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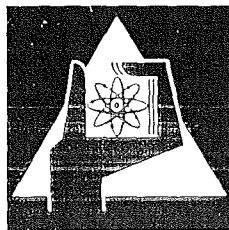
Dezember 1969

KFK 1150

Institut für Strahlenbiologie

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H. Jung, U. Hagen, M. Ullrich, E. E. Petersen



GESELLSCHAFT FÜR KERNFORSCHUNG M. B. H.

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## Structural and Functional Changes in DNA after Exposure to Hydrogen Atoms and Gamma Radiation

H. JUNG, U. HAGEN, M. ULLRICH, and E. E. PETERSEN \*

Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe

(Z. Naturforschg. 24 b, 1565—1573 [1969]; eingegangen am 2. August 1969)

The action of hydrogen atoms — generated in an electrodeless high frequency gas discharge — on calf thymus DNA in aqueous solution was investigated. The loss of priming activity was compared with the appearance of single strand breaks in native and denatured DNA, double strand breaks, denatured zones, base damage and rupture of hydrogen bonds. The primary lesions after exposure to H atoms and gamma radiation, respectively, are single strand breaks and base damage. Double strand breaks originating from accumulation of single breaks, and rupture of hydrogen bonds caused by single breaks and base damage, were identified as secondary lesions. In relation to strand breaks arising from radical attack on the sugar-phosphate backbone of the DNA molecule, base damage is about 12.5 times more frequent after H-exposure than after  $\gamma$ -irradiation. It is concluded from this observation, that single strand breaks are the predominant critical lesions responsible for the loss of the functional activity of DNA.

Radiation damage to cells and, consequently, also to higher organisms is mainly caused by alterations in their genetic material. Thus, one of the principal tasks of molecular radiation biology consists in investigating the effects which occur in irradiated nucleic acids. Since cellular radiation damage is caused at approximately equal parts by "direct" and "indirect" effects<sup>1</sup>, it is necessary to include both types of radiation effects in the investigations. Both the direct and the indirect effects are initiated by a number of different primary processes: In dry systems excitations, ionizations, elastic nuclear collisions, and thermal hydrogen atoms are of importance, whilst the changes observed in irradiated aqueous solutions of biomolecules are caused by attack of H and OH radicals and hydrated electrons. Having examined the biological effectiveness of the various primary processes of energy absorption in highly purified preparations of lyophilized DNA in a series of earlier experiments<sup>2-4</sup>, the present paper deals with the action of hydrogen atoms on diluted aqueous solutions of DNA describing both the change of various

biological functions of DNA and the changes in its physico-chemical properties. To eliminate the contribution of OH radicals and hydrated electrons also present in irradiated solutions we adopted the technique of externally generating atomic hydrogen in a high frequency gas discharge used by STEIN and collaborators<sup>5-7</sup> in a number of radiation chemical investigations.

### Experimental Procedure

#### Generation of Hydrogen Atoms

To expose diluted aqueous solutions of DNA to hydrogen atoms we used a setup of which the main parts are given in Fig. 1. In a glass tube of 8 mm inside diameter a gas discharge is maintained in hydrogen gas at a pressure of 50 Torr by applying a high voltage of high frequency (20 kV, 16 kc/sec). Since no internal electrodes were used, the deposition of metal vapors in the discharge tube is avoided which would affect the recombination of H atoms and, hence, impair reproducibility<sup>8</sup>. The reproducibility of our method was better than  $\pm 15\%$  and is quite comparable with the accuracy achieved in earlier experiments. The hydrogen gas, which contains a constant amount of H

\* Hygiene-Institut, Universität Freiburg/Br.

<sup>1</sup> H. DERTINGER and H. JUNG, *Molekulare Strahlenbiologie*. Springer-Verlag, Berlin-Heidelberg-New York 1969.

<sup>2</sup> H. JUNG and K. KÜRZINGER, *Radiat. Res.* **36**, 369 [1968].

<sup>3</sup> K. KÜRZINGER and H. JUNG, *Int. J. Radiation Biol.* **14**, 493 [1968].

<sup>4</sup> K. G. ZIMMER, G. HOTZ, K. O. HERMANN, H. JUNG, K. KÜRZINGER, A. MÜLLER, J. UENZELMANN, H. DERTINGER, U. HAGEN, L. BOHNE, TH. COQUERELLE, and K. U. BERGER, in: *Current Topics in Radiation Research* (M. EBERT and A. HOWARD, eds.), Vol. 5, p. 1. North-Holland, Amsterdam 1969.

<sup>5</sup> G. CZAPSKI and G. STEIN, *Nature [London]* **182**, 598 [1958].

<sup>6</sup> G. CZAPSKI, J. JORTNER, and G. STEIN, *J. physic. Chem.* **65**, 956 [1961].

<sup>7</sup> B. E. HOLMES, G. NAVON, and G. STEIN, *Nature [London]* **213**, 1087 [1967].

<sup>8</sup> G. CZAPSKI and G. STEIN, *J. physic. Chem.* **63**, 850 [1959].

