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Persistent Chromosomal Aberrations Following Radioiodine Therapy[1]

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Significant chromosomal abberrations have been demonstrated in white blood cells following radiation from x-rays and from radioisotopes (1).

In 1960, Tough *et al* reported the presence of chromosomal abnormalities in peripheral leukocytes of two patients given x-ray therapy to the spine for ankylosing spondylitis (2). Buckton *et al* showed that these abnormalities persist for as long as 18 years after x-irradiation (3).

Bender and Gooch demonstrated persistent chromosomal damages in leukocytes of eight men, two and one-half to three and one-half years after accidental whole body exposure to mixed gamma-ray and fission neutrons with absorbed doses calculated to vary from 22.8 to 365 rads (4,5).

In 1961, Boyd *et al* found acute chromosomal effects in the leukocytes of four patients treated for thyrotoxicosis and two treated for thyroid cancer with different doses of I^{131} ranging from 7-150 mc (6). MacIntyre and Dobyns reported abnormalities persisting in the leukocytes of one patient about six years after exposure to a total of 475 mc of I^{131} and 400 r of x-irradiation (7). Numerous abnormalities of number and structure of chromosomes were observed in this patient at frequent intervals during the next 14 days after an additional dose of 167 mc of I^{131}. Abnormalities have also been described after diagnostic doses of x-irradiation (8,9).

We have studied the abnormalities induced in chromosomes of peripheral leukocytes for periods up to 14 years after I^{131} treatment of hyperthyroidism and thyroid cancer. This study was designed to investigate the possible correlation between the incidence or degree of induced chromosomal abnormalities occurring acutely or chronically after I^{131} therapy and the development of any possible late hazardous effect, especially in the hemopoietic system.

MATERIAL AND METHODS

Patient material

Four groups of subjects were studied. Group I. 21 controls, 18-78 years of age, having no history of irradiation or thyroid disease and matched for age and sex with Group II.

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Group II. 48 patients 24-79 years of age, studied 3-14 years after I^{131} treatment for hyperthyroidism; the total dose of I^{131} ranging from 8-54 mc.

Group III. 11 hyperthyroid patients, 27-61 years of age, studied before and ½ hour after 8.3–12.7 mc of I^{131}.

Group IV. 11 thyroid cancer patients, ranging in age from 16-49 years, treated with doses of I^{131} between 150-200 mc. These patients were also studied before and at different intervals following the therapy dose (½, 2, 24 and 48 hours).

Leukocyte culture

The technique used for white blood cell culture was essentially of Moorehead *et al* (10). Ten ml of heparinized blood was centrifuged and the "buffy coat" and serum cultured in 10 ml culture medium,[1] 0.5 ml bactophytohemagglutinin M, 1000 units penicilin G and 1.0 mg streptomycin. After incubation for 66 hours at 37°C, 0.3 ml colcemid (10^{-7}M) was added to each culture, the cultures were incubated for four more hours, then sacrificed. The contents of the cultures were then mixed thoroughly and centrifuged. All but the bottom 0.5 ml of supernatant were discarded and this was resuspended in 5.0 ml Hanks solution, centrifuged, and the supernatant removed. Cells were then resuspended in 5 ml distilled water at 37°C for 10 minutes, centrifuged and the supernantant removed. To the "button" of cells left, 5 ml of fresh fixative was added for 30 minutes (absolute methanol and glacial acetic acid 3:1). The fixed button was washed three times with fixative and then the cells were resuspended in 0.5 ml. One or two drops of cell suspension were then placed on clean chilled slides which were dried first by fan then by flame. After drying overnight, slides were stained in 2% acetoorecin for 20 minutes and then mounted in mounting medium.[2]

In order to avoid radiation during culture in patients given large doses of I^{131}, only the buffy coat and top mm of erythrocytes were withdrawn from the serum and used for culture. Control sera with added I^{131} in concentration of 0.4 μc/ml, stimulating the concentration found in sera within two hours after a treatment dose of I^{131} for hyperthyroidism also served as controls for the effect of I^{131} irradiation of cells during culture.

All patients had complete blood counts performed at the time of the culture of leukocytes.

RESULTS

Coded slides were scanned for the presence of mitotic figures, without knowledge of their origin. Chromosomes were then analyzed for any abnormality in number or structure. Fifty countable metaphases were considered necessary for scoring in each culture, but in many cases 100 cells were counted. Doubtful cases were scored as normal.

