FROM THE RADIUM CENTRE (DIRECTOR: PROF. JENS NIELSEN) AND THE DEPARTMENT OF EXPERIMENTAL GENETICS AND CYTOLOGY (DIRECTOR: J. SCHULTZ-LARSEN), THE UNIVERSITY INSTITUTE FOR HUMAN GENETICS, COPENHAGEN, DENMARK.

RADIATION-INDUCED CHROMOSOME ABERRATIONS

Persisting aberrations in long-term cultures from human skin irradiated in vivo

by

JAKOB VISFELDT

Recent advances in the field of chromosome research have opened new possibilities of quantitative as well as qualitative investigations on radiation-induced chromosome aberrations in human cells.

Reports are numerous of in vitro irradiated cell cultures (e.g. ref. 1, 2, 8, and 15). It has been generally accepted that cells must be examined during the primary mitosis following exposure since radiation-damaged cells in long-term cultures are eliminated quickly in the successive divisions. Plant experiments have shown that cells presenting chromosome aberrations are rapidly and progressively lost, and because of selection against damaged cells nothing but normal cells persist after a certain interval (ref. 5).

Consequently, the publication of studies describing chromosome aberrations in leucocytes from patients who long previously had been exposed to radiation attracted much attention (ref. 3, 4, 6, 13, and 17). Short-term cultures of peripheral blood were used in all of these studies and the mitoses observed were probably those which occurred first upon establishment of the culture. The finding

Submitted for publication 29 January 1964.

Table 1

Chromosome numbers

	Total of analysed mitoses	40—44	45	46	47	48—52
Culture H 673						
Non-irradiated skin biopsy	144	2	8	134	0	0
Culture H 672						
Skin biopsy 3 hrs after irradiation	365	14	30	314	5	2
Culture H 674						
Skin biopsy 24 hrs after irradiation	146	3	10	133	0	0
Culture H 675						
Skin biopsy 72 hrs after irradiation	69	1	9	58	1	0

of aberrations that hitherto had been considered incapable of survival through several cell generations was accounted for by postulating that the damaged cells had entered into their first mitosis during the brief in vitro cultivation (ref. 7).

It was therefore considered essential to investigate whether human cells irradiated in vivo and presenting chromosome aberrations might persist and reproduce in our cultures. As fibroblasts have been found highly suitable for long-term cultivation the choice in the present study has been to use biopsies from irradiated skin in which such cells as grow during cultivation are supposed to be fibroblasts. The experiments were primarily directed to establishing: (1) whether cells presenting abnormal chromosome patterns were demonstrable after long-term cultivation, and (2) if so, what types of chromosome aberrations may be found after long-term cultivation, (3) if any qualitative and/or quantitative differences were demonstrable between aberrations observed in biopsies taken 3, 24 and 72 hours, respectively, after exposure to radiation.

Material and methods. A patient who was due for heavy radiotherapy (4 000 to 5 000 r) for laryngeal cancer was selected for the preliminary experiment. Prior to therapy, an isolated dose of 100 r was administered to a field (5 cm) on the volar surface of right forearm with a Muller RT 100 unit at 100 kV, 12 mA, FSD 10 cm, and a 1.7 mm Al filter. Skin biopses were taken from the irradiated area at intervals of 3, 24, and 72 hours, respectively, after exposure. A skin biopsy from the left forearm served as control. The lines laid down by FRØLAND (1961) for the cultivation and histologic preparation of skin biopsies were followed. Slides were prepared from a great number of subcultures in which growth was stopped by colchicine 2 to 5 weeks after the establishment of the cultures; they were studied in random order, and as usual the cells were analysed in late

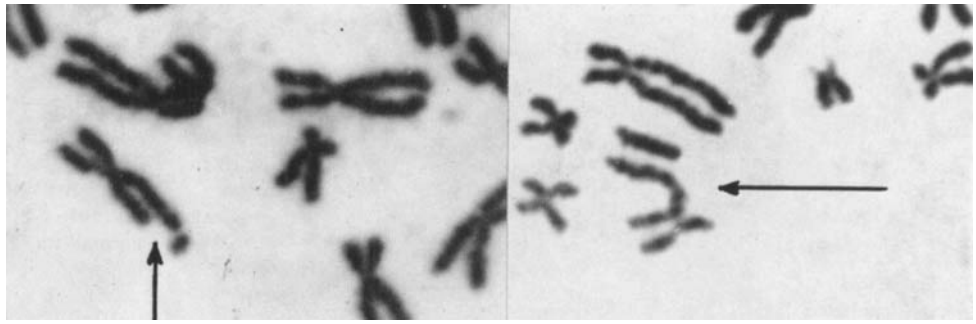

Fig. 1. Details of 2 mitoses from culture H 673 of (non-irradiated) human skin, each showing a chromatid fracture. Giemsa stain.

metaphase. Mitoses of supreme quality were applied exclusively and no more than one slide from the individual subcultures was studied. The numbering of chromosomes followed the Denver classification (12). Furthermore, the small acrocentrics (21—22 and y) were classified as group A; small metacentrics (16, 19—20) as group B; small sub-acrocentrics (17—18) as group C; large acrocentrics as group D; and the middle group (6—12) as group M.

