

# Release of $K^+$ and $H^+$ from Poly U in Aqueous Solution upon $\gamma$ and Electron Irradiation.

## Rate of Strand Break Formation in Poly U

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Conductivity changes were found which followed the reaction of radiolytically generated OH radicals with the potassium salt of polyuridylic acid (poly U) in aqueous solution. After  $^{60}\text{Co}$ - $\gamma$ -irradiation the observed increase of conductivity at pH = 6.8 was shown to consist of the liberation of  $K^+$  ions from the stock of  $K^+$  ions electrostatically bound to the polyanion. The initial  $G(K^+)$  is 36 and hence 6 times higher than the  $G$  value of OH radicals in  $\text{N}_2\text{O}$  saturated solutions. At a poly U concentration of  $60 \text{ mg l}^{-1}$  half of the ion release occurred at  $12 \text{ J kg}^{-1}$  and nearly all ions are released at  $40 \text{ J kg}^{-1}$ . The liberation of  $K^+$  is explained to be a consequence of the formation of chain breaks leading to an increase of the degree of dissociation.

The rate of the ion release was studied under pulse radiolysis conditions. Because of the high  $G$ -value of counterion liberation and the use of conductivity as analytical quantity the method is very sensitive. With  $6 \text{ mg l}^{-1}$  poly U the rate could be measured even at a dose per pulse of  $0.25 \text{ J kg}^{-1}$ . The kinetics of the ion release can be described in terms of two parallel first order reactions of comparable contribution with an average rate constant of  $0.8 \text{ s}^{-1}$  at  $20^\circ\text{C}$ ,  $60 \text{ mg l}^{-1}$  poly U and pH = 6.8 with a small contribution of slower components. In more acidic solutions, besides  $K^+$  ions  $H^+$  are also liberated since at low pH values bound  $K^+$  is replaced by  $H^+$ . The rate of the ion release was found to increase with increasing replacement of  $K^+$  by  $H^+$  ( $k_{\text{obs}} = 100 \text{ s}^{-1}$  at pH = 3.4,  $60 \text{ mg l}^{-1}$  poly U and  $T = 18^\circ\text{C}$ ). With potassium ion free polyuridylic acid the corresponding rate constant amounted to  $220 \text{ s}^{-1}$ , nearly independent of pH. From the temperature dependence activation parameters for the ion release were derived ( $E_a = 57 \text{ kJ mol}^{-1}$ ,  $A = 1.0 \times 10^{10} \text{ s}^{-1}$  at pH = 6.8). Addition of p-benzoquinone at pH = 3.7 and dithiothreitol (DTT) at pH 6.8 were found to decrease the size of the conductivity changes and to increase the rate. The results show that p-benzoquinone and DTT react with poly U radicals and that these reactions prevent chain breaks and ion liberation.

It is concluded that the rate determining step of the conductivity increase is the formation of strand breaks by a cleavage of poly U radicals and that this reaction is pH dependent. The pH dependence and the observed value for the activation energy was found to be in agreement with the behaviour of a model system for the earlier postulated C-4' mechanism for strand break formation of polynucleotides and DNA.

## Introduction

In aqueous solution hydroxyl radicals induce strand breaks in DNA [1]. The first step in the reaction of the OH radicals with DNA is addition to the bases ( $\sim 80\%$ ) and H abstraction at the sugar moiety ( $\sim 20\%$ ) [2]. The cleavage of the main chain must originate from one or more of these radicals. Mechanisms leading to cleavage of the main chain by OH radicals have been discussed in several reviews [3]. In particular it has been shown that the C-4' radical of the sugar moiety is responsible at least for a significant part of the strand breaks (C-4'-mechanism) [4]. Base damage possibly also

leads to main chain breaks but evidence of this is not available and detailed mechanisms have not yet been advanced.

In the present work poly U was used as substrate instead of DNA since poly U is available in high purity and since it is a simpler compound than DNA because it contains only one kind of heterocyclic base. Furthermore poly U in aqueous solution at  $\geq 20^\circ\text{C}$  exists only in the single-stranded form and complications arising from the presence of double strands are not expected [5]. Moreover helix-coil transitions as well as other types of superstructures do not occur with poly U at least at room temperature and at higher ionic strength. Only at temperatures below  $20^\circ\text{C}$  a helix-coil transition was recognized with a transition temperature of  $4^\circ\text{C}$  [6]. Thus at room temperature poly U may be

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regarded as a model system representing a base line for further studies on more complex polyanions [5]. However, there is one further difference between poly U and DNA. The sugar in DNA is 2-deoxyribose and the sugar in poly U is ribose, therefore poly U is a closer model for RNA than DNA.

Surprisingly it has now been found that the reaction of OH radicals with poly U leads to changes in conductivity that are much larger than expected on the basis of known effects of OH on low molecular weight compounds and that at pH = 6.8 they are mainly due to the liberation of counter ions. It is well known that polyanionic salts are not fully dissociated as was shown for the first time by Hammersten for DNA as polyanion [7]. Part of the cations are electrostatically bound to the polyanion so that the degree of dissociation [7, 11] and the electrical conductivity [7–13] are reduced. For polyphosphates Schindewolf has found that the degree of dissociation increases with decreasing chain length [8]. Since it is known that irradiation of polynucleotides with  $\gamma$  or electron rays in aqueous,  $N_2O$  saturated solution (which means reaction with  $\cdot OH$  radicals) leads to a reduction of chain length [1], it is now suggested that the liberation of the potassium ions is the result of an increase of dissociation due to the reduction of chain length induced by radiolytically generated  $\cdot OH$  radicals.

Lindenau, Hagen and Schnabel [14] used a light scattering method in order to investigate the formation of strand breaks of DNA and of other phenomena associated with it.

The conductivity effect observed now turns out to be a further method for this purpose. In the first part of the present paper the increase of conductivity by  $^{60}Co$ - $\gamma$ -irradiation is investigated as a function of dose and concentration of poly U. In the second part the rate of the conductivity increase is studied after pulse irradiation as a function of various parameters. The results show, that the rate determining step of the conductivity increase is the strand break of poly U radicals.

## Results

### 1. Abbreviations used

$\kappa_0$  and  $\Delta\kappa$  denote the conductivity of the solutions before irradiation and its increase after absorbance of a dose  $D$  respectively.  $Y(\kappa) = \Delta\kappa \cdot D^{-1}$  is the

relative "yield" of conductivity with respect to the dose absorbed. The plateau conductivity of the solutions achievable with increasing dose is named limiting conductivity and abbreviated  $\kappa_{lim}$ . Poly U denotes the potassium salt of polyuridylic acid (polyribouridylic acid),  $H^+$  poly U the potassium ion free acid of the polymer. The  $G$  value is defined as the number of molecules formed per 100 eV absorbed dose.

### 2. $^{60}Co$ - $\gamma$ -irradiations

$N_2O$  saturated solutions of the potassium salt of poly U ( $60 \text{ mg l}^{-1}$ ) were irradiated at pH = 6.6 and  $20^\circ C$  with  $^{60}Co$ - $\gamma$ -rays (dose rate  $0.35 \text{ J kg}^{-1} \text{ s}^{-1}$ , range 5–100  $\text{J kg}^{-1}$ ) and the conductivity before, during and one hour after irradiation was measured as a function of dose, see Fig. 1. It is seen, that the conductivity increases steeply on irradiation with smaller doses ( $\leq 15 \text{ J kg}^{-1}$ ) and levels off at higher doses towards  $\kappa_{lim}$  (dose  $> 40 \text{ J kg}^{-1}$ ). The conductivity did not change within six hours after irradiation. Quantitatively, at a dose of  $10 \text{ J kg}^{-1}$ , the conductivity of the solution increases by  $2.5 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$ . The pH value of the solutions after absorption of  $10 \text{ J kg}^{-1}$  shifted from 6.6 to 6.3. This shift accounts for an increase in conductivity of only  $7.0 \times 10^{-8} \Omega^{-1} \text{ cm}^{-1}$ , (corresponding to  $G(H^+) = 0.2$ ) an amount which is far to small to explain the increase in conductivity actually observed. Hence, in near neutral solutions, only a minor part of the observed conductivity increase after irradiation can

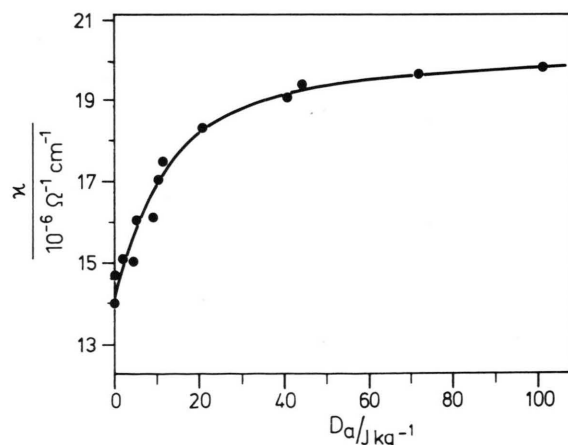


Fig. 1. Conductivity  $\kappa$  as a function of dose measured one hour after  $^{60}Co$ - $\gamma$ -irradiation (dose rate  $22 \text{ J kg}^{-1} \text{ min}^{-1}$ ). Aqueous  $N_2O$ -saturated solutions of poly U ( $60 \text{ mg l}^{-1}$ ) at  $18^\circ C$  and pH = 6.5.

