52. *Chemical Action of Ionising Radiations in Solution. Part XIX.* Some Aspects of the Reduction of Methylene-blue by X-Rays in Aqueous Systems.*

By E. Hayon, G. Scholes, and J. Weiss.

Aqueous solutions of methylene-blue containing an excess of a second organic solute (*e.g.*, ethanol, lactate, formate, benzoate, DL-alanine, phenyl-β-alanine) have been irradiated with *X*-rays (200 kv) in the absence of oxygen. The extent of decolorisation of the dye, and the yields of hydrogen and of the oxidation products from the added solute, have been determined, and, in selected systems, the effects of pH and of concentration of solute. The results have been interpreted on the basis of reactions with the free radicals (H, OH) produced from the water. Methylene-blue (Mb) can be reduced (*via* the semiquinone radical) by hydrogen atoms and also by radicals (RH·) produced from the added organic compounds (RH_2). Further, even in the presence of methylene-blue (10^{-4}M), hydrogen atoms dehydrogenate some of the organic solutes (*e.g.*, ethanol, lactate, formate) : $RH_2 + H \longrightarrow RH\cdot + H_2$.

It has been known for some time that aqueous solutions of a number of dyes (*e.g.*, methylene-blue,[1,2] 2 : 6-dichlorophenol-indophenol,[3] chlorophenol-red [4]) are decolorised by ionising radiations. When methylene-blue is irradiated in oxygen-free solutions,[2] most

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* Part XVIII, *J.*, 1957, 235.

[1] Clark, Pickett, and Johnson, *Radiology*, 1930, **15**, 245; Stenstrom and Lohmann, *ibid.*, 1931, **16**, 332; Shekhtman, Krasnovskii, and Vereshchinskii, *Doklady Akad. Nauk S.S.S.R.*, 1950, **74**, 767.

[2] Seitz, *Strahlentherapie*, 1938, **61**, 140; Zimmer, *Naturwiss.*, 1944, **32**, 375; Day and Stein, *Nature*, 1950, **166**, 146; *Nucleonics*, 1951, **8**, II, 34.

[3] Gallico and Camerino, *Experientia*, 1948, **4**, 109.

[4] Weber and Schuler, *J. Amer. Chem. Soc.*, 1952, **74**, 4415.

of the decolorisation can be reversed on admission of oxygen, and this has been taken as indicating radiation-induced formation of *leuco*methylene-blue. Collinson [5] has, in fact, obtained the spectrum of the *leuco*-dye on irradiation of methylene-blue solutions *in vacuo*. It has been assumed that the *leuco*-base is formed by reduction by hydrogen atoms and that irreversible decolorisation can occur by oxidation of the dye by hydroxyl radicals, in accord with the recognised processes,[6, 7] $H_2O \rightsquigarrow H + OH$, and (" molecular yield process ") $2H_2O \longrightarrow H_2 + H_2O_2$.

Day and also Stein have shown [8] that presence of an excess of a substance which will accept OH radicals, *e.g.*, benzoate, ethanol or succinate, during irradiation of methylene-blue solutions leads to a decrease in the extent of irreversible decolorisation and an increase in the yield of *leuco*-base. This has also been observed by Daniels [9] who, in addition, has investigated several other second solutes, *e.g.*, alanine, serine, glycerol, and formate. Unfortunately, there has been some discordancy in the reported values of the yields in the various systems.

It seemed of interest, therefore, to investigate, in addition, the yields of the gaseous products and of the oxidation products. Below are presented some of our results, obtained with ethanol, lactate, benzoate, formate, DL-alanine, and phenyl-β-alanine as added solutes.

RESULTS

In the following experiments, unless otherwise stated, 10^{-4}M-methylene-blue solutions were used. The added solutes were present in a concentration of 10^{-1}M, this being considered sufficient to use up practically all the OH radicals produced by irradiation. The pH of the (unbuffered) solutions was, in all cases, between pH 4·5 and 5·0. Irradiations were carried out with *X*-rays (200 kv), *in vacuo*, in a vessel to which was sealed on a 1 cm. optical cell; in this way, the optical densities and spectra of the evacuated solutions could be measured directly and the decolorisation could be determined as a function of radiation dose. For spectrophotometric purposes, the corresponding unirradiated methylene-blue solutions were used as blanks. This procedure gave a " difference " spectrum of the methylene-blue, which exhibited maxima at 596 and 690 mμ (cf. Fig. 1, curve *a*). These two wavelengths were used throughout for the quantitative estimation of the dye. For each system, calibration curves were constructed at both these maxima.

On irradiation of methylene-blue solutions containing added solutes, it was found, in most cases, that the estimate of total decolorisation varied slightly according to which of the two maxima was used for its assessment. The greatest differences were observed for lactic and benzoic acid, when these amounted to about 5% and 15% respectively. Over the dose range employed, *viz.*, up to about 2×10^{-6} ev/*N* per ml., the yield–dose plots were in all cases linear, *i.e.*, the initial yields were maintained. Fig. 2, for example, shows typical yield–dose plots measured at 596 and at 690 mμ, in this case for the 10^{-1}M-sodium benzoate–10^{-4}M-methylene-blue system. In Table 1 are given the initial yields, expressed as *G* values (molecules/100 ev), of the total decolorisation observed in several systems. In all these cases, the " difference " spectra of the irradiated solutions were measured *in vacuo*. The absorption maxima were identical with those given in Fig. 1 for pure methylene-blue solutions, showing that we were actually determining methylene-blue itself throughout the whole period of irradiation.