All the mitoses were analysed by the author and the aberrations observed were submitted to the inspection of one or more cytogeneticians who in turn have given their opinion; there has been a consensus of opinion as to the correctness of the interpretation in all cases. Whenever an aberration gave rise to doubt the mitosis was recorded as normal. The distribution of the 733 mitoses analysed was as follows:

H 673 Skin biopsy from the left arm (non-irradiated)	145 mitoses
H 672 Skin biopsy from the right arm 3 hours after exposure	370 mitoses
H 674 Skin biopsy from the right arm 24 hours after exposure	148 mitoses
H 675 Skin biopsy from the right arm 72 hours after exposure	70 mitoses

Results

The experiment was briefly reported in a previous communication (ref. 18).

All details of the morphologic chromosome deviations have been filed, thus making it a simple matter to compare quantitatively the aberrations in the various cultures.

The chromosome numbers are listed in Table 1.

Chromatid aberrations in cells were rare. Two of the three fractures identified in non-irradiated, analysed cells are shown in Fig. 1. A well-known phenomenon was encountered, i.e. that although the peripheral portion of the fractured chromatid is dislocated it remains 'adherent' to the homologous chromatid. Besides fractures, the chromatid aberrations included single fragments and, on an isolated occasion, a deleted chromatid was observed in which the lost

Table 2

Chromosome aberrations

	Total of analysed mitoses	Deletions	Abnormal numerical distribution assumed to arise from deletion	Trans-locations	Abnormal numerical distribution assumed to arise from translocation	Abnormal numerical distribution in the M-B-C groups without other abnormalities
Culture H 673						
Non-irradiated skin biopsy	145	0	0	0	0	1
Culture H 672						
Skin biopsy 3 hrs after irradiation	370	2	2	24	3	7
Culture H 674						
Skin biopsy 24 hrs after irradiation	148	2	1	19 (18)	3	6
Culture H 675						
Skin biopsy 72 hrs after irradiation	70	2	0	1	1	1

material could not be traced in the mitosis. Neither qualitative nor quantitative differences between irradiated and non-irradiated aberrations were demonstrable, but the figures were very small. Achromatic lesions and isolated twin fragments were noted and filed although such anomalies have not been classified nor have they been quantitatively compared in the present study.

The chromosome aberrations are listed in Table 2. Some of these are included in the form of photos of mitoses and kariograms. A cell originating from the biopsy taken three hours after exposure is represented in Fig. 2. The aberration is listed in the table under 'translocations'. The arms of a chromosome in the 13—15 group are seen to be extraordinarily long. Classification of this chromosome in the D group is correct taking into consideration the distinctness with which the satellites are demonstrable in the microscope. Otherwise the kariogram presents normal findings; in particular no other chromosome is seen to have lost any material.

The cell depicted in Fig. 3 is derived from the same biopsy. The aberration is listed in Table 2 under 'translocations'. One chromosome is also in this case seen to present extraordinarily long arms. The kariogram suggests that it is a chromosome belonging to the 4—5 group. The cell presents no other anomalies, nor can material be seen to be absent in any other chromosome.

The cell depicted in Fig. 4 is derived from the biopsy obtained 24 hours after exposure. The aberrations are listed in Table 2 under 'translocations'. In slides from 4 out of the 6 subcultures examined a total of 18 cells were found to present identical aberrations. The position of the centromere in a chromosome in the 4—5 group is abnormal. The length of this chromosome was measured

