%)							Chromatid Abnormalities		Total Number of Cells
			<44	44	45	46	47	48	92	Gap	Deletion	
E.B.	39	F	4	2	2	92	0	0	0	0	0	50
B.C.	33	F	0	4	0	96	0	0	0	0	0	50
H.C.	35	F	0	0	0	100	0	0	0	0	0	50
V.C.	46	F	6	4	0	86	2	2	0	0	0	50
J.C.	45	F	0	2	0	96	2	0	0	0	0	50
M.D.	40	F	2	2	0	94	0	0	2	0	0	50
M.E.	28	M	0	0	0	96	0	0	4	1	0	25
I.G.	32	F	0	0	2	98	0	0	0	0	0	50
B.H.	39	F	4	0	0	96	0	0	0	0	0	50
B.H.	32	F	0	0	2	96	0	0	2	1	0	50
I.H.	53	F	0	0	2	98	0	0	0	0	0	50
N.H.	41	F	4	0	0	96	0	0	0	0	0	50
D.J.	22	M	0	2	8	88	0	0	2	1	0	50
K.K.	45	F	0	4	0	96	0	0	0	0	0	25
R.M.	56	F	0	0	0	100	0	0	0	0	0	50
L.M.	24	F	0	2	2	92	2	0	2	1	0	50
H.M.	24	M	2	4	2	90	2	0	0	0	0	50
H.S.	68	F	0	0	0	100	0	0	0	0	0	50
J.S.	46	F	0	0	0	98	0	2	0	0	0	50
R.S.	45	F	0	2	0	98	0	0	0	0	0	50
E.S.	78	F	0	0	2	98	0	0	0	1	0	50
C.T.	23	M	0	2	2	96	0	0	0	0	0	50
M.T.	26	F	0	0	4	94	2	0	0	0	0	50
S.T.	34	M	0	0	6	90	2	2	0	0	0	50
J.V.	43	F	0	4	0	96	0	0	0	0	0	50
A.V.	32	F	0	0	2	98	0	0	0	0	0	50
D.W.	48	F	0	0	0	100	0	0	0	0	0	25

Appendix II

Follow Up Group

Initials & registration#	Sex	age (yrs)	dose (mC)	R/ date (yr)	Chromosome Count (%)							Chromatid Gap Br.		Chromosome Dic. Br.	Total No. of Cells Counted	Remarks
					<44	44	45	46	47	48	92					
VB 701222	M	49	12.0	52			7	90	2		1	1			100	
HB 710905	F	54	24.0	53,54			2	90		6	2	1			50	
MB 760364	F	61	13.0	53		2	2	96							50	
AB 760213	F	79	12.0	53			3	96	1						100	
JB 890440	F	40	11.0	55		2	6	90	2						50	
FB 834044	F	46	12.0	56		1	10	88			1	2		1	100	
EB 897247	F	76	6.0	58		4		96							50	
IB 776832	F	68	24.3	60		2	8	78		2	10	1			50	polycythemia 10 mc ^{32}P "54,58"
WB 328103	M	54	9.4	60	2	2	2	94							50	
RB 869029	F	70	15.0	57			12	84	2		2	1	1		50	
IC 938260	F	62	11.0	59			2	98				1			50	
HC 922808	F	61	41.7	59,60			2	94	2	2		1			50	
VC 606724	F	42	12.0	53			2	96			2				50	
AC 840968	F	43	11.6	56		4		92			4				25	4% endo
VD 796849	F	61	45	54,55			2	98				1			100	
CD 739996	F	60	12.0	52			2	98				3	1		100	
MD 792584	F	47	10.4	54		12		86			2	1			50	
EE 825341	M	65	30	56			3	97							100	
GF 785047	F	62	33.7	54		1	2	95			2				100	
EF 155379	F	41	8.3	59			3	97				6			100	
JG 849875	M	35	8.2	56		1	4	94			1	3			100	
DG 841824	M	46	11.5	56		4	2	92	2						50	
AG 787944	F	56	35.2	54,55		2	4	92	2						50	

Appendix II

Follow Up Group, cont.