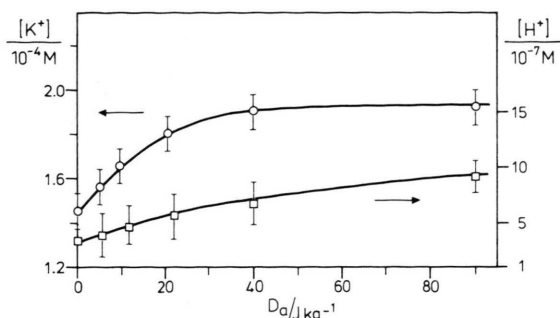


Fig. 2. Concentration of free K<sup>+</sup> (○) and of H<sup>+</sup> (□) as a function of dose measured one hour after <sup>60</sup>Co- $\gamma$ -irradiation (dose rate 20 J kg<sup>-1</sup> min<sup>-1</sup>). Aqueous, N<sub>2</sub>O-saturated solutions of poly U (60 mg l<sup>-1</sup>) and at 20 °C and pH = 6.5.

be explained by the formation or removal of H<sup>+</sup> or OH<sup>-</sup>.

The concentration of free potassium ions in the solution was measured at pH = 6.6 with a potassium ion sensitive electrode before and after irradiation (Fig. 2). Again an increase at small doses and a plateau at higher doses is observed, and this in the same dose range as in the conductivity measurement experiments above.

In several experiments with a K<sup>+</sup>-sensitive electrode we observed a significant scatter ( $\pm 25\%$ ) in the differences between the reading with the unirradiated solution and with a  $2 \times 10^{-4} \text{ M}$  standard solution of potassium chloride ( $\pm 4 \text{ mV}$  corresponding to  $\pm 5 \times 10^{-5} \text{ M KCl}$ ). These differences may be due to the fact, that we did not add any ionic strength adjuster to the solutions. The differences in the ion concentration due to irradiation, however, did not show such scatter (see errors indicated in Fig. 2). The experimental error of the conductivity measurements under  $\gamma$  irradiation is around  $\pm 10\%$ .

The smallness of the pH-change and the similarity of the curves for the changes of  $\kappa$  (Fig. 1) and K<sup>+</sup> concentration (Fig. 2) suggests that both increases have the same origin, i.e. that the increase of conductivity is caused mainly by the increase of free K<sup>+</sup> ions. Comparing the increments in Figs. 1 and 2 quantitatively, one concludes: (i) after absorption of 80 J kg<sup>-1</sup> within 5%, a relative increase of 37% is observed for both quantities. (ii) for comparison of the absolute increases one has to calculate the increase of conductivity from the increase of potassium ion concentration at the same dose. Using the equivalent conductances of  $64 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$  for K<sup>+</sup> [15] and  $48 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$  for the counterion at

18 °C (the value of 48 will be discussed further below), one calculates a conductivity increase of  $2.6 \times 10^{-6} \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$  from the increase of potassium ion concentration of  $2.3 \pm 0.8 \times 10^{-5} \text{ M}$  at 10 J kg<sup>-1</sup>. This agrees with the measured value of  $2.5 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$  within the limits of experimental error and demonstrates, that the increase of conductivity at pH = 6.6 is mainly due to an increase in concentration of free K<sup>+</sup> ions. Expressing  $Y(x)$  in terms of  $G$ -values for the formation of free K<sup>+</sup> ( $G(K^+)$ ), one obtains  $G(K^+) \sim 22$  from the conductivity increase of  $2.5 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$  at a dose of 10 J kg<sup>-1</sup> and pH = 6.6. The corresponding calculation for the initial slope in Fig. 1 gives  $G(K^+) \sim 36$ .

#### a) Dependence on concentration of poly U

Fig. 3 shows the results of conductivity measurements after <sup>60</sup>Co- $\gamma$ -irradiation at lower concentrations of poly U (6–60 mg l<sup>-1</sup>, pH = 6.8,  $T = 20^\circ \text{C}$ ). The difference between the upper curve (conductivity of solutions after irradiation with doses high enough to reach the plateau values  $\kappa_{\text{lim}}$ ) and the curve in the middle (conductivity of solutions before irradiation) corresponds to an increase of 40% independent on concentration of poly U, i.e.  $\kappa_{\text{lim}} \times \kappa_0^{-1} = 1.4$ . The dependence of  $D_{1/2}$  (the dose, at which half of the total increase is measured), on the substrate concentration is also presented in Fig. 3 (lower curve).

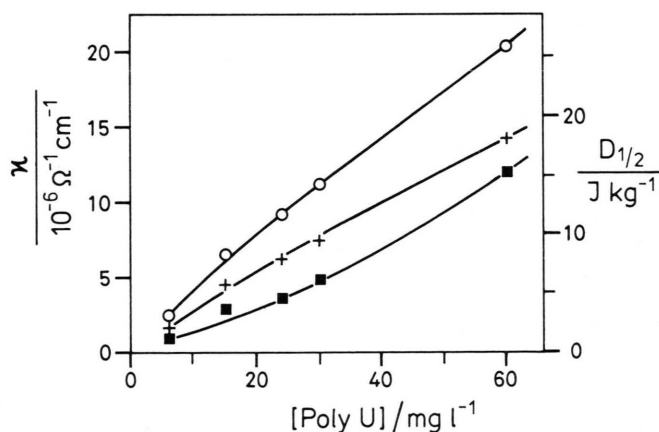


Fig. 3. Dependence on concentration of poly U of (i) conductivity of solutions before <sup>60</sup>Co- $\gamma$ -irradiation (+), (ii) conductivity of solutions after irradiation when the maximum conductivity is achieved (○) and (iii) dose required to obtain half of the maximum conductivity increase ( $D_{1/2}$ ; ■) at 20 °C, pH = 6.8.

## b) Dependence on pH

In contrast to the findings at pH = 6.6 the shift in pH was not negligible after  $^{60}\text{Co}$ - $\gamma$ -irradiation at lower pH values. In Table I the pH changes at four pH values are presented together with the  $G$  values of proton liberation calculated from it (columns 1–3). From column 4 it can be seen, that the formation of protons accounts for 26%, 40% and 75% of the observed  $\Delta\kappa$  at pH 5.06, 4.56 and 3.88 respectively (in the calculation of the latter fractions use was made of the dependence of  $\Delta\kappa$  on pH found in pulse radiolysis (see further below and Fig. 6) since measurements of conductivity are more difficult under steady state compared to pulse conditions at lower pH values. From comparison of columns 3 and 5 in Table I one calculates, that with decreasing pH 1.8%, 10%, 17% and 46% of the liberated counterions are protons. Hence with decreasing pH more and more  $\text{H}^+$  are liberated instead of  $\text{K}^+$  after irradiation. This result gives evidence, that with decreasing pH more and more of the bound potassium ions are replaced by protons according to mass action law (compare Fig. 13 and Discussion). From column 5 in Table I it can be seen, that at pH = 3.88 (when 46% of the liberated counterions are protons) the  $G$  value for formation of free counterions is slightly lower than at pH = 6.6 where mainly  $\text{K}^+$  is liberated.

A few experiments were performed with solutions of potassium free polyuridylic acid ( $\text{H}^+$  poly U, isoionic solutions) without addition of perchloric acid (Table II). From the  $\kappa$  values given in Table II, one calculates a relative increase of conductivity of  $\kappa_{\text{lim}} \times \kappa_0^{-1} = 1.37 \pm 0.05$  for  $\text{H}^+$  poly U which is very

Table I. Yields of protons per 100 eV absorbed energy (third column) as found after  $^{60}\text{Co}$ - $\gamma$ -irradiation ( $D = 20 \text{ J kg}^{-1}$ ) of  $60 \text{ mg l}^{-1}$  poly U at  $20^\circ\text{C}$  and four different pH values. The conductivity increase due to  $\text{H}^+$  liberation induced by  $^{60}\text{Co}$ - $\gamma$ -irradiation ( $\Delta\kappa(\text{H}^+)$ ) was calculated from column 3 and divided by the total conductivity increase measured in pulse radiolysis (column 4). Finally, in column 5, the total  $G$ -value for the liberation of counterions is presented (calculated from columns 3 and 4).

pH before irradiation	pH after irradiation	$G(\text{H}^+)$	$\frac{\Delta\kappa(\text{H}^+)}{\Delta\kappa(\text{P.R.})}$	$G(\text{K}^+ + \text{H}^+)$
6.60	6.11	0.3	0.06	17
5.06	4.90	2.0	0.26	19
4.56	4.48	3.0	0.40	18
3.88	3.84	6.5	0.75	14

Table II. Changes of pH and conductivity  $\kappa$  observed after  $^{60}\text{Co}$ - $\gamma$ -irradiation with aqueous isoionic solutions of potassium free polyuridylic acid ( $\text{H}^+$  poly U) at  $22^\circ\text{C}$  and substrate concentrations given in column 1.  $\text{pH}_0$  and  $\kappa_0$  denote the pH value and conductivity before irradiation.  $\text{pH}_{\text{lim}}$  equals the smallest pH and  $\kappa_{\text{lim}}$  the highest conductivity achievable with increasing dose. The doses required to reach half of  $\kappa_{\text{lim}}$  are denoted as  $D_{1/2}$  and presented in the last column (values in brackets refer to the potassium salt; see Fig. 3).