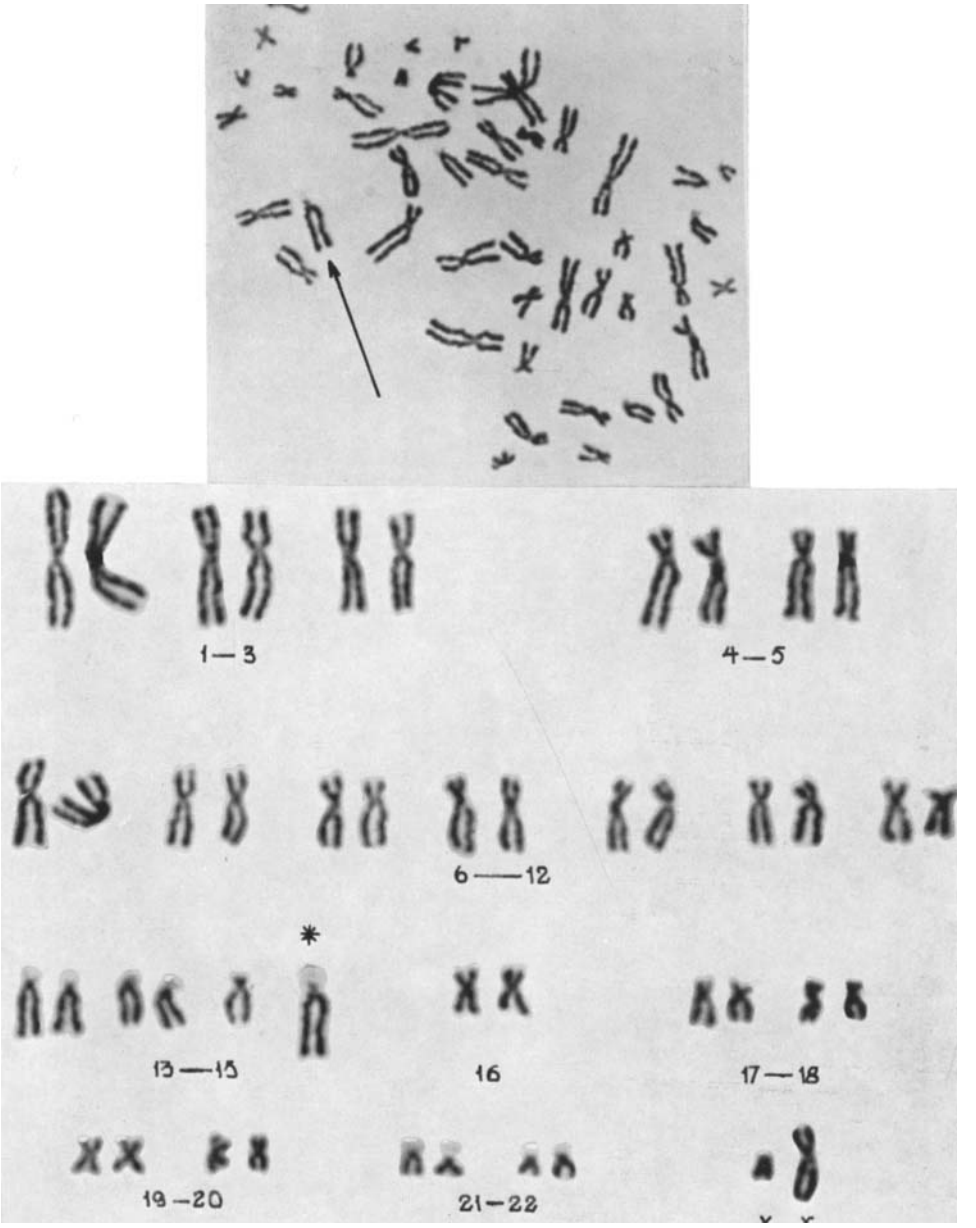

Fig. 2. Mitosis from culture H 672 of irradiated human skin. Kariogram showing abnormal long arms on a chromosome in the 13—15 group. Giemsa stain.

