DNA REPAIR SYNTHESIS IN HUMAN HETEROKARYONS

II. A TEST FOR HETEROZYGOSITY IN XERODERMA PIGMENTOSUM AND SOME INSIGHT INTO THE STRUCTURE OF THE DEFECTIVE ENZYME

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SUMMARY

Heterokaryons between fibroblasts from patients with classical Xeroderma pigmentosum (X.P.) and fibroblasts from normal or heterozygous subjects have indicated that normal cells contain at least three- to fourfold the amount of 'X.P.-enzyme' necessary for maximal unscheduled DNA synthesis and that the reduced enzyme levels in heterozygotes can be detected using heterokaryons with a high ratio of X.P. to heterozygous nuclei. Furthermore, a kinetic study of complementation in heterokaryons suggests that the 'X.P.-enzyme' is, probably, not a monomer and that its function may be dependent upon binding to an acceptor and formation of a stable complex which turns over slowly. Patients with the same clinical form of X.P. (classical type) may carry defective enzymes which do or do not bind to the acceptor.

Our findings and the evidence in favour of a close correlation in the kinetics of the different aspects of the excision repair of DNA prompt us to suggest that the enzymes of this repair system may assemble to form a 'repair organelle'.

INTRODUCTION

It is now thought that the excision DNA repair system of higher organisms follows broadly the same enzymic steps of bacteria (Kelly, Atkinson, Huberman & Kornberg, 1969; Kelly, Cozzarelli, Deutscher, Lehman & Kornberg, 1970; Setlow, Setlow & Carrier, 1970; Kushner, Kaplan, Ono & Grossman, 1971; Cleaver, 1968, 1969; Setlow, Regan, German & Carrier, 1969; Cleaver & Trosko, 1970) and that therefore the unscheduled DNA synthesis expressing this process is the result of a number of enzymic reactions each one of which could be rate limiting.

Recently we have shown that in fibroblasts of normal individuals the enzyme defective in Xeroderma pigmentosum ('X.P.-enzyme') is present in amounts larger than needed for maximum DNA repair synthesis but its concentration, relative to that of the other enzymes, can be decreased by fusing normal and X.P. cells together (Giannelli, Croll & Lewin, 1973).

Cells from heterozygotes for X.P. mutations usually synthesize enough X.P.-enzyme to show normal DNA repair synthesis. Therefore, in order to set up a test of heterozygosity, it seemed appropriate to dilute the X.P.-enzyme down to rate-limiting levels by producing heteropolykaryons containing one heterozygous and one or
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more homozygous mutant X.P. nuclei (Giannelli et al. 1973). These would then be compared with similar heteropolykaryons containing one normal nucleus. Such investigation has shown that it is possible to test for heterozygosity in X.P. and has provided some insight into the structure of the defective enzyme.

MATERIALS AND METHODS

A family where a boy with classical X.P. (case no. 1) was born to first degree cousins was chosen for study together with two other non-related cases of classical X.P. (one male, no. 2, and one female, no. 3), the mother of patient 3 and a normal male and normal female controls. The third case, which had shown the lowest level of repair, and the two controls had also been used in our earlier experiments (Giannelli et al. 1973). Heterokaryons were produced by a modification of the Harris and Watkins technique (Harris & Watkins, 1965) using skin fibroblasts treated with $\beta$-propiolactone-inactivated Sendai virus. The following crosses were made: case 1 v. his mother and v. the normal female control (experiment 1); case 2 v. case one's mother and v. the normal female control (experiment 2); case 3 v. case 1's father and v. the normal male control (experiment 3); and case 1 v. case 3's mother (experiment 4).