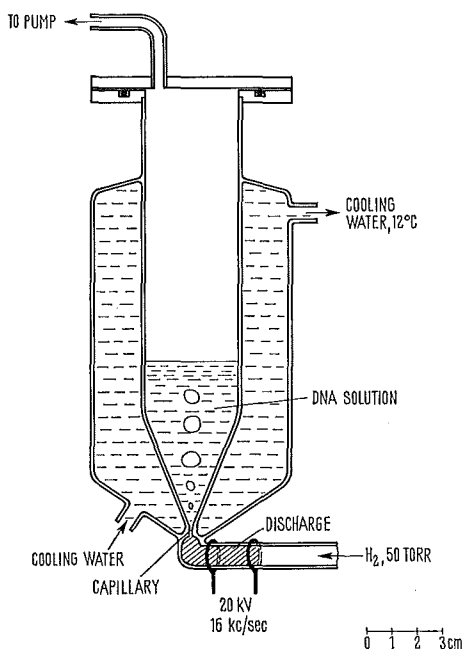


Fig. 1. Experimental setup for exposing aqueous solutions to atomic hydrogen generated in a high frequency gas discharge.

atoms, enters the reaction vessel cooled to 12 °C through a capillary and bubbles through the DNA solution to be treated. Before the discharge voltage was applied oxygen had been removed from the DNA solution by hydrogen gas flowing through the vessel. Though it is possible to determine the number of H atoms entering the solution by means of  $K_3[Fe(CN)_6]$  (CZAPSKI and STEIN<sup>9</sup>) we used infectious DNA of bacteriophage  $\Phi X174$  for this purpose, for it cannot be excluded that part of the reactions between H atoms and biomolecules takes place already at the surface of the gas bubbles flowing through the reaction solution. In this case, in  $K_3[Fe(CN)_6]$  and DNA solutions, respectively, different fractions of the H atoms will react with the dissolved molecules before entering the solution. Thus the yield of molecules affected will become dependent on concentration and, possibly, on other parameters. Moreover, ferricyanide dosimetry only measures the number of H atoms entering the solution<sup>9</sup> but not the fraction which reacted with the biomolecules. In highly diluted solutions generally used for work on bacteriophages or infectious DNA only a negligible fraction of the H atoms introduced into the solution reacts with the dissolved macromolecules (e. g.,  $10^{-5}$  to  $10^{-8}$ ; DEWEY and STEIN<sup>10</sup>), whilst the majority of H atoms recombine. For this reason, we preferred a "biological" dosimetry system using infectious  $\Phi X$ -DNA rather than conventional

methods. In all experiments 15 ml of solution were exposed containing 200  $\mu\text{g/ml}$  of thymus DNA in 0.01 M NaCl at pH 7.

#### Gamma Irradiation

The irradiation of DNA also was carried out at concentrations of 200  $\mu\text{g/ml}$  in 0.01 M NaCl, pH 7.0, in a  $^{60}\text{Co}$ - $\gamma$  source (Gammacell 220, Atomic Energy of Canada, Ltd.), the dose rate being 1.2 Mrad/h. Prior to irradiation nitrogen was passed through the solution for 10 minutes to expel the oxygen of the air.

#### Infectious $\Phi X$ -DNA

One of the objects investigated was infectious DNA of bacteriophage  $\Phi X174$ , as this single stranded and circular molecule can be used to test the integrity of the genetic information with respect to the formation of complete bacteriophages. Phage  $\Phi X174$  was prepared and purified by the method of SINSHEIMER<sup>11</sup>. The infectious DNA was isolated by three successive extractions with hot phenol saturated with buffer according to GUTHRIE and SINSHEIMER<sup>12</sup> and, finally, after removal of phenol, it was dialyzed against NCE buffer. For determination of the infectivity of  $\Phi X$ -DNA we used a modification of the method of GUTHRIE and SINSHEIMER<sup>12</sup> the details of which have already been communicated<sup>2</sup>. The number of  $\Phi X$ -phages liberated from spheroplasts of *E. coli* K12 is proportional to the quantity of DNA used over several orders of magnitude (cf. l. c.<sup>2</sup>).

#### Isolation of Thymus DNA

It is difficult and expensive to prepare  $\Phi X$ -DNA in such quantities as to permit detailed investigations of its physico-chemical properties. Therefore, most of our experiments were performed using calf thymus DNA, since this nucleic acid, too, allows the measurement of structural and functional damage. DNA was isolated from calf thymus either by the use of Duponol according to KAY et al.<sup>13</sup> or phenol according to COLTER et al.<sup>14</sup>, depending on the problems to be solved. For investigations of strand breaks and base damage DNA was degraded to a molecular weight of 1–2 millions by ultrasonics (Branson Sonifier), so that thymus DNA and  $\Phi X$ -DNA attained comparable hydrodynamic properties.

#### Priming Activity of Thymus DNA

When testing the biological function of thymus DNA we used its property of acting as a primer in enzymatic RNA synthesis with RNA nucleotidyl transferase (E.C.2.7.7.6). The enzyme was isolated from *E. coli* by the method of CHAMBERLIN and BERG<sup>15</sup>. The biological activity of DNA was determined by the amount of AMP incorporated from the offered [ $8\text{-}^{14}\text{C}$ ]-ATP

<sup>9</sup> G. CZAPSKI and G. STEIN, *J. physic. Chem.* 64, 219 [1960].  
<sup>10</sup> D. L. DEWEY and G. STEIN, *Nature [London]* 217, 351 [1968].

<sup>11</sup> R. L. SINSHEIMER, *J. molecular Biol.* 1, 37 [1959].

<sup>12</sup> G. D. GUTHRIE and R. L. SINSHEIMER, *Biochim. biophysica Acta [Amsterdam]* 72, 290 [1963].

(Schwarz Bioresearch Inc.) into the RNA. Details on the procedure are given by ZIMMERMANN et al.<sup>16</sup>.