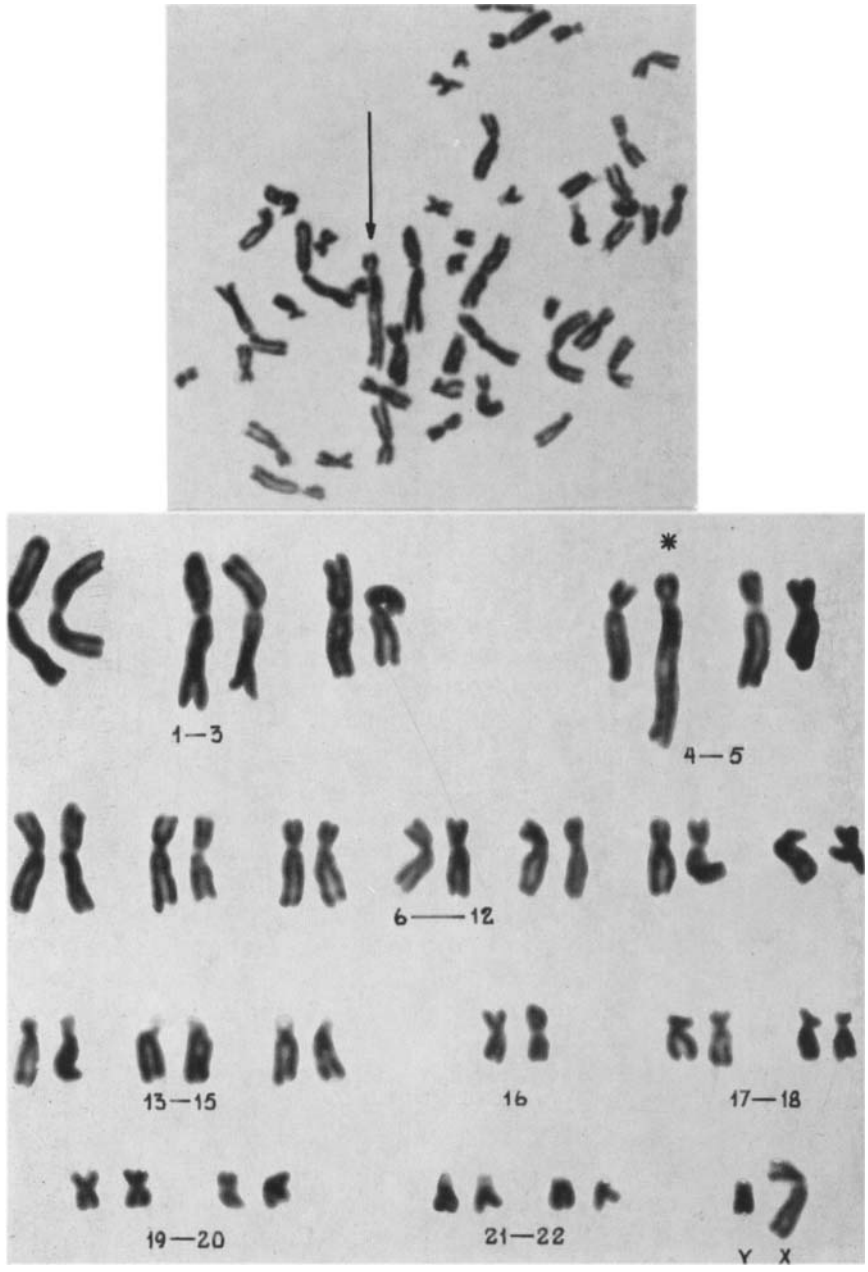

Fig. 3. Mitosis from culture H 672 of irradiated human skin. Kariogram showing abnormal long arms on a chromsome in the 4—5 group. Giemsa stain.

in all 18 cells and found to correspond to a classification in the 4—5 group as was also suggested by the analysis. Moreover, anomaly of a chromosome No. 16 represents a constant finding, the major portion of one set of arms being absent. Inspection of the kariogram reveals, however, that one of the small group C chromosomes is absent and that an extra chromosome, which is somewhat larger, appears in the middle group.

The cell shown in Fig. 5 is also derived from the biopsy taken 24 hours after exposure. The aberrations are listed in Table 2 as 'abnormal numerical distribution assumed to arise from translocation'. Absence of one chromosome in the D group, including 5 only, is demonstrated by the analysis and an extra chromosome is found in group B. Several mitoses are deficient by one group D chromosome and present one extra chromosome in groups M, B, or C.

Discussion

Aneuploids are listed in Table 1, which records the chromosome numbers of the analysed cells. It should be noted that with the routine technique in this laboratory, the number of aneuploid cells is about 10 per cent. Whether the slightly increased percentage of aneuploids in irradiated cells is accidental or genuine cannot be decided until the material has become more comprehensive. Analysed tetraploids and near tetraploids have been precluded from the material since tetraploid mitoses capable of being analysed admittedly represent the exceptions. If these were included, a false impression might be obtained from the table which allows for nothing outside the recording of mitoses capable of being analysed. The percentage of tetraploid mitoses is actually higher than could be calculated from the table.

Very few chromatid aberrations were observed, these including exclusively the well-known fractures (break on a chromatid with dislocation of a fragment), and the presence of isolated fragments. The figures being very small, a comparison of irradiated and non-irradiated cells serves no reasonable purpose until the material has become larger. It should be noted, however, that the incidence of chromatid aberrations per number of analysed cells, derived from the non-irradiated biopsy, was found to be relatively higher than the incidence in the irradiated biopsies. For the time being the assumption seems to be preferable that the chromatid aberrations encountered in the present material probably have developed during the cultivation or the histologic preparation of the slides.

