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ONE-ELECTRON REACTIONS IN BIOCHEMICAL SYSTEMS AS STUDIED BY PULSE RADIOLYSIS

I. NICOTINAMIDE-ADENINE DINUCLEOTIDE AND RELATED COMPOUNDS

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SUMMARY

1. Some univalent oxidations and reductions of NAD and related compounds have been studied by means of pulse radiolysis.

2. Both hydrated electrons and the COO^- radical will reduce NAD^+ with rate constants of $k = 2.5 \cdot 10^{10}$ $M^{-1} \cdot sec^{-1}$ and $1.6 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$ respectively.

3. The reduction leads to a single free radical whose unpaired electron is located at the nicotinamide end of the molecule. By using AMP it has been shown that the addition of an electron to the adenine group is followed by efficient electron transfer to the nicotinamide group.

4. The absorption spectra of the free radicals formed from NAD and 1-methylnicotinamide chloride have been determined, and shown to resemble published absorption spectra of related free radicals.

5. The NAD (or 1-methylnicotinamide chloride) free radicals have been shown to react with each other with a rate constant of $k = 5.6 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ ($k = 6.9 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ for 1-methylnicotinamide chloride), probably to give a dimer, as in previous experiments using X-irradiation.

INTRODUCTION

The role of one-electron reactions (free-radical reactions) in biological oxidations and reductions has been of interest since the classic work of Michaelis[1]. Ionizing radiations are a convenient source of free radicals, and have been used to provide information about some of the free-radical reactions of certain biological oxidation–reduction compounds[2]. The invention of the technique of pulse radiolysis[3] has now made it possible to study some of the reactions in an improved manner.

When the oxidized form of NAD (NAD^+) is irradiated in the presence of an excess of an organic substance such as ethanol or formate, it becomes reduced *via* the formation of free radicals, probably according to the equations:

$$H_2O \text{—}\wedge\!\vee\!\text{→} e^-_{aq} (G^* = 2.60) + H(G = 0.55) + OH(G = 2.65) + H_2(G = 0.45) + H_2O_2 (G = 0.7) \quad (1)$$

* G may be defined as the number of molecules changed per 100 eV of energy absorbed in the system.

$$H + HCOO^- \longrightarrow H_2 + COO^- \quad (2)$$

$$OH + HCOO^- \longrightarrow H_2O + COO^- \quad (3)$$

$$e^-_{aq} + NAD^+ \longrightarrow NAD\cdot \quad (4)$$

$$COO^- + NAD^+ \longrightarrow NAD\cdot + CO_2 \quad (5)$$

The product finally obtained differs from the reduced form obtained enzymatically (NADH), and is probably a dimer formed by the combination of two pyridinyl radicals[2,4-7]:

$$2\,NAD\cdot \longrightarrow (NAD)_2 \quad (6)$$

Pyridinyl radicals are also implicated in the electrolytic reduction of nicotinamide derivatives[8-11]. The present study is concerned with some of the properties of these radicals, as studied spectrophotometrically. The methodology employed is similar to that already used in a study of methylene blue[12]. Preliminary results have been published elsewhere[13,14]. Later papers in this series will be concerned with other components of the mitochondrial electron transport chain, and with one-electron interactions between them.

METHODS

NAD^+ and NADH were used as supplied by Koch–Light or Boehringer. The samples supplied by both firms behaved identically. 1-Methylnicotinamide chloride and AMP were used as supplied by K and K (U.S.A.) and Koch–Light, respectively. Water was re-distilled from alkaline permanganate in an atmosphere of nitrogen. Air was removed from solutions by bubbling with argon, stated to contain about 2 p.p.m. oxygen, obtained from Air Products. N_2O, where used, was medical grade from the British Oxygen Co.

The pulse-radiolysis equipment used was basically that described by KEENE[15]. Two different electron accelerators were used during the present work. One was a 4-MeV linear accelerator giving pulses of 2 μsec duration (A.E.I.). The other was a 8–14-MeV linear accelerator giving pulses of 0.5 or 5 μsec (Vickers). Solutions were contained in cells of optical path length 1.6 or 2.5 cm. The effects of the radiation pulse on the optical transmission at selected wavelengths, band width 100 Å, were observed on a cathode-ray oscilloscope, the picture being recorded where necessary using a Polaroid camera.