One third of the fused cells were treated with 5 $\mu$g/ml of cycloheximide for the 7-h interval which separated fusion from harvest, while, of the rest, half the cells were harvested 7 h and half 25 h after fusion. One hour before harvesting the cells were irradiated with 120 J m$^{-2}$ of ultraviolet light (254 nm), so as to produce saturating numbers of repairable lesions (Giannelli et al. 1973), and then incubated in a medium containing 10 $\mu$Ci/ml of $^3$H-thymidine ($^3$H-Tdr, sp. act. 125 Ci/mmol) and $2 \times 10^{-3}$ M of hydroxyurea. In order to distinguish between cells undergoing replicative and repair DNA synthesis the cultures were injected with 10 $\mu$Ci/ml of $^3$H-Tdr (sp. act. 25 Ci/mmol) 1 h before u.v. irradiation. This resulted in differential labelling of the cells that were in $S$ at the time of u.v. irradiation. The cells were harvested, fixed in 9:1 methanol-acetic acid mixture and stained with Acranil (1 [(6-chloro-2-methoxy-9-acradinyl) amino]-3-(diethylamino)-2-propanol) so that nuclei of different origins could be identified by their sex-specific markers. The following cells were selected for study; parental mono-karyons, hetero-di-karyons, hetero-tri-, -tetra-, -penta-, and -hexa-karyons each containing one normal or heterozygous nucleus and 2, 3, 4, and 5 homozygous mutant X.P. nuclei, respectively. Care was taken to choose cells with nuclei of similar size not overlapping each other. These were photographed and the preparations then coated with autoradiographic stripping film. DNA repair synthesis was measured by counting the autoradiographic silver grains over nuclei, presumably, in $G_1$ or $G_2$.

RESULTS

Table 1 summarizes the results obtained from the various crosses and clearly shows that heteropolykaryons can provide a test for heterozygosity. In fact normal and heterozygous cells differ markedly in their ability to complement their abnormal partners in any section of experiments 1 and 2 and in those parts of experiment 3 where cycloheximide or 24-h incubation was used. Nevertheless, while in experiments 1 and 2 complementation appears to occur slowly and shows a steady increase from the cycloheximide to the 24-h part of the experiment, crosses involving patient 3 show, in keeping with our earlier findings (Giannelli et al. 1973), maximum complementation in the presence of cycloheximide whilst incubation in the absence of this inhibitor appears to have a negative effect. Furthermore, while in experiments 1, 2 and 4 the normal and heterozygous nuclei tend to maintain their normal level of repair, in the third all the nuclei show the same repair level, be that normal or not.

These observations may raise the following questions: Do the mutations in patients
Table 1. Deviations from normal levels of DNA repair synthesis†, observed over the normal, heterozygous, and X.P. nuclei of heterokaryons, and their comparisons†

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dikaryons</th>
<th>Trikaryons</th>
<th>Tetrikaryons</th>
<th>Pentikaryons</th>
<th>Hexakaryons</th>
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<td>XP or XP'</td>
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<td>42</td>
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<td>XP-XP'</td>
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<td>24 h</td>
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<td>77</td>
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<tr>
<td>XP × N</td>
<td>6 h</td>
<td>42</td>
<td>1.63</td>
<td>-14.73</td>
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<td>24 h</td>
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<td>XP × H</td>
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<tr>
<td></td>
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<td>XP × N</td>
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<td>16</td>
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<td>17.96</td>
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<td>24 h</td>
<td>26</td>
<td>8.7</td>
<td>3.21</td>
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continued on page 166
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<td>XP - XP'</td>
<td></td>
<td>N - H</td>
<td>XP - XP'</td>
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<td>XP - XP'</td>
<td></td>
<td>N - H</td>
<td>XP - XP'</td>
</tr>
</tbody>
</table>

† The normal level of DNA repair synthesis was considered that of the phenotypically normal parental monokaryons scored in the same microscopic field as the heterodikaryons. The data have been adjusted by multiplying each deviation in a given experiment section by the ratio of the experiment overall normal level of repair to the normal level of repair of the section. The normal level of repair was 60 grains/nucleus for experiments 1 and 4; 98 for experiment 2 and 50 for experiment 3.

†† The comparison indicated in the table has been done by the two samples 't' test and statistically significant differences have been indicated by asterisks according to the level of significance: i.e. *5 %, **1 % and ***0·1 % level.

XP = nucleus of an X.P. fibroblast in an heterokaryon containing a normal nucleus.
XP' = nucleus of an X.P. fibroblast in an heterokaryon containing an heterozygous nucleus.
H = heterozygous nucleus. N = normal nucleus.
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1, 2 and 3 affect the same enzyme and, if so, is it or is it not a monomer? What may limit the movement of enzyme from and to the nucleus in some individuals? Is (are) the wild-type allele(s) capable of responding to the presence of abnormal nuclei by stepping up production?

These questions may find answers - some firm and others tentative - in our experiments.

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**Fig. 1.** This model assumes that the X.P. enzyme is a monomer (see text) and describes the expected relationship between the deviations from normal DNA repair synthesis of nuclei in heterokaryons formed by one or more X.P. and one normal or heterozygous fibroblast and the dose of wild-type ‘X.P.’ alleles present in such cells. Note that the points relative to heterokaryons containing a normal or heterozygous nucleus fall on the same regression line.