#### Single and Double Strand Breaks

The number of breaks caused in the double helix or in the single strand by H-exposure or  $\gamma$ -irradiation was assessed through determination of the molecular weights of native and denatured DNA samples, respectively. An analysis of the distribution of DNA concentration within the cell of an analytical ultracentrifuge yields the distribution of molecular weights within a DNA preparation. Knowing the distribution of molecular weights, one can compute the number average of the molecular weight and, hence, the average chain length of the molecules exposed. The latter is compared to the chain length of an untreated sample, resulting in the number of breaks per molecule. In order to be independent of the molecular weight of the control samples we give the frequency of breaks, i. e. the number of single strand breaks per nucleotide ( $A_1$ ) and the number of double strand breaks per nucleotide pair ( $A_2$ ), respectively. Further details of our experimental and mathematical procedures were described in detail by HAGEN<sup>17</sup> and COQUERELLE et al.<sup>18</sup>. Applying the method described to native DNA one obtains the frequency of double strand breaks. In order to determine the number of single breaks, DNA was denatured before centrifugation with alkali and formaldehyde according to DAVISON et al.<sup>19</sup>. The DNA samples irradiated or hydrogen-treated in the single stranded state, were denatured by thermal treatment, since the presence of formaldehyde would change the experimental conditions. In this procedure the DNA solution is heated to 90 °C for 10 minutes and then quickly cooled to 0 °C in an ice bath.

#### Denatured Zones

The appearance in double stranded thymus DNA of small regions with unpaired nucleotide chains can be determined by chromatography on a column of methylated albumin on kieselgur (MAK). Native DNA molecules are eluted from the column at concentrations of 0.6–0.8 M NaCl, whilst the molecules containing a denatured zone are firmly bound to the column. Subsequent elution with 1.5 M NaCl in 0.1 M Tris buffer (pH 8) and then with 1.5 M NaCl in 2 N NH<sub>4</sub>OH permits complete recovery of the bound DNA fraction<sup>20</sup>. Details of our procedure were published by ULLRICH and HAGEN<sup>21</sup>.

#### Base Damage

The number of DNA bases destroyed can be determined by the decrease in their optical absorption at 2,600 Å. During exposure to H atoms or  $\gamma$ -radiation double stranded DNA is denatured which results in an increase in extinction. As this increase is superimposed to the decrease due to base destruction our experiments were conducted with DNA denatured by thermal treatment. As the secondary products formed in the destruction of bases do not show any remarkable absorption at 2,600 Å (WEISS<sup>22</sup>), the extinction of denatured DNA at 2,600 Å may be taken as a direct measure of the number of bases unchanged.

#### Rupture of Hydrogen Bonds

To determine the extent of DNA denaturation caused by exposure to H atoms or  $\gamma$ -rays the so-called "hypochromicity" may be used. It is due to the fact that the optical absorption of native double stranded DNA is smaller than would be expected from the number of bases present. During denaturation of undamaged DNA the extinction therefore increases by about 30% at 2,600 Å. With increasing time of exposure the increase in extinction observed during thermal denaturation of DNA (10 min. at 90 °C in 0.01 M NaCl) approaches zero as a consequence of the growing number of hydrogen bonds already ruptured during exposure. From the change in extinction the amount of bases no longer paired by hydrogen bonds is calculated using a semi-empirical formula given by APPLEQUIST<sup>23</sup>.

## Results and Discussion

#### Infectious $\Phi$ X174-DNA

When treating a solution of thymus DNA (200  $\mu$ g/ml in 0.01 M NaCl) to which about 10<sup>-8</sup> grams of  $\Phi$ X-DNA per milliliter had been added the number of infectious  $\Phi$ X-DNA molecules decreases exponentially with increasing exposure time. A 37% exposure time ( $D_{37}^{\Phi X}$ ) of 11 seconds is determined from the dose-effect curve represented in Fig. 2. An experiment conducted with  $\gamma$ -radiation under identical conditions also yielded an exponential dose-effect curve with  $D_{37}^{\Phi X} = 225$  rad. To compare the relative effectiveness of hydrogen atoms to that of

<sup>13</sup> E. R. M. KAY, N. S. SIMMONS, and A. L. DOUNCE, J. Amer. chem. Soc. **74**, 1724 [1952].

<sup>14</sup> J. S. COLTER, R. A. BROWN, and K. A. O. ELLEM, Biochim. biophysica Acta [Amsterdam] **55**, 31 [1962].

<sup>15</sup> M. CHAMBERLIN and P. BERG, Proc. nat. Acad. Sci. USA **48**, 81 [1962].

<sup>16</sup> F. ZIMMERMANN, H. KRÖGER, U. HAGEN, and K. KECK, Biochim. biophysica Acta [Amsterdam] **87**, 160 [1964].

<sup>17</sup> U. HAGEN, Biochim. biophysica Acta [Amsterdam] **134**, 45 [1967].

<sup>18</sup> T. COQUERELLE, L. BOHNE, U. HAGEN, and J. MERKWITZ, Z. Naturforsch. **24b**, 885 [1969].

<sup>19</sup> P. F. DAVISON, D. FREIFELDER, and B. W. HOLLOWAY, J. molecular Biol. **8**, 1 [1964].

<sup>20</sup> M. G. SMITH and K. BURTON, Biochem. J. **98**, 229 [1966].

<sup>21</sup> M. ULLRICH and U. HAGEN, Z. Naturforsch. **23b**, 1176 [1968].

<sup>22</sup> J. J. WEISS, Progr. Nucleic Acid Res. **3**, 103 [1964].

<sup>23</sup> J. APPLEQUIST, J. Amer. chem. Soc. **83**, 3158 [1961].

$\gamma$ -radiation, the H-exposure times and  $\gamma$ -doses, respectively, are subsequently related to the 37%-values for the inactivation of infectious  $\Phi$ X-DNA (11 sec or 225 rad) and, therefore, all doses are given in the form of  $D/D_{37}^{\Phi X}$ .

