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# Reduced DNA Repair Synthesis in Healthy Women Having First Degree Relatives with Breast Cancer

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**Abstract**—*The DNA repair synthesis induced by UV-C irradiation was studied in unstimulated lymphocytes of* 64 *healthy women whose mothers, or sisters, or mothers and sisters had had breast cancer. For comparison we took* 48 *control women. As the parameter for the determination of DNA repair synthesis the incorporation of* [$^3$H]*thymidine in the presence of* 2 mM *hydroxyurea was taken. The levels of* [$^3$H]*thymidine incorporation were reduced by* 19 *of the* 29 *women whose mothers, in* 17 *of the* 25 *women whose sisters and in nine of the* 10 *women whose mothers and sisters had had breast cancer. By comparison a decreased level was found in only seven of the* 48 *control women. This difference between the controls and women having first degree relatives with breast cancer was significant in each group. In an earlier study a redued DNA repair synthesis in breast cancer patients was established. The present findings suggest that DNA repair synthesis may be one of the factors involved in the genesis of breast cancer.*

## INTRODUCTION

SEVERAL studies have shown that the risk of developing breast cancer is higher in first degree female relatives of patients than in controls. Having a mother or sister with breast cancer increases the risk for relatives by a factor of 1.8 or 2.5, respectively [1]. However the risk of breast cancer varies according to whether the disease is diagnosed in premenopausal or postmenopausal women and whether it is unilateral or bilateral [1–3]. The risk is the highest in women whose mothers and sisters have bilateral premenopausal breast cancer; they have a 30% probability of developing breast cancer [3].

Deficiencies in DNA repair synthesis have been correlated with autosomal recessive genetic disorders [4–6]. Furthermore Pero *et al.* [7] reported that there are deficiencies in the DNA repair capacity of mononuclear leukocytes of persons genetically predisposed to colorectal cancer. In a previous study [8] we found that significantly more breast cancer patients than age-matched controls had a reduced UV-C induced DNA repair capacity.

Therefore to find out if reduced DNA repair is involved in a familial tendency to breast cancer, the DNA repair synthesis in 64 healthy women with first degree relatives with this disease was investigated.

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## MATERIALS AND METHODS

*Subjects*

The 64 healthy women came from the Frauenspital Basel (Maternity Hospital). The Frauenspital examines women who are first degree relatives of breast cancer patients every six months. These women had never had breast cancer or any other tumor disease. They were divided into three groups. In the *first* group there were 29 women (aged between 24 and 69 years) whose mothers had breast cancer (in 18 cases the breast cancer was diagnosed premenopause and in 11 cases postmenopause). Only one mother (40 years old) had bilateral breast cancer. In the *second* group there were 25 women (aged between 31 and 74 years) who had sisters with breast cancer. Twenty-one of these had only one sister with this disease (in 17 cases premenopausal and in four postmenopausal) and four had two or more sisters. The *third* group included 10 women (aged between 34 and 78 years) who had both mother and sister(s) with breast cancer. In five cases the breast cancer was diagnosed pre- and in five cases postmenopause. Three of the subjects had two sisters having breast cancer.

*Controls*

The blood of 38 healthy women (aged between 20 and 68 years) was obtained from the Basel Blood Center. For ethical reasons it was not possible to get information about the family cancer history. Therefore we took 10 additional control women (aged between 26 and 66 years) without any positive family history of tumor disease.

*Measurement of DNA repair synthesis*

As the parameter for DNA repair synthesis [$^3$H]thymidine incorporation was taken. The separation and irradiation of lymphocytes from fresh 20 ml heparinized blood samples and the process of [$^3$H]thymidine incorporation were carried out as already described [9]. The cells were irradiated with doses of 2, 4 ,8 and 16 J/m$^2$ at 253.7 nm (Philips lamp, fluence: 0.2 J/m$^2$).

To inhibit the DNA replicative synthesis of unstimulated lymphocytes hydroxyurea was added to a final concentration of 2 mM [10]. Immediately after exposure the cell cultures were incubated for 2 h with 10 μCi methyl-[$^3$H]thymidine (Amersham, sp. act. 25 Ci/mmol). The radioactivity was measured as counts per minute incorporated per $10^6$ cells. The degree of DNA repair synthesis was calculated as the difference btween the amounts of incorporated thymidine (in the presence of hydroxyurea) in irradiated and unirradiated cells.

The methodological variation lay between 13.4 and 15.1% [9].