(a) Describes either a situation where the fusion of abnormal and phenotypically normal cells does not detectably induce enzyme production or the position prior to induction.

(b) Describes a change from (a) which could result from enzyme induction.

 opral deviation from normal DNA repair synthesis of polykaryons containing one normal nucleus; opral deviation from normal DNA repair synthesis of polykaryons containing one heterozygous nucleus.

The first, for example, can be answered in the affirmative because if patients 1 and 3 had mutations affecting different enzymes, crosses between each patient and a parent of the other should not have revealed the heterozygous state of the parents or produced results different in any other way from those of crosses between the patients and the normal controls (see Figs. 3–5 and below for details).

An answer to the second question can be approached by plotting the DNA repair synthesis of individual nuclei against the overall proportion of wild-type alleles present in the cells since the outcome of these plots is in part dependent on whether the enzyme is monomeric or not.

Two model situations can be envisaged (Figs. 1 and 2). (1) If the enzyme is a monomer its basic concentration in any one cell is a direct expression of the dose of wild-type alleles. Consequently the DNA repair synthesis of nuclei in heterokaryons with X.P.
and normal, or heterozygous nuclei should be distributed along the same regression line. This should reach the normal level of repair when the X.P. enzyme ceases to be rate limiting and DNA repair synthesis becomes independent of gene dosage. The shape and elevation of the regression line should be independent of the age of the heterokaryons unless cell fusion were followed by enzyme induction. Were this the case,

![Diagram](image)

**Fig. 2.** This model assumes that the X.P. enzyme is not a monomer and describes, as in Fig. 1, the expected relationship between the deviations from normal DNA repair synthesis and the dose of wild-type 'X.P.' alleles in the heterokaryons considered in our experiments (see text). I, immediately after fusion: a, regression relative to heterokaryons containing one normal and one or more X.P. nuclei; b, regression relative to heterokaryons containing one heterozygous and one or more X.P. nuclei, all carrying the same mutant allele, in cases where the hybrid enzyme of the heterozygous cell is functionally good; b', regression relative to heterokaryons similar to those in b but where the hybrid enzyme is functionally poor. II, after complete enzyme turnover: c, regression relative to heterokaryons carrying a mutant allele such that the newly formed hybrid enzyme is functionally good; c', regression relative to heterokaryons producing hybrid enzyme which is functionally poor. Note that in II the points relative to heterokaryons containing a normal or an heterozygous nucleus fall on the same regression line.

○, deviation from normal DNA repair synthesis in heterokaryons containing one normal nucleus; ●, deviation from normal DNA repair synthesis in heterokaryons containing one heterozygous nucleus.
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the regression line should increase in elevation until a new equilibrium is established. This model does not take into account the possibility that the X.P. enzyme may not be able to move readily between nuclei. Were this the case, we should predict that the normal and the heterozygous nuclei of heterodikaryons would maintain, initially, their level of repair while the X.P. nuclei would fall on the same regression line irrespective of the type of phenotypically normal nucleus sharing their cytoplasm, as indicated above, but this line would increase in elevation with the age of the cells until equilibrium was reached.

(2) If the enzyme is a dimer or higher polymer, its concentration in a cell does not depend simply upon the proportion of wild-type alleles but also upon their possible interplay with the mutant counterparts, and on the activity of the hybrid enzyme molecules which may be formed.