It was soon realized that a series of chromosome aberrations were demonstrable in the irradiated biopsies but not in the non-irradiated control, thereby providing evidence that radiation-induced chromosome aberrations might persist in our long-term cultures, a feature that has hitherto been doubted.

Radiation-induced chromosome aberrations may theoretically develop in two basically different ways. Breaks on one or more chromosomes, occasionally

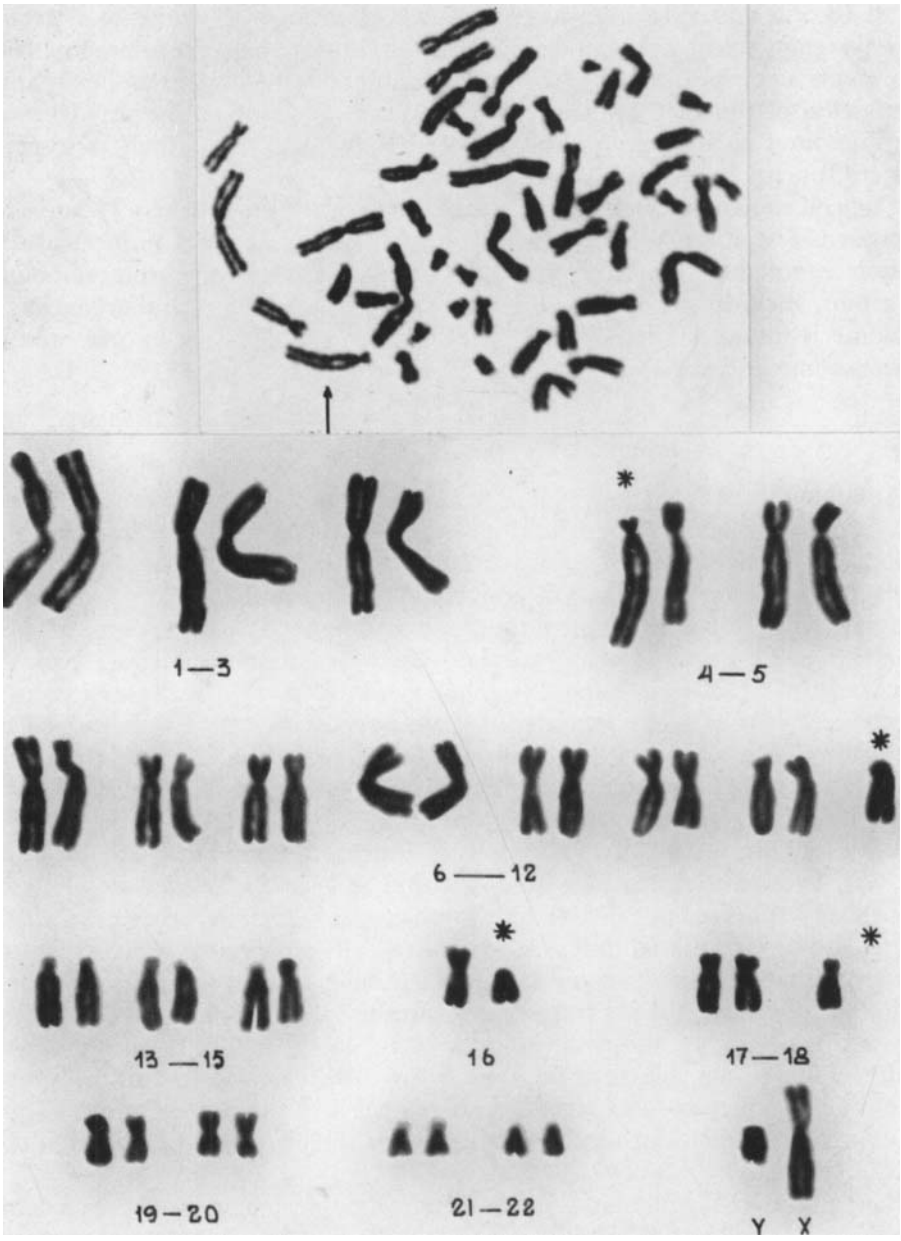

Fig. 4. Mitosis from culture H 674 of irradiated human skin. Kariogram showing abnormal position of the centromere on a chromosome in the 4—5 group. One pair of arms on a chromosome No. 16 is too short and the numerical distribution between the 6—12 group (15 chromosomes) and the 17—18 group (3 chromosomes) is abnormal. Giemsa stain.

with translocation of the chromosome material, may mean that the lesions in viable cells appear in the form of chromosome aberrations during mitoses; this is always provided that the breaks occur prior to the effective duplication of the chromosome. Radiation-induced chromatid aberrations may disappear during division or be interpreted as chromosome aberrations following the primary division. Similar theoretical considerations have been advocated previously by BENDER & GOOCH (1962).