*Statistical analysis*

For comparison of the values of spontaneous and hydroxyurea-inhibited DNA synthesis in non-irradiated cells the Wilcoxon $U$ test was used. Linear regression analysis was performed using $y$ (cpm/$10^6$ cells) as the dependent variable and $x$ (log dose) as the independent variable. The confidence range of the regression line of the 38 controls was calculated for a significance level of 99%. For the evaluation of the DNA repair synthesis in irradiated cells the Fisher exact 2 × 2 table probability test was employed. The significance analysis was calculated one-tailed at a significance level of 2.5%. The Wilcoxon $U$ test was used additionally for comparison of the values at various doses in controls and subjects.

## RESULTS

To establish the values of thymidine incorporated after UV exposure the spontaneous and hydroxyurea-inhibited DNA synthesis was measured in the unirradiated cells.

*Spontaneous DNA synthesis and its inhibition by hydroxyurea*

Table 1 shows the mean values of DNA synthesis with and without hydroxyurea in unstimulated unirradiated lymphocytes. There were no significant differences between women having first degree relatives with breast cancer and the controls. Hydroxyurea (2 mM) depressed spontaneous DNA synthesis to a similar extent in all groups: residual activity in the controls 56%, in the subjects 61, 60, 63%.

*DNA-repair synthesis. [$^3$H]Thymidine incorporation after UV-C exposure*

To determine the degree of repair synthesis, the cpm values (mean of duplicate determinations) of hydroxyurea-inhibited, unirradiated samples were subtracted from the cpm values of the irradiated samples.

Thymidine incorporation is dose-dependent for 2, 4, 8 and 16 J/m$^2$ (doses plotted logarithmically). Saturation in this dose range was not reached. Although our result curves became progressively less steep, they did not flatten completely. Preliminary results showed that in our investigation the saturation dose is about 20 J/m$^2$ (unpublished data).

Figure 1(A–D) presents the measured values of the incorporated thymidine for the control women and for subjects with positive family history. Comparing Fig. 1(A) with 1(B), 1(C) and 1(D) it can be seen that the [$^3$H]thymidine incorporation was generally lower in subjects than in controls.

To establish individual variation of DNA repair synthesis in the dose range 2–16 J/m$^2$, the regression line for each person investigated was calculated. The regression lines of the subjects were compared with the regression lines of the 38 control women. The evaluation was based on the following statistical criterion: DNA repair synthesis was taken to be reduced or increased if the value of the incorporated thymidine on the individual regression line at 6 J/m$^2$ (median dose) lay beyond the 99% confidence range of the regression line from the 38 controls. For statistical analysis we used the Fisher exact 2 × 2 table probability test. To justify 6 J/m$^2$: the median dose was taken, since the effect of discriminating between patient controls is expected to be largest at the median dose and the error is lowest in this range. This type of evaluation considers the response to UV exposure within the definite dose range (2–16 J/m$^2$).

Table 2 presents the mean values of thymidine incorporation and the evaluated DNA repair synthesis in the probands and in the 38 controls. There were significant differences ($P < 0.05$) between values in all three groups and in the controls at each dose. A reduced repair capacity was found in five out of 38 control women.

Among the 29 daughters whose mothers had breast cancer, DNA repair synthesis was reduced in 19 women; the number of subjects and controls

*Table* 1. *The effect of hydroxyurea on spontaneous DNA synthesis in non-irradiated unstimulated lymphocytes of women having first degree relatives with breast cancer and female controls*

| Group (number of subjects) | Family member with breast cancer | Incorporation of [$^3$H]thymidine (cpm/$10^6$ cells, mean ± S.E.M.) No HU | 2 mM HU |
|---|---|---|---|
| 1 ($n$ = 29) | Mother | 1922 ± 367 | 1085 ± 218 |
| 2 ($n$ = 25) | Sister(s) | 2255 ± 266 | 1328 ± 182 |
| 3 ($n$ = 10) | Mother + sister(s) | 2376 ± 505 | 1710 ± 593 |
| Controls ($n$ = 38) | | 1878 ± 195 | 1103 ± 169 |

HU: hydroxyurea.