X-irradiation was obtained from a 300-kV Resomax set, and the radiation was delivered to argon-flushed solutions contained in glass syringes. H_2 was determined after extraction from the irradiated solutions. A Perkin–Elmer gas chromatograph model 452 was used (molecular sieve No. 5A/2412). Radiation doses were measured by $FeSO_4$ dosimetry.

RESULTS

The rate constants for reaction of the hydrated electron e^-_{aq} with NAD^+ and 1-methylnicotinamide chloride were obtained by following the rates of disappearance of the hydrated-electron absorption at 7000 Å in solutions containing low concentrations of NAD^+ or 1-methylnicotinamide in aqueous 10^{-1} M sodium formate. Reaction

of hydrated electrons with formate is very slow* ($2.4 \cdot 10^4$ $M^{-1} \cdot sec^{-1}$) and would be negligible in this system. The results are given in Table I. It was found that the disappearance of the electron was accompanied by a corresponding increase in transient absorption around 4000 Å. There was also an additional but slower increase in transient absorption around 4000 Å, presumably caused by reaction of COO^- with NAD^+ or 1-methylnicotinamide. The rate of the reaction of COO^- could be estimated from this rate of increase but a more accurate determination was made by saturating the solutions with N_2O in order to convert e^-_{aq} into COO^-:

$$e^-_{aq} + N_2O \xrightarrow{H_2O} OH + N_2 + OH^- \quad 7$$

$$OH + HCOO^- \longrightarrow COO^- + H_2O \quad 3$$

Such solutions gave a single first-order build up at 4000 Å from which the required rate (Reaction 5) was obtained (see Table I).

The rate of reaction of the hydrated electron with AMP:

$$e^-_{aq} + AMP \longrightarrow AMP\cdot^- \quad 8$$

was measured in the same way as for NAD^+. The value obtained, $4.0 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$, agrees with a previous measurement of $3.8 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$ (see ref. 16). After the electron had reacted, the solution showed transient absorptions with peaks around 3100, 3500, 5100 and 5600 Å. If the solution was saturated with N_2O before giving the pulse, little if any transient absorption was formed, showing that the transient was formed by the reaction of the hydrated electron, and that COO^- reacts slowly if at all with AMP.

Rate constants for reaction of the hydrated electron with NADH and pyrophosphate were also obtained and are shown in Table I. In the case of sodium pyrophosphate, a 10^{-2} M solution was used adjusted to pH 7.7 with HCl.

TABLE I

REACTION RATE CONSTANTS OF e^-_{aq} AND COO^- WITH VARIOUS COMPOUNDS

Compound	e^-_{aq} *rate* (M^{-1} sec^{-1})	COO^- *rate* ($M^{-1}sec^{-1}$)
NAD^+	$2.5 \cdot 10^{10}$	$1.6 \cdot 10^9$
1-Methylnicotinamide chloride	$4.1 \cdot 10^{10}$	$4.6 \cdot 10^9$
AMP	$4.0 \cdot 10^9$	$< 10^6$
NADH	$5.2 \cdot 10^9$	—
Pyrophosphate	$< 3 \cdot 10^6$	—

Fig. 1 shows the change in spectrum in the range 3000–6000 Å immediately (approx. 10–100 μsec) and 10 msec after giving a pulse to an aqueous solution of $2 \cdot 10^{-4}$ M NAD^+ containing 10^{-1} M sodium formate. The time after the pulse at which the immediate spectrum was measured was such that all e^-_{aq} and COO^- had reacted with NAD^+, but a negligible number of the NAD· radicals thus formed had decayed. The dose per pulse was approx. 1600 rads. The rate of disappearance of the transient

* J. H. BAXENDALE, M. EBERT, J. P. KEENE AND A. J. SWALLOW, unpublished results, quoted in ref. 14.

absorption at 4000 Å followed accurate second-order kinetics (Fig. 2). The rate constant for the reaction, defined by $-d[R]/dt = 2k[R]^2$ where R is the transient radical species, is found from the slope of Fig. 2 to be given by $k = 3.03 \cdot 10^4 (\varepsilon_R - \varepsilon_P/2)$ $M^{-1} \cdot sec^{-1}$ where ε_R is the molar extinction coefficient of the transient

