- Levitow, L. & Novelli, G. D. (1954). *J. biol. Chem.* **207**, 761.
- Mollwain, H. & Hughes, D. E. (1944). *Biochem. J.* **38**, 187.
- McRorie, R. A. & Williams, W. L. (1951). *J. Bact.* **61**, 737.
- Nossal, P. M. (1951). *Biochem. J.* **49**, 407.
- Novelli, G. D. (1953). *Physiol. Rev.* **33**, 525.
- Partridge, S. M. & Westall, R. G. (1949). *Biochem. J.* **44**, 418.
- Pierpoint, W. S. & Hughes, D. E. (1952). *2nd Int. Congr. Biochem. Paris*, p. 91.
- Pierpoint, W. S. & Hughes, D. E. (1954). *Biochem. J.* **56**, 130.
- Thompson, R. Q. & Bird, O. D. (1954). *Science*, **120**, 763.
- Wang, T. P. & Kaplan, N. O. (1954). *J. biol. Chem.* **206**, 311.

The Reduction of Diphosphopyridine Nucleotide by Ethanol under the Influence of X-Irradiation

By A. J. SWALLOW*

Department of Radiotherapeutics, University of Cambridge

(Received 28 February 1955)

Two types of mechanism have been suggested to explain dehydrogenase action. On the one hand, it has been postulated that the oxidations and reductions may be univalent in character, with free radicals as intermediates (Waters, 1948; Swallow, 1953, 1954*a*; Barron & Levine, 1952). On the other hand, an ionic mechanism has been proposed (Burton & Kaplan, 1954). Recent work on the reduction of diphosphopyridine nucleotide (DPN) by sodium dithionite (Yarmolinsky & Colowick, 1954; Colowick, 1954; Swallow, 1955) supports the ionic mechanism.

In this paper are presented some further experiments on the reduction of DPN by ethanol under the influence of ionizing radiations, a reaction which is believed to proceed through the agency of free radicals. Some of the results have been briefly reported elsewhere (Stein & Swallow, 1954; Swallow, 1954*b*).

EXPERIMENTAL

Irradiation arrangements. Two Victor Maximar X-ray sets were used. One was operated at 220 kVp and the radiation filtered through 1 mm. aluminium, while the other was operated at 190 kVp with the radiation unfiltered. Doses were determined by the FeSO_4 method (Miller & Wilkinson, 1952), the value for the yield being taken as 15.6 molecules of Fe^{3+} produced/100 eV of energy absorbed (Farmer, Rigg & Weiss, 1955). The dose rate with the 220 kVp set was 640 r/min. and with the 190 kVp set 4000–5000 r/min. The temperature of irradiations was not controlled, and was normally 20–30°. Irradiation vessels were similar to those previously employed (Swallow, 1952), two sizes of tube being used, to take 1 or 3 ml. of solution.

Deoxygenation of solutions was by the method previously described (Swallow, 1952). When 3 ml. of solution

were to be irradiated in the absence of dissolved oxygen, 1 ml. of water + volatile solute was first evaporated off by the deoxygenation procedure, and allowance made for changes in concentration. It was assumed that the rate of evaporation of each constituent was proportional to its vapour pressure and to its mole fraction in the liquid.

Chemicals. Oxidized DPN (DPN^+) was obtained from Sigma Chemical Co., U.S.A. (90% pure) and C. F. Boehringer and Soehne, Germany (100% pure). Reduced DPN (DPNH) was from Sigma Chemical Co. Pig-heart lactic dehydrogenase was kindly given by members of the Biochemistry Department, University of Cambridge. Crystalline yeast alcohol dehydrogenase was obtained from C. F. Boehringer and Soehne. Other chemicals were of A.R. purity.

Determination of hydrogen. This work was done at King's College, Newcastle upon Tyne, by kind permission of Dr J. Weiss. The author wishes to thank Dr R. G. A. Johnson, Mr G. G. Jayson and other members of the radiation chemistry group for help with the apparatus. Irradiations of 100 ml. solution (air-free) were performed with 200 kVp unfiltered X-rays at a dose rate of 3100 r/min. Gas analysis was by the modified method of Stein & Weiss (see Johnson, Scholes & Weiss, 1953) and results were accurate to within 10%.