Initials & registration#	Sex	age (yrs)	dose (mC)	R/ date (yr)	Chromosome Count (%)							Chromatid Gap Br.		Chromosome Dic. Br.		Total No. of Cells Counted	Remarks
					<44	44	45	46	47	48	92						
SG 839831	F	63	12.0	56			6	92	2							50	
PG 713147	F	40	17	51,53	4	2		94								50	
VH 906449	F	50	10.3	58			2	90	6	2		1				50	
FH 579834	F	47	12.0	57		3	4	93				1		1		100	
CH 797466	M	53	11.0	54			4	94			2	6	1			50	
CH 781468	M	69	12.0	54			2	98								100	
HH 799876	F	67	33.0	54,55	2		4	90	2		2	1				50	
AH 945134	F	82	11.5	60		4		94			2	1				50	
JH 268483	F	69	24.5	54,55		1	4	92	2	1		2				100	
FH 821545	F	42	11.9	55		2	4	92			2	1				50	
AJ 821100	F	64	21.0	55,56		3	3	90		1	3					100	
MJ 865596	F	64	11.7	57			4	95	1			1		1		100	
MJ 829893	F	33	11.0	55		1		99								100	
HK 935364	M	50	11.5	59	5	1	1	91	1	1				1		100	
HK 865888	M	74	11.4	59		3	10	87				3		1	2	100	
FK 909516	F	50	11.0	58	2	1	3	92		1	1	2				100	
JK 691424	M	56	10.0	58	2		2	90	4		2	1				50	
HK 910182	F	49	11.0	58		1	2	97				1				100	
MK752993	F	46	10.0	53		8	3	87	1		1					100	
FK 632058	F	50	12.0	53		3	4	92	1							100	
MK706882	F	29	10.0	52	2	2	4	88	2		2	2				50	
LK 776000	F	48	54.0	54		1	2	94		1	2	3	3	2		100	22% short G
GK 695781	F	50	22.0	53			1	98			1					100	
DK 834494	M	52	10.0	56			1	94			5					100	
NL 891074	F	21	11.6	58				100								50	

Appendix II

Follow Up Group, cont.

Initials & registration#	Sex	age (yrs)	dose (mC)	R/ date (yr)	Chromatid Count (%)							Chromatid Gap Br.	Chromosome Dic. Br.		Total No. of Cells Counted	Remarks
					<44	44	45	46	47	48	92					
ML 636906	F	70	18.0	54		6	2	84	2	4	2		1		50	
KL 886817	M	57	11.5	57			2	94			4	2			50	
BL 960498	F	53	12.0	53		1	3	95			1				100	
ML 711035	F	49	10.7	55		½	1	95			3½				200	1½% endo
JM 810657	M	37	28.5	55		1	1	97			1	1			100	
JM 825301	M	67	11.3	55				98			2		1	1	50	2% endo, iso chromatid gap
HM 936838	F	31	10.0	59	1		3	95	1			1	2		100	
JM 943126	M	24	28.3	59,60	2		2	94	2					1	50	
BM 966358	F	26	10.0	60			3	93			4				100	
DO 777742	F	44	12.0	54		1	3	95	1			3			100	
FR 008683	M	29	11.0	62		2		98				1			50	
RS 680961	F	41	8.0	50	1			98	1			9			100	
BS 979109	F	56	9.5	61		4	2	88	4	2		1			50	
HT 724289	M	40	30.2	61			1	97			2				100	
AT 808998	F	36	41.1	55,56	2		3	90	3	2	2		1		100	
JT 929566	M	58	6.8	59		2		94	4						50	
CW 692597	F	44	9.0	51		2	4	92			2	1			50	