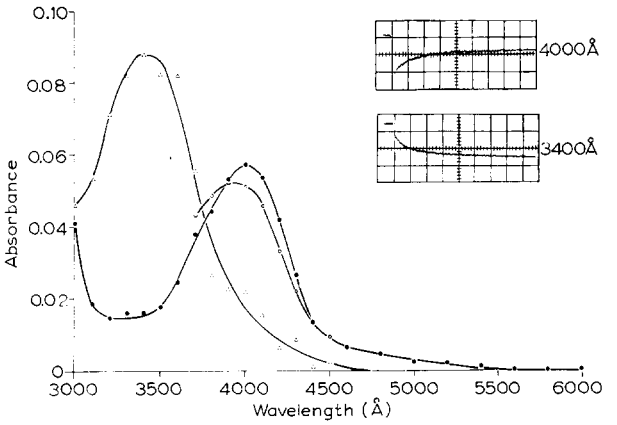


Fig. 1. Absorption in the range 3000–6000 Å following pulse radiolysis of aqueous NAD solutions. (a) ●—●, $2 \cdot 10^{-4}$ M NAD in 10^{-1} M sodium formate (pH 6.4), immediately (approx. 10–100 μsec) after pulse; (b) △—△, the same solution as in (a), 20 msec after pulse; (c) ○—○, 10^{-3} M AMP + $2 \cdot 10^{-5}$ M NAD in 10^{-1} M sodium formate (pH 7.5), 150 μsec after pulse. Dose: 1600 rads. Inset: Typical oscilloscope traces used for kinetic analysis. Vertical displacements correspond to increased transmission (1 large division = 5% transmission at 4000 Å and 10% transmission at 3400 Å), horizontal displacements correspond to time (1 large division = 2 msec).

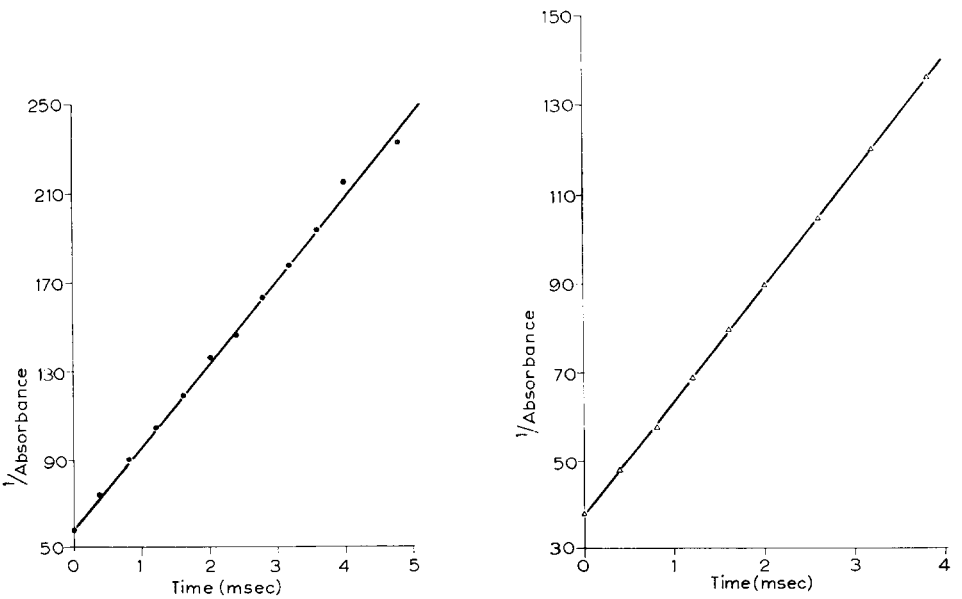


Fig. 2. Second-order plot of decay of transient absorption at 4000 Å observed on pulse radiolysis of $2 \cdot 10^{-4}$ M NAD in 10^{-1} M sodium formate (pH 6.4).

Fig. 3. Second-order plot of appearance of product absorption at 3400 Å observed on pulse radiolysis of $2 \cdot 10^{-4}$ M NAD in 10^{-1} M sodium formate (pH 6.4).

radical R at 4000 Å and ε_P is the molar extinction coefficient of the product P at the same wavelength. The rate of appearance of absorption at 3400 Å was consistent with its being formed in the second-order reaction by which the 4000-Å absorption disappeared (Figs. 1 (inset), 2 and 3). The rate constant for formation of the product defined by $d[P]/dt = 4k([P_\infty]-[P])^2$ where P is the product, is found from the slope of Fig. 3 to be $k = 1.03 \cdot 10^4$ $(\varepsilon_P - 2\,\varepsilon_R)$ $M^{-1} \cdot sec^{-1}$ where ε_P is the molar extinction coefficient of P at 3400 Å and ε_R is the molar extinction coefficient of R at 3400 Å. The product absorbing at 3400 Å was stable indefinitely. Almost identical changes in spectrum were obtained when sodium formate was replaced by ethanol ($5 \cdot 10^{-1}$ M) or when the NAD was replaced by NADP. Also there were no significant differences in the spectral changes observed if, instead of neutral solutions, acid conditions (10^{-1} M formic acid instead of 10^{-1} M sodium formate; pH 2.4) or alkaline conditions ($5 \cdot 10^{-4}$ M NaOH added; pH 9.9) were used.