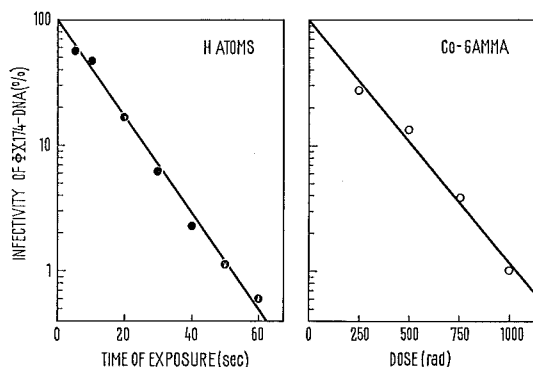


Fig. 2. Inactivation of  $10^{-8}$  g infectious  $\Phi$ X174-DNA in a solution of 200  $\mu$ g/ml calf thymus DNA (in 0.01 M NaCl, pH 7.0) by hydrogen atoms and gamma radiation respectively. ( $D_{37}^{\Phi X}$ : 11 seconds and 225 rads, respectively).

#### Priming Activity

Fig. 3 shows the decrease of the amount of AMP incorporated into RNA following exposure or irradiation of thymus DNA acting as primer in the

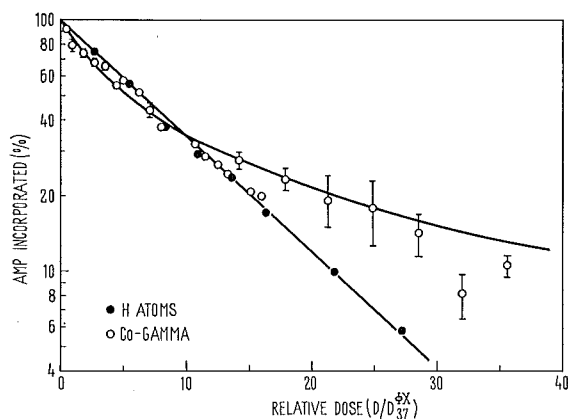


Fig. 3. Priming activity of calf thymus DNA after exposure to hydrogen atoms and gamma radiation, respectively.

RNA-polymerase system. Upon treatment with H atoms the priming activity decreases exponentially with exposure time. The 37% exposure amounts to  $9.4 \cdot D_{37}^{\Phi X}$ ; i.e., in relation to the same molecular weight, the priming activity of thymus DNA is 9.4 times less sensitive than the infectivity of  $\Phi$ X-DNA. This result is not unexpected, considering that the

synthesis of mRNA takes place probably along small sections on the DNA molecule delimited by defined binding and stopping points. These sections represent smaller targets as in the case of infectivity of  $\Phi$ X-DNA, where the entire DNA molecule is to be regarded as radiosensitive target<sup>1</sup>. After treatment of thymus DNA with  $\gamma$ -radiation the slope of the inactivation curve in a semi-logarithmic plot decreases steadily with increasing dose. The underlying reasons were discussed in detail by HAGEN and collaborators<sup>24</sup>. However, it is still uncertain why the action of hydrogen atoms and  $\gamma$ -radiation follow different kinetics. It is an interesting finding that in spite of the obviously different inactivation kinetics H atoms initially show approximately the same effectiveness in destroying the priming activity of thymus DNA as do the radiolytic products of water. While, under the influence of atomic hydrogen, the ratio of sensitivities determined for either DNA function is 9.4, the priming activity in the case of  $\gamma$ -irradiation is 8 to 13 times more resistant than the infectivity of  $\Phi$ X-DNA, depending on dose.

#### Single Strand Breaks

According to Fig. 4, the number of single strand breaks determined in native DNA by ultracentrifugation increases linearly with dose, the experimental points obtained after exposure to H atoms and  $\gamma$ -rays, respectively, falling on a common straight line. This means that atomic hydrogen and the

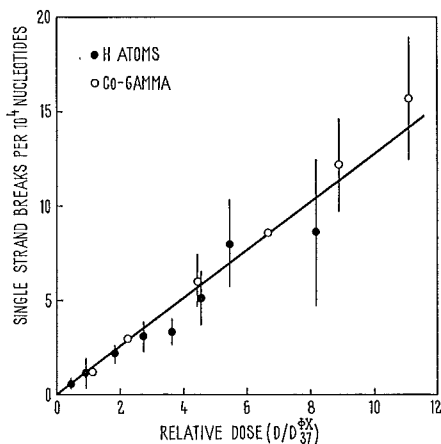


Fig. 4. Number of single strand breaks in native calf thymus DNA after exposure to hydrogen atoms and gamma radiation, respectively.

<sup>24</sup> U. HAGEN, M. ULLRICH, E. E. PETERSEN, E. WERNER, and H. KRÖGER, *Biochim. biophysica Acta* [Amsterdam], in press.

radiolytic products of water are equally efficient in producing breaks in one of the two polynucleotide chains of double stranded calf thymus DNA. The number of single strand breaks per nucleotide ( $A_1$ ) can be represented by

$$A_1 = k \cdot D \quad (1)$$

which permits to compute the probability of breaks  $k$  to be  $1.26 \cdot 10^{-4}$  per nucleotide and per  $D_{37}^{\phi X}$ . For  $\gamma$ -radiation using  $D_{37}^{\phi X} = 225$  rads a probability for breaks of  $k = 5.6 \cdot 10^{-7}$  is found per nucleotide and rad. This figure is in approximate agreement with the value of  $k = 4.15 \cdot 10^{-7}$  per nucleotide and rad found by HAGEN<sup>17</sup> in solutions of the same concentration. It can be deduced from the above probability of breaks that in a DNA molecule with a molecular weight of  $1.7 \cdot 10^6$  Dalton (corresponding to 5,500 nucleotides) after a dose of  $D_{37}^{\phi X} 0.7$  single strand breaks occur both after treatment with H atoms and with  $\gamma$ -radiation.