In the case of benzoate, if the commercial sodium salt was used, high total decolorisation values ($G \sim 7$) were obtained in the initial stages of the reaction (cf. Daniels [9]). The value quoted in Table 1 ($G \sim 3·5$) refers to solutions made up from " AnalaR " benzoic acid and " AnalaR " sodium hydroxide. From an experimental point of view, it is of interest that with the benzoate–methylene-blue system, there is a slow change in the absorption spectrum of the solutions at room temperature. This occurs both in the blank and in the irradiated solutions and consists of a change of the absorption spectrum, with a maximum at 580 mμ (measured in a 5 mm. cell against a water control).

[5] Collinson, *Discuss. Faraday Soc.*, 1952, **12**, 285.

[6] Weiss, *Nature*, 1944, **153**, 748; *Brit. J. Radiol.*, 1947, Suppl. 1, 56.

[7] Allen, *Ann. Rev. Phys. Chem.*, 1952, **3**, 57.

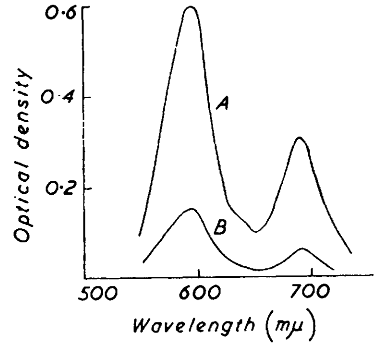
[8] Day, *Discuss. Faraday Soc.*, 1952, **12**, 280; Stein, *J. Chim. phys.*, 1955, **52**, 634.

[9] Daniels, *J. Chim. phys.*, 1955, **52**, 638.

Also included in Table 1 are some *G* values for the total decolorisation of methylene-blue on irradiation of aqueous 10^{-4}M-solutions *in vacuo* at pH 4·5—5·0. Here, however, measurements of the decolorisation at the two absorption maxima gave values which varied by as much as about 40%. Determination of the " difference " spectra of the irradiated solutions *in vacuo* showed that there was no change in the positions of these maxima. There was, however, a marked difference in the ratios of the extinction coefficients at the two peaks.

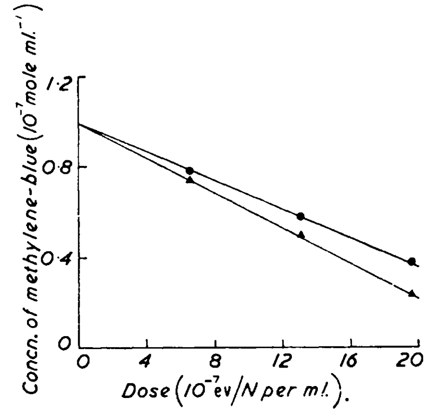
After irradiation, the solutions were equilibrated with air and sufficient time allowed for complete autoxidation of the *leuco*-base (at pH 4·5—5·0, with frequent shaking, 20 min. were usually sufficient). The extent of *leuco*-base formation (reversibility) could then be determined at both wavelengths. The results obtained are shown in Table 1 (columns 5 and 6). It can be seen that, in the presence of the various solutes (except for alanine), the production of *leuco*-base accounts for not less than about 94% of the total decolorisation. A typical " difference " spectrum of the irreversible change in the presence of solute, *e.g.*, ethanol, is given in Fig. 1, curve *B*. Since this is similar to the " difference " spectrum of pure methylene-blue, we may conclude that this irreversible change is a real loss of methylene-blue, due, probably, to destruction of the conjugated system of double bonds of the dye molecule.

FIG. 1. " *Difference* " *spectra of methylene-blue solutions* (1 *cm. cell*).



A, 8 × 10^{-5}M-*Methylene-blue versus* 1 × 10^{-4}M-*methylene-blue as control.*
B, Spectrum of the irreversible decolorisation in 10^{-4}M-*methylene-blue*–10^{-1}M-*ethanol, irradiated with a total dose of* 1·95 × 10^{-6} ev/*N per ml. at* pH 4·5—5·0. *Solutions measured against unirradiated blank.*

FIG. 2. *Typical yield–dose plots of the extent of total decolorisation of methylene-blue solutions containing an added second organic solute.*



10^{-4}M-*Methylene-blue*–10^{-1}M-*sodium benzoate irradiated with* X-*rays* (200 kv) in vacuo *at* pH 4·5—5·0.

● *Measured at* 596 mμ.
▼ *Measured at* 690 mμ.

In each system, the degree of irreversible change was also determined by spectrophotometric

TABLE 1. *Yields* (*G values*) *of reversible and irreversible decolorisation on irradiation of aqueous solutions of methylene-blue* (10^{-4}M) *in the presence of added solutes* (10^{-1}M) *with* X-*rays* (200 kv), in vacuo. pH 4·5—5·0.