Fig. 4 shows the changes in spectrum in the same wavelength range obtained immediately (approx. 10–100 μsec) and 2 msec after pulse radiolysis of aqueous $2 \cdot 10^{-4}$ M 1-methylnicotinamide chloride containing 10^{-1} M sodium formate. It can be seen that both the initial and final absorption maxima were shifted to longer wavelengths by about 100 Å. From the oscilloscope traces in the inset it can be seen that the decay of transient around 4200 Å ($k = 1.86 \cdot 10^5$ $(\varepsilon_R - \varepsilon_P/2)$ $M^{-1} \cdot sec^{-1}$) is consistent with the second-order rate of formation of product at 3400 Å ($k = 1.33 \cdot 10^5$ $(\varepsilon_P - 2\varepsilon_R)$ $M^{-1} \cdot sec^{-1}$). According to previous work[4,7], the X-irradiation of NAD in the presence of an excess of an organic substance led to a permanent decrease in absorption below 3000 Å, whereas the irradiation of 1-methylnicotinamide chloride solutions led to an increase. In order to see whether the same thing happened under the present conditions, the initial and final changes below 3000 Å have been investigated. In the case of NAD it was necessary to use a lower solute concentration than for the experiments for Fig. 1 in order to allow light to be transmitted where the parent itself absorbs. The differences between the final changes in absorption at 2600 Å

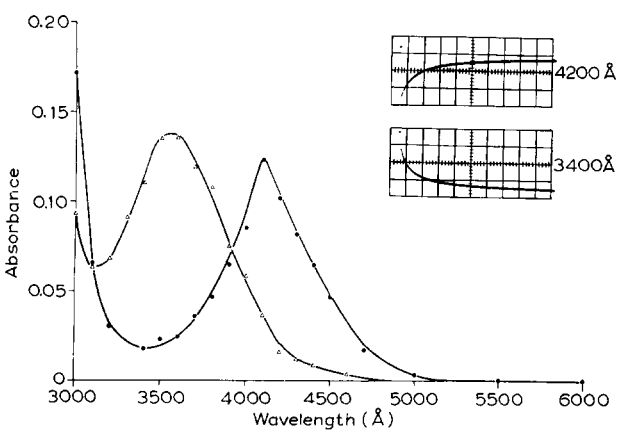


Fig. 4. Absorption changes in the range 3000–6000 Å following pulse radiolysis of aqueous 1-methylnicotinamide solutions. ●—●, $2 \cdot 10^{-4}$ M 1-methylnicotinamide in 10^{-1} M sodium formate (pH 8.5), immediately (approx. 10–100 μsec) after pulse; △—△, the same solution, 2 msec after pulse. Dose: 1850 rads. Inset: Typical oscilloscope traces used for kinetic analysis. Vertical displacements correspond to increased transmission (1 large division = 15% transmission at 4200 Å and 3400 Å), horizontal displacements correspond to time (1 large division = 100 μsec).

found for the two compounds (Fig. 5) are in agreement with those found in the previous low dose-rate studies.

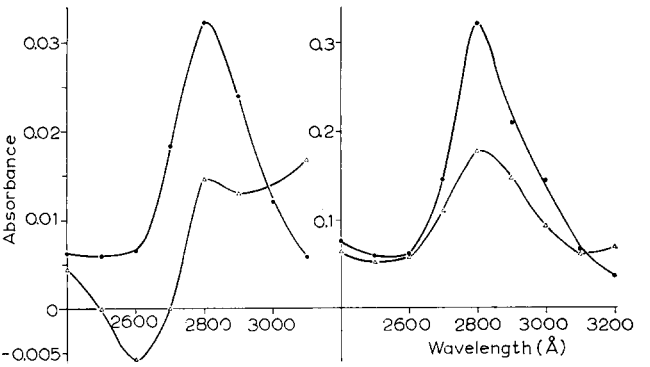


Fig. 5. Absorption changes below 3000 Å following pulse radiolysis of aqueous NAD and 1-methylnicotinamide solutions. (a) $3 \cdot 10^{-5}$ M NAD in 10^{-1} M sodium formate (pH 7.8): ●—●, 100 μsec after pulse; △—△, 20 msec after pulse. Dose: 535 rads. (b) $2 \cdot 10^{-4}$ M 1-methylnicotinamide in 10^{-1} M sodium formate (pH 8.5); ●—●, immediately (approx. 10–100 μsec) after pulse; △—△, 2 msec after pulse. Dose: 1850 rads.