If thymus DNA is denatured prior to treatment with atomic hydrogen or to irradiation, respectively, the number of single strand breaks increases linearly with dose and, moreover, an identical effectiveness for H atoms and "water radicals" is found, too (Fig. 5). Since the probability for breaks is  $k = 1.74 \cdot 10^{-4}$  per nucleotide and  $D_{37}^{\phi X}$ , 0.95 single strand breaks will occur per DNA molecule (mol.wt.

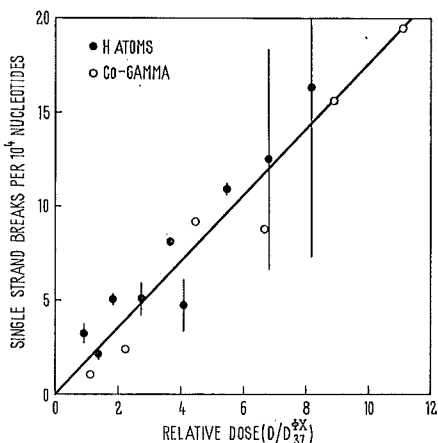


Fig. 5. Number of single strand breaks in denatured calf thymus DNA after exposure to hydrogen atoms and gamma radiation, respectively.

$= 1.7 \cdot 10^6$  Daltons) upon application of the dose  $D_{37}^{\phi X}$ . Consequently, the generation of single strand breaks is almost 40% more effective in denatured DNA than in native DNA. This increased frequency

of single strand breaks possibly depends on the fact that in denatured DNA the number of molecules is twice as high as in native DNA of the same concentration and that the molecular configuration changes in such a way that a more effective utilization of radicals offered in either system is achieved. On the other hand, it could be considered that in native DNA part of the primary single strand breaks is restituted by reunion of the broken ends, whilst this process is less probable in the case of single stranded DNA, since the broken ends move away from each other by diffusion.

### Double Strand Breaks

The finding that the number of double strand breaks detected in thymus DNA increases with the square of dose (Fig. 6) indicates that double strand breaks are caused not by one single event but by

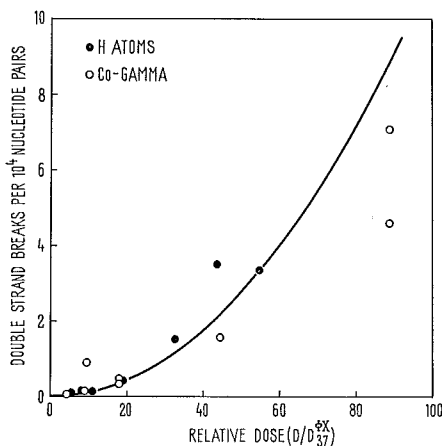


Fig. 6. Number of double strand breaks in calf thymus DNA after exposure to hydrogen atoms and gamma radiation, respectively.

accumulation of single breaks. Since, according to Eq. (1), single strand breaks increase linearly with dose, the probability of a DNA chain being broken at two opposite nucleotides is calculated to be  $(kD)^2$ . However, if the change in one of  $n$  nucleotides opposite to a single break results in the formation of a double strand break, the number of double strand breaks per nucleotide pair equals

$$A_2 = n \cdot (kD)^2 \quad (2)$$

Actually, in addition to the single strand breaks caused by exposure also those should be considered which exist already at the beginning of the experiment in untreated DNA. However, their number is

so small in our preparations as to be negligible at intermediate and higher doses<sup>17</sup>. The curve drawn in Fig. 6 was calculated from Eq. (2) for  $n=7$  with the value determined from Fig. 4,  $k=1.26 \cdot 10^{-4}$  per nucleotide and  $D_{37}^{\phi X}$ , being used. This means, a double strand break is generated when a single break exists on one of the polynucleotide chains and the break of the other strand occurs at the opposite nucleotide or not farther away than 3 nucleotide pairs. This value is identical for H atoms and  $\gamma$ -radiation; it is in good agreement with the results obtained by HAGEN<sup>17</sup> who also determined  $n=7$  in an earlier investigation using X-rays.

### Denatured Zones

The quantity of  $\gamma$ -irradiated thymus DNA eluted from the MAK-column decreases exponentially with dose (Fig. 7). From the slope of the dose-effect curve one determines  $D_{37}=0.8 \cdot D_{37}^{\phi X}$ . For technical reasons<sup>21</sup>, DNA with a molecular weight of  $10^7$  was

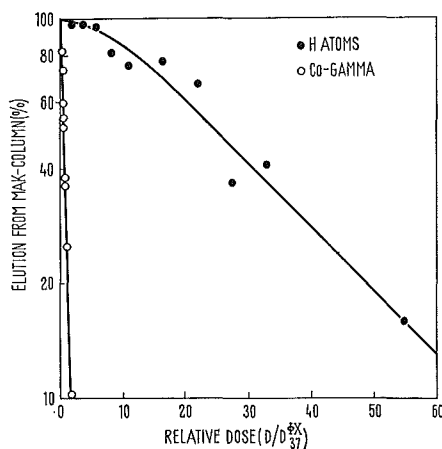


Fig. 7. Fraction of DNA molecules containing denatured zones after exposure to hydrogen atoms and gamma radiation, respectively, as determined from the amount of DNA eluted from a MAK-column.