For example: if the enzyme is a dimer, the mutant polypeptide ($\beta$) is produced and degraded at the same rate as its normal counterpart ($\alpha$) and $\alpha$ and $\beta$ complex at random, two cells with the same proportion of wild-type alleles: i.e. a newly formed heterodikaryon with a normal and an X.P. nucleus on the one hand, and a heterozygous cell on the other, should contain different complements of potential enzyme molecules (i.e.: $\frac{1}{2}\alpha^2 + \frac{1}{2}\beta^2$ and $\frac{1}{4}\alpha^2 + \frac{3}{8}\alpha\beta + \frac{1}{8}\beta^2$ respectively). If $\beta^2$ is completely inactive, the first compound cell has half the enzyme activity of a normal fibroblast while the second has three-quarters to one-quarter of this activity according to whether the hybrid molecule ($\alpha\beta$) is fully active or inactive. This discrepancy between the functional enzyme content of the cells considered above will tend to disappear with time as the heterodikaryon replaces its old enzyme with newly formed molecules and the proportion $\frac{1}{2}\alpha^2 + \frac{1}{2}\beta^2$ gradually changes into $\frac{1}{4}\alpha^2 + \frac{3}{8}\alpha\beta + \frac{1}{8}\beta^2$. At this point the quantity of functional enzyme becomes, as in the previous model, a simple function of the proportion of wild-type alleles. This type of argument can be extended to all the heterokaryons formed by X.P. with normal and heterozygous cells. It becomes obvious then that the crosses involving heterozygous and normal cells should tend to contribute regression lines of different elevations in the part of the experiment where cycloheximide is used; unless either the mutation(s) result(s) in ‘absence’ of polypeptide synthesis or the heterozygous cells achieve just half the activity of normal cells by using their wild type and hybrid enzyme. Furthermore, de novo enzyme synthesis and turnover should bring about two changes: a decrease or increase in the slope of the regression lines, according to whether the hybrid molecules are active or inactive; and the convergence on to the same regression line of the points relative to the crosses X.P. x normal and X.P. x heterozygote.

Obviously in experiments where the homozygous and heterozygous mutant cells carry different mutations the situation is more complex. In this case the regression lines relative to the two types of crosses used in our experiments may diverge from each other even more after normal incubation than after cycloheximide treatment if the hybrid molecules, formed by the normal with the different mutant polypeptides, differ markedly in enzymic activity.

For example, let us postulate that the X.P.-enzyme is a dimer and that the synthesis and degradation of the mutant polypeptides ($\beta$, $\gamma$) is equal to that of their normal
Fig. 3. For legend see facing page.
counterpart \((a)\) and that all polypeptides combine at random. Let us then consider the enzyme complements of cells with the same dose of wild-type alleles, e.g.: tetra-
karyons with one normal and three homozygous mutant nuclei and dikaryons with an
X.P. nucleus identical to the latter and the other nucleus heterozygous for a different
X.P. allele. These, respectively, could be represented as: \(\frac{1}{4}a^2 + \frac{3}{4}b^2\) and \(\frac{1}{8}a^2 +
\frac{2}{8}a\gamma + \frac{1}{8}b^2 + \frac{4}{8}b\gamma\) at the time of fusion and as \(\frac{1}{16}a^2 + \frac{6}{16}a\beta +
\frac{9}{16}b^2\) and \(\frac{1}{16}a^2 + \frac{2}{16}a\gamma + \frac{4}{16}b^2 + \frac{4}{16}ab + \frac{4}{16}b\gamma\) after complete enzyme turnover.
It is easy to see that if the mutant homo- and hetero-polymers are inactive and the
hybrid molecules containing normal and mutant polypeptides vary in their enzyme
activity: i.e. \(a\beta\) is nearly inactive and \(a\gamma\) is nearly normal, the two cells have respec-
tively \(\frac{4}{16}\) and \(\frac{6}{16}\) of the normal enzyme content at the time of fusion and \(\frac{1}{16}\) and
\(\frac{3}{16}\) after complete turnover.

Finally, enzyme induction in this model should produce similar effects to those
discussed in relation to monomeric enzyme while restriction of enzyme movement
may result in greater differences between the crosses involving normal and hetero-

\[\text{Fig. 3. Experiments 1 and 4. Regressions of the deviations from normal DNA repair}
\text{synthesis}^{*} \text{ (i.e. 60 grains/nucleus) on dose of wild-type alleles. The data have been}
\text{standardized by multiplying the deviation in each experimental section by the ratio of}
\text{the overall normal level of repair to the normal level of the individual sections.}
\]

\(\text{O, mean deviations of normal nuclei in heterokaryons of experiment 1; O, mean}
\text{deviations of X.P. nuclei in heterokaryons of experiment 1 containing a normal nucleus;}
\]

\(\text{□, mean deviations of heterozygous nuclei in heterokaryons of experiment 1; ■, mean}
\text{deviations of X.P. nuclei in heterokaryons of experiment 1 containing a heterozygous}
\)

\(\)nucleus; \(\triangle\), mean deviations of heterozygous nuclei in heterokaryons of experiment 4;

\(\text{▲, mean deviations of X.P. nuclei in heterokaryons of experiment 4 containing a}
\text{heterozygous nucleus.}
\]

\(\)In the presence of cycloheximide (I) the deviations of normal and heterozygous
nuclei scatter about the normal level of repair while the deviations of X.P. nuclei in
experiment 1 fall on two regression lines which differ significantly in elevation
\((P < 0.05)\) according to whether the X.P. nuclei belong to heterokaryons with a normal
or heterozygous nucleus\(\dagger\) and those in experiment 4 on a line very close to the homo-

logous one from experiment 1.