Conc. of $\text{H}^+$ poly U [ $\text{mg l}^{-1}$ ]	$\text{pH}_0$	$\text{pH}_{\text{lim}}$	$\kappa/10^{-6} \Omega^{-1} \text{ cm}^{-1}$		
			$\kappa_0$	$\kappa_{\text{lim}}$	$D_{1/2}$ [ $\text{J kg}^{-1}$ ]
60	3.94	3.80	4.9	6.7	17.0 (15.0)
30	4.19	4.07	2.5	3.3	7.8 (6.5)
16	4.46	4.31	1.3	1.8	3.3 (2.9)

similar to 1.40 found for poly U. The same value is also obtained from the change of pH values after irradiation (see Table II) using  $335 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$  and  $52.8 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$  for the equivalent conductances of  $\text{H}^+$  and the polyanion at  $22^\circ\text{C}$  respectively. This demonstrates, that in the case of  $\text{H}^+$  poly U only protons are liberated. The  $G$ -values for the liberation of protons were found to be  $G(\text{H}^+) = 16$  after  $10 \text{ J kg}^{-1}$  and  $G(\text{H}^+) = 22$  for initial slope. These values are smaller than those for poly U (22 and 36 respectively). This is in line with the finding, that the doses  $D_{1/2}$  which are required to reach  $\kappa_{\text{lim}}/2$  are 10–20% larger for  $\text{H}^+$  poly U (see Table II) than for poly U (see Fig. 2 and values in brackets in Table II).

## 3. Pulseradiolysis

Fig. 4 shows the time dependence of the conductivity increase observed after a  $1 \mu\text{s}$  electron pulse of  $10 \text{ J kg}^{-1}$  in a  $\text{N}_2\text{O}$ -saturated solution of poly U ( $60 \text{ mg/l}$ ) at pH = 6.8 and  $21^\circ\text{C}$ . The conductivity increases after the pulse to a constant level which is  $\Delta\kappa = 2.2 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$  higher than the value prior to the pulse. This amount agrees roughly with the value found under  $^{60}\text{Co}$ - $\gamma$ -irradiation conditions at the same total absorbed dose and pH-value ( $2.5 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$ ). Under pulse condition the relative error in  $Y(\kappa)$  is  $\pm 10\%$ , corresponding to  $\pm 2.5 \times 10^{-6} \text{ M}$  free  $\text{K}^+$  ions, at  $10 \text{ J kg}^{-1}$  absorbed dose, pH = 6.6 and  $60 \text{ mg l}^{-1}$  poly U. If the concentration of poly U is lowered to  $6 \text{ mg l}^{-1}$ , a well detectable signal is observed even at a dose of  $0.15 \text{ J kg}^{-1}$  with an error



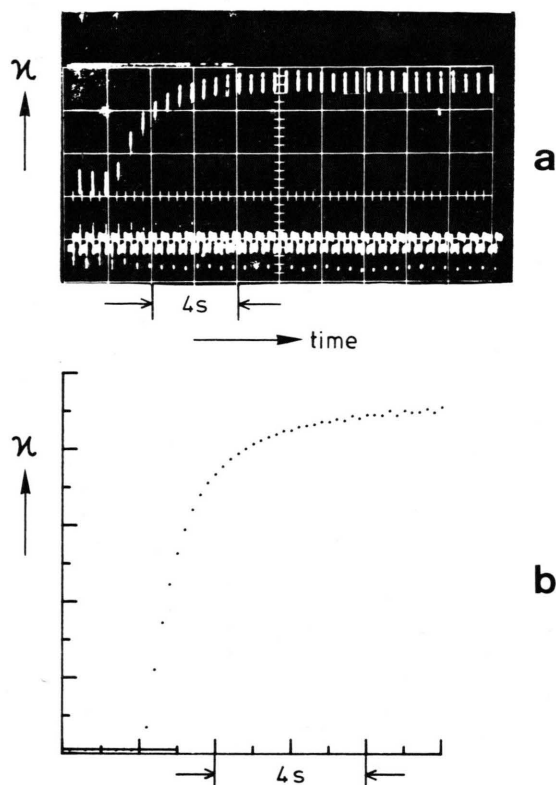


Fig. 4. Conductivity  $\kappa$  in relative units as a function of time measured in pulse radiolysis of an aqueous,  $\text{N}_2\text{O}$ -saturated solution of poly U ( $60 \text{ mg l}^{-1}$ ) after a  $1 \mu\text{s}$  pulse of  $10 \text{ J kg}^{-1}$ . a: measured with the DC method at  $18^\circ\text{C}$  (see experimental section) and  $\text{pH} = 6.8$ ; b: Computer print-out (50 points) of a conductivity signal trace measured with the AC bridge at  $10^5 \text{ Hz}$  at  $20^\circ\text{C}$  and  $\text{pH} = 6.6$ .

of again  $\pm 10\%$  corresponding in this case roughly to  $\pm 2.2 \times 10^{-7} \text{ M}$  free  $\text{K}^+$  ions.

#### a) Dependence on dose and dose rate

The rate of the build-up was found to be independent of dose per  $1 \mu\text{s}$  pulse (range  $2\text{--}20 \text{ J kg}^{-1} \mu\text{s}^{-1}$ ) with a first half life of  $1. t_{1/2} = 0.8 \pm 0.1 \text{ s}$  at  $20^\circ\text{C}$ ,  $\text{pH} = 6.8$  and  $60 \text{ mg/l}$  poly U. This reveals that the conductivity increase is governed by first order reactions. However, kinetic analysis of the build up showed, that the rate of the change of the conductivity increase became slower at longer times. The third half life of the build up was  $\sim 30\%$  greater compared to the first one ( $3. t_{1/2} = 1.05 \text{ sec}$ ). Unless otherwise stated, the half lives in this work refer always to the first ones. We have attempted to

fit the build up to the kinetics of two parallel first order reactions. Best fits were obtained with two components of comparable contribution with rate constants differing by a factor or roughly 2.5. The value  $k_{\text{obs}} = 1. t_{1/2}^{-1} \cdot \ln 2$  represented approximately the average of the rate constants for the two components. It is generally difficult however to obtain precise data from curve fittings if the rate constants are separated only by a factor of two or three. Therefore the conductivity build up may be fitted also to types of composite first order reactions others than described above. Additionally to the kinetics mentioned before, a much slower component could be detected ( $t_{1/2} \sim 5.0 \text{ sec}$ ) which accounted for approximately 10% of the total conductivity increase. The slow component will not be discussed in the present paper.

In Fig. 5 the dependence of  $\Delta\kappa$  on dose at various concentrations of poly U is given. It is seen that  $\Delta\kappa$

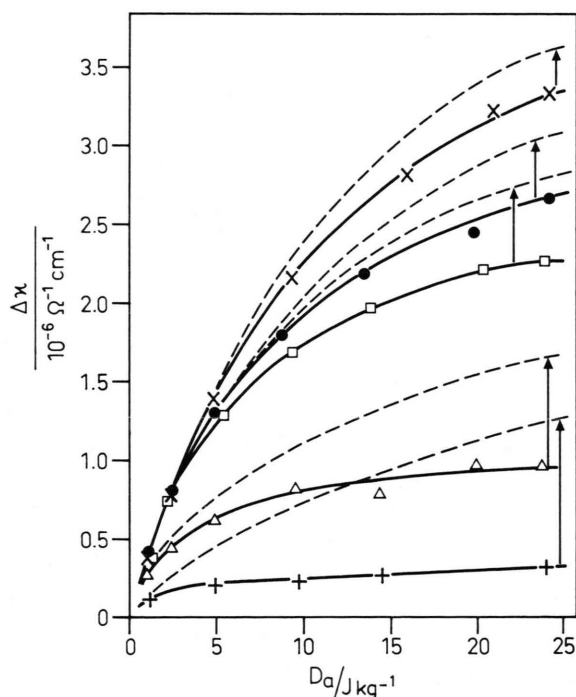


Fig. 5. Dependence of the final conductivity increase  $\Delta\kappa$  on dose measured 15 s after electron pulses in  $\text{N}_2\text{O}$ -saturated aqueous solutions of poly U.  $\text{pH} = 6.5 \pm 0.3$ ,  $T = 18^\circ\text{C}$ , concentrations of poly U:  $\times$ :  $85 \text{ mg l}^{-1}$ ;  $\bullet$ :  $60 \text{ mg l}^{-1}$ ;  $\square$ :  $35 \text{ mg l}^{-1}$ ;  $\Delta$ :  $15 \text{ mg l}^{-1}$ ;  $+$ :  $6 \text{ mg l}^{-1}$ . Dashed lines indicate the hypothetical results after correction for the recombination reaction  $\text{OH} + \text{OH} \rightarrow \text{H}_2\text{O}_2$  on the basis  $K(\text{OH} + \text{OH}) = 6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  and  $k(\text{OH} + \text{poly U}) = 1.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , however not accounting for limited concentration of bound  $\text{K}^+$ .

becomes smaller with increasing dose per pulse and therefore  $Y(\infty)$  decreases with increasing dose. The  $G$  values for the liberation of  $K^+$  calculated from Fig. 5 are 20 at  $10 \text{ J kg}^{-1}$  and 37 for initial slope ( $\text{pH} = 6.5$  and  $60 \text{ mg l}^{-1}$  poly U). The dose dependence of  $\Delta\kappa$  found in pulse radiolysis (Fig. 5) and in  $^{60}\text{Co}$ - $\gamma$ -irradiation (Fig. 1) are similar, and because of the great differences in the dose rate (factor  $10^7$ ) pronounced dose rate effects are absent.