Observable abnormalities were scored and recorded. Less obvious abnormalities were photographed and karyotypes prepared according to the Denver

[1]TC 199–Difco Laboratories.

[2]Euparal–Flatters and Garnett Ltd.

classification (11). Chromosomes were grouped according to their length and the arm ratio, into 22 somatic pairs and one sex pair, as shown in Fig. 1.

Group I: Table I shows the chromosome count distribution (a) and the chromosomal aberrations (b) recorded in the control group.

Ninety-seven per cent of all cells counted had a modal count of 46 chromosomes. The only type of aberration observed in this group was achromatic lesions in 14 per cent of cases, present only as 2 per cent or less of the cells counted (chromatid type), as shown in Table Ib.

Group II: As shown in Table II, in patients treated 3-14 years previously for hyperthyroidism, 93.9 per cent of cells counted had a normal chromosome count. Twenty-one per cent of the 48 patients studied in this group had achromatic lesions of more than the 2 per cent incidence observed in the control group. Group II patients also showed an abnormal incidence of chromatid deletions, chromosome deletions, dicentrics and breaks, as shown in Table IIb.

Group III. The chromosome count distribution in 11 hyperthyroid patients immediately after treatment showed an incidence of 95.5 per cent modal count as compared to 98.1 per cent before therapy (Table IIIa). The chromosome aberrations were significantly increased in the post therapy samples as compared to the pre-therapy ones (Table IIIb).

Group IV: In the 11 thyroid cancer patients, the modal chromosome count was 94.3 per cent ½ to 2 hours and 90.9 per cent 24 to 48 hours after the I^{131} dose as compared to 96.3 per cent before treatment (Table IVa). All types of chromosomal aberrations were more strikingly increased as shown in Table IVb, especially in the 24-48 hour samples.

No significant hematological abnormality was observed in the blood count of any of these patients. None of the patients other than those treated for thyroid cancer had neoplastic disease at the time of this study.

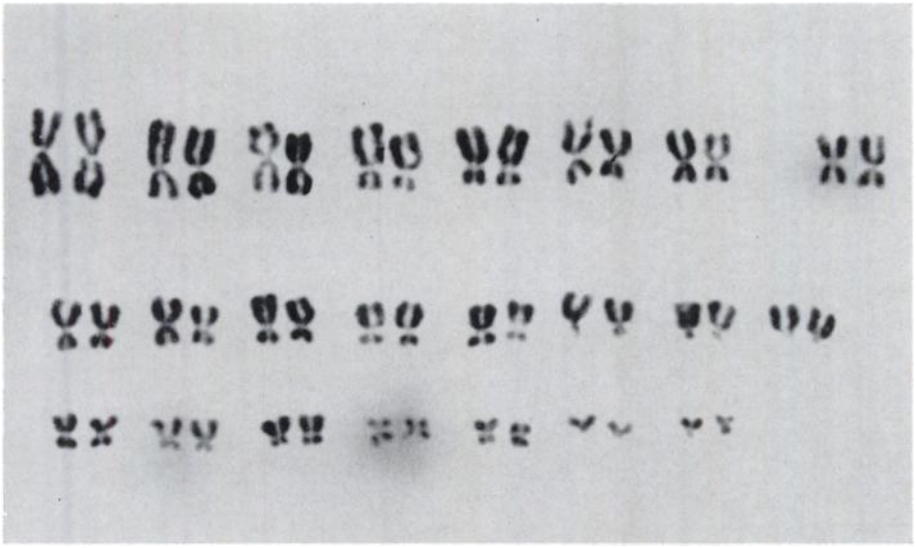

Fig. 1. Normal karotype of female patient; constructed by "Paste-up" method, according to Denver Classification.

TABLE I

a—CHROMOSOME COUNT DISTRIBUTION IN 21 CONTROLS

Chromosome Count	*<44*	*44*	*45*	*46*	*47*	*48*	*Polyploids*	*Total*
Number of Cells Counted	10	11	6	941	2	2	3	975
Percentage	1.0	1.1	0.6	96.6	0.2	0.2	0.3	100%

b—CHROMOSOME ABERRATIONS IN 21 CONTROLS

Type	*Number of Cases*	*Percentage*
Achromatic Lesions	3 (2% or less)	14
Chromatid Deletions	0	0
Chromosome Deletions	0	0
Dicentrics	0	0
Breaks	0	0