used for this experiment. Conversion of the experimentally obtained 37 per cent dose to a molecular weight of  $1.7 \cdot 10^6$  will result in  $D_{37}=4.6 \cdot D_{37}^{\phi X}$ . As was determined from Fig. 4, the energy required in native DNA to generate a single strand break is  $1.4 \cdot D_{37}^{\phi X}$  per molecule. Thus, single strand breaks are 3.3 times more frequent than

denatured zones. This value is in good agreement with the ratio of single strand breaks to denatured zones of 4:1 determined by ULLRICH and HAGEN<sup>21</sup>. As is further shown by Fig. 7, hydrogen atoms are much less effective in producing denatured zones than  $\gamma$ -radiation. The dose-effect curve determined with hydrogen atoms initially shows a shoulder. From the slope of the straight part of the dose-effect curve obtained after prolonged exposure a  $D_{37}=26 \cdot D_{37}^{\phi X}$  is read off, which corresponds to a value of  $155 \cdot D_{37}^{\phi X}$  when related to a molecular weight of  $1.7 \cdot 10^6$ . Hence, denatured zones are 33 times less frequent after exposure to H atoms than to  $\gamma$ -radiation.

As is shown by the different shapes of the dose-effect curves obtained with H atoms and  $\gamma$ -radiation, respectively, the formation of denatured zones in either system is due probably to different mechanisms the nature of which, however, has not yet been fully elucidated. One reason for the increased effectiveness of  $\gamma$ -radiation in local denaturation of double stranded DNA might be that the  $H_3O^{\oplus}$  ions formed in a relatively high concentration within the track of secondary electrons result in a major decrease of the pH of the solution in small volume elements<sup>25</sup> with a local acid denaturation of DNA occurring. Another mechanism capable of explaining the change in a number of hydrogen bonds without break of a polynucleotide strand is the effect of a "polarization wave" discussed by PLATZMAN and FRANCK<sup>26</sup>. According to this model, the sudden appearance of an electrostatic charge within a macromolecule causes a rearrangement of polar groups in the resulting inhomogeneous electric field with several hydrogen bonds possibly being ruptured. Since these regions of single stranded DNA form "false" hydrogen bonds afterwards, the original structure on the double helix is changed at this point. Either mechanism discussed is more probable to occur in systems irradiated with  $\gamma$ -rays than after exposure to hydrogen atoms.

### Base Damage

Fig. 8 shows that the action of H atoms on native thymus DNA will damage the nucleic acid bases with higher efficiency than irradiation in aqueous

<sup>25</sup> D. R. SMITH and W. H. STEVENS, *Nature* [London] **200**, 66 [1963].

<sup>26</sup> R. L. PLATZMAN and J. FRANCK, in: *Symposium on Information Theory in Biology* (H. P. YOCKEY, R. L. PLATZMAN, and H. QUASTLER, eds.) p. 262. Pergamon Press, London—New York 1958.



solution. Linear extrapolation of the initial part of the curves on a semilogarithmic scale results in a  $D_{37} \approx 400 \cdot D_{37}^{\phi X}$  for hydrogen atoms and a  $D_{37} \approx 5000 \cdot D_{37}^{\phi X}$  for  $\gamma$ -radiation. Hence, atomic hydrogen is about 12.5 times more effective in destroying the chromophore groups of DNA bases than the radio-

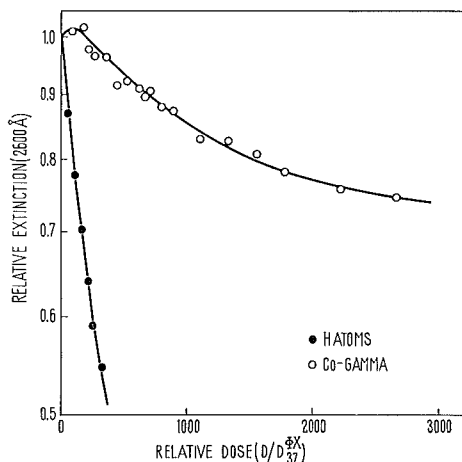


Fig. 8. Base damage in denatured calf thymus DNA after exposure to hydrogen atoms and gamma radiation, respectively, as determined from the decrease in relative extinction at 2600 Å. The small increase in optical absorption at small gamma doses originates from incomplete denaturation.

lytic products of water. From the  $D_{37}$  values it is possible to calculate the number of bases ruptured after the dose  $D_{37}^{\phi X}$  in a DNA molecule consisting of 5,500 nucleotides (mol.wt. =  $1.7 \cdot 10^6$ ); this results in 14 bases damaged per molecule after exposure to the H atoms and 1.1 bases changed after  $\gamma$ -irradiation.