In our long-term cultures, in which cells have been seen to persist through several divisions, radiation-induced lesions may be expected to appear in the form of chromosome aberrations, provided that such lesions are compatible with the permanent viability of the cells.

The interpretation of the morphologic findings as regards the chromosome aberrations described has been made as follows.

Figs 2 and 3 illustrate the translocation of material to a chromosome; the material is apparently not absent in any other chromosome.

Fig. 4 illustrates two features: a pericentric inversion is visible in a chromosome in the 4—5 group, and the material lost from chromosome No. 16 has evidently been translocated to a chromosome in the 17—18 group, which consequently has become larger and may be interpreted as an extra chromosome in the middle group. Hence, the two last-mentioned aberrations are interpreted as translocations. The numeral 18 is bracketed in the column translocations in cultures 24 hours after exposure in Table 2. This indicates that 18 out of the 19 translocations were identified in cells of the kariotype discussed, presenting two marker chromosomes (one in the 4—5 group, and the other one in one of the No. 16 chromosomes). This may be a matter of a clon originating in a radiation-damaged chromosome, and in addition this cell must have been highly viable. The material probably included many clon developments, among which this particular clon exclusively presents two marker chromosomes.

The presence of five group D chromosomes and five chromosomes belonging to the 19—20 group (Fig. 5) is accounted for by suggesting that part of a chromosome in the middle group has been translocated to the apex of a group D chromosome. This would reduce the size of the chromosome from the middle group, meaning that this chromosome would probably be registered in group B, and the group D chromosome in group M. Other explanations may be possible, e.g. a pericentric inversion of a group D chromosome might have occurred. It might be mentioned that a diploid cell with five group D and five group C chromosomes has been reported in the bone marrow from a case of leukaemia in quiescent phase treated by cytotoxic drugs (PEDERSEN, personal communication).

The findings included in the individual groups listed in Table 2 will be only briefly reviewed.