Ultraviolet absorption curves. The curves shown in Figs. 1 and 2 were obtained by diluting the solution with 2 vol. of water and measuring optical densities with a Unicam SP. 500 spectrophotometer with distilled water as blank. Curves shown in Fig. 3 were on undiluted solutions.

RESULTS

Absorption spectra

The effect of X-irradiation on the absorption curve of oxygen-free aqueous solutions of DPN^+ (0.27 mM) containing ethanol (0.5 M) is shown in Fig. 1. This result was for solutions 0.01 M in sodium pyrophosphate, adjusted to pH 7.8 with HCl. It was found that the effect of a dose of 39 000 r was the same when the buffer was 0.01 M borax–boric acid

* Imperial Chemical Industries Fellow.

at pH 7.8, or 0.01M borax-sodium hydroxide or 0.01M sodium carbonate-sodium bicarbonate at pH 10.4. Attempts were made to irradiate at pH 12, but variable results were obtained. In some experiments there was an increase in optical density at 340 m μ ., but in others only a strong increase at about 280-290 m μ .. As DPN⁺ itself

is unstable in alkaline solution it was not considered desirable to investigate this point further.

The substance absorbing at 340 m μ . is formed by the action of X- or γ -rays on DPN⁺ under the same conditions as DPNH is formed by the action of alcohol dehydrogenase, except that for the formation of the irradiation product it is essential for oxygen to be absent (Swallow, 1953). As DPNH also absorbs at 340 m μ ., it is natural to compare the two products. The full absorption curves of the two are shown in Fig. 2, together with the absorption curve of DPN⁺. The irradiated solution still contains some unchanged DPN⁺, and it is apparent that the absorption curve of the product is not very different from that of DPNH. Fig. 1 shows that the irradiation product changes in a characteristic fashion on prolonged irradiation, and it was also found that 0.27 mM DPNH in oxygen-free aqueous 0.5M ethanol at pH 7.8 changed in the same way on X-irradiation with a dose of 117 000 r.

Fig. 1. Absorption curves of oxygen-free DPN solutions irradiated with X-rays in 0.5M ethanol, pH 7.8. +, No irradiation; x, dose, 39 000 r; O, dose, 78 000 r; ●, dose, 156 000 r.

Effect of adding acid to irradiated solutions

It is known that when DPNH solutions are made acid, the absorption maximum at 340 m μ . disappears and is replaced by an increased ab-

Fig. 2. Comparison of irradiation product with DPNH and DPN⁺. x, Absorption curve of 0.27 mM DPN⁺; +, 0.27 mM DPN, oxygen-free, in 0.5M ethanol, irradiated with a dose of 39 000 r; O, 0.27 mM DPNH.

Fig. 3. Effect of adding dilute acid to reduced DPN solutions. +, DPNH, pH 7.8; x, DPNH after acidification; O, irradiated solution, pH 7.8; ●, irradiated solution after acidification.

sorption at 295 $m\mu$. (Haas, 1936). Irradiated solution (3 ml.; in 0.01M pyrophosphate buffer, pH 7.8) was acidified with 0.1 ml. N-HCl. DPNH solution (3 ml.) with the same initial optical density at 340 $m\mu$. was similarly treated. The changes in absorption spectrum are recorded in Fig. 3. Addition of 0.1 ml. 10N- instead of N-HCl gave a similar change initially, but this was followed by a fairly rapid decrease in absorption at 290 $m\mu$. in both cases, which made measurement of the absorption curve difficult. Apparently the substances which absorb at 290 $m\mu$. are themselves attacked in strongly acid solutions.