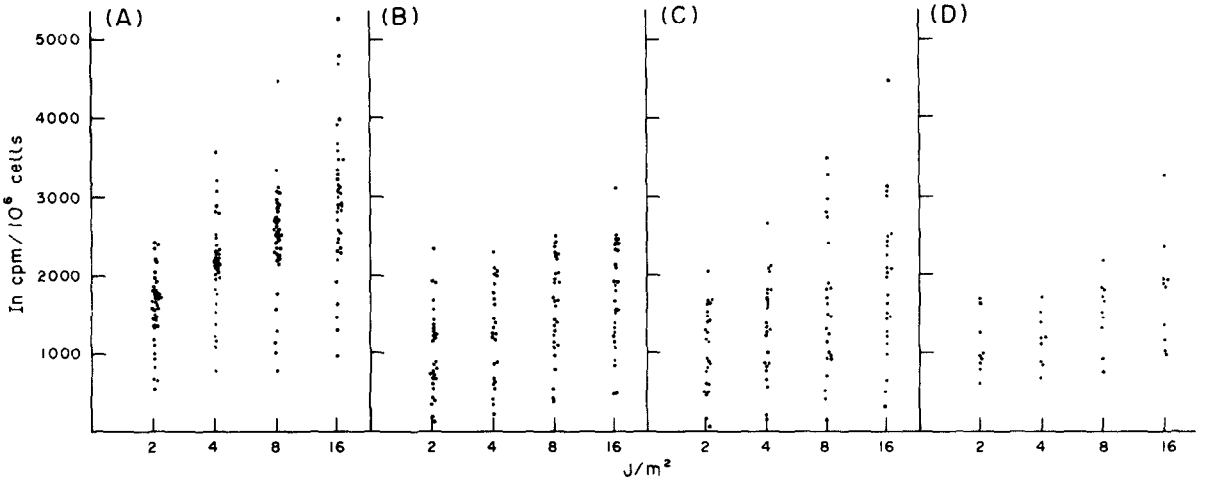


*Fig.* 1. *DNA repair synthesis in* 38 *control women* (*A*) *and in* 64 *women having mother* (*B*), *sister* (*C*), *both mother and sister* (*D*) *with breast cancer. The unstimulated lymphocytes were preincubated for* 30 min *with* 2 mM *hydroxyurea, UV-C irradiated and incorporated for* 2 h *with* 10 μCi *methyl-*[$^3$H]*thymidine*/2 × $10^6$ *cells. Each point represents the measured value of the incorporated thymidine after subtraction of hydroxyurea values. Ordinate: UV-induced DNA repair synthesis* (cpm/$10^6$ *cells*). *Abscissa: UV fluence* (J/m$^2$).

*Table* 2. *UV-C induced DNA repair synthesis in healthy subjects with positive family history and in control women*

| Group (number of subjects) | Family member with breast cancer | [$^3$H]Thymidine incorporation in cpm/$10^6$ cells (mean ± S.E.M.) at various UV doses 2 J/m$^2$ | 4 J/m$^2$ | 8 J/m$^2$ | 16 J/m$^2$ | Repair synthesis reduced in |
|---|---|---|---|---|---|---|
| 1 ($n$ = 29) | Mother | 995 ± 104* | 1164 ± 124* | 1561 ± 115* | 1729 ± 123* | 19/24† |
| 2 ($n$ = 25) | Sister(s) | 1034 ± 100* | 1294 ± 117* | 1576 ± 175* | 1844 ± 179* | 17/25† |
| 3 ($n$ = 10) | Mother + sister(s) | 1065 ± 124* | 1125 ± 112* | 1506 ± 133* | 1768 ± 218* | 9/10† |
| Control ($n$ = 38) | | 1572 ± 76 | 2121 ± 98 | 2464 ± 116 | 2919 ± 146 | 5/38 |

*$P < 0.05$ compared to the controls (Wilcoxon $U$ test).
†Significantly different compared to the controls (Fisher exact 2 × 2 table probability test).

with a reduced activity was significantly different ($P < 0.01\%$). The results for daughters of mothers with pre- or postmenopausal breast cancer were 11/18 and 8/11, respectively (Table 3).

A reduced repair capacity was found in 17 of the 25 subjects whose sister(s) had breast cancer. The

*Table 3. UV-C induced DNA repair synthesis in daughters and sisters of breast cancer patients according to menopausal status at diagnosis*

| Family member with breast cancer | Diagnosis | Number of subjects | Repair synthesis reduced in |
|---|---|---|---|
| Mother | Premenopausal | 18 | 11/18 |
| | Postmenopausal | 11 | 8/11 |
| Sister | Premenopausal | 17 | 10/17 |
| | Postmenopausal | 4 | 3/4 |