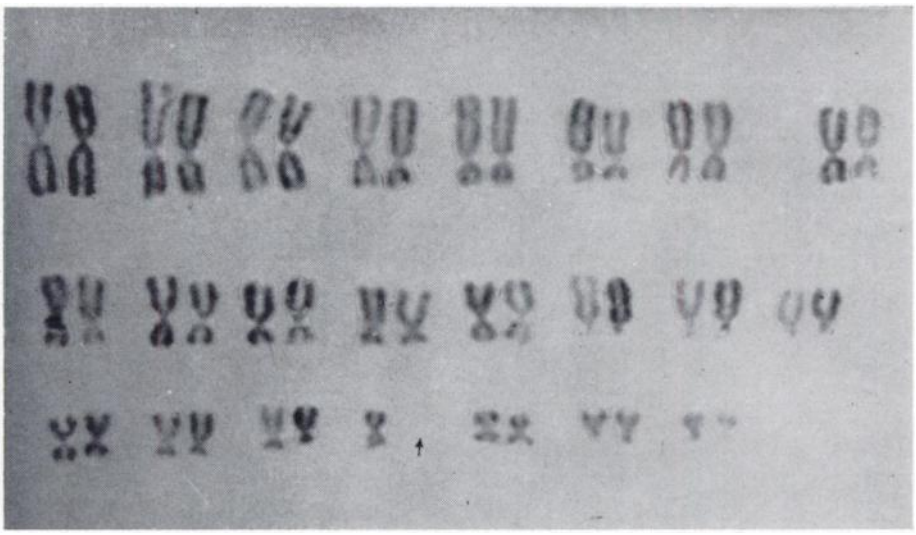

Fig. 2. Karotype of patient treated with 54 mc of I^{131} in 1954, showing a 45 chromosome set; missing chromosome is in pair 19. (arrow).

DISCUSSION

Our data demonstrate that there is a higher incidence of nonmodal chromosome counts in the patients treated for hyperthyroidism with I^{131} up to 14 years previously as compared to the control group (Group II vs Group I), p value = 0.001 (Chi Square test)

Group III and IV patients served as their own controls since their blood was analyzed before and shortly after the I^{131} therapy doses. Here, again, the Chi Square test showed a significant difference in the modal chromosome count before and after treatment, (p value 0.01 in Group III and 0.005 in Group IV).

The highest incidence of aneuploidy in the I^{131} treated groups was found in the 45 chromosome sets (Fig. 2) with an abnormal incidence of polyploidy (Fig. 3). However, it is probable that counts lower than 46 may be due to cell breakage during preparation as a result of hypersensitivity of the irradiated cells to osmotic and mechanical stress (5).

Several kinds of chromosomal aberrations could be induced by radiation as shown in the tables. If the chromosome is broken after duplication, a chromatid-

TABLE II

a—CHROMOSOME COUNT DISTRIBUTION IN 48 HYPERTHYROIDS TREATED 3–14 YEARS PREVIOUSLY

Chromosome Count	*<44*	*44*	*45*	*46*	*47*	*48*	*Polyploids*	*Total*
Number of Cells Counted	8	34	114	3499	22	8	39	3725
Percentage	0.2	0.9	3.1	93.9	0.6	0.2	1.1	100%

b—CHROMOSOME ABERRATIONS IN 48 HYPERTHYROIDS TREATED 3–14 YEARS PREVIOUSLY

Type	*Number of Cases*	*Percentage*
Achromatic Lesions > 2%	10	20.8
Chromatid Deletions	4	8.3
Chromosome Deletions	5	10.4
Dicentrics	3	6.3
Breaks	1	2.1

type lesion is produced (one of the two chromatids affected). If, on the other hand, the effective hit occurred before duplication, then chromosomal aberrations will appear in the next division (both chromatids affected). Thus chromatid-type aberrations (achromatic lesions or gaps and chromatid deletions) can occur during the short-term leukocyte culture, while chromosome-type aberrations (chromosome deletions, dicentrics and breaks) are induced in the circulating