#### b) Dependence on concentration of poly U

On lowering the concentration of poly U at  $\text{pH} 6.8$  from  $60 \text{ mg l}^{-1}$  ( $1.7 \times 10^{-4} \text{ M}$  nucleotides) to  $3.0 \text{ mg l}^{-1}$  we observed a decrease of the half life of the conductivity build up from  $0.8 \text{ s}$  to  $0.3 \text{ s}$ . The reason for this decrease is very likely due to a gradual exchange of bound potassium ions versus protons on lowering the concentrations of poly U (protons increase the rate of the conductivity increase; see below). The exchange is expected to be of relatively greater importance in more diluted solutions. This exchange is not reflected strongly in a change of the concentration of free  $\text{H}^+$  in the bulk of the solution, since the  $\text{pH}$  was found not to shift by more than  $0.3$  units after dilution.

#### c) Dependence on pH

The dependence of the yield of conductivity and of the rate of its build-up are both presented in Fig. 6 for the range  $3.7 < \text{pH} < 10$ .  $\Delta\kappa$  increases with decreasing  $\text{pH}$  in the entire  $\text{pH}$  range investigated. This increase is explained to be mainly due to the higher mobility of  $\text{H}^+$  ions which are liberated in greater proportions instead of  $\text{K}^+$  on lowering the  $\text{pH}$  value. The decrease of  $\Delta\kappa$  at  $\text{pH} \geq 8$  is probably caused partially by an ionic strength effect of the added potassium hydroxide, because  $\Delta\kappa$  was found to become smaller on addition of  $\text{KCl}$  and  $\text{KClO}_4$  [26] and from analogy we expect a similar behaviour for  $\text{KOH}$ . A further explanation may be found in an influence of the  $\text{pK}$  value of the uracil group. These aspects will not be discussed in the present paper.

The rate of the build-up shows a marked increase in the range from  $6.5$  to  $3.7$  (Fig. 6) and is constant for  $\text{pH} \geq 8$ . The greatest value of the rate constant  $k_{\text{obs}}$  measured with  $60 \text{ mg l}^{-1}$  poly U at  $18^\circ\text{C}$  amounted to  $100 \text{ s}^{-1}$  at  $\text{pH} = 3.4$ , the smallest  $\text{pH}$

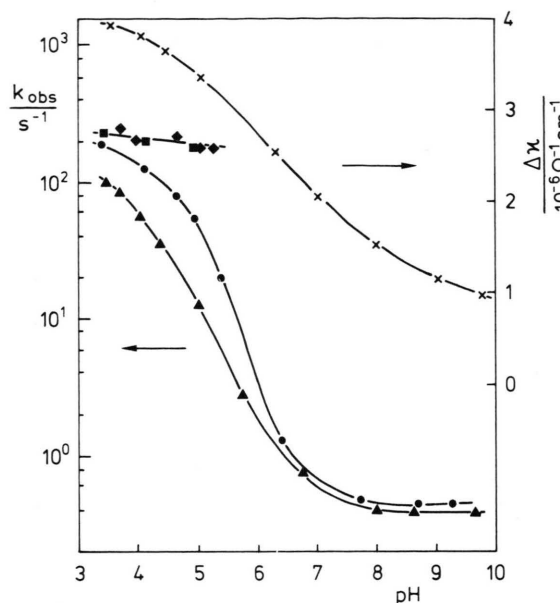


Fig. 6. pH-Dependence of the conductivity-increase  $\Delta\kappa$  (right ordinate) and of the logarithm of its rate constant (left ordinate) measured after  $1 \mu\text{s}$  electron pulses of  $10 \text{ J kg}^{-1}$  dose in aqueous  $\text{N}_2\text{O}$ -saturated solution of poly U at  $18^\circ\text{C}$ .  $\times \times$  refers to the right ordinate and was measured with the potassium salt of polyribouridylic acid (= poly U) at  $60 \text{ mg l}^{-1}$ . The filled symbols refer to the left ordinate:  $\blacktriangle \blacktriangle = 60 \text{ mg l}^{-1}$  poly U;  $\bullet \bullet = 12 \text{ mg l}^{-1}$  poly U;  $\blacksquare \blacksquare = 6 \text{ mg l}^{-1}$  potassium ion free polyribouridylic acid with  $\text{HClO}_4$  added;  $\blacklozenge \blacklozenge =$  stock solution of  $120 \text{ mg l}^{-1}$  potassium ion free polyribouridylic acid diluted with distilled water.

value which allowed conductivity measurements. At smaller concentrations of poly U, the increase of the rate with decreasing  $\text{pH}$  is steeper compared to higher concentrations for  $6 > \text{pH} > 5$  and levels off for  $\text{pH} < 5.0$  (see Fig. 6). With  $6 \text{ mg l}^{-1}$  poly U, the greatest rate constant measured was  $190 \text{ s}^{-1}$  at  $\text{pH} = 3.4$ . Using isoionic solutions of potassium-free polyuridylic acid, the  $\text{pH}$  values of the solutions were adjusted by dilution of the acid (Fig. 6). The obtained rate constant is  $k_{\text{obs}} = 220 \text{ s}^{-1}$  at  $18^\circ\text{C}$  and is nearly independent of  $\text{pH}$  ( $3.8 > \text{pH} > 5.3$ ). The effect of  $\text{pH}$  on the rate constant was also found to be very small, when a solution of  $6 \text{ mg l}^{-1}$  isoionic polyribouridylic acid (starting  $\text{pH} = 5.0$ ) was brought to  $\text{pH} = 3.7$  by adding perchloric acid (Fig. 6).

The absence of dose rate effects on the half life of the conductivity build-up revealed that the conductivity increase is due to first order processes in the whole  $\text{pH}$  range studied. However, also at lower  $\text{pH}$  values the third half life of the conductivity build-

up was  $\sim 30\%$  greater than the first one, and a much slower component was found at  $3.6 < \text{pH} < 5.7$ . It accounted again for  $\approx 10\%$  of the increase and will not be discussed in this work.

#### d) Dependence on temperature

The influence of temperature was studied in the range of  $5^\circ\text{C} < T < 80^\circ\text{C}$  at  $\text{pH} = 4.5, 6.8$  and  $8.0$ . Plotting  $\ln k_{\text{obs}}$  versus  $T^{-1}$  we obtained straight lines for the full temperature range. The calculated activation energies and frequency factors are  $E_a = 50 \text{ kJ mol}^{-1}$  and  $A = 4 \times 10^{10} \text{ s}^{-1}$  for  $\text{pH} 4.5$  (Fig. 7),  $E_a = 57 \text{ kJ mol}^{-1}$ ,  $A = 1 \times 10^{10} \text{ s}^{-1}$  at  $\text{pH} 6.8$  and  $E_a = 54 \text{ kJ mol}^{-1}$ ,  $A = 2 \times 10^9 \text{ s}^{-1}$  at  $\text{pH} 8.0$ .

$\Delta\kappa$  increases strongly with temperature at  $\text{pH} = 7.0$  (range  $2^\circ\text{C} - 80^\circ\text{C}$ ) (Fig. 8, full line). A larger part of this increase is due to the known temperature dependency of the mobility of ions in aqueous solutions. Using the known mobilities of potassium ions a correction was made, but nevertheless a net increase of conductivity with tempera-

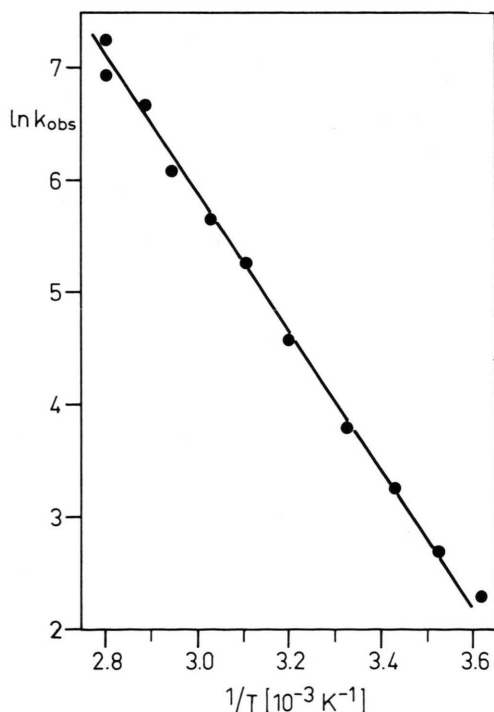


Fig. 7. Arrhenius diagram ( $\ln k$  versus  $T^{-1}$ ) for the rate of conductivity build-up measured in an aqueous  $\text{N}_2\text{O}$ -saturated solution of poly U ( $60 \text{ mg l}^{-1}$ ) after  $1 \mu\text{s}$  electron pulses of  $10 \text{ J kg}^{-1}$  at  $\text{pH} = 4.5$ .