	Total decolor. of methylene-blue at			Extent of reversibility at			Extent of irreversibility
Added solute	596 mμ	690 mμ	Mean *	596 mμ	690 mμ	Mean	Mean
Ethanol	3·03	3·03	3·03	2·85	2·85	2·85	0·18
Benzoate	3·25	3·83	3·54	3·09	3·64	3·36	0·18
Lactate	3·03	3·23	3·13	2·97	3·09	3·03	0·10
Formate	3·28	3·38	3·33	3·21	3·31	3·26	0·07
DL-Alanine	2·72	2·62	2·67	2·39	2·31	2·35	0·32
Phenyl-β-alanine	3·12	3·12	3·12	3·00	3·06	3·03	0·09
No solute added (10^{-4}M-dye)	1·12	1·81	—	—	—	—	—

* In all cases where the mean is given the values at the two different absorption peaks differ only by a few per cent.

measurements against a water control, in 5 mm. cells; the values thus obtained compared well with those observed with the 1 cm. cell. Fig. 3 shows some typical spectra, *e.g.*, of the ethanol–methylene-blue system, with water as control. Under these conditions, the absorption maxima were at 615 and at 670 mμ, in the unirradiated as well as in the re-oxidised irradiated solutions, in agreement with the reported absorption peaks of methylene-blue solutions.[10]

With pure methylene-blue solutions, although the extent of total decolorisation was reproducible, considerable irreproducibility was observed in the results obtained *after* the introduction of atmospheric oxygen. It was not possible, therefore, to determine the relative extent of reversible and irreversible change in this system. That some irreversible (oxidative) damage to the dye had indeed taken place, under these conditions, was apparent from the observation of a positive reaction for carbonyl compounds (on treatment of the irradiated methylene-blue solutions with 2 : 4-dinitrophenylhydrazine); in terms of acetaldehyde, the yield of carbonyl compound amounted to G 0·4—0·5.

The yields of hydrogen, on irradiation of these various systems, are given in Table 2. In the case of pure 10^{-4}M-methylene-blue the amounts of hydrogen produced, $G(H_2) \sim 1$, are greater than the " molecular " yield ($G \sim 0{\cdot}5$) and indicate that, at this dye concentration, there is some recombination of hydrogen atoms. This view is supported by the observation that increasing the methylene-blue concentration can lower the yield of hydrogen; in 10^{-2}M-solutions, for example, $G(H_2)$ is reduced to values of the order of the molecular yield (cf. Table 2).

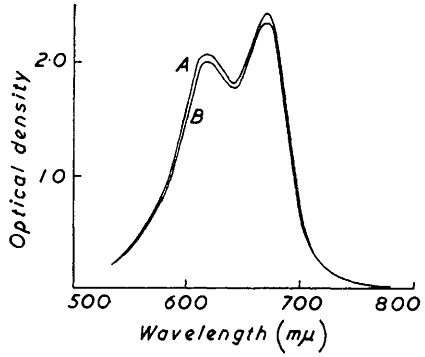


FIG. 3. *Spectra of methylene-blue* (10^{-4}M)*–ethanol* (10^{-1}M) (*water control, 5 mm. cell*). pH 4·5—5·0.
A, Unirradiated solution.
B, Irradiated solution, after complete re-oxidation in air (*total dose* 1·95 × 10^{-6} ev/*N per ml.*).

It has been found that the hydrogen yields on irradiation of 10^{-4}M-methylene-blue containing certain solutes, *e.g.*, formate, lactate, ethanol, were higher than those observed on irradiation of 10^{-4}M-solutions of pure methylene-blue. These results seem to indicate that hydrogen atoms can dehydrogenate these particular solutes, even in the presence of methylene-blue. The question of dehydrogenation of organic compounds by hydrogen atoms is of general interest from the point of view of the mechanism of the action of ionising radiations in aqueous solutions. We have, therefore, investigated, in more detail, the stoicheiometry of selected systems under various experimental conditions. In addition, the yields of the oxidation products of the added solutes have been determined. In these experiments, special attention has been given to ethanol and lactic acid, the main oxidation products of which are acetaldehyde [11] and pyruvic acid [12] respectively.

TABLE 2. *Yields of hydrogen* (G-*values*) *on irradiation of aqueous solutions of pure methylene-blue and of methylene-blue* (10^{-4}M) *in the presence of added solutes* (10^{-1}M) *with* X-*ray* (200 kv) in vacuo. pH 4·5—5·0. Total dose, 1·95 × 10^{-6} ev/*N* per ml. Mb = methylene-blue.

Solution	H_2	Solution	H_2	Solution	H_2	Solution	H_2
Ethanol + Mb	2·30	Benzoate + Mb...	0·64	Phenyl-β-Alanine + Mb	0·88	Mb (10^{-4}M)	1·01
Lactate + Mb	1·29	DL-Alanine + Mb	0·84	Benzene • + Mb ...	0·69	Mb (2 × 10^{-2}M)	0·62
Formate + Mb	1·90						

• Saturated benzene–water solutions.

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[10] Michaelis and Granick, *J. Amer. Chem. Soc.*, 1945, **67**, 1212.
[11] Jayson, 1955, Ph.D. Thesis, Durham.
[12] Johnson, Scholes, and Weiss, *J.*, 1953, 3091.