TABLE III

a—CHROMOSOME COUNT DISTRIBUTION IN 11 TREATED HYPERTHYROIDS

Chromosome Count	*<44*	*44*	*45*	*46*	*47*	*48*	*Polyploids*	*Total*
			BEFORE THERAPY					
Number of Cells Counted	0	1	8	510	0	0	1	520
Percentage	0	0.2	1.5	98.1	0	0	0.2	100%
			20–30 MIN AFTER THERAPY					
Number of Cells Counted	0	2	20	548	2	0	3	575
Percentage	0	0.3	3.5	95.3	0.3	0	0.5	100%

b—CHROMOSOME ABERRATIONS IN 11 TREATED HYPERTHYROIDS

	Before Therapy		*20-30 Min After Therapy*	
	Number of Cases	*Percentage*	*Number of Cases*	*Percentage*
Achromatic Lesions > 2%	1	9.1	5	45.5
Chromatid Deletions	0	0	0	0
Chromosome Deletions	0	0	3	27.3
Dicentrics	0	0	1	9.1
Breaks	0	0	0	0

TABLE IV

a—CHROMOSOME COUNT DISTRIBUTION IN 11 CANCER THYROID PATIENTS

Chromosome Count	*<44*	*44*	*45*	*46*	*47*	*48*	*Polyploids*	*Total*
			BEFORE THERAPY					
Number of Counted Cells	1	2	13	457	0	0	2	475
Percentage	0.2	0.4	2.7	96.3	0	0	0.4	100%
			½–2 HOURS AFTER THERAPY					
Number of Counted Cells	4	6	13	519	0	1	7	550
Percentage	0.7	1.1	2.4	94.3	0	0.2	1.3	100%
			24–48 HOURS AFTER THERAPY					
Number of Counted Cells	0	8	18	409	6	0	9	450
Percentage	0	1.8	4	90.9	1.3	0	2.0	100%

b—CHROMOSOME ABERRATION IN 11 CANCER THYROID PATIENTS

	Before Therapy		*½-2 Hours After Therapy*		*24-48 Hours After Therapy*	
	Number of Cases	*Percentage*	*Number of Cases*	*Percentage*	*Number of Cases*	*Percentage*
Achromatic Lesions > 2%	3/10	30	4/8	50	4/6	67
Chromatid Deletions	0	0	5/8	63	3/6	50
Chromosome Deletions	0	0	2/8	25	1/6	17
Dicentrics	0	0	5/8	62	6/6	100
Breaks	0	0	0	0	0	0

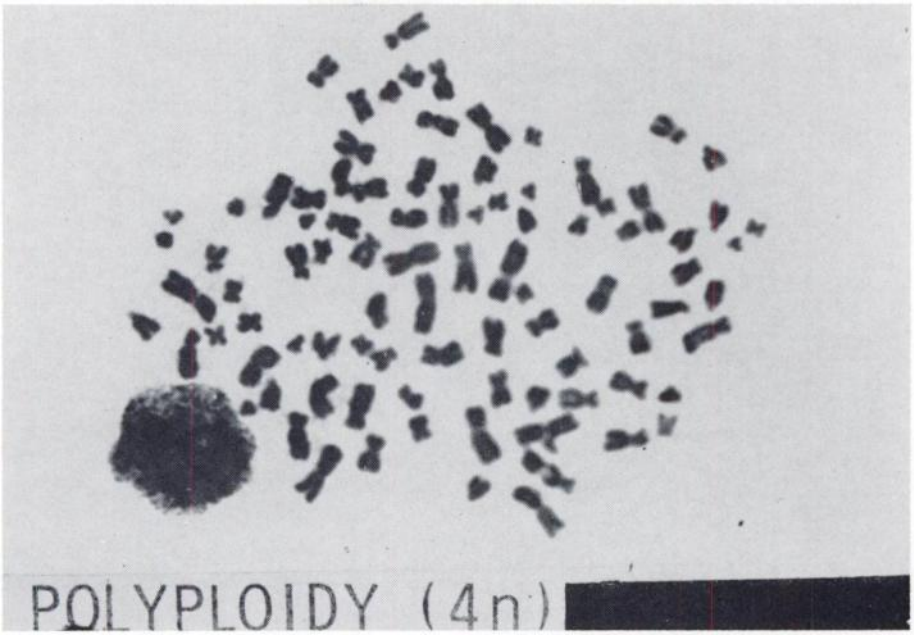

Fig. 3. Cell from patient treated with 11.9 mc of I^{131} in 1956, showing tetraploidy.