Appendix III

Treated Hyperthyroid Group

(a) Pre-therapy

Initials & Registration#	Sex	age (yrs)	dose (mC)	Chromosome Count (%)							Chromatid Gap Br.	Chromosome Dic. Br.	Total No. of Cells Counted	Remarks
				<44	44	45	46	47	48	92				
LA 037120	F	43	9.8			2	98						50	
CB 052013	F	40	10.0			2	98				1		50	
LC 808684	F	33	12.0			4	96				2		50	
JD 055861	F	31	9.5	2	6	4	84	2	2			1	50	
JD 050051	F	44	9.0		6	2	92				2		50	
FH 295054	F	58	10.6				100						50	
HK 035292	M	59	11.0			2	98				1		50	
AL 025152	M	53	11.9				100						50	
BMc 039119	F	35	10.0		2		98				1		50	
RM 035430	M	27	8.3				100				1		50	
LR 641406	F	54	8.4			5	95						20	
GT 037992	F	40	12.7				100				1		50	
GU 042385	M	66	7.2		2	4	92			2			50	
CW 032918	M	38	11.9			2	98						50	
BW 748446	F	61	8.8			2	96			2			50	

Appendix III

Treated Hyperthyroid Group

(b) Post-therapy

Initials & Registration#	Sex	age (yrs)	dose (mC)	Chromosome Count (%)							Chromatid Gap Br.	Chromosome Dic. Br.	Total No. of Cells Counted	Remarks
				<44	44	45	46	47	48	92				
LA 037120	F	43	9.8			2	98				4		50	
CB 052013	F	40	10.0				100						75	
LC 808684	F	33	12.0			2	98				1	1	50	1 ring
JD 055861	F	31	9.5		2	8	84	4		2			50	
JD 050051	F	44	9.0	2	2	6	86	2	2		1	1	50	
FH 295054	F	58	10.6	6		2	86			6	1 1		50	
HK 035292	M	59	11.0			2	94			4	3		50	
AL 025152	M	53	11.9			8	86	4		2	2		50	
BMc 039119	F	35	10.0			2	98				5	1 2	50	
RM 035430	M	27	8.3			2	98						50	
LR 641406	F	54	8.4		4	14	82					2	50	
GT 037992	F	40	12.7				100						50	
GU 042385	M	66	7.2	2	2	2	92		2				50	
CW 032918	M	38	11.9			4	96						50	
BW 748446	F	61	8.8			4	96				2		50	

Appendix IV

Treated Thyroid Cancer Group

(a) Pre-therapy

Initials & Registration#	Sex	age (yrs)	dose (mC)	Chromosome Count (%)							Chromatid Gap Br.	Chromosome Dic. Br.	Total No. of Cells Counted	Remarks
				<44	44	45	46	47	48	92				
BA 043051	F	42	150			2	98						50	
LB 038721	F	37	198				100						50	
OB 067867	M	30	190	-	-	-	-	-	-	-	- -	- -	0	
DG 675732	F	47	154			6	94				1		50	
MH 033622	F	31	151		4	2	94				2		50	
EM 913184	F	23	200			2	98						50	
CM 031690	F	16	195			10	90						50	
KP 051415	F	45	200	2	4	2	90			2			50	2% endo
NR 044645	F	28	200				98			2			50	
JT 039458	F	37	208	2		2	96				3		50	
JvH 040606	M	25	196			2	96			2	2		50	2% endo
EW 049036	F	49	175				100						25	

Appendix IV

Treated Thyroid Cancer Group

(b) Within 2 hours Post-therapy

Initials Registration#	Sex	age (yrs)	dose (mC)	Chromosome Count (%)							Chromatid Gap Br.		Chromosome Dic. Br.		Total No. of Cells Counted	Remarks
				<44	44	45	46	47	48	92						
BA 043051	F	42	150	4			96				2	2			50	
LB 038721	F	37	198			2	96			2	1	1	1		50	2% endo
OB 067867	M	30	190	-	-	-	-	-	-	-	-	-	-	-	0	
DG 675732	F	47	154	4	4	6	86				1		2		50	
MH 033622	F	31	151		6		94				1	2			50	
EM 913184	F	23	200			3	97				2		1	1	100	
CM 031690	F	16	195	-	-	-	-	-	-	-	-	-	-	-	0	
KP 051415	F	45	200	2	2	8	88						1		50	
NR 044645	F	28	200	-	-	-	-	-	-	-	-	-	-	-	0	
JT 039458	F	37	208		1	4	95				28	4	4	12	100	
JvH 040606	M	25	196			1	93		1	5	5		3		100	5% endo
EW 049036	F	49	175			2	96			2	1		2		50	