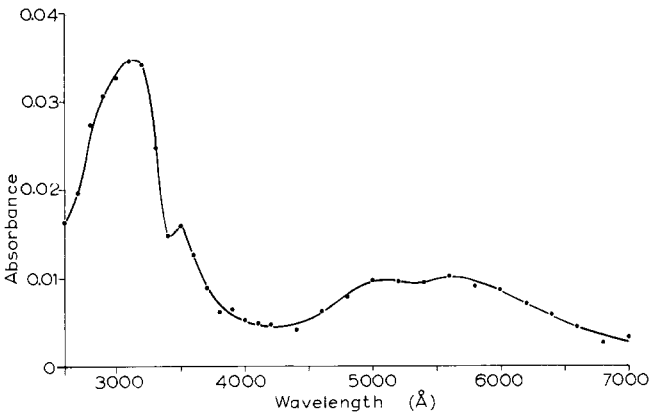


Fig. 6. Absorption changes in the range 2600–7000 Å, 20 μsec after pulse radiolysis of an aqueous solution of $2 \cdot 10^{-4}$ M AMP in 10^{-1} M sodium formate (pH 8.3). Dose: 1400 rads.

Fig. 6 shows the change in absorption in the range 2600–7000 Å 20 μsec after giving a pulse to $2 \cdot 10^{-4}$ M AMP containing 10^{-1} M sodium formate. The dose was about 1400 rads. The transient spectrum in this case was quite different from that obtained with the nicotinamide derivatives and showed little absorption around 4000 Å where the nicotinamide-containing radicals absorb strongly. The kinetics of decay of the transient at 3200 Å and 3500 Å were dissimilar showing that at least two different species were present.

A number of experiments were carried out on mixtures of AMP and NAD. Fig. 1(c) shows the spectrum 150 μsec after giving a pulse to a mixture of 10^{-3} M AMP and $2 \cdot 10^{-5}$ M NAD in 10^{-1} M formate. The spectrum of Fig. 1(c) resembles Fig. 1(a). In this solution practically all of the hydrated electrons react with AMP, although the COO^- radicals react with NAD. It was found that the disappearance of

the absorption at 5500 Å where only $AMP\cdot^-$ absorbs, was much faster than for AMP alone where $k_I = 2.6\cdot10^3$ sec^{-1}. The faster decay at 5500 Å also followed first-order kinetics and the rate increased with the concentration of NAD (10^{-5} M NAD, k_I approx. $1.3\cdot10^4$ sec^{-1}; $2\cdot10^{-5}$ M NAD, k_I approx. $3.6\cdot10^4$ sec^{-1}). Although the oscilloscope traces were rather noisy these pseudo first-order rates in the presence of NAD lead to an estimate of approx. $1.5\cdot10^9$ $M^{-1}\cdot sec^{-1}$ for the rate constant for the reaction of $AMP\cdot^-$ with NAD^+. This is very similar to the rate constant obtained for Reaction 5, electron transfer from COO^- to NAD^+. The formation of $NAD\cdot$ absorption at 4000 Å due to the reaction of both COO^- and $AMP\cdot^-$ with NAD^+ gives quite a good single first-order plot which is practically unaffected when the solution is flushed through with N_2O instead of argon.

Experiments on mixtures containing large amounts of NAD ($2\cdot10^{-4}$ M) relative to AMP (2 and $4\cdot10^{-5}$ M) in 10^{-1} M formate showed that the decay of $NAD\cdot$ is unaffected by small amounts of AMP, so that electron transfer from $NAD\cdot$ to AMP is unlikely.

Solutions of $2\cdot10^{-4}$ M NADH in N_2O-saturated solutions were studied briefly in order to determine whether the one-electron oxidation of NADH by OH radicals would give the same radical as found on one-electron reduction of NAD^+. Although there was some formation of a transient which absorbed in the same region as the $NAD\cdot$ free radical, the decay at 4000 Å was different from that obtained with the solutions containing NAD^+ and formate.