#### Rupture of Hydrogen Bonds

If one determines the number of bases no longer paired by hydrogen bonds after different exposure times, the shapes of the dose-effect curves obtained with H atoms and  $\gamma$ -radiation, respectively, are equal, but hydrogen atoms are 1.7 times more effective than the radiolytic products of water (Fig. 9). From the initial slope of the dose-effect curves 0.244% unpaired bases are determined per  $D_{37}^{\phi X}$  after exposure to H atoms and 0.143% after exposure to  $\gamma$ -radiation. In the case of linear extrapolation rupture of all hydrogen bonds present in

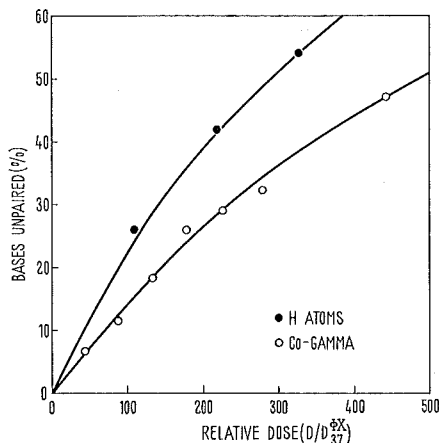


Fig. 9. Fraction of bases unpaired after exposure of native calf thymus DNA to hydrogen atoms and gamma radiation, respectively, as calculated from the hypochromicity in denaturation.

the DNA molecule requires the following doses: with H atoms  $D_0 = 410 \cdot D_{37}^{\phi X}$ , with  $\gamma$ -radiation  $D_0 = 710 \cdot D_{37}^{\phi X}$ . At a base ratio of (adenine + thymine) : (cytosine + guanine) of 60:40 (CHARGAFF and LIPSHITZ<sup>27</sup>) thymus DNA on the average contains 2.4 hydrogen bonds per base pair. A DNA molecule with a mol.wt. of  $1.7 \cdot 10^6$  thus contains a total of 6,600 hydrogen bonds. Thus, after the dose  $D_{37}^{\phi X}$  per DNA molecule 16.1 hydrogen bonds are ruptured by H atoms and 9.4 hydrogen bonds are opened by  $\gamma$ -radiation. From these values it is possible to calculate the energy expenditure necessary to break one hydrogen bond. However, such procedure would have a purely formal meaning, since it is known that hydrogen bonds are not broken individually. After SCHOLLES et al.<sup>28</sup>, about 15 hydrogen bonds are opened per single strand break. From the frequency of 0.7 single strand break per  $D_{37}^{\phi X}$  (cf. Fig. 4) 10.5 hydrogen bonds ruptured per  $D_{37}^{\phi X}$  should therefore be anticipated. This value is in rather good agreement with the result of the experiment using  $\gamma$ -radiation while the effect of the H atoms yielding 16.1 hydrogen bonds broken per molecule and  $D_{37}^{\phi X}$  is somewhat higher.

This difference could be due to the fact that hydrogen bonds are opened not only in the vicinity of single strand breaks but also at the sites of damaged or eliminated DNA bases. Since H atoms, as is shown in Fig. 8, are much more effective than

<sup>27</sup> E. CHARGAFF and R. LIPSHITZ, J. Amer. chem. Soc. 75, 3658 [1953].

<sup>28</sup> G. SCHOLLES, J. F. WARD, and J. J. WEISS, J. molecular Biol. 2, 379 [1960].

$\gamma$ -radiation producing base damage, this finding might explain the greater capability of H atoms in rupturing hydrogen bonds. Moreover, it is known from irradiation of aqueous solutions of DNA that bases are liberated from the polynucleotide chain, the ratio between destroyed and liberated bases being 1.01:0.23 under anaerobic experimental conditions<sup>29</sup>. If it is assumed that this ratio applies also to H atoms (which still requires experimental verification), it is possible to calculate from the frequency of single strand breaks (0.7) and base damage (14 and 1.1, respectively) the number of opened hydrogen bonds to be anticipated. For H atoms the result is  $0.7 \cdot 15 + 14 \cdot 0.23 \cdot 2.4 = 18.2$ , for  $\gamma$ -radiation it is  $0.7 \cdot 15 + 1.1 \cdot 0.23 \cdot 2.4 = 11.1$ . The calculated numbers of 18.2 and 11.1 hydrogen bonds ruptured per  $D_{37}^{\Phi X}$  and per DNA molecule correspond to the experimentally measured values of 16.1 and 9.4, respectively.

### Concluding Remarks

The results of our experiments are summarized in Table I. The second and third columns indicate the doses required (in units of  $D_{37}^{\Phi X}$ ) in an aqueous DNA solution of 200  $\mu\text{g}/\text{ml}$  to reduce the various properties tested to 37% in the case of exponential kinetics ( $D_{37}$ ) and to zero in the case of linear kinetics ( $D_0$ ). It is shown that the properties determined by the entire DNA molecule or major fractions of it, such as infectivity, priming activity and denatured zones, have smaller  $D_{37}$  values than the other changes restricted to DNA components. More-

over, Table I lists the respective number of various changes per DNA molecule and per  $D_{37}^{\Phi X}$ . After exposure to hydrogen atoms at a dose reducing the infectivity of  $\Phi X$ -DNA (mol.wt. =  $1.7 \cdot 10^6$ ) to 37% of its original value, the following changes are encountered in double stranded thymus DNA of the same molecular weight: 0.7 single strand breaks when exposed in the native state; 0.95 single strand breaks when DNA is denatured before exposure to H atoms;  $3 \cdot 10^{-4}$  double strand breaks the number of which increases with the square of dose; moreover, 0.007 denatured zones, 14 damaged bases, and 16 ruptured hydrogen bonds. After exposure to  $\gamma$ -radiation one obtains partly the same values, while some of them are different. This may be seen from the last column of Table I showing that H atoms and  $\gamma$ -radiation have the same effectiveness with respect to the change in the two functional properties of DNA tested, i.e. infectivity of  $\Phi X$ -DNA and priming activity of thymus DNA, as well as with respect to the induction of single and double strand breaks. For the generation of denatured zones H atoms are much less efficient than  $\gamma$ -radiation, while the destruction of DNA bases occurs much more frequently after exposure to H atoms than by the action of the radiolytic products of water. Finally, H atoms are about 70 per cent more effective in rupturing the hydrogen bonds in double stranded DNA than  $\gamma$ -radiation, probably because of their higher effectiveness in causing base damage.