Appendix IV

Treated Thyroid Cancer Group

(c) 24-48 Hours Post-therapy

Initials Registration#	Sex	age (yrs)	dose (mC)	Chromosome Count (%)							Chromatid Gap Br.		Chromosome Dic. Br.		Total No. of Cells Counted	Remarks
				<44	44	45	46	47	48	92						
BA 043051	F	42	150		2	2	94			2			2		50	
LB 038721	F	37	198	-	-	-	-	-	-	-	-	-	-	-	0	
OB 067867	M	30	190		4	7	85	4			3	1		1	100	
DG 675732	F	47	150	-	-	-	-	-	-	-	-	-	-	-	0	
MH 033622	F	31	151	-	-	-	-	-	-	-	-	-	-	-	0	
EM 913184	F	23	200			2	98				1		1	1	100	
CM 031690	F	16	195			10	88	2					2		50	
KP 051415	F	45	200		2	6	86	4		2					50	2% endo
NR 044645	F	28	200		3	1	90	1		5	3	2	2		100	1 cell with 65
JT 039458	F	37	208	-	-	-	-	-	-	-	-	-	-	-	0	
JvH 040606	M	25	196	-	-	-	-	-	-	-	-	-	-	-	0	
EW 049036	F	49	175			2	94			4	2	1	2	1	50	

Appendix V

Treated Hyperthyroid Group: Re-evaluation

(a) Pre-therapy

Initials & Registration	Sex	age (yrs)	dose (mC)	Chromosome Count 43	44	45	46	47	48	±92	Chromatid Lesions Gap.	Br.	Chromosome Lesions Br.	Dic.	Ring	Total # of Cells	Remarks
CB 052013	F	40	10.0	-	1	-	48	1	-	-	1	1	-	2		50	Dic. in 47
LC 808684	F	33	12.0	-	-	-	10	-	-	-	-	-	-	-	-	10	
JD 055861	F	31	9.5	-	-	3	42	-	-	-	-	-	3	-	-	45	-E, -E
JD 050051	F	44	9.0	-	-	2	47	-	1	-	1	1	-	-	1	50	47 + ring, - isogap
FH 295054	F	58	10.6	-	-	4	35	-	1	1	-	-	2	-	-	40	one 59, -D, -E, -F, -G
HK 035292	M	59	11.0	-	-	-	-	-	-	-	-	-	-	-	-	-	
AL 025152	M	53	11.9	1	-	2	25	-	-	-	-	1	1	-	-	28	-C, -G, -CCD
BMc 039119	F	35	10.0	-	-	1	12	-	-	-	-	-	-	-	-	13	
RM 035430	M	27	8.3	1	1	1	32	-	-	-	1	-	-	-	-	35	One ?46 ?45 -FC, -FCB
LR 641406	F	54	8.4	-	-	1	29	-	-	-	-	-	-	-	-	30	-D-E+C
GT 037992	F	40	12.7	-	-	5	45	-	-	1	1	-	1	-	-	50	-E, -C, -D
GU 042385	M	66	7.2	-	-	2	48	-	-	-	-	-	-	-	-	50	-C
BW 748446	F	61	8.8	-	1	4	43	2	-	-	1	-	3	-	-	50	-C One ?46 ?47