Measurements were made of the yield of H_2 from irradiated solutions of NAD^+ ($2.7\cdot10^{-4}$ M) in 10^{-2} M pyrophosphate–HCl buffer at pH 7.8, in the presence of $5\cdot10^{-1}$ M ethanol or 10^{-1} M sodium formate. Solutions were given 30000 rads of X-rays. After irradiation the hydrogen was determined gas-chromatographically. The yield in the presence of ethanol was found to be $G = 0.89$ and in the presence of formate $G = 0.82$. The former result contrasts with the previous finding[4] using a combustion technique that the yield was $G = 3.2$. In view of the unambiguity of the gas-chromatographic method the present value is considered to be the more reliable.

Previous work had shown that the product of the irradiation of NAD in the presence of ethanol would reduce methylene blue[7]. It has now been shown that solutions of AMP ($3\cdot10^{-4}$ M) which have been given 20000 rads of X-rays in the presence of $5\cdot10^{-1}$ M ethanol or 10^{-1} M sodium formate will also reduce methylene blue. Quantitative measurements have not been made.

DISCUSSION

The result given in Table I shows that hydrated electrons react very rapidly with NAD^+ presumably according to Equation 4. Earlier experiments had indicated that there was a high yield of hydrogen ($G = 3.2$) in the X-radiolysis of a pyrophosphate-buffered solution of NAD^+-containing ethanol[4]. Such a result would be inconsistent with a fast reaction of hydrated electrons with NAD^+ unless electrons could be converted by a rapid reaction with the pyrophosphate into hydrogen atoms, which could then abstract hydrogen atoms from ethanol to give the molecular hydrogen[17]. However, Table I shows that hydrated electrons do not in fact react rapidly with pyrophosphate. The hydrogen measurements of G approx. 1 now reported are consistent with the rapid reaction of hydrated electrons with NAD^+ and

with the expected formation of hydrogen only by a molecular process (G approx. 0.45) and from H atoms (G approx. 0.55).

Comparison of some of the rates in Table I provides evidence about the site of reduction in NAD^+. The reported rate of reaction of the electron with adenine ($1.2 \cdot 10^{10}$, see ref. 18) is about one half the measured electron rate of NAD^+ and that of AMP is only one fifth of the NAD^+ rate suggesting that in NAD^+ the nicotinamide ring is the main point of attack. This is supported by the fact that the 1-methylnicotinamide-electron rate is about the same as the NAD^+ rate, or if anything a little faster. The rate of reaction with NADH is only one fifth of that with NAD^+, confirming still further that in NAD^+ the oxidized nicotinamide end of the molecule is the major site of reduction by the hydrated electron. The fact that AMP is reduced slowly if at all with COO^-, whereas NAD^+ is reduced with $k = 1.6 \cdot 10^9\ M^{-1} \cdot sec^{-1}$ shows that in reduction with COO^-, as with hydrated electrons, the nicotinamide ring is the major site of reaction.

Although the nicotinamide ring appears to be the major site of reduction in NAD^+, the fast reaction of the hydrated electron with adenine does suggest that at least some initial attack may occur at other positions. It is noted that the product of the irradiation of AMP in the presence of formate possesses reducing properties which would be consistent with this view. On the other hand the decay of the absorption at 4000 Å and the formation of the product at 3400 Å both follow accurate second-order kinetics, suggesting that only one free-radical intermediate is involved. It is therefore postulated that if an electron reacts with the adenine part of NAD^+ it transfers rapidly to the nicotinamide ring. It is already known that fluorescence energy can be transferred between these two groupings in NADH[19]. Furthermore the conformation of pyridine nucleotides in solution is favourable for such an electron transfer, since NMR evidence[20] has shown that the two rings are stacked in parallel. Comparison of curves (a) and (c) of Fig. 1 shows that quite rapid electron transfer can take place from $AMP^{\cdot -}$ to the nicotinamide end of NAD. The kinetics at 5500 Å and 4000 Å in solutions containing both AMP and NAD^+, and the effect of N_2O, still further confirm the reaction, which may be written:

$$AMP^{\cdot -} + NAD^+ \longrightarrow AMP + NAD^{\cdot} \qquad 9$$

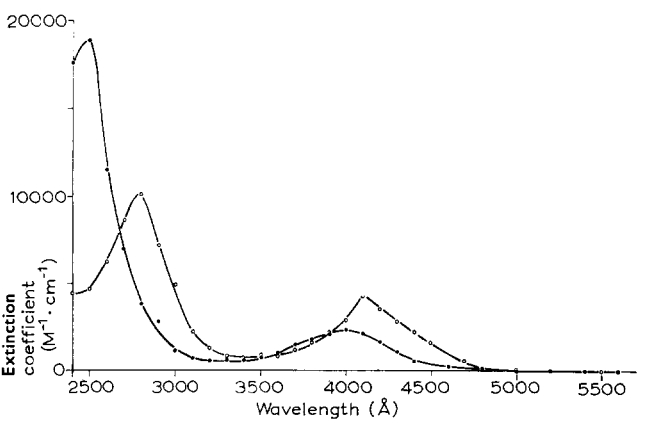


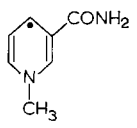
Fig. 7. Absorption spectra of radicals derived from NAD (●—●) and 1-methylnicotinamide (O—O).

and show that the rate constant is approx. $1.5 \cdot 10^9\ M^{-1} \cdot sec^{-1}$. If such a reaction can occur in free solution it seems highly probable that electron transfer from adenine to nicotinamide can occur intramolecularly in NAD.

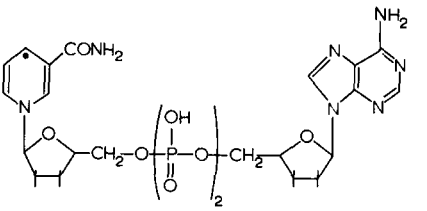
The data of Figs. 1, 4 and 5 were used to obtain the full spectra of the radicals derived from NAD and 1-methylnicotinamide in the range 2400–6000 Å. The mechanism was taken to be that described by Reactions 1–6 and primary yields were taken to be $G_{OH} = 2.65$, $G_H = 0.55$ and $G_e = 2.6$ (see ref. 21). For the region below 3000 Å, it was necessary to make allowance for the absorption of the parent nicotinamide derivative. The full spectra of both radicals are given in Fig. 7. They are characterised by 2 maxima, around 4000 Å and 3000 Å. The spectra bear a strong resemblance to those of a number of pyridinyl radicals of general formula I (where X = $COOCH_3$,

I

$COCH_3$, $CONH_2$ or CN, and R = CH_3 or C_2H_5, for example) which have been prepared by metallic reduction of the corresponding pyridinium halides[22,23]. In the case of the 1-ethyl-4-carbomethoxypyridinyl radical in acetonitrile[22], the extinction of the bands at 3950 and 3040 Å are 4700 and 11 300 $M^{-1} \cdot cm^{-1}$, respectively, as compared with $\varepsilon_{4100} = 4300$ and $\varepsilon_{2800} = 10200$ as found here for the 1-methylnicotinamide radical. In view of the similarity between the spectra of the chemically prepared pyridinyl radicals and the transient spectra in the pulse radiolysis of 1-methylnicotinamide, and NAD^+, it is suggested that the latter belong to the corresponding pyridinyl radicals II and III:



II



III

The variation in wavelength maxima and intensities in going from the *N*-alkyl-substituted pyridinyl radicals to NAD· may be due to the influence of the adenine group.

The radicals derived from NAD and 1-methylnicotinamide both react to form products with absorption maxima around 3400–3500 Å. Similar products were found in the low dose-rate irradiations of these solutions[4,7]. In the case of NAD the product was enzymatically inactive and so could not be NADH. It was therefore identified[5,7] as a dimer formed from the interaction of two pyridinyl radicals. The present results are consistent with this view. The data of Figs. 1, 4 and 5 were used to derive the full spectra of the final products of irradiation of NAD and 1-methylnicotinamide as already done for the radical spectra. The derived spectra are shown in Fig. 8.

Since all the radical and product extinction coefficients are now known the rate of dimerisation of the radicals can be deduced from the k/ε values obtained from the kinetic plots. For NAD, the k/ε value for decay at 4000 Å, together with the extinction coefficients of the radical and dimer product at this wavelength, led to a dimerisation rate of $k = 5.15 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$. The data at 3400 Å led to a value of $k = 5.97 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ in reasonable agreement. A mean value of $5.6 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ was taken.