After 6 h incubation in normal medium (II) the situation within experiment 1 has
not changed much and the two regression lines differ significantly in elevation
\((P < 0.05)\) while the difference in slope just reaches the 5 %, significance level. However,
the relationship between the data of experiments 1 and 4 is clearly changing.

After 24 h incubation in normal medium (III) the points relative to X.P. nuclei in
the two types of heterokaryons in experiment 1 fall on to regression lines which do
not differ significantly either in slope or elevation, while the points relative to X.P.
nuclei in experiment 4 fall on a regression line of distinctly higher elevation \((P < 0.01)\).
Note that now also the points relative to the normal and heterozygous nuclei begin to
show a regression on gene dose.

None of the regressions shown in the figure depart significantly from linearity and
those relative to the same cross do not show a statistically significant temporal change
in slope from the cycloheximide to the 24-h incubation while they show a sig-
nificant increase in elevation \((P < 0.001)\).

\(\)The level of DNA repair synthesis of phenotypically normal parental monokaryons which
were in the microscopic field of the heterokaryons has been taken as the norm.

\(\dagger\) The data from dikaryons have been excluded from the calculations of regressions relative
to the heterokaryons containing a normal nucleus since they should have an optimal amount of
X.P. enzyme and possibly lay outside the straight part of the line.
Fig. 4. Experiment 2. Regressions of the deviations from normal DNA repair synthesis (i.e. 98 grains/nucleus) on dose of wild-type alleles. Symbols and treatment of data as in Fig. 3. The deviations of normal and heterozygous nuclei scatter about the normal level of repair in all sections of the experiment while those of X.P. nuclei fall on a regression line which seems to fit the data from heterokaryons containing a normal as well as those from heterokaryons with a heterozygous nucleus. The regressions do not show a statistically significant temporal change in slope from the cycloheximide to the 24-h part of the experiment but their elevation increases significantly ($P < 0.001$).
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Fig. 5. Experiment 3. Regressions of the deviations from normal DNA repair synthesis (i.e. 50 grains/nucleus) on dose of wild-type alleles. Symbols and standardization of data as in Fig. 3. The deviations of normal and heterozygous nuclei are similar to those of their abnormal partners in any section of the experiment but the points relative to heterokaryons with one normal nucleus and to those with one heterozygous nucleus fall on separate regression lines. These lines show a significant change in slope from the cycloheximide (I) to the 24-h (III) part of the experiment ($P < 0.005$). If the cycloheximide part of the experiment is considered as time $0$, the regression slope is a linear function of time for heterokaryons containing an heterozygous nucleus, but not for those containing a normal nucleus. The time-dependent change in regression slope may be interpreted as evidence that the hybrid enzyme synthesized by the heterokaryons is functionally poor.

N.B. In I, the deviation for pentakaryons with a normal nucleus falls outside the 99% confidence limits of the linear regression fitted to the other data of the same experiment section. A curved line has therefore been also fitted to these points. This line has not been calculated and represents a subjective interpretation of the data.
zygous cells particularly in the cycloheximide part of the experiment, since wild-type and hybrid polymers might differ in their ability to leave or enter nuclei.

Fig. 3 shows that the results of experiment 1 do not fit the first model but that they are in keeping with the second since the points relative to the abnormal cells in crosses with normal and heterozygous partners fall at first (i.e. in the presence of cycloheximide) on different lines, but tend to converge on to the same line with time. In addition the behaviour of the heterozygous and normal nuclei indicates that the enzyme does not move freely since these nuclei do not rapidly share the enzyme present with their affected partners. Therefore the time-dependent increase in elevation may be due to the gradual entry of enzyme into the abnormal nuclei rather than to increased enzyme production.