If one leaves aside the denatured zones, whose mechanism of generation is still unknown anyway, the structural changes observed in DNA can be

Effects investigated	Relative doses related to the inactivation of infectious $\Phi X 174$ -DNA		Number of changes per DNA molecule <sup>a</sup> after a dose $D = D_{37}^{\Phi X}$		Ratio of the efficiency of H atoms to that of $\gamma$ -rays
	$D_{37}/D_{37}^{\Phi X}$ H atoms	$D_0/D_{37}^{\Phi X}$ Co-gamma	H atoms	Co-gamma	
Inactivation of infectious $\Phi X 174$ -DNA	1	1	1	1	1
Priming-activity of thymus DNA	9,4	8-13	— <sup>b</sup>	— <sup>b</sup>	$\approx 1$
Single strand breaks in native thymus DNA	7900	7900	0,7	0,7	1
Single strand breaks in denatured thymus DNA	5700	5700	0,95	0,95	1
Double strand breaks in thymus DNA	— <sup>b</sup>	— <sup>b</sup>	0,0003 <sup>c</sup>	0,0003 <sup>c</sup>	1
Denatured zones in thymus DNA	155	4,7	0,007	0,21	0,03
Base damage in thymus DNA	400	5000	14	1,1	12,5
Rupture of hydrogen bonds in thymus DNA	410	700	16	9,5	1,7

Table I. Efficiency of hydrogen atoms and <sup>60</sup>Co-gamma radiation for the generation of various functional and structural changes in calf thymus DNA. Exposure in aqueous solution at a concentration of 200  $\mu\text{g}/\text{ml}$ . <sup>a</sup> Related to a molecular weight of  $1.7 \cdot 10^6$  Dalton. <sup>b</sup> Not to be given in this form. <sup>c</sup> Increasing with  $D^2$ .

<sup>29</sup> G. HEMS, Nature [London] 186, 716 [1960].

subdivided into primary and secondary lesions. The primary lesions caused in diluted aqueous solution are single strand breaks, and base damage, while double strand breaks are caused by the accumulation of single breaks and rupture of hydrogen bonds is a consequence of single strand breaks and base damage. As has already been discussed in detail, the frequency of primary lesions, i.e. the relationship between single strand breaks and base damage, determines the extent of the secondary reactions. From the values listed in Table 1 the ratio of single strand breaks to base damage can be calculated considering that the extent of base destruction is identical for native and denatured DNA when  $\gamma$ -irradiated in aqueous solution (KECK<sup>30</sup>). In native DNA this ratio amounts to 1:1.6 for  $\gamma$ -radiation and to 1:20 for H atoms; for denatured DNA the corresponding values are 1:1.15 and 1:14.5, respectively. OH radicals produced in irradiated aqueous solution thus react with about equal frequencies with bases and with the sugar residues of DNA, while the H atoms have a particular affinity to the bases<sup>31</sup> with which they probably undergo addition reactions in most cases hydrating the 5–6 double bond.

It is surprising that the priming activity of thymus DNA has the same relative sensitivity after exposure to H atoms as after  $\gamma$ -irradiation (in both cases in relation to the infectivity of  $\Phi$ X-DNA). This finding could be interpreted by stating that the molecular lesions decisive for the destruction of the two DNA functions are to a large extent identical after exposure to H atoms and  $\gamma$ -radiation. Since single strand breaks occur with the same frequency in both systems, our experiments support the conclusions drawn by HAGEN et al.<sup>24</sup> on the basis of other measurements, i.e., that single strand breaks induced by radical attack should be regarded as criti-

cal lesions with respect to the destruction of priming activity. Whether the decrease in the priming activity observed in thymus DNA is due exclusively to single strand breaks or whether other types of lesion play a minor part in addition cannot yet be decided with certainty. However, in view of the different kinetics of inactivation in both experimental systems the second possibility must not be excluded.

It is known that a DNase induced break in single stranded and circular  $\Phi$ X-DNA will always result in inactivation<sup>32</sup>. Assuming the probability for breaks to be the same in  $\Phi$ X-DNA as in thymus DNA, one arrives at the conclusion that 70 to 95% of the inactivation observed is due to breaks, depending on whether  $\Phi$ X-DNA is compared with native or denatured thymus DNA. These values are slightly higher than the results communicated by BLOK<sup>33</sup> according to which 50–65% of the  $\Phi$ X-DNA molecules inactivated in solution contain a break in the polynucleotide chain. Both findings indicate that breaks have a greater significance than base damage in inactivating the infectivity of  $\Phi$ X-DNA. Exposure to  $\gamma$ -radiation produces 0.7 to 0.95 single breaks and 1.1 base changes per inactivated DNA molecule, while exposure to atomic hydrogen will bring about the same number of single strand breaks but 14 base changes, which clearly shows that base damage has only a small relevance for destructing the genetic information of phage-DNA. The question this result raises, i.e., whether the bases damaged do not affect DNA replication at all or whether they result in the inclusion of false complementary bases which, however, do not prevent the formation of functioning phages, will be investigated in another series of experiments.

We wish to thank Miss G. KRONIMUS for her help in preparing the manuscript.

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<sup>31</sup> G.SCHOLES and M. SIMIC, Biochim. biophysica Acta [Amsterdam] 166, 255 [1968].

<sup>32</sup> W. FIERS and R. L. SINSHEIMER, J. molecular Biol. 5, 424 [1962].

<sup>33</sup> J. BLOK, in: Radiation Research (G. SILINI, ed.) p. 423. North-Holland, Amsterdam 1967.