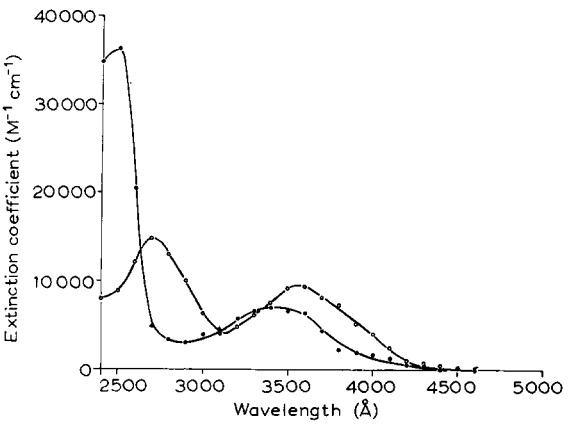


Fig. 8. Absorption spectra of final products of NAD (●—●) and 1-methylnicotinamide (○—○) irradiation.

The kinetic data for 1-methylnicotinamide were treated in the same manner, estimates of $k = 5.8 \cdot 10^7$ and $8.0 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ being obtained for the rate of dimerisation of the 1-methylnicotinamide radical, from the data at 4000 and 3400 Å, respectively. The mean value of $6.9 \cdot 10^7$ $M^{-1} \cdot sec^{-1}$ was taken as the best estimate for the dimerisation rate constant.

REFERENCES

1 B. CHANCE, in M. S. BLOIS, W. H. BROWN, R. M. LEMMON, R. O. LINDBLOM AND M. WEISSBLUTH, *Free Radicals in Biological Systems, Stanford, 1960*, Academic Press, New York, 1961, p. 1.
2 A. J. SWALLOW, *Biochem. J.*, 54 (1953) 253.
3 J. P. KEENE, *Nature*, 188 (1960) 843.
4 A. J. SWALLOW, *Biochem. J.*, 61 (1955) 197.
5 A. J. SWALLOW, in J. S. MITCHELL, B. E. HOLMES AND C. L. SMITH, *Progress in Radiobiology, Cambridge, 1955*, Oliver and Boyd, Edinburgh, 1956, p. 317.
6 G. STEIN AND A. J. SWALLOW, *Nature*, 173 (1954) 937.
7 G. STEIN AND A. J. SWALLOW, *J. Chem. Soc.*, (1958) 306.
8 S. J. LEACH, J. H. BAXENDALE AND M. G. EVANS, *Australian J. Chem.*, 6 (1953) 395.
9 B. KE, *J. Am. Chem. Soc.*, 78 (1956) 3649.
10 Y. PAISS AND G. STEIN, *J. Chem. Soc.*, (1958) 2905.
11 A. J. CUNNINGHAM AND A. L. UNDERWOOD, *Biochemistry*, 6 (1967) 266.
12 J. P. KEENE, E. J. LAND AND A. J. SWALLOW, in M. EBERT, J. P. KEENE, A. J. SWALLOW AND J. H. BAXENDALE, *Pulse Radiolysis, Manchester, 1965*, Academic Press, London, 1965, p. 227.
13 E. J. LAND, *Intern. Radiation Biol.*, 10 (1966) 302.
14 A. J. SWALLOW, *Photochem. Photobiol.*, 7 (1968) 683.
15 J. P. KEENE, *J. Sci. Instr.*, 41 (1964) 493.
16 G. SCHOLES, P. SHAW, R. L. WILLSON AND M. EBERT, in M. EBERT, J. P. KEENE, A. J. SWALLOW AND J. H. BAXENDALE, *Pulse Radiolysis, Manchester, 1965*, Academic Press, London, 1965, p. 151.

17 M. EBERT AND A. J. SWALLOW, in R. F. GOULD, *Solvated Electron, Atlantic City, 1965*, Am. Chem. Soc. Washington, D.C., 1965, p. 289.
18 G. SCHOLES AND M. SIMIC, *J. Phys. Chem.*, 68 (1964) 1731.
19 G. WEBER, *Nature*, 180 (1957) 1409.
20 O. JARDETZKY AND N. G. WADE-JARDETZKY, *J. Biol. Chem.*, 241 (1966) 85.
21 L. M. DORFMAN AND M. S. MATHESON, in G. PORTER, *Progress in Reaction Kinetics*, Vol. 3, Pergamon, Oxford, 1965, p. 237.
22 E. M. KOSOWER AND E. J. POZIOMEK, *J. Am. Chem. Soc.*, 86 (1964) 5515.
23 M. ITOH AND S. NAGAKURA, *Tetrahedron Letters*, (1965) 417.