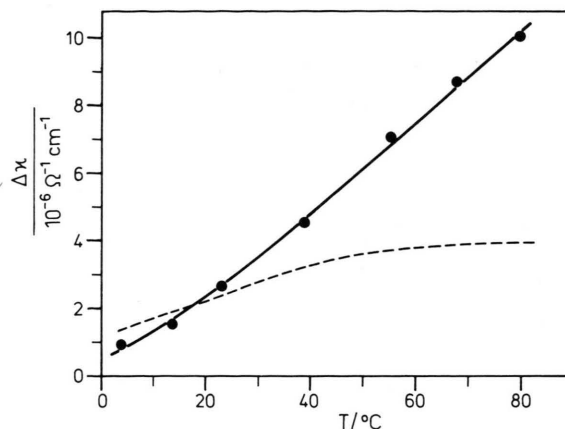


Fig. 8. Dependence of the conductivity increase  $\Delta\kappa$  on temperature measured in aqueous  $\text{N}_2\text{O}$ -saturated solutions of poly U ( $60 \text{ mg l}^{-1}$ ) after  $1 \mu\text{s}$  electron pulses of  $10 \text{ J kg}^{-1}$  at  $\text{pH} = 7.0$  (solid line); the dashed line is obtained by correcting the solid line for the temperature dependence of the mobility of  $\text{K}^+$  and of the anion (assuming the same dependence of the latter as for  $\text{K}^+$ ).

ture was obtained (Fig. 8, dashed line). This may result from an increase in the yield of ions, from a change in the kind of ions, or from a change of the mobility of the ions due to the presence of poly U. We measured that the mobility of alkali cations is not significantly influenced by the presence of  $70 \text{ mg l}^{-1}$  poly U in aqueous solutions.

Similar temperature effects concerning  $Y(\kappa)$  were obtained at  $\text{pH} = 8.9$ . At  $\text{pH} = 4.5$ , however,  $Y(\kappa)$  decreased strongly within some minutes at temperatures above  $60^\circ\text{C}$ . After cooling the solution down to room temperature the yield after pulse remained small. This effect could be due to a thermal degradation of poly U which occurs probably more easily in solutions with low ionic strength as used here. Another reason may be found in the acid catalyzed formation of 2',3' cyclic phosphates, which could possibly be formed with ribose derivatives at low pH values and higher temperatures.

#### e) Dependence on addition of scavenger

*Parabenzquinone (pBQ).* The presence of small amounts of p-benzoquinone (pBQ) in a solution of poly U leads to a decrease of  $\Delta\kappa$  at  $\text{pH} = 3.75$  (Fig. 9, solid line). The dashed line indicates the calculated decrease of  $\Delta\kappa$  expected for scavenging of OH radicals by pBQ. The line was calculated using rate constants given in the legend of Fig. 9. As

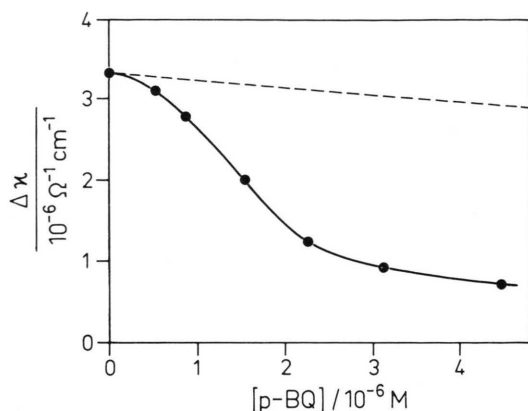


Fig. 9. Dependence of the final conductivity change  $\Delta\kappa$  on the concentration of added para-benzoquinone (pBQ) observed after pulse radiolysis of aqueous  $\text{N}_2\text{O}$ -saturated solutions of poly U ( $60 \text{ mg l}^{-1}$ ), dose =  $11 \text{ J kg}^{-1}$ , pH = 3.7,  $T = 20^\circ \text{C}$  (solid line). The dashed line shows the expected conductivity change if only those OH radicals contribute to the conductivity increase which react with poly U and not with pBQ calculated on the basis of  $k(\text{OH} + \text{poly U}) = 1.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  and  $k(\text{OH} + \text{pBQ}) = 6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .

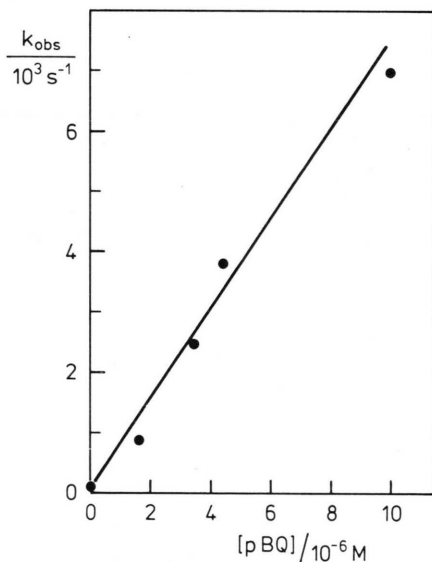


Fig. 10. Dependence of the rate constant of the conductivity build-up on the concentration of pBQ. The dose was increased successively with increasing concentration of pBQ from  $2 \text{ J kg}^{-1}$  to  $5 \text{ J kg}^{-1}$  in order to compensate for smaller signals at higher concentrations of pBQ. The other experimental conditions as in Fig. 9.

can be seen from a comparison of the full line and the dashed line in Fig. 9, the observed decrease is much larger than is explained by the mere scavenging of OH radicals by pBQ. A similar decrease of  $\Delta\kappa$  on addition of pBQ is observed at pH = 6. This indicates that at both pH values a poly U radical is

scavenged by pBQ and prevented from giving rise to conductivity.

It is further observed that in the presence of pBQ also the rate of the conductivity build up is increased: the rate constant  $k_{\text{obs}} = 1.41_{1/2} \cdot \ln 2$  is a linear function of the concentration of pBQ at

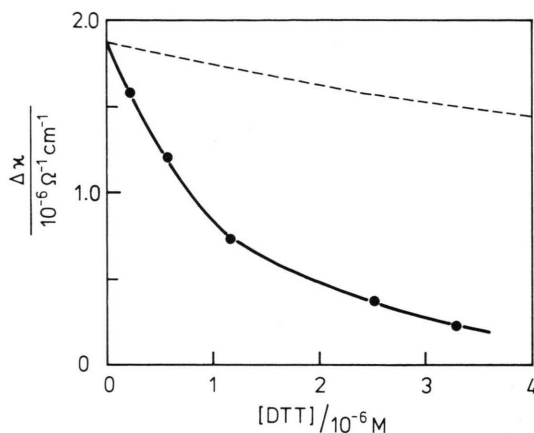


Fig. 11. Dependence of the final conductivity change  $\Delta\kappa$  on the concentration of added Dithiothreitol (DTT) observed after pulse radiolysis of aqueous  $\text{N}_2\text{O}$  saturated solutions of poly U ( $60 \text{ mg l}^{-1}$ ), dose =  $8.5 \text{ J kg}^{-1}$ , pH = 6.8,  $T = 20^\circ \text{C}$  (solid line). The dashed line shows the expected conductivity change if only those OH radicals contribute to the conductivity increase which react with poly U and not with DTT calculated on the basis of  $k(\text{OH} + \text{poly U}) = 1.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  and  $k(\text{OH} + \text{DTT}) = 1.5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ .

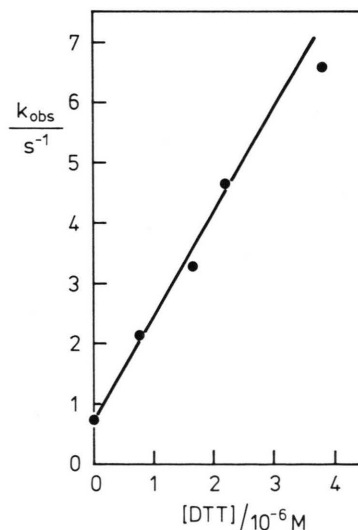


Fig. 12. Dependence of the rate constant of the conductivity build-up on the concentration of DTT. The dose was increased successively with increasing concentration of DTT from  $2 \text{ J kg}^{-1}$  to  $5 \text{ J kg}^{-1}$  (see legend of Fig. 10). The other experimental conditions as in Fig. 11.



pH = 3.7 (Fig. 10). At higher pH values ( $4.5 < \text{pH} < 7.0$ ) a similar decrease of conductivity with increasing concentration of p-benzoquinone is observed, however, the linear relationship between  $k_{\text{obs}}$  and the concentration is not found. The change in the rate constant is relatively smaller than the change of the size of the conductivity increase.

**Dithiothreitol (DTT).** At pH = 6.8, addition of DTT leads to a decrease of  $\Delta\kappa$ , which is also too large to be assignable merely to a reaction of  $\cdot\text{OH}$  with DTT (Fig. 11). Similarly to pBQ, this is explained by a reaction of DTT with poly U radicals which prevents their contribution to the conductivity increase.

Like in the case with pBQ at pH = 3.7, it is further observed that the rate constant  $k_{\text{obs}}$  of the conductivity build up is increased on addition of DTT at pH = 6.8; Fig. 12 shows that there is a linear relationship between  $k_{\text{obs}}$  and the concentration of DTT.

## Discussion

Gamma and electron irradiation of aqueous solutions of poly U leads to chain breaks in the substrate. This is known already from analogy with the behaviour of other poly nucleic acids and mainly from that of DNA. For poly U we have demonstrated the cleavage of the main chain by measuring the decrease of molecular weight of poly U as a function of dose with the help of light scattering at small angles [16] and sedimentation rate change in an ultra centrifuge. The result is that chain breaks in poly U are produced in  $\text{N}_2\text{O}$  containing solutions with a  $G$  value between 1.0–2.5. Our results have shown on the other hand that at neutral pH an increase of conductivity some seconds after the pulse is observed, which is mainly due to an increase in mobile potassium ion concentration. We suggest that the formation of free potassium ions is a consequence of the strand breaks introduced by radiolytically generated OH radicals (the solvated electrons are converted into OH radicals by  $\text{N}_2\text{O}$ ) and that these strand breaks cause the observed increase of mobile cation concentration.