*Table 4. Measured values of [$^3H$]thymidine incorporation in women having mother and sister(s) with breast cancer and in control women without previous cancer cases in the family*

| Initials of subjects/controls | Age | Age of family member at diagnosis: Mother | Sisters | [$^3$H]Thymidine incorporation (cpm/$10^6$ cells) at various UV doses*: 2 J/m$^2$ | 4 J/m$^2$ | 8 J/m$^2$ | 16 J/m$^2$ | Repair synthesis |
|---|---|---|---|---|---|---|---|---|
| *Subjects* | | | | | | | | |
| Sch. E. | 60 | 73 | 39,54 | 1690 | 1700 | 1815 | 1871 | Reduced |
| L. M. | 45 | 56 | 41 | 605 | 642 | 771 | 1016 | Reduced |
| F. D. | 42 | 36 | 40 | 1222 | 1185 | 1802 | 2366 | Reduced |
| G. M. | 50 | 44 | 43 | 835 | 857 | 1299 | 1153 | Reduced |
| S. A. | 48 | 72 | 37,47 | 943 | 1110 | 1655 | 1923 | Reduced |
| G. A. | 57 | 45 | 32 | — | 1380 | 2170 | 3237 | Normal |
| F. M. | 66 | 79 | 40 | 988 | — | 1509 | 1916 | Reduced |
| Z. A. | 78 | 40 | 80 | 922 | 1180 | 1438 | 1365 | Reduced |
| E. A. | 34 | 48 | 33 | 1619 | 1493 | 1678 | 1839 | Reduced |
| K. H. | 56 | 54 | 62,60 | 767 | 854 | 931 | 996 | Reduced |
| *Controls* | | | | | | | | |
| K. E. | 44 | | | 1296 | 1801 | 1988 | 2983 | Normal |
| M. Y. | 49 | | | 838 | 1009 | 2801 | 2592 | Normal |
| M. G. | 60 | No previous cancer cases | | 969 | 1582 | 2072 | 2569 | Normal |
| L. H. | 54 | in the family | | 2193 | 2273 | 2680 | | Normal |
| A. R. | 50 | | | 1726 | 1735 | 1963 | 1975 | Normal |
| B. H. | 26 | | | 243 | 650 | 914 | 1055 | Reduced |
| H. E. | 45 | | | 1825 | 2160 | 2234 | 2433 | Normal |
| F. M. | 66 | | | 759 | 913 | 1056 | 1370 | Reduced |
| E. E. | 61 | | | 1311 | 1725 | 2009 | 2414 | Normal |
| F. M. | 42 | | | 1677 | 2474 | 2449 | 2783 | Normal |

*Values after subtraction of the hydroxyurea values.

number of subjects and controls with reduced repair capacity was significantly different ($P = 0.01\%$). All the four women having two or more sisters with breast cancer had a reduced repair capacity. The results for sisters of women with pre- or postmenopausal breast cancer are 10/17 and 3/4 respectively (Table 3). It is of interest that these ratios allowing for the small numbers, are about the same as those for the daughters.

Table 4 gives the family history of the 10 women having mother and sister(s) with breast cancer, the measured values at four irradiation doses and the evaluated repair synthesis. In addition the experimental data are given for the 10 control women who had no previous cancer case in the family. In the 10 women who had both mother and sister(s) with breast cancer a decreased DNA repair level was found in nine cases. This is significantly different from the 38 controls ($P = 0.01\%$), but not different from the women of the first and second groups ($P = 22$ and $39\%$). By comparison of the 10 control women without any previous cancer case in the family only two showed a reduced capacity.

## DISCUSSION

Autosomal recessive genetic disorders are associated with a high incidence of cancer. Firstly Cleaver [11] reported defective DNA repair in xeroderma pigmentosum patients. Pero *et al.* [7] found a decreased level of unscheduled DNA synthesis induced by *N*-acetoxy-*n*-2-fluoroenyl-acetamide in leukocytes of individuals with colorectal cancer and in individuals predisposed to colorectal cancer. Recently, we found a reduced repair capacity in breast cancer patients [8]. Similar results were established earlier in patients with various kinds of

cancer (unpublished data).

In this study we compared two populations: healthy women with a positive family history for breast cancer and age-matched controls. The level of DNA repair synthesis was significantly lower in the women with a family history for cancer.

It is known that the DNA repair mechanism depends on the cell type, cell cycle, dose and duration of exposure to the damaging agent.

The proportion of lymphocytes in the cell cultures was over 97% (the rest: monocytes, granulocytes, plasmocytes). In our cell culture the T–B cell population was heterogenously distributed. Lewensohn *et al.* [12] found no difference in the repair capacity between T and B cell fractions. In an earlier pilot study [8] no difference in the distribution of lymphocyte subpopulation between healthy controls and breast cancer patients was found.