Experiment 2 agrees with the above in suggesting that the enzyme does not move freely between nuclei (Fig. 4). The points relative to the abnormal cells in crosses involving normal and heterozygous cells fall on to the same regression line in keeping with either model 1 or 2. Experiment 3 fulfils the expectations of model 2 and in particular those of the situation where the enzyme is free to move but the hybrid molecules formed with the contribution of the mutant alleles of patient 3 are functionally poor (Fig. 5). The latter point is brought out by the observation that normal or heterozygous nuclei complement their partners better in the presence than in the absence of cycloheximide. Furthermore, since in experiments 1 and 2 the regression lines intersect the normal level of repair at doses of wild-type alleles higher than one-quarter while in experiment 3 one normal cell appears to have enough enzyme to share it with up to four abnormal fibroblasts and achieve nearly maximal repair, the time-dependent increase in elevation of the regression lines in experiments 1 and 2 is probably not due to enzyme induction.

The comparison of crosses involving only the X.P. alleles of patient 1 or 3 with those crosses involving both alleles (i.e. heterokaryons made of fibroblasts from patient 3 plus a normal control compared with heterokaryons of patient 3 plus patient 1’s father, and heterokaryons of patient 1 plus his mother or a normal control compared with heterokaryons of patient 1 plus patient 3’s mother) indicates that the latter do somewhat better (see Figs. 3, 5, and Table 1) and suggests that intragenic complementation may possibly occur between these alleles.

DISCUSSION

The present experiments seem to provide results of both practical and theoretical interest. On the practical side they show that it is possible to detect heterozygotes for X.P. mutations by producing heteropolykaryons with fibroblasts from a potential heterozygote and a related affected individual of opposite sex. If this is not available, unrelated patients may be used but, then, intragenic complementation may result in false negative tests. Negative results should therefore be confirmed by repeating the experiments using a panel of unrelated patients and, if possible, by studying the behaviour of heterokaryons formed by a patient related to the presumptive heterozygote and those used in the test. Whatever the type of the cross, it is necessary to study the behaviour of heteropolykaryons under cycloheximide inhibition and after different
incubation periods in inhibitor-free medium since the optimal conditions for heterozygote detection may vary with the alleles involved in the test.

On the theoretical side, our results suggest that the X.P. enzyme is not a monomer, that it is possible to establish the functional allelism of different mutations and that these may differ qualitatively in their effect.

Since one of the consequences of the polymeric nature of the enzyme could be that heterozygotes may show greater intra-individual variation in enzyme activity than they would otherwise, the rare occasions in which presumptive heterozygotes for X.P. mutations show reduced rates of DNA repair synthesis after high doses of u.v. irradiation (Cleaver, 1972) may be in keeping with our findings.

Of particular interest are the rather unexpected qualitative differences observed in the behaviour of non-related mutants (i.e. patients 1, 2 and 3). These warrant further speculation. In fact, since the cells of patients 1 and 2 do not benefit from fusion with normal partners as quickly as those of patient 3, their enzyme appears to compete with the normal more effectively than that of patient 3. Such competition, however, should not involve the reaction of the enzyme with the u.v. lesions because, in view of the rapid transfer of X.P.-enzyme observed in crosses involving patient 3, it can be argued that soon after fusion all the nuclei of heterokaryons should have the same concentration of mutant and normal enzyme and therefore show similar rates of DNA repair synthesis. This is not so in heterokaryons involving fibroblasts from patients 1 and 2 even 24 h after fusion. It seems therefore reasonable to postulate that competition occurs with respect to other factors: e.g. an acceptor molecule which, presumably, forms a stable complex with the X.P. enzyme and enables it to function. Patients 1 and 2 should therefore have an enzyme capable of binding to the acceptor but ineffective against u.v. lesions and patient 3 one which does not complex with the acceptor. If that were true the progressive improvement of X.P. nuclei in the heterokaryons of experiments 1 and 2 and the slow convergence of normal and affected nuclei towards the same levels of DNA repair synthesis may be the expression of complex turnover. This in our experiments is not complete even after 24 h.

The hypothetical acceptor could have important functions in that it could provide a mechanism for the regulation of the X.P. enzyme, which is normally produced in large excess (Giannelli et al. 1973) and could explain its co-integration with the other enzymes of the excision repair system (Gautschi, Young & Cleaver, 1973; Cleaver, Thomas, Trosko & Lett, 1972; Kleijer, Hoeksema, Sluyter & Bootsma, 1973). This, for instance, could be achieved easily if the acceptor itself were another enzyme or multiple enzyme complex of the DNA repair system. Thus it could be that the whole set of enzymes, though not produced in a strictly cointegrated fashion, assembled to form a 'repair organelle' made up exclusively of enzymic units or such units connected to an independently produced structural core.

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