The reason for this suggestion is the following: it is well documented that alkali cations are bound electrostatically to polyanionic chains due to the high density of negative charges in polyanions [11] and that this effect reduces the conductivity. An

example is DNA. Single stranded DNA with high molecular weight dissociates only to  $\sim 55\%$  in aqueous solution at room temperature [12], whereas mononucleotides are fully dissociated.

The poly U which we used is also not fully dissociated. A calculation of the degree of dissociation ( $\alpha$ ) by Manning's theory [12] gives  $\alpha = 0.63$ . On the other hand the potassium salt of 5'-uridylic acid is fully dissociated. Moreover, for polyphosphates Schindewolf has shown that the ion binding decreases with decreasing chain length [8]. This provides evidence, that also with poly U the degradation of the polymer chain causes  $\alpha$  (and hence the concentration of free counterions) to increase. The question arises now whether a complete dissociation can be achieved with increasing dose. This is indeed possible.

### 1. Size of the conductivity increase

The conductivity in aqueous salt free solution containing poly U is increasing with dose to a plateau (from  $\kappa_0$  to  $\kappa_{\text{lim}}$ ) with  $\kappa_{\text{lim}} \times \kappa_0^{-1} = 1.40 \pm 0.05$ , independent of the concentration of poly U in the range 6–60  $\text{mg l}^{-1}$  (e.g. from 14.0 to  $20.0 \times 10^{-6} \Omega^{-1} \text{cm}^{-1}$  at 60  $\text{mg l}^{-1}$  poly U, see Fig. 1). We interpret  $\kappa_{\text{lim}}$  as representing the conductivity of fully dissociated poly U. This interpretation allows firstly to calculate the equivalent conductivity of the anion of poly U and secondly to estimate the degree of dissociation  $\alpha$  before irradiation. The equivalent conductance calculated using  $\kappa_{\text{lim}}$  results in a value of  $48 \text{ cm}^2 \Omega^{-1} \text{mol}^{-1}$  for the poly anion at 60  $\text{mg l}^{-1}$  poly U and  $18^\circ \text{C}$  ( $64 \text{ cm}^2 \Omega^{-1} \text{mol}^{-1}$  was used for the equivalent conductance of  $\text{K}^+$  [15]).

Assuming as a first approximation that the equivalent conductance of the polyanion does not depend on the chain-length and that the activity of the free counterions is equal before and after irradiation, a degree of dissociation of  $\alpha = 0.71$  is calculated from the factor 1.40 of the conductivity increase mentioned above, independent on concentration of poly U. For the potassium ion free polyuridylic acid ( $\text{H}^+$  poly U) the corresponding values were found to be  $\kappa_{\text{lim}} \times \kappa_0^{-1} = 1.37$  and hence  $\alpha = 0.73$ . Manning's theory [12] predicts  $\alpha = 0.63$ , which is based on an average charge spacing of 0.45 nm for poly U. It is expected that our  $\alpha$  value is larger, because Manning's theory predicts that the equivalent conductance of a long polyanionic chain is greater than the equivalent conductance of the respective monomer,

*i.e.* that our assumption of the independence of the mobility of the chain length does not fully hold down to the small chain length considered here.

The average chain length below which poly U is fully dissociated can be estimated: a solution of  $60 \text{ mg l}^{-1}$  contains  $1.0 \times 10^{-7} \text{ M}$  poly U chains with a uniform chain length of 860 nucleotides. After absorption of a dose of  $40 \text{ J kg}^{-1}$  (when the conductivity increase is nearly finished) each strand is attacked  $\sim 20$  times by OH radicals. If one assumes that 20% of the OH radicals produce a strand break (this assumption agrees roughly with the above mentioned estimation of a  $G$  value for main chain scission of poly U of 1.0–2.5 [16] measured by light scattering), one calculates that the average chain consists of  $\sim 36$  nucleotides.

With  $\text{H}^+$  poly U of  $60 \text{ mg l}^{-1}$  we found that after  $60 \text{ J kg}^{-1}$   $\alpha_{\text{lim}}$  is nearly reached, from which it follows by an analogous estimation as for poly U, that proton condensation starts to become important at chain lengths of approximately 24 nucleotides. This is somewhat lower than for the potassium salt and correspondingly the  $D_{1/2}$  values are slightly higher for  $\text{H}^+$  poly U compared to poly U (see Table II). The reason for this difference is presently not known. The degree of condensation ( $1-\alpha$ ) at higher chain length however is not greatly influenced as can be seen from the similarity of the  $\alpha$  values for poly U and  $\text{H}^+$  poly U presented above (0.71 and 0.73).

With decreasing pH, we have shown in the results for poly U that the bound counterions  $\text{K}^+$  are increasingly exchanged against  $\text{H}^+$ . Therefore at lower pH not only  $\text{K}^+$  but also protons should be liberated. It may be assumed that the replacement of  $\text{K}^+$  by  $\text{H}^+$  is governed mainly by mass action law similar as it was found for association of ethidium bromide to DNA [18]. With  $60 \text{ mg l}^{-1}$  poly U (corresponding to  $1.7 \times 10^{-4} \text{ M}$  nucleotide units) at  $\text{pH} = 3.88$  (corresponding to  $1.3 \times 10^{-4} \text{ M H}^+$ ) 44% of the total counterions in the solution are  $\text{H}^+$  and 46% of the liberated counterions are protons (calculated from data in Table I). The agreement of the two percentages supports the assumption of ion exchange. Because of the higher mobility of protons compared to  $\text{K}^+$ ,  $\Delta\kappa$  is expected to increase with decreasing pH, in accordance with experimental results, see Fig. 6. Total exchange of  $\text{K}^+$  versus  $\text{H}^+$  should result in an increase of conductivity by a factor of  $(325 + 48)/(65 + 48) = 3.2$ . With  $60 \text{ mg l}^{-1}$

poly U *e.g.*, the conductivity should increase continuously with decreasing pH until it is 3.2 times greater at  $\text{pH} \approx 2.8$  compared to  $\text{pH} = 6.5$  (at  $\text{pH} = 2.8$  the concentration of protons exceeds the concentration of  $\text{K}^+$  by a factor of 10).

However, at  $\text{pH} < 4.0$  the increase of  $Y(\kappa)$  with decreasing pH is smaller than expected. This agrees with the smaller  $G(\text{H}^+)$  values found with  $\text{H}^+$  poly U ( $G(\text{H}^+) = 16$ ) compared to  $G(\text{K}^+)$  measured with poly U ( $G(\text{K}^+) = 20$ ) at doses of  $10 \text{ J kg}^{-1}$ . A further reason is likely an ionic strength effect of the added perchloric acid because  $Y(\kappa)$  was found to decrease with increasing ionic strength of the solution [26].

## 2. Rate of the conductivity increase

With decreasing pH the rate of the conductivity increase with poly U was found to become faster (Fig. 6). It is therefore concluded that the process leading to the liberation of ions is proton catalysed. However, the rate constant is not directly determined by the proton concentration in the bulk of the solution. This follows from the result obtained on plotting the relative rate constants ( $k_{\text{obs}}/k_{\text{H}^+\text{poly U}}$ ;  $k_{\text{obs}}$  taken from Fig. 5 and  $k_{\text{H}^+\text{poly U}} = 220 \text{ s}^{-1}$ ) versus the proton fraction of the counterions (Fig. 13). The measured relative rate constants are well represented in Fig. 13 by a straight line with a slope of unity. This clearly indicates together with the counterion exchange described above that is the condensed part of the protons which is responsible for the catalytic activity and not simply the concentration of  $\text{H}^+$  in the bulk of the solution.

The rate constants especially at  $6 \text{ mg l}^{-1}$  poly U tend to reach a limiting value with decreasing pH (Fig. 6). This limiting value is assumed to indicate complete exchange of  $\text{K}^+$  by  $\text{H}^+$ . The position of this limiting value can be obtained by using metal cation free polyuridylic acid ( $\text{H}^+$  poly U). The obtained value of  $220 \text{ s}^{-1}$  at  $18^\circ\text{C}$  seems indeed to be the value which is approached by poly U with decreasing pH (Fig. 5). An interesting phenomenon is the result that despite of the  $\text{H}^+$  catalysis of the ion liberation the influence of the pH on the rate constant in case of metal cation free polyuridylic acid is very small (Fig. 6). However, this result is expected from the theory [12] since the local concentration of condensed protons in the immediate vicinity of poly anions is not greatly influenced by

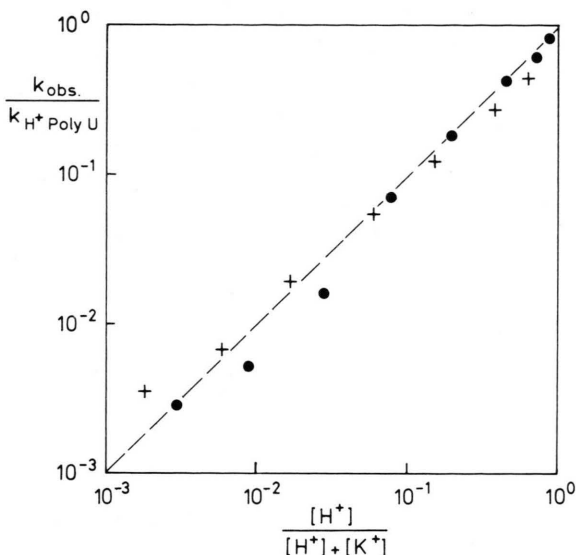


Fig. 13. Dependence of the relative rate of conductivity increase on the fraction of protons of the total amount of counterions.  $k_{\text{obs}}$  at various concentrations of  $\text{H}^+$  is taken from Fig. 6.  $k_{\text{H}^+ \text{poly U}}$  is the rate constant of conductivity increase observed with potassium ion free polyuridylic acid ( $k_{\text{H}^+ \text{poly U}} = 220 \text{ s}^{-1}$ ); ++ =  $60 \text{ mg l}^{-1}$  poly U, ●● =  $12 \text{ mg l}^{-1}$  poly U.

the pH in the bulk of the solution if protons are the only counterions present. The local concentration in the vicinity of the poly U chain for long chain lengths was calculated to be  $0.17 \text{ M}$  [12] for monovalent counterions, and hence also for protons in the case of metal cation free polyuridylic acid at pH values below 7. The influence of the poly U concentration on the rate constants at  $\text{pH} \approx 6.0$  shown in Fig. 6 is then explained by the different fraction of protons of the total amount of counterions in the solution at fixed pH values but different concentrations of poly U, and therefore of  $\text{K}^+$ .