The effects of pH on the yields of acetaldehyde, hydrogen, and *leuco*methylene-blue on irradiation of 10^{-1}M-ethanol–10^{-4}M-methylene-blue (Fig. 4) show marked pH-dependence: in all cases the yields were highest in acid solution. Throughout the whole range of pH investigated, the extent of irreversibility was small, about 5% of the total decolorisation. A somewhat similar pH-dependence of the yields was also observed in the irradiation of 10^{-4}M-methylene-blue–10^{-1}M-lactic acid (see Table 3). It seems, therefore, that dehydrogenation by hydrogen atoms occurs in these systems at all pH's over the range pH ~1—10; the extent of this process, however, falls off with increasing pH.

TABLE 3. *Irradiation of aqueous solutions of methylene-blue* (10^{-4}M) *in the presence of lactic acid* (10^{-1}M) *with* X-*rays* (200 kv) in vacuo. pH-*Dependence of the yields of hydrogen and pyruvic acid, and of the extents of reversible and irreversible decolorisations* (G-*values*). *Total dose* 1·95 × 10^{-6} ev/*N per ml.*

pH	H_2	Pyruvic acid	Extent of reversibility at 596 mμ	690 mμ	Mean	Extent of irreversibility at 596 mμ	690 mμ	Mean
1·9	2·96	4·46	3·11	3·15	3·13	0·16	0·20	0·18
5·0	1·36	4·20	2·95	3·15	3·05	0·08	0·08	0·08
9·9	1·10	2·50	2·74	2·84	2·79	0·08	0·03	0·06

TABLE 4. *Irradiation of aqueous solutions of methylene-blue* (10^{-4}M) *in the presence of ethanol with* X-*rays* (200 kv) in vacuo. *Effect of ethanol concentration on the yields of hydrogen and acetaldehyde and on the extents of reversible and irreversible decolorisations* (G-*values*). *Solutions irradiated at* pH 1·8 *and* 4·5. *Total dose* 1·95 × 10^{-6} ev/*N per ml.*

Ethanol (mole/l.)	H_2 pH: 1·8	4·5	Acetaldehyde 1·8	4·5	Reversibility * 1·8	4·5	Irreversibility * 1·8	4·5
10^{-1}	3·66	2·30	5·07	3·60	3·70	2·85	0·11	0·15
10^{-2}	1·80	1·59	3·20	2·68	3·10	2·72	0·15	0·11
10^{-3}	1·0	0·90	1·90	1·90	3·00	2·28	0·12	0·16

* Mean of values at 596 and 690 mμ.

TABLE 5. pH-*Dependence of the yields of hydrogen and of the extent of total decolorisation* (G-*values*) *on irradiation of aqueous solutions of methylene-blue* (10^{-4}M) *with* X-*rays* (200 kv) in vacuo. *Total dose* 1·95 × 10^{-6} ev/*N per ml.*

pH	1·9	4·5	10·0
Total decolorn. at 596 mμ	1·29	1·12	1·10
,, ,, 690 mμ	2·03	1·81	2·05
H_2	0·81	1·01	0·80

The dependence of the yield on the concentration of the added solute has also been investigated. Table 4 shows some results obtained with ethanol. It was found that the yields of all the products showed a concentration-dependence, this being greatest in the acid solutions.

The effect of pH on the total decolorisation yields in pure 10^{-4}M-methylene-blue was also investigated. The results obtained (Table 5) show that, in this case, the total decolorisation of methylene-blue and the hydrogen yields are almost independent of pH.

DISCUSSION

The chemical action of ionising radiations in aqueous systems can be ascribed to reactions of hydrogen atoms and of hydroxyl radicals produced from the water.[6] Reduction of methylene-blue (Mb) by hydrogen atoms proceeds through the semiquinone radical (MbH) as follows:

$$\mathrm{Mb} + \mathrm{H} \longrightarrow \mathrm{MbH} \qquad (1)$$

$$\mathrm{MbH} + \mathrm{H} \longrightarrow \mathrm{MbH_2} \qquad (2a)$$

$$2\mathrm{MbH} \rightleftharpoons \mathrm{Mb} + \mathrm{MbH_2} \qquad (2b)$$

Evidence for the intermediary rôle of semiquinone radicals in oxidation–reduction reactions of methylene-blue and other dyes has been discussed in some detail by Michaelis and his co-workers.[13]

In the irradiation of solutions containing methylene-blue only, possible reactions of the OH radicals are as follows:

Back-reactions occur with the semiquinone radicals:

$$\mathrm{MbH + OH \longrightarrow Mb + H_2O} \qquad (3a)$$

or with the *leuco*-base:

$$\mathrm{MbH_2 + OH \longrightarrow MbH + H_2O} \qquad (3b)$$

Reactions (3*a* and *b*) will become predominant at higher doses of radiation, *i.e.*, when the concentrations of the reduction products have built up.

Further, oxidation of the dye may or may not lead to an irreversible decolorisation, *viz.*:

$$\mathrm{Mb + OH \longrightarrow Oxidation\ products} \qquad (4)$$

OH-radical attack on the ring systems of the dye molecule would presumably consist of a hydroxylation, with the production of phenols. It seems, however, that an irreversible decolorisation, *i.e.*, destruction of the conjugation, by this type of reaction would require multiple OH-radical attack, since a monohydroxylated methylene-blue should still exhibit light absorption in the visible region. The nature of the oxidation processes leading to the irreversible decolorisation is unknown; in this respect, it is of interest that on irradiation of pure methylene-blue solutions a positive reaction for carbonyl compounds was observed.