Deletions. The group comprises chromosomes in which identical amounts of

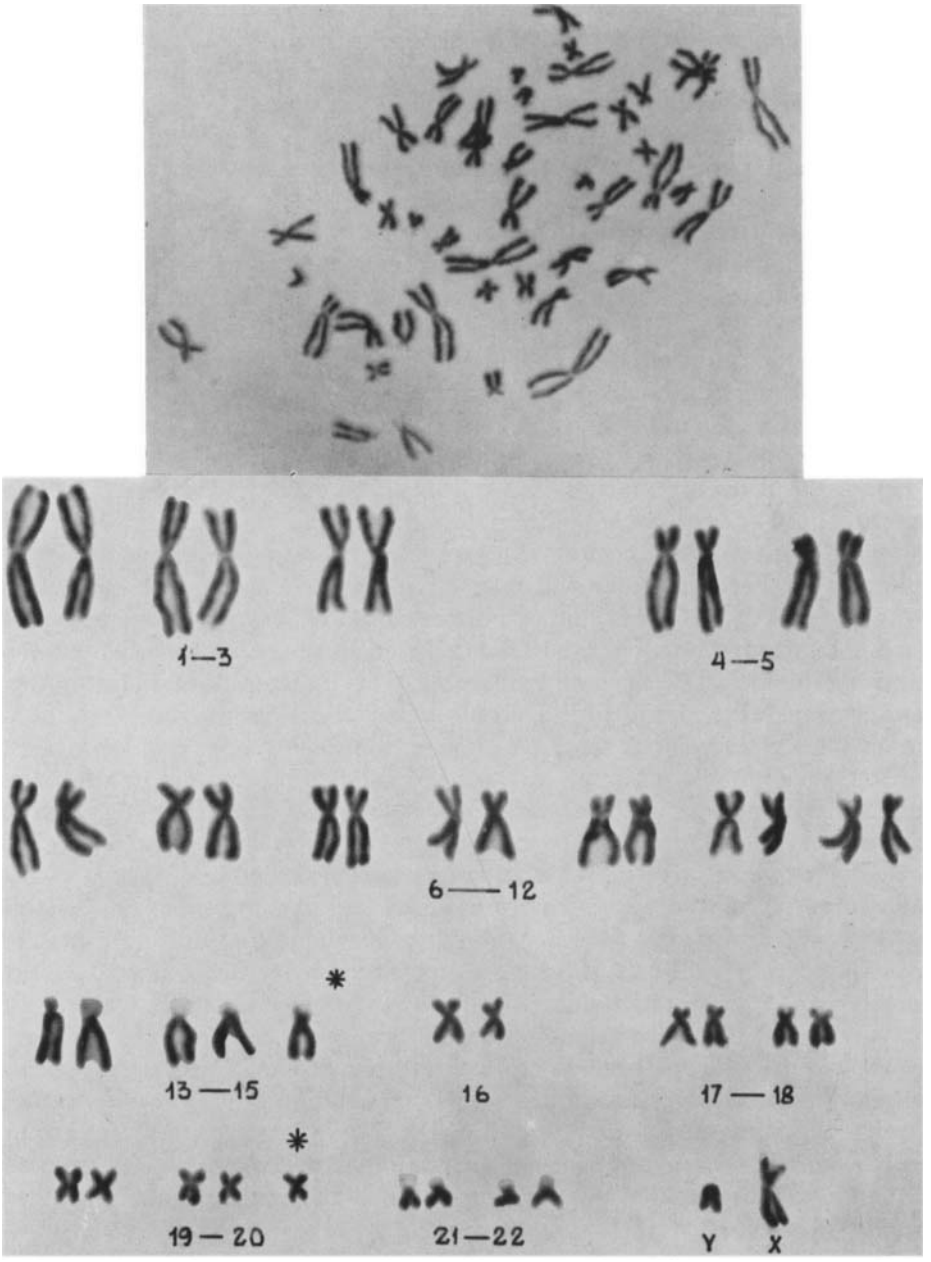

Fig. 5. Mitosis from culture H 674 of irradiated human skin. Kariogram showing abnormal numerical distribution between the 13—15 group (5 chromosomes) and the 19—20 group (5 chromosomes). Giemsa stain.

material are absent on the homologous chromatids; the deleted material is not traceable in other chromosomes although this fact does not preclude the possibility that such material may have been translocated to some other chromosome.

Abnormal numerical distribution assumed to arise from deletion. The diploid cells presenting increased numbers of chromosomes in the groups A and D serve as example.

Translocations. The group comprises mitoses in which the translocation of surplus material to a chromosome is directly visible (Figs 2 and 3). Some large chromosomes presenting pericentric inversion are also included (Fig. 4).

Abnormal numerical distribution assumed to arise from translocation. The mitosis, depicted in Fig. 5, and discussed in the above, serves as an example of this feature.

Abnormal numerical distribution between groups M, B, and C is recorded as 'chromosome aberrations' although it is questionable so far whether the abnormal distribution is attributable to radiation damage; if so, it might be a matter of reciprocal translocation. One mitosis from the non-irradiated biopsy, however, presented the same anomaly. To arrive at an answer to the question whether these should be considered as 'normal deviations' would not seem possible, however, until a larger material is available.

It is apparent from Table 2 that deletions are few as compared with other types of aberrations encountered in the cells. The most common, irreparable radiation-damage to develop after 'one hit' is presumably a break involving loss of the deleted material; whenever translocations are observed, they must have been preceded by 'two hits' and, from a statistical point of view, this represents a much more unusual phenomenon. The rare occurrence of chromosome aberrations in the form of deletion makes it tempting to advance the following hypothesis. Among the radiation-damaged cells the ones presenting balanced chromosome aberrations and the ones to which extra chromosome material has been translocated are more likely to survive than cells presenting loss of chromosome material.

The question as to whether any qualitative and/or quantitative differences exist between chromosome aberrations in the three irradiated cultures cannot be definitely answered until a more comprehensive material is available.

The possibilities of comparison between the present and previous studies are reduced because of the scarcity of available reports of chromosome aberrations in human cells irradiated in vivo. TOUGH et coll. (1960) and BUCKTON et coll. (1962) have described aberrations in short-term cultures of leucocytes from the

peripheral blood of patients exposed to irradiation to the spine for ankylosing spondylitis. In the latter study the cells were classified into three main categories, i.e. A, B, and C cells. Type A cells present no evidence of structural chromosome anomalies, chromosome analyses revealing the presence of diploid or aneuploid cells. Type B cells are structurally normal apart from showing a chromatid gap, a chromatid break, or an isochromatid gap. Type C cells were further subdivided into C-1 cells presenting fragments, dicentrics, tricentrics, or rings; C-2 cells contain abnormal chromosomes other than those of the C-1 type (a photo serves as example). The most likely explanation for their origin is the rearrangement of chromosome material after multiple breaks leading to inversions, deletions, and translocations. In the type C-3 cells the distribution of chromosomes between the various subgroups is found to be abnormal, the explanation being the same as the one given for the C-2 anomalies. The authors have regarded the aberrations in the types C-2 and C-3 cells as stable and the remaining aberrations as unstable, first and foremost because of the more constant and marked decline of unstable aberrations with time after exposure.