### 3. Rate determining step of the conductivity increase

The question now arises whether the rate determining step for the conductivity increase has to be attributed (i) to the reaction of OH radical with poly U, (ii) to the establishment of an equilibrium between poly U bound and free (mobile)  $\text{K}^+$  and  $\text{H}^+$  ions, (iii) to the diffusive separation of the degradation fragments or changes of a hitherto unknown superstructure of poly U, or (iv) to the formation of strand breaks due to a splitting of the poly U radicals.

i) The first possibility, the reaction of OH with poly U as rate determining step can be ruled out since OH radicals add to the double bond of uracil with a rate constant of  $k = 6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  [19] and react with ribose by H abstraction with  $k = 2.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  [20]. We obtained a rate constant for the reaction of OH with poly U of  $k = 1.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  at  $\text{pH} = 7$  measured at a concentration of  $60 \text{ mg l}^{-1}$  (the rate constant was obtained by measuring the rate of increase of optical absorption at  $380 \text{ nm}$ ). These values would lead to rate constants for the conductivity increase three orders of magnitude too fast.

ii) The establishment of the ionic equilibrium after chain break as rate determining step is unlikely because with DNA it was measured, that the ionic equilibrium is achieved in the  $\mu\text{s}$ -range [21]. A slow process to be considered was found with DNA in neutral aqueous solution [22, 23]. The authors measured a dielectric relaxation time of  $2 \times 10^{-2} - 2 \times 10^{-1} \text{ s}$  with native DNA, however this slow contribution was absent in heat-denatured single-stranded DNA. Poly U can be regarded to be similar to denatured DNA since it is single-stranded and by analogy it is assumed that these effects are absent in poly U. Moreover, the rate of relaxation processes should not be influenced by addition of so small amounts of an organic substrate, as used in our experiments with DTT and pBQ (Figs. 10 and 12).

iii) The third possibility, the rate of diffusion of degradation fragments into the bulk of the solution as rate-determining step is not probable: The measured activation energy of  $\sim 50 \text{ kJ mol}^{-1}$  is four times larger than expected for purely diffusion-controlled processes ( $\sim 12 \text{ kJ mol}^{-1}$ ).

In order to further distinguish diffusion of degradation products (or changes of an unknown superstructure) from strand breaks by cleavage of poly U radicals as rate determining step, the influence of a scavenger on the rate of the conductivity increase has been measured. If the diffusion of degradation products is rate determining, then the rate for the increase of conductivity should not change with increasing concentration of scavenger (that is with decreasing  $\Delta\epsilon$ ). However, if the rate of the cleavage of poly U radicals is rate determining (which implies that the diffusion of degradation products is faster than the conductivity build up), then the rate for the conductivity build-up should increase with in-

creasing scavenger concentration. This case is expressed by Eqn. (A7), see Appendix.

$$k_{\text{obs}} = k_1 + k_2[S]. \quad (\text{A7})$$

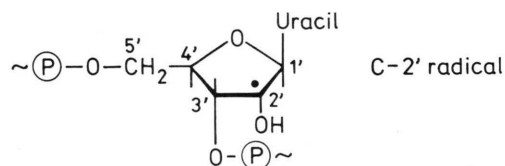
Equation (A7) predicts a linear relationship between the observed rate constant  $k_{\text{obs}}$  and the concentration of scavenger [S]. The experimental results (Figs. 10 and 12) show that such a linear dependence indeed exists at pH = 3.7 with pBQ and at pH = 6.8 with DTT. This suggests that the rate determining step for the conductivity increase is at least mainly governed by the rate for the cleavage of poly U radicals at pH = 3.7 and 6.8 and not by the diffusion of degradation products. See also [27].

From the slope of the straight lines in Figs. 10 and 12 average rate constants  $k_2$  can be calculated for the reaction of poly U radicals with pBQ and DTT respectively. One obtains  $k_2(\text{pBQ}) = 7.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_2(\text{DTT}) = 1.7 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ . These values have to be considered as lower limits, since due to the low concentration of scavenger in the solutions, these concentrations do not remain constant during the decay of the poly U radicals. Low doses have been used in these experiments to minimize this effect (see legend of Figs. 10 and 12). However it is not excluded that the true values of  $k_2$  are greater by up to a factor of two. In this context it is noteworthy, that the concentration of scavenger at which  $\Delta\kappa$  has decreased to one half of the value without scavenger is significantly smaller than one half of the concentration of OH radicals. This may be explained either by the fact that only one kind of the poly U radicals react with the scavenger or by the assumption that one molecule of scavenger can prevent more than one poly U radical from producing a strand break.

Concerning the molecular mechanism of the main chain cleavage, the observed pH dependence of the rate constant is in agreement with the C-4' mechanism postulated earlier [4] for the OH radical-induced splitting of the sugar phosphoric acid ester bonds in DNA and thus for strand break formation [24, 25]. Studies with the model radical  $\text{CH}_3\text{O}\dot{\text{C}}\text{HCH}_2\text{OPO}_3\text{H}_2$  show, that the rate constant for the cleavage of the phosphoric acid ester bond in that radical (carrying the free spin in  $\beta$  position to the ester bond) increases with decreasing pH to the same extent as observed with the rate constant for conductivity increase with poly U [25]. Furthermore, we have measured that the activation energy

for the elimination of acetic acid from the radical  $\text{CH}_3\text{O}\dot{\text{C}}\text{HCH}_2\text{OCO}_2\text{CH}_3$  at pH 5.1 is  $54 \pm 5 \text{ kJ mol}^{-1}$  which is close to the value found for poly U in the present work. Since  $-\text{OCOCH}_3$  and  $-\text{OPO}_3\text{H}^-$  are leaving groups which eliminate with the same mechanism and with similar rates, also a similar activation energy is expected [28].

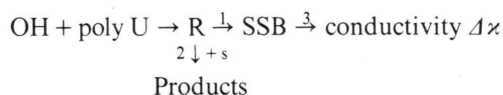
The two different half lives observed with the conductivity build up may be explained by assuming that besides the C-4' mechanism also a C-2' mechanism operates (*i.e.* elimination of phosphoric acid from the C-2' radical), because in poly U the sugar moiety carries one OH group at position 2'.



The presence of the OH group should lead to a higher probability of H abstraction by OH at position 2'. The results then suggest that the C-4' and the C-2' radicals are formed in poly U with comparable yields and decay with similar rates.

## Appendix

The reaction pathways in the presence of a scavenger may be summarized by the scheme:



R denotes those poly U radicals which form single-strand breaks (SSB) by reaction 1 with average rate constant  $k_1$ , or are scavenged by a scavenger S to form uncharged products, reaction 2, with average rate constant  $k_2$ . Reaction 3 represents either of the reactions described under ii and iii in the Discussion, *e.g.* establishment of equilibria or diffusion.

The reaction scheme is described by the equations

$$\frac{d[R]}{dt} = -(k_1 + k_2[S])[R]; \quad (\text{A1})$$

$$\frac{d[\text{SSB}]}{dt} = k_1[R] - k_3[\text{SSB}]; \quad (\text{A2})$$

$$\frac{d\Delta\kappa}{dt} = +k_3[\text{SSB}]. \quad (\text{A3})$$



Solving (A1) and substituting the solution into (A2), one obtains after integrating ( $[R]_0$  equals radical concentration immediately after the pulse):

$$[SSB] = \frac{k_1[R]_0}{k_3 - (k_1 + k_2[S])} [e^{-(k_1 + k_2[S])t} - e^{-k_3t}]. \quad (A4)$$

If reaction 3 is rate determining, i.e.  $k_3 \ll k_1 + k_2[S]$ , equations (A3) and (A4) give

$$\Delta\kappa = \frac{k_1[R]_0}{k_1 + k_2[S]} [1 - e^{-k_3t}]. \quad (A5)$$

The observed rate constant in (A5) is simply  $k_3$ , independent of scavenger concentration.

If reaction 1 is rate determining, i.e.  $k_3 \gg k_1 + k_2[S]$ , then

$$\Delta\kappa = \frac{k_1[R]_0}{k_1 + k_2[S]} [1 - e^{-(k_1 + k_2[S])t}]. \quad (A6)$$

In Eq. (A6) the observed rate constant  $k_{obs}$  is

$$k_{obs} = k_1 + k_2[S] \quad (A7)$$

i.e. a linear function of the scavenger concentration.