We may now consider the effects of the presence of a suitable OH-radical acceptor in the irradiation of methylene-blue solutions. If all the OH radicals go to the acceptor (RH_2), according to:

$$\mathrm{RH_2 + OH \longrightarrow RH\cdot + H_2O} \qquad (5)$$

and the methylene-blue is attacked only by hydrogen atoms, then no irreversible decolorisation should take place. Further, if the *leuco*-base is formed only by reactions with hydrogen atoms (*viz.*, reactions 1, 2*a*, 2*b*), the yield of this compound should be equivalent to half the total number of available hydrogen atoms produced in the system. If for dosimetric purposes [14] we take $G(\mathrm{Fe^{3+}}) = 15{\cdot}5$, the maximum yield of hydrogen atoms produced from water according to the primary process should be $G(\mathrm{H}) \simeq 3{\cdot}6$. Therefore, G(*leuco*-base) should not be greater than $\sim 1{\cdot}8$. It has been found, however (Table 1, column 7), that the yields of *leuco*-base formed on irradiation of methylene-blue solutions containing different OH-radical acceptors are, in general, well above this value. Hence, we may conclude that other reductive processes must take place in these systems. It is reasonable to suppose that this involves reaction of the organic radicals (RH·) with methylene-blue and possibly also with the semiquinone radical, *viz.* (R = stable oxidation product):

$$\mathrm{Mb + RH\cdot \longrightarrow MbH + R} \qquad (6a)$$

$$\mathrm{MbH + RH\cdot \longrightarrow MbH_2 + R} \qquad (6b)$$

In most of the systems investigated, there was almost complete reversibility (in the region of 94% or more of the total decolorisation) indicating that, in fact, the majority of the OH radicals were consumed in reaction (5). With alanine as added solute, however, the extent of reversibility was noticeably lower (~88%); this seems to indicate that, under these conditions, methylene-blue is competing with alanine for the available OH radicals. It is worth noting that the introduction of a phenyl group, which is known to be a good OH-radical acceptor, into the alanine molecule (*viz.*, phenyl-β-alanine) somewhat lowers

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[13] Cf. Michaelis, Schubert, and Granick, *J. Amer. Chem. Soc.*, 1940, **62**, 204.
[14] Farmer, Rigg, and Weiss, *J.*, 1955, 582.

this degree of irreversiblity. This is in conformity with the idea that irreversible oxidation of methylene-blue is connected with OH-radical attack on the dye molecule.

If all the available hydrogen atoms go to the methylene-blue, the yields of hydrogen gas should correspond to the " molecular " yield. In pure 10^{-4}M-methylene-blue, $G(H_2)$ was about 1·0 and this value was, more or less, independent of the pH of the solution (Table 5). Even though methylene-blue is readily reduced, it is apparent from these results that, at this relatively low dye concentration, some recombination of the primarily formed hydrogen atoms must be taking place, *viz.* :

$$H + H \longrightarrow H_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

This is supported by the fact (cf. Table 2) that when the methylene-blue concentration is increased, $G(H_2)$ falls to a value of the order of the molecular yield.

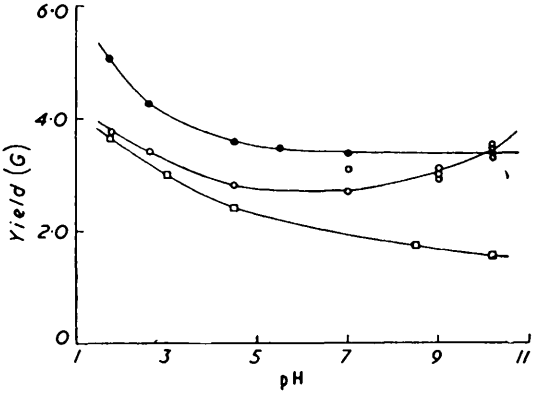

FIG. 4. pH-*Dependence of the yields of hydrogen, acetaldehyde and* leuco*methylene-blue, on irradiation of aqueous solutions of methylene-blue* (10^{-4}M)–*ethanol* (10^{-1}M) *with* X-*rays* (200 kv) in vacuo. *Total dose* 1·95 × 10^{-6} ev/*N per ml.*

○ leuco*Methylene-blue.*
● *Acetaldehyde.*
□ *Hydrogen.*

In the presence of some of the solutes, on the other hand, relatively high yields of hydrogen were observed. This can only be explained if one assumes that the added solute can compete with methylene-blue for hydrogen atoms, by a dehydrogenation process of the type :

$$RH_2 + H \longrightarrow RH\cdot + H_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

Some evidence for the dehydrogenation of simple organic compounds by radiation-produced hydrogen atoms in aqueous systems has also been provided by Hart [15] for formic acid, and by Garrison *et al.*[16] for acetic acid solutions.

In the experiments with benzene and benzoate, the hydrogen yields were of the order of the " molecular " yield, *i.e.*, lower than those obtained by irradiation of pure 10^{-4}M-methylene-blue. We may therefore conclude that the reactions of hydrogen atoms with benzene or with benzoate do not proceed by a dehydrogenation process. This, in fact, is not unexpected, since hydrogen atoms can react with the benzenoid system by an addition mechanism.[17] In this connection it has been found that in the irradiation of *pure* aqueous benzene solutions *in vacuo*, again only the " molecular yield " of hydrogen is produced; [18, 19] this is explained by the observation that, under these conditions, *cyclo*hexadiene is formed, showing that the hydrogen atoms saturate the benzene ring.