Appendix V

Treated Hyperthyroid Group: Re-evaluation

(b) Post-therapy

Initials & Registration	Sex	age (yrs)	dose (mC)	Chromosome Count							Chromatid Lesions		Chromosome Lesions			Total # of Cells	Remarks
				43	44	45	46	47	48	±92	Gap	Br.	Br.	Dic.	Ring		
CB 052013	F	40	10.0	-	1	2	47	-	-	-	-	1	1	-	-	50	-C, -D
LC 808684	F	33	12.0	-	-	-	2	-	-	-	-	-	-	-	-	2	
JD 055861	F	31	9.5	-	-	-	30	-	-	1	1	-	-	-	-	30	
JD 055051	F	44	9.0	-	1	2	47	-	-	1	2	1	3	-	-	50	-E, -DG isogap
FH 295054	F	58	10.6	-	1	4	43	-	-	-	2	1	1	-	-	48	
HK 035292	M	59	11.0	-	-	-	5	-	-	-	-	-	-	-	-	5	
AL 025152	M	53	11.9	1	-	1	29	-	-	-	-	-	-	-	-	31	-C, -CEG
BMc 039119	F	35	10.0	-	-	1	38	1	-	1	1	-	-	-	-	40	+D
RM 035430	M	27	8.3	-	-	1	22	-	-	-	-	1	-	-	-	23	
LR 641406	F	54	8.4	-	-	-	11	-	1	-	1	-	1	-	-	12	
GT 037992	F	40	12.7	-	-	-	-	-	-	-	-	-	-	-	-	0	
GU 042385	M	66	7.2	-	-	-	50	-	-	1	1	-	2	-	-	50	
BW 748446	F	61	8.8	-	-	1	7	-	-	-	1	-	1	-	-	8	-G

Appendix VI

Treated Thyroid Cancer Group: Re-evaluation

(a) Pre-therapy

Initials & Registration	Sex	age (yrs)	dose (mC)	Chromosome Count 43	44	45	46	47	48	±92	Chromatid Lesions Gap	Br.	Chromosome Lesions Br.	Dic.	Ring	Total # of Cells	Remarks
BA 043051	F	42	150	-	-	1	48	-	-	-	-	-	-	-	-	49	-C
LB 038721	F	37	198	-	1	-	11	-	-	-	-	-	-	-	-	12	
OB 067867	M	30	190	-	-	-	4	-	-	-	1	-	-	-	-	4	
DG 675732	F	47	154	-	1	2	46	1	-	-	1	-	-	1	-	50	-G, -E, -CC
MH 033622	F	31	151	-	1	2	41	-	-	-	1	-	1	-	-	44	-C, -CG
EM 913184	F	23	200	-	-	-	6	-	-	-	-	-	-	-	-	6	
KP 051415	F	45	200	-	-	3	35	1	-	3	-	-	-	?	-	39	One ?46 ?45 Three endo
NR 044645	F	28	200	-	-	3	45	2	-	1	2	-	-	-	-	50	2 iso-gaps +B, +C, -F, -G, -G
JT 039458	F	37	208	-	1	1	48	-	-	-	-	-	3	1	-	50	Cri du chat B in cell ē 44
JvH 040606	M	25	196	-	-	1	27	-	-	3	-	-	-	?	-	28	Three endo
EW 049036	F	49	175	-	1	-	42	-	-	-	2	-	1	-	-	43	-EE, Iso-gap

Appendix VI

Treated Thyroid Cancer Group: Re-evaluation

(b) Post-therapy

Initials & Registration	Sex	age (yrs)	dose (mC)	Chromosome Count							Chromatid Lesions		Chromosome Lesions			Total # of Cells	Remarks
				43	44	45	46	47	48	±92	Gap	Br.	Br.	Dic.	Ring		
BA 043051	F	42	150	-	-	2	43	-	1	2	-	-	-	-	-	46	+EE, -F, -C
LB 038721	F	37	198	-	-	-	-	-	-	-	-	-	-	-	-	0	
OB 067867	M	30	190	-	-	1	48	-	-	-	-	-	1	-	-	49	
DG 675732	F	47	154	-	-	-	19	-	-	2	-	-	-	-	-	19	
MH 033622	F	31	151	-	-	1	29	-	-	-	-	-	-	-	-	30	-E
EM 913184	F	23	200	-	-	1	47	2	-	-	-	-	-	-	-	50	
KP 051415	F	45	200	-	-	-	30	-	-	-	-	-	-	-	-	30	
NR 044645	F	28	200	-	1	5	35	-	-	3	-	-	1	1	-	41	-C, -D, -E -G, -CG
JT 039458	F	37	208	-	1	3	31	-	-	-	-	-	-	-	-	35	
JvH 040606	M	25	196	-	-	-	30	-	-	3	-	-	-	-	-	30	Three endo
EW 049036	F	49	175	1	2	2	39	2	-	1	-	1	1	-	-	46	