As the parameter for the excision DNA repair we chose the method of [$^3$H]thymidine incorporation. Several authors [13–15] used different methods to investigate the excision repair and found that each method gave similar results. To measure thymidine incorporation in irradiated cells, the replicative synthesis of the few cells in S phase was inhibited by 2 mM hydroxyurea. The proportion of unstimulated lymphocytes in S phase was determined by cytofluorometry in four persons. There was 0.25–0.4% without hydroxyurea and 0.17–0.25% in the presence of hydroxyurea. The incorporation values of the present study show that spontaneous DNA synthesis is not completely inhibited by 2 mM hydroxyurea. A higher concentration of hydroxyurea could not be used as it would impair UV-C induced repair synthesis [10–16]. The values of spontaneous DNA synthesis and hydroxyurea-inhibited DNA synthesis must be the same level in subject and control groups. If this is not the case, there is a risk of underestimating repair synthesis. In this investigation there were no significant differences in spontaneous and hydroxyurea-inhibited DNA synthesis between the two groups. However, in some of the subjects of the second and third groups both values were higher than the average. Some of these subjects also had decreased [$^3$H]thymidine incorporation. In these cases the decreased [$^3$H]thymidine incorporation could be due to a high rate of spontaneous DNA repair a high rate of spontaneous DNA synthesis inhibited by hydroxyurea. In the first case, because of the high rate of spontaneous DNA repair, the cells might have a lower capacity to repair the additional UV-C induced damage. In the second case, the high rate of spontaneous DNA synthesis in the presence of hydroxyurea could be explained by either a lower uptake of hydroxyurea by the cells or an altered inhibition of ribonucleoside diphosphate reductase.

The measurements of DNA repair synthesis may also be influenced by variation in the thymidine cellular pools. The results of Lambert *et al.* [17] and Kathleen *et al.* [18] show that an increased or reduced level in the DNA repair synthesis is not due to different pool sizes.

The extent of the damage could not be measured: it was assumed to be about the same in all persons investigated.

A reduced capacity for the repair of UV-C induced DNA damage is a hallmark of xeroderma pigmentosa. Patients with disease—using our method—have a 60–70% reduction of repair synthesis (unpublished data). In the present study 45 out of 64 subjects having first degree relatives with breast cancer had a reduced repair capacity and 13 out of these had a reduction in capacity similar to that of xeroderma pigmentosum patients. Hayakawa *et al.* [19] found that the kinetics of UV-induced unscheduled DA synthesis in xeroderma pigmentosum patients was markedly different from that in controls. Our preliminary results show that persons with reduced thymidine incorporation 2 h after irradiation reach a normal level between and 8 h after exposure. This investigation is still in progress.

During previous studies ([8, 9] and unpublished data), and intraindividual variation was investigated in both patients and healthy controls. Certain people have very stable values whereas others show variations of up to 20–25%. Cerutti also found a similar variation (personal communication). However, in the present investigation we compared a control population with a population with a family history of breast cancer and these variations might not influence the evaluation.

It can be assumed that there is a reduced repair capacity not only in the lymphocytes but also in the fibroblasts of the patients [20]. The damage caused by UV irradiation is corrected by excision repair. This mechanism is one of the most important repair mechanisms and the enzymes could be also involved in the correction of damage caused by other agents.

Pross *et al.* [21] found an increased NK-cell activity in women with a family history of breast cancer. It was suggested that the elevated NK-cell activity is a reaction to hormonal factors. The total estrogen and pregnanediol levels in daughters of breast cancer patients were found to be higher than in controls without previous familial cases of breast cancer [22]. Hyperprolactinemia was also present in teenage daughters of women having breast cancer [23]. The results obtained in our study suggest that a decrease in the DNA repair synthesis may be important in the genesis of breast cancer. Bain *et al.* [1] found that the risk of developing breast cancer is higher in sisters of breast cancer patients than in daughters. However Tulinius *et al.* [24] reported that when other factors are also considered the risk

is the same. We found no difference in repair capacity between daughters and sisters of breast cancer patients.

The frequency of reduced repair capacity was the same in the 38 control women and in the 10 women without previous familial cancer cases (5/38 and 2/10).

In an earlier study a reduced DNA repair synthesis as measured by [$^3$H]thymidine incorporation in 20 out of 41 breast cancer patients was established. We have now found a reduction in 45 out of 64 healthy women with a positive family history. For the interpretation of these data the repair values of the mothers and sisters with breast cancer are missing. Therefore we plan a further investigation in breast cancer patients and their first degree relatives (including fathers) to show whether a reduced DNA repair synthesis correlates with a hereditary cancer susceptibility.

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