The hydrogen yields in the solutions containing alanine and phenyl-β-alanine are also low (cf. Table 2); hence, in these systems too, dehydrogenation by hydrogen atoms is of no importance.

The effect of pH on the yields of hydrogen (Fig. 4, Table 3) is rather striking, particularly

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[15] Hart, *J. Phys. Chem.*, 1952, **56**, 594.
[16] Garrison, Bennett, Cole, Haymond, and Weeks, *J. Amer. Chem. Soc.*, 1955, **77**, 2720.
[17] Cf. Augood, Hey, Nechvatal, Robinson, and Williams, *Research*, 1951, **4**, 356.
[18] Stein and Weiss, *J.*, 1949, 3245.
[19] Milling and Weiss, A.E.R.E. Report, 1952.

in the more acid solutions. Under acid conditions, hydrogen atoms are present, to a greater or smaller extent, in the form of H_2^+ radical-ions, according to the equilibrium process:[20]

$$H + H^+ \rightleftharpoons H_2^+ \qquad (9)$$

Since H_2^+ is an effective oxidising species, it is possible that, at low pH, oxidation of the solute according to:

$$RH_2 + H_2^+ \longrightarrow RH\cdot + H^+ + H_2 \qquad (10)$$

may be more favourable than other reactions involving the hydrogen atoms, *e.g.*, reactions (1) and (8). It is, of course, apparent from the results that at higher pH (even up to pH 10) dehydrogenation according to reaction (8) must still be taking place.

The yields of hydrogen depend markedly on the solute concentration, as shown for ethanol in Table 4. At relatively low ethanol concentrations (10^{-3}M), both in acid and in neutral solutions, $G(H_2)$ was about the same as that observed on irradiation of pure 10^{-4}M-methylene-blue. Hence, at this low dye concentration, the hydrogen atoms react predominantly according to processes (1) and (7). The finding that $G(H_2)$ increases with increasing ethanol concentration, particularly in acid solution, supports our general conclusions on the dehydrogenation of ethanol by H atoms (reaction 8) and by H_2^+ ions (reaction 10). As a result of these reactions, the yields of acetaldehyde should follow a trend similar to the yields of hydrogen, with regard to the change both of concentration and of pH; this has been confirmed by experiment (cf. Fig. 4 and Table 4).

On the basis of the above discussion, we can account for the stoicheiometry of the reaction processes on irradiation of solutions of 10^{-1}M-ethanol–10^{-4}M-methylene-blue in neutral solutions as follows: in addition to reactions (1), (2*a*), and (4) we can have, in the presence of ethanol, the following processes:

$$OH + CH_3\cdot CH_2\cdot OH \longrightarrow CH_3\cdot\dot{C}H\cdot OH + H_2O \qquad (11)$$

$$H + CH_3\cdot CH_2\cdot OH \longrightarrow CH_3\cdot\dot{C}H\cdot OH + H_2 \qquad (12)$$

$$Mb + CH_3\cdot\dot{C}H\cdot OH \longrightarrow MbH + CH_3\cdot CHO \qquad (13a)$$

$$MbH + CH_3\cdot\dot{C}H\cdot OH \longrightarrow MbH_2 + CH_3\cdot CHO \qquad (13b)$$

The experimental values of the yields at pH 4·5 (unbuffered solutions) were $G(MbH_2) = 2{\cdot}85$, $G(H_2) = 2{\cdot}3$, $G(CH_3\cdot CHO) = 3{\cdot}6$. If we take maximum yields of $G(H) = G(OH) = 3{\cdot}6$ and of $G(Mb) = 0{\cdot}6$ (from the minimum hydrogen yields obtained, *e.g.*, in the presence of benzoate), then, if the G values of reactions (11), (12), (13*a* plus *b*) and of reaction (1 plus 2*a*) are 1·9, 1·7, 3·6, and 1·9 respectively, this would lead to the calculated yields $G(MbH_2) = 2{\cdot}75$, $G(H_2) = 2{\cdot}3$, $G(CH_3\cdot CHO) = 3{\cdot}6$, which agree well with the observed values given above.

This mechanism accounts for all the available hydrogen atoms, but not for all the hydroxyl radicals, as only about one half of the latter are used up according to reactions (11) and (13). The extent of irreversible decolorisation of methylene-blue in this system is small and could not possibly account for this OH-radical deficiency. On the other hand, since it has been shown[11] that irradiation of pure aqueous ethanol with *X*-rays *in vacuo* leads to the formation of some butane-2 : 3-diol, *viz.*:

$$2CH_3\cdot\dot{C}H\cdot OH \longrightarrow (CH_3\cdot CH\cdot OH)_2 \qquad (14)$$

it seemed to us, that, if this reaction also occurred in the methylene-blue–ethanol system, the above OH-radical deficiency could be acounted for. Butane-2 : 3-diol has, in fact, been detected in the irradiated methylene-blue–ethanol solutions (see Experimental section), in the yields of the expected order [G(diol) $\sim 0{\cdot}5$].