Similar results were obtained in the present investigation in a fundamentally different way. Our results may serve as a strong indication supporting the theoretical opinions on stable and unstable radiation-induced aberrations. Indeed, cells presenting translocations (including pericentric inversions) or abnormal distribution between the groups have been seen to persist through many generations in our cultures. For the time being cells presenting deleted chromosomes should be considered with a certain reservation. It is apparent from our results that dicentrics, tricentrics and rings have not been evident at all in the present investigation. Other authors (ref. 3, 4, 6, 9, 10, 13, 16, and 17) have described the said aberrations after in vivo irradiation of human tissue. BUCKTON et coll. found that these aberrations were of an unstable character and, indeed, from a theoretical point of view, it is hardly possible to explain how these chromosomes may be able to persist through mitosis, in particular through the uncoiling phase.

WOLFF (1963), citing plant experiments, declared that dicentric chromosomes have persisted through several generations. Cells in our cultures have undergone several divisions in the interval between the in vivo radiation and the time of analysis. It may therefore be assumed that cells presenting ring-shaped, dicentric, or tricentric chromosomes in our cultures are unable to survive mitosis but are eliminated. The finding of these chromosome aberrations in short-term cultures of leucocytes from patients exposed to radiation long previously has been explained rather often on the basis of the theory that cells may have entered into their first mitosis during the short-term in vitro cultivation to become visible in the initial division following exposure (cf. e. g. CHU (1963)). In the frequently cited works of BENDER & GOOCH (1962, 1963) comprising eight individuals who received whole-body irradiation in a 'criticality accident', dicentric and ring-shaped aberrations were demonstrated in the leucocytes cultured 29 months

after exposure; 42 months after the accident re-examination failed to demonstrate any ring-shaped aberrations.

The experiments are to be continued in our laboratory with other patients exposed to varying, but small, roentgen doses. A quantitative correlation of dosage to incidence of aberrations is hardly obtainable, primarily because such incidence has been markedly influenced quantitatively by the development of the clon. Finally it should be noted that the persisting chromosome aberrations apparently are of a type (balanced translocations and translocations presenting partial trisomi) that is similar to the one encountered in patients with certain congenital malformations and in their nonaffected relatives as well as in patients suffering from certain malignant diseases.

Acknowledgement

This work was supported by grants from the Danish Anti-Cancer League and the Danish Atomic Energy Commission.

SUMMARY

A small area of a patient's forearm was exposed to 100 r to investigate whether cells presenting chromosome aberrations attributable to radiation in vivo persisted in long-term cultures. Skin biopsies were taken at intervals after exposure and cultured for up to about five weeks. Several aberrations, mostly translocations, including pericentric inversion, deletions, and an abnormal distribution between the chromosome groups, were detected. It appeared that cells with certain aberrations were capable of reproduction by mitotic division.

ZUSAMMENFASSUNG

Ein kleines Feld auf dem Vorarm eines Patienten wurde mit 100 r bestrahlt um herauszufinden, ob Zellen mit strahlenbedingter Chromosomenabweichung in einer Zellkultur überleben. Probeexcisionen von der Haut wurden in Abständen nach der Bestrahlung entnommen und bis zu 5 Wochen im Brutschrank gelassen. Verschiedene Aberrationen, meistens Umstellungen einschliesslich perizentrische Inversion, Auslöschungen und abnorme Verteilung unter den Chromosomengruppen wurden gefunden. Es scheint, dass Zellen mit gewissen Aberrationen fähig sind sich durch Kernteilung zu vermehren.

RÉSUMÉ

Une petite surface de l'avant-bras d'un malade a été exposé à 100 r pour chercher si les cellules présentant des aberrations chromosomiques attribuables à l'irradiation in vivo persistent dans des cultures de longue durée. Des biopsies de peau ont été prélevées à différents intervalles de temps après l'irradiation et cultivées pendant environ cinq semaines. Plusieurs aberrations ont été détectées, surtout des translocations, des inversions péricentriques, des délétions et une distribution anormale entre les groupes de chromosomes. Il semble que des cellules portant certaines aberrations sont capables de se reproduire par division mitotique.

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