## Experimental

Most experiments were done using poly U (the potassium salt of polyuridylic acid) obtained from Boehringer, Mannheim. Some of the experiments were performed with poly U from P.L. Biochemicals, Milwaukee. The results were the same within the limits of experimental error (15%). According to supplier specification, the substrate of Boehringer had a molecular weight of  $3 \times 10^5$  ( $S_{20w} = 8.0$ ). No small oligomers could be detected by us by anion exchange chromatography. The substrate (Boehringer) lost 15% of its weight when kept in vacuo at 60°C for 6 h and the dry material had a molar absorptivity of  $9700 \text{ l mol}^{-1} \text{ cm}^{-1}$  per nucleotide unit.

Poly U was dissolved in water which had been distilled five times, including two times in the presence of  $\text{KMnO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  and kept in quartz vessels thereafter. The solutions were allowed to stand for a minimum time of four hours. After a longer period of standing (up to 40 h) we obtained the same results in our conductivity experiments within the limits of experimental error. After passing  $\text{N}_2\text{O}$  through the solution for 45 min, the pH value of the solution was measured to be  $6.8 \pm 0.2$  at the standard concentration of  $60 \text{ mg l}^{-1}$  poly U.

Solutions of lower concentrations were obtained by diluting the standard solution with the above distilled water.

Solutions were concentrated by a factor of 10 in a membran filtration apparatus (Amicon, filter UM 10) and then rediluted with distilled water to the desired concentration. Without this additional treatment, the changes of conductivity during pulse radiolysis were found to be approximately 35% smaller. The pH was adjusted with perchloric acid or potassium hydroxide solutions. In the pulse radiolysis experiments the conductivity changes were measured with a DC operated Wheatstone-bridge normally at 100 V between the platinum electrodes of the conductivity cell. On varying the applied voltage in the range of 20–200 V, we observed no effect on the size as well as on the kinetics of the conductivity changes.

At recording times greater than 0.1 s the conductivity was measured by applying 5 ms voltage pulses to the cell. These pulses were separated by time intervals without voltage between the electrodes, the duration of which could be chosen to suit the respective recording time (Fig. 4a). A greater part of the measurements were repeated with an AC bridge powered by an AC generator of 40 V (peak to peak) with frequencies tunable from 50 Hz to 1 MHz (Fig. 4b). The AC measuring set up was supplied by Dr. Janata from the Hahn Meitner Institut für Kernforschung, Berlin. In the range  $10^2$ – $10^5$  Hz the size of the conductivity changes was measured to be  $\sim 15\%$  smaller in most cases compared to the results obtained with the DC method. The size of the conductivity changes presented in this paper are averages of results obtained with the DC and AC method. The rates of the conductivity increases, however, were observed to be the same with both methods. With the AC method a small initial decrease of conductivity was observed at frequencies  $10^3 < f < 10^5$  Hz. It amounted to approximately 5% of the conductivity increase under typical experimental conditions ( $10 \text{ J kg}^{-1}$ ,  $60 \text{ mg l}^{-1}$  Poly U, pH = 6.8) and was not found with the DC method. This initial decrease did not influence the rate of the increase for doses  $\geq 2 \text{ J kg}^{-1}$  and will not be discussed in the present paper. During and after  $^{60}\text{Co}$ - $\gamma$ -irradiation the conductivity changes were measured using a commercial conductivity meter (Radiometer, CDM 3) operated with AC voltage of 0.2 V and 75–350 Hz.

Solutions of free polyuridylic acid ( $H^+$  poly U) were obtained by passing solutions of the potassium salt through a column containing a strongly acidic cation exchanger (Merck, Type I).

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- [1] a) J. Blok and H. Loman, *Curr. Top. Radiat. Res.* **9**, 165 (1973).  
b) J. F. Ward, *Advan. Radiat. Biol.* **5**, 181 (1975);  
c) K. W. Held, R. W. Synek, and E. L. Powers, *Int. J. Radiat. Biol.* **33**, 317 (1978).
- [2] a) G. Scholes, J. F. Ward, and J. Weiss, *J. Mol. Biol.* **2**, 379 (1960);  
b) H. Jung, U. Hagen, M. Ullrich, and E. E. Petersen, *Z. Naturforsch.* **24 b**, 1565 (1969);  
c) D. E. Hoard, F. N. Hayes, and W. B. Goad, *Intern. J. Radiat. Biol.* **25**, 603 (1974).
- [3] a) D. Schulte-Frohlinde, in: *Radiation Research*, (S. Okada *et al.*, eds.), p. 407, Maruzen, Tokyo 1979;  
b) C. v. Sonntag and D. Schulte-Frohlinde, in: *Effects of Ionizing Radiation on DNA* (A. J. Bertinchamp, ed.), p. 204, Springer Verlag, Berlin, Heidelberg, New York 1978;  
c) C. v. Sonntag, U. Hagen, A. Schön-Bopp, and D. Schulte-Frohlinde, *Adv. Radiat. Biol.* **9**, 109 (1981).
- [4] M. Dizdaroglu, C. v. Sonntag, and D. Schulte-Frohlinde, *J. Amer. Chem. Soc.* **97**, 2277 (1975).
- [5] a) E. G. Richards, C. P. Flessel, and J. R. Fresco, *Biopolymers* **1**, 431 (1963);  
b) R. Steiner and D. B. S. Miller, in: *Biological Polyelectrolytes* (A. Veis, ed.), p. 65, Dekker, New York 1970.
- [6] M. N. Lipsett, *Proc. Natl. Acad. Sci. USA* **46**, 445 (1960).
- [7] E. Hammarsten, *Biochem. Z.* **144**, 383 (1924).
- [8] U. Schindewolf, *Z. Phys. Chem. N.F.* **1**, 134 (1954).
- [9] I. A. Kuznetsov, S. M. Filippov, and O. V. Vorontsova, *Mol. Biol.* **13**, 408 (1979).
- [10] A. Katchalsky, *Pure Appl. Chem.* **26**, 27 (1971).
- [11] F. Oosawa, *Polyelectrolytes*, p. 160, Dekker, New York 1971; T. Onishi, N. Imai, and F. Oosawa, *J. Phys. Soc. Jap.* **15**, 896 (1960); N. Imai and T. Onishi, *J. Chem. Phys.* **30**, 1115 (1959); N. Imai, *J. Phys. Soc. Jap.* **16**, 746 (1961).
- [12] G. S. Manning, *Ann. Rev. Phys. Chem.* **23**, 117 (1972), *J. Phys. Chem.* **79**, 262 (1975), *Quart. Rev. Biophys.* **11**, 179 (1978), *Acc. Chem. Res.* **12**, 443 (1979), *J. Phys. Chem.* **85**, 1506 (1981).
- [13] I. A. Kuznetsov, N. V. Apolonnikh, and L. F. Maznikova, *Mol. Biol.*, **13**, 546 (1978).
- [14] D. Lindenau, U. Hagen, and W. Schnabel, *Z. Naturforsch.* **31 c**, 484 (1976); K. Washino and W. Schnabel, *Makromol. Chem.* **183** (1982); K. Washino and W. Schnabel, *Int. J. Radiat. Biol.* **41**, 271 (1981); W. Schnabel, O. Denk, U. Gröllmann, I. A. Raap, and K. Washino, private communication, submitted to *Radiat. Phys. Chem.*
- [15] R. A. Robinson and R. H. Stokes, *Electrolyte Solutions*, Butterworths, London 1959.
- [16] D. Lemaire, unpublished results.
- [17] P. Debye and E. Hückel, *Physical. Z.* **24**, 185 and 305 (1923).
- [18] J. Pauluhn and H. W. Zimmermann, *Ber. Bunsenges. Phys. Chem.* **82**, 1265 (1978).
- [19] G. Scholes, P. Shaw, R. L. Willson, and M. Ebert, in: *Pulse Radiolysis*, (M. Ebert *et al.*, eds.), p. 151, Academic Press, London, New York 1965; C. L. Greenstock, M. Ng, and J. W. Hunt, *Advan. Chem. Ser.* **81**, Radiation Chemistry (R. F. Gould, ed.), p. 397 (1968); C. L. Greenstock, J. W. Hunt, and M. Ng, *Trans. Faraday Soc.* **65**, 3279 (1969); I. Kraljic, in: *Chemistry of Ionization and Excitation* (G. R. A. Johnson and G. Scholes, eds.), p. 303, Taylor and Francis, London 1967.
- [20] G. Scholes, J. Weiss, and C. M. Wheeler, *Nature* **178**, 157 (1956).
- [21] T. Vreugdenhil, F. van der Touw, and M. Mandel, *Biophys. Chem.* **10**, 67 (1979).
- [22] M. Hanss and J. C. Bernengo, *Biopolymers* **12**, 2151 (1973).
- [23] M. Hanss, *Biopolymers* **4**, 1035 (1966).
- [24] G. A. Qureshi, E. Bothe, and D. Schulte-Frohlinde, in preparation.
- [25] G. Behrens, G. Koltzenburg, A. Ritter, and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.* **33**, 163 (1978).
- [26] E. Bothe and D. Schulte-Frohlinde, unpublished results.
- [27] E. Bothe and D. Schulte-Frohlinde, *Radiat. Environ. Biophys.* **17**, 310 (1980).
- [28] G. Koltzenburg, G. Behrens, and D. Schulte-Frohlinde, unpublished results.