With regard to the intermediary rôle of the semiquinone radical in the reaction

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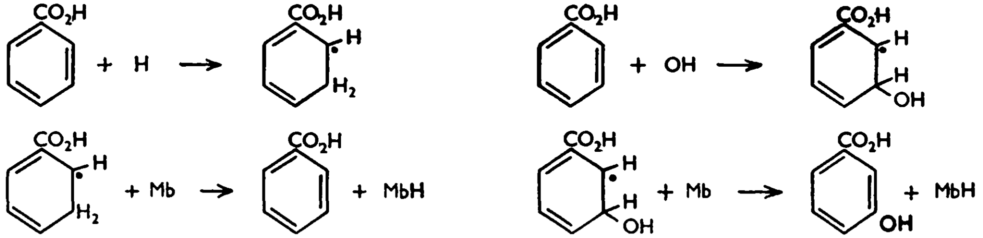
[20] Weiss, *Nature*, 1950, **165**, 728.

mechanism, some recent experiments by Swallow[21] are of considerable interest. He irradiated methylene-blue in the presence of excess of ethanol in strongly acid solutions (~25N-sulphuric acid) and found the ultraviolet absorption spectrum of the semiquinone radical, stated by Michaelis *et al.* to be stable under these conditions.[13]

The above sequence of reactions (11—13*b*) also accounts quantitatively for the observations concerning the dependence of the yield on ethanol concentration in solutions irradiated at pH 4·5—5·0 (cf. Table 4).

Although the overall stoicheiometry of the other methylene-blue–solute systems has not been investigated in such detail, it appears that such a mechanism would, in several instances (*e.g.*, lactate, formate), explain the results obtained in neutral solution. The high decolorisation yields in the benzoate–methylene-blue solutions can be accounted for only if we assume that all the available hydroxyl radicals and hydrogen atoms lead eventually to a reduction of the dye. In this case, reaction of hydrogen atoms and of hydroxyl radicals with the benzene ring can effect the reduction of methylene-blue according to the reactions annexed.



The effect of pH on the radiation yields is rather more complicated and it is difficult to account for the stoicheiometry at the extreme pH values. Among other things, one has to consider the rôle played by the H_2^+ ions, as discussed above. Since the total decolorisation of pure solutions of methylene-blue is independent of pH (Table 5), it seems that it is the presence of the added solutes which gives rise to these particular pH effects.

EXPERIMENTAL

Irradiations.—The source of *X*-rays was a Victor Maximar therapy tube operating at 200 kv and 15 mA. The solutions (100 ml.) were irradiated in a Pyrex-glass vessel to which a 1 cm. optical " Spekker " cell was sealed on (see Fig. 5). The absorption cell was protected during irradiation by a lead sheath. The dose rate was $3{\cdot}25 \times 10^{-7}$ ev/*N* per ml. per min., as measured by the ferrous sulphate dosimeter, (GFe^{3+}) being taken [14] as 15·5.

Evacuation of the solutions was effected by pumping out with a mercury diffusion pump backed by a two-stage oil-pump; the pressure of residual gas amounted to $\ngtr 4 \times 10^{-3}$ mm. Hg. It was important to ensure practically *complete* removal of oxygen before irradiation, otherwise errors due to some re-oxidation of the *leuco*-base were observed.

Triply distilled water was used throughout, obtained by distilling ordinary distilled water from potassium permanganate and then from sulphuric acid.

All materials used were of " AnalaR " grade, except methylene-blue chloride and phenyl-β-alanine (Hopkin and Williams Ltd.) and also DL-alanine (Roche Products Ltd.). Sodium lactate, formate, and benzoate were prepared from the respective " AnalaR " acids and " AnalaR " sodium hydroxide. In the case of lactic acid, distillation from 2 : 4-dinitrophenylhydrazine (to remove any pyruvic acid) was followed by refluxing in order to hydrolyse any anhydrides present.

Reproducibility depended on the method of cleaning the irradiation vessels. " AnalaR " concentrated nitric acid was found to be very satisfactory for this purpose and the vessels were cleaned before each irradiation.

A stock methylene-blue solution (10^{-3}M) was used and found to be quite stable when stored in Pyrex-glass in the dark.[22] All methylene-blue solutions containing the added solutes were prepared immediately before irradiation.

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[21] Swallow, personal communication.

[22] Rabinowitch and Epstein, *J. Amer. Chem. Soc.*, 1941, **63**, 69.

Purified samples of methylene-blue were kindly provided by Mr. M. J. Day and Dr. J. Swallow. They gave results identical with those obtained by using the Hopkin and Williams product.

Adjustments of pH, where necessary, were carried out with sulphuric acid or with sodium hydroxide. The use of buffers was avoided.

Determinations of Acetaldehyde and Pyruvic Acid.—These were based on Friedman and Haugen's method, but the modified form reported by Johnson and Scholes[23] was adopted. Methylene-blue did not interfere in these determinations, which were accurate within about ±3%.

Identification of Butane-2 : 3-diol in Irradiated Ethanol–Methylene-blue Solutions.—Butane-2 : 3-diol was detected by the liberation of acetaldehyde on treatment with periodate.[11] However, methylene-blue itself was found to be oxidised by periodic acid, giving a large yield of aldehyde and, therefore, the dye had to be removed before the oxidation. This was done by passing the solutions (both irradiated and control) twice through a cation-exchange column—Amberlite IRC-50(H), which was reactivated by washing with 2N-hydrochloric acid, followed by distilled water until neutral, then by 2N-sodium hydroxide, and finally by water until again neutral. In the presence of ethanol, hydrogen peroxide interferes in the periodate oxidation (giving acetaldehyde) and correction must be made for this. The peroxide contents of the eluates were determined by the titanium sulphate reagent.[24] (The yields of peroxide measured were of the order of magnitude expected from autoxidation of *leuco*-base in the amounts formed on irradiation.)