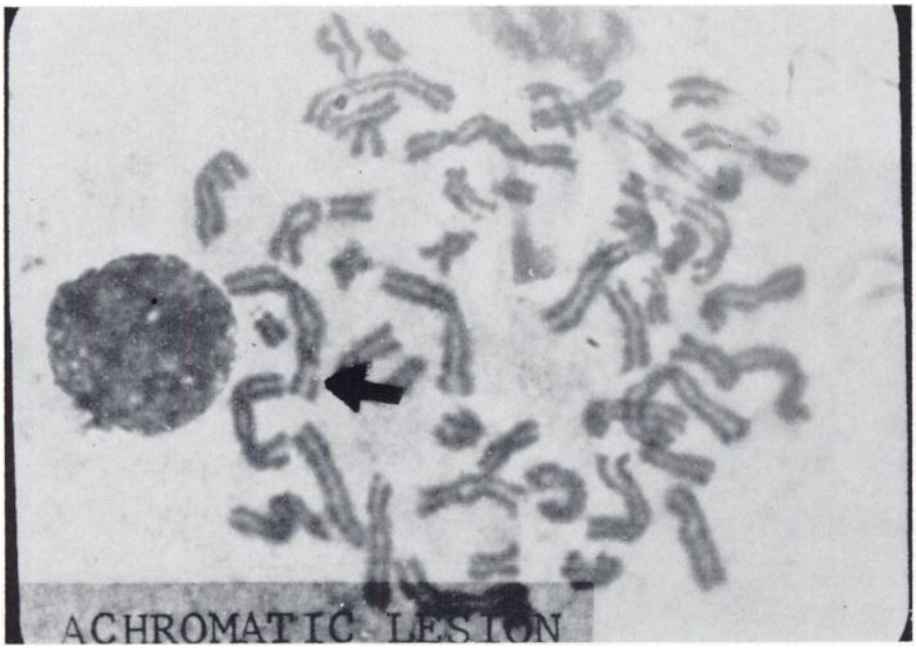

Fig. 4. Achromatic lesion detected in patient treated with total dose of 42 mc of I^{131} in 1960.

leukocytes (4). One-hit aberrations were considered to be the result of single chromosome breaks; while two-hit aberrations produced by the interaction of two chromosome breaks were manifested as dicentrics. Chromatid-type lesions (Figs. 4 and 5) tend to be either lost completely or later involve the whole chromosome after the first division.

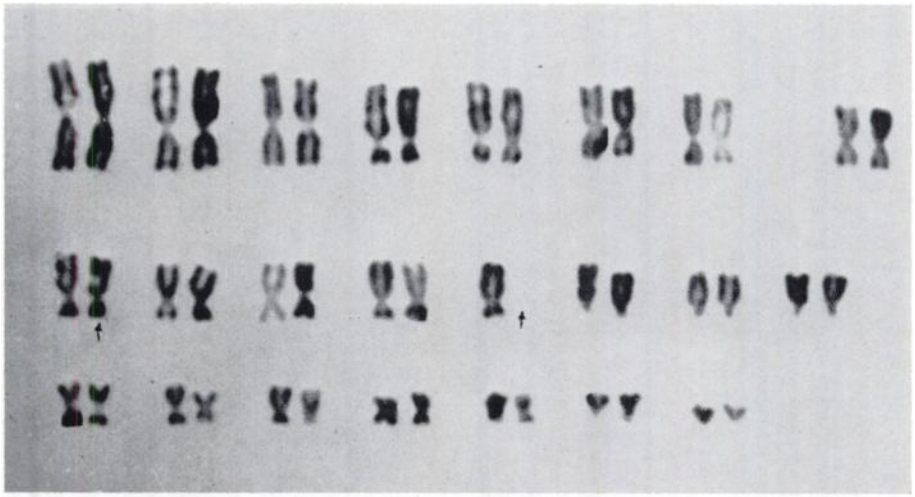

Fig. 5. Karyotype of patient treated with 200 mc of I^{131} showing achromatic lesion (pair 8) in 45 chromosome set.