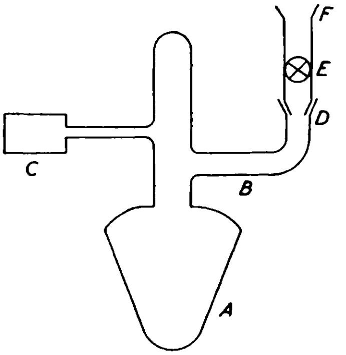

FIG. 5. *Radiation vessel. Solution contained in bulb (A) to which is sealed optical cell (C) and side-arm (B) connected to tap (E) through joint (D). Evacuation effected* via *joint (F).*

The oxidation was carried out by addition of potassium periodate (0·5 g.) and concentrated sulphuric acid (2 ml.) to the eluate (50 ml.). The aldehyde was then steam-distilled *in vacuo* at 40° and the distillate collected in a solution (20 ml.) of 0·25% w/v 2 : 4-dinitrophenylhydrazine in 30% v/v perchloric acid, as used in the aldehyde determination.

Four " runs " were needed to establish the presence of glycol in the irradiated solutions : (*A*) Steam-distillation of the acetaldehyde already formed during irradiation. (*B*) Steam-distillation of unirradiated solutions containing an equivalent amount of hydrogen peroxide as in (*A*). (*C*) Periodate oxidation of the irradiation solution and steam-distillation of total acetaldehyde. (*D*) Periodate oxidation of the unirradiated solution containing an equivalent amount of peroxide to that in (*A*), and steam-distillation. It was found ($D - C$) was greater than ($A - B$), thus confirming the formation of butane-2 : 3-diol on irradiation of methylene-blue–ethanol solutions. This method was only semiquantitative and gave only approximate values for the yields of butane-2 : 3-diol.

Gas Analysis.—After irradiation, the gas was pumped from the vessel, by means of a Töpler pump, through two traps containing solid carbon dioxide–methanol. The volume of gas was first measured on a semimicroburette and then introduced into the analytical part of the apparatus, where the pressure of the gas was measured by a McLeod gauge. Hydrogen was determined by diffusion through a palladium thimble at 700°, and carbon dioxide after replacement of a solid carbon dioxide–methanol trap by one of liquid oxygen.

The results obtained by this procedure were checked, on the same gas sample, by mass spectrometry; agreement within ±1—2% was found between the two methods. (We are

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[23] Johnson and Scholes, *Analyst*, 1954, **79**, 217.

[24] Eisenberg, *Ind. Eng. Chem. Analyt.*, 1943, **15**, 327.

greatly obliged to Mr. P. Kelly who carried out the analyses on the mass spectrometer.) In all experiments 2—6% of nitrogen was found to be present in the gases collected after irradiation.

Solid carbon dioxide–methanol traps rather than liquid-oxygen traps were used for collecting the gas, since it was found that some adsorption of hydrogen occurred in the latter, leading to losses of about 10%.

Spectrophotometric Determination of Methylene-blue.—In preliminary experiments extinctions were measured on a " Spekker " photoelectric absorptiometer, with an Ilford 608 filter. However, these results did not agree with those obtained by measurements at the absorption maxima (Unicam S.P. 500 spectrophotometer). Solutions of methylene-blue do not obey Beer's law in the visible spectral region and this has been attributed to the reversible polymerisation of the dye molecule.[10,22,25] The deviation is more marked when non-monochromatic incident light is used, as for example when using the " Spekker " instrument. In view of this, all determinations were made on the Unicam instrument.

Since the absorption was too strong when the 1 cm. cell was attached to the vessel, a water control could not be used and all measurements were referred to a methylene-blue control. Optical densities were determined at 596 and at 690 mμ, *i.e.*, at the peaks corresponding to the dimeric and the monomeric form of the dye, respectively. For each system, calibration was made at these two wavelengths (which, in all cases, were found to be the absorption maxima) with unirradiated solutions as the respective cell controls. The accuracy of the spectrophotometric determinations was within ±2%. In these experiments, *in vacuo*, there were no noticeable effects due to the hydrogen peroxide present in solution.

The extent of irreversibility was determined, in the same way, after the vessel had been opened to the air. In neutral solutions, the rate of autoxidation was such that, with frequent shaking, about 20 min. were required for completion. Under alkaline conditions, precipitation of the *leuco*-base took place during irradiation; here the rate of autoxidation after opening to the air was rapid. In acid solutions, on the other hand, oxidation of *leuco*-base was much slower and the solutions were set aside overnight for complete oxidation.

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King's College, University of Durham,
Newcastle upon Tyne, 1.

[*Received, July* 11*th*, 1956.]

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[25] Lewis, Goldschmid, Magel, and Bigeleisen, *J. Amer. Chem. Soc.*, 1943, **65**, 1150; Sheppard and Geddes, *ibid.*, 1944, **66**, 2003.