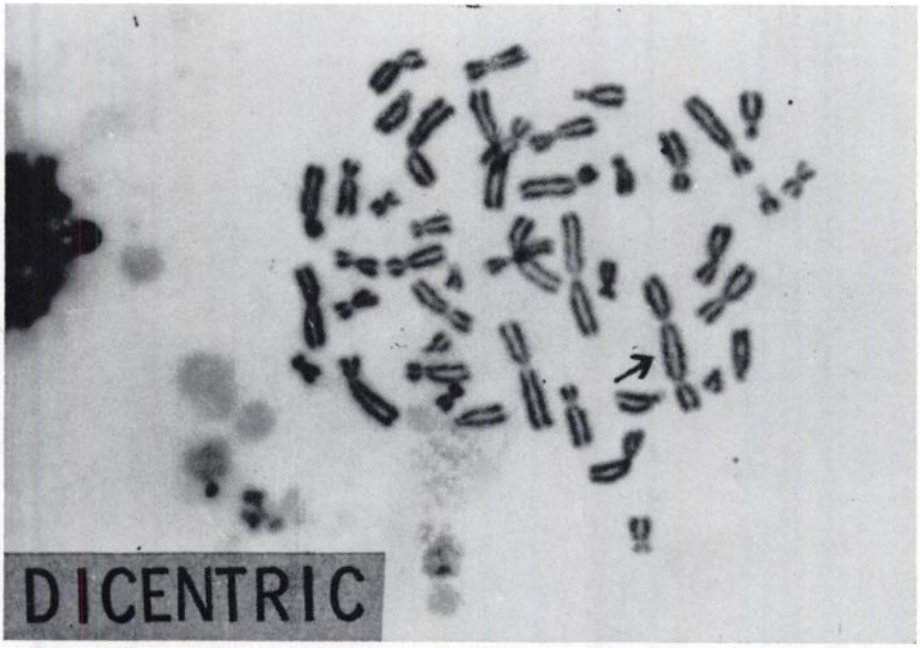

Fig. 6. Dicentric chromosome detected in patient treated with 12 mc of I^{131} in 1957.

Chromosome aberrations (Fig. 6) and some of the later chromatid aberrations are either lost, if lethal, or may persist for long periods in the hemopoietic cells which give rise to the circulating leukocytes. It is noted that dicentrics occurred in cells without the presence of fragments.

Blood radioactivity was measured after a 10 mc I^{131} therapy dose for thyrotoxicosis. The plasma concentration was about 0.4 mc/ml at ½ hour giving a calculated whole body dose of 20 rads.[1] Following a 200 mc I^{131} therapy dose for thyroid cancer, plasma concentration was about 3-4 μc/ml at ½ hour, giving a calculated whole body radiation dose of 60 rads.

No chromosomal changes were found that could be attributed to diagnostic doses of radioiodine as shown by comparing the chromasomal pictures before and ½ hour after a 2-10 μc tracer dose.

No chromosomal abnormality was associated with the original disease as shown from the chromosomal pre-therapy analysis in Group III (proved thyrotoxicosis) and in Group IV (myxedematous following total thyroidectomy). Also, no correlation was established between the thyroid status of patients at the time of the study and the chromosomal abnormality; 50 per cent of the cases with "abnormal" chromosome picture in Group II were hypothyroid.

It was also shown that no abnormalities were associated with the use of antithyroid medications. The pre-treatment blood of six patients in Group III who had had propylthiouracil therapy did not differ from the blood of the other five patients with no exposure to such drugs.

Pochin *et al* (12) reviewing over 10,000 cases treated with radioiodine for thyrotoxicosis, concluded that there was no indication that this treatment has produced an increased incidence of leukemia. In our series, no hematological abnormality was detected in any of the patients either clinically or by blood studies.

As the incidence of chromosomal abnormalities was higher shortly after irradiation as compared to those present after periods up to 14 years, it might be assumed that the aberrations are being gradually lost and that the circulating leukocytes may eventually become normal. Recently, Curtis *et al* (13) 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 significance of these findings cannot be interpreted in relation to the future health of these patients. Genetically, it seems reasonable to consider that the persistence of chromosomal aberrations in the somatic cells is not a great hazard and that man can tolerate a certain quantity of these abnormalities without observable ill effects.

SUMMARY

A statistically significant increased incidence of chromosomal abnormalities both in count and morphology was observed acutely and to a lesser extent chronically after radioiodine therapy for hyperthyroidism. These abnormalities

[1]Hine, G. J. and Brownell, G. L.—Editors: Radiation Dosimetry, Academic Press Inc., N.Y., 1956, p. 867.

were detected as early as ½ hour after the therapy dose and were found to persist for at least 14 years after the treatment.

The incidence and severity of abnormalities were greater after the larger treatment doses of I^{131} for thyroid carcinoma, and in the period shortly after treatment. No hematological phenomena accompanied the chromosomal abnormalities.

Apparently, no chromosomal aberrations could be attributed to the use of antithyroid drugs or to the thyroid status at the time of the study. Further work is in progress to evaluate other factors which might affect the chromosomal analysis.

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