Reduction of methylene blue

An important property of DPNH is that of reducing methylene blue to the colourless leuco form. Diaphorase increases the rate of decolorization, but was not used in the present experiments. 0.27 mM DPN⁺ (3 ml.; pH 7.8) in oxygen-free 0.5M aqueous, ethanol was irradiated with an X-ray dose of 39 000 r. A stream of nitrogen was then bubbled through at 23° for 20 min. to remove acetaldehyde. It was found that this solution would decolorize 0.05 ml. of mM methylene blue in 18 min. In a control experiment it was found that 1 ml. of 0.27 mM DPNH at pH 7.8 in 0.5M ethanol would decolorize the same amount of methylene blue in 20 min.

Fluorescence

DPNH, in common with many other nicotinamide derivatives, has the property of fluorescing in the visible region of the spectrum under illumination with Wood's light (Sumner & Somers, 1947). It was found that 0.1 mM DPNH in aqueous 0.5M ethanol at pH 7.8 exhibited an intense pale-green fluorescence, but a 0.1 mM DPN⁺ in aqueous ethanol did not fluoresce. Solutions of the irradiation product, whether prepared at pH 7.8 or 10.2, exhibited only a barely detectable fluorescence. The lack of fluorescence was not due to quenching by acetaldehyde, for when a small quantity of the latter was added to a DPNH solution the fluorescence was unaffected. More conclusively, it was found that the addition of a small quantity of DPNH to an irradiated solution instantly produced the characteristic fluorescence. The lack of fluorescence proves that the irradiation product cannot be identical with DPNH.

Coenzyme activity

That the irradiation product differs from DPNH was confirmed by an experiment with lactic dehydrogenase. 0.27 mM DPN⁺ (3 ml.) in oxygen-free aqueous 0.5M ethanol at pH 7.8 was irradiated with an X-ray dose of 39 000 r. 10 mM Sodium pyruvate (0.1 ml.) was added, and, at $t=0$, 0.1 ml.

of a lactic dehydrogenase solution was added (Fig. 4). The optical density at 340 $m\mu$. remained constant, showing that the enzyme was unable to catalyse the oxidation of the irradiation product by pyruvate. In order to prove that this could not be due to the presence of an enzyme inhibitor in the solution, a small amount of solid DPNH was added at $t=15$ min. The optical density at 340 $m\mu$. increased as a result of adding DPNH, and instantly began to decrease at the rate usual for pure DPNH solutions. The final pH of the solution was about 7.0, as the enzyme solution added was buffered to about this pH. Similar results were obtained for solutions irradiated at pH 10.2.

Fig. 4. Action of lactic dehydrogenase on a solution of the irradiation product containing sodium pyruvate. At $t=0$ min. the lactic dehydrogenase was added, and at $t=15$ min. DPNH was added.

A test with yeast alcohol dehydrogenase gave the same result. 0.36 mM DPN⁺ (40 ml.) in aqueous, oxygen-free 0.46M ethanol at pH 7.7 was irradiated with an X-ray dose of 45 000 r. When 3 ml. were placed in a spectrophotometer cuvette the optical density at 340 $m\mu$. was found to be 0.77. Alcohol dehydrogenase solution (0.15 ml.) was added, and the optical density increased to 0.96, due to the enzyme-catalysed reduction of residual DPN⁺ by ethanol. 0.5M Acetaldehyde (0.15 ml.) was then added and the optical density found to decrease immediately to 0.74, showing that the DPNH was completely oxidized by the enzyme, but that the irradiation product was unaffected. The final pH of the solution was found to

be 6.9, the explanation being that the enzyme was dissolved in a buffer of about this pH and the acetaldehyde had an acid reaction.

Reduction of DPN⁺ by substances other than ethanol

Experiments were performed to discover whether substances other than ethanol would cause the production of an irradiation product absorbing at 340 m μ . Oxygen-free aqueous solutions of DPN⁺ (0.27 mM) were prepared in 0.01M sodium pyrophosphate-HCl buffer in presence of other substances at either 0.5M or 0.01M concentration (pH 7.8). 0.5M solutions were irradiated with X-ray doses of 39 000 and 78 000 r, and 0.01M solutions with doses of 39 000 r only. The absorption curve of each solution was examined for the presence of a maximum at 340 m μ . In some cases definite absorption bands appeared at 340 m μ . as with 0.5M ethanol. In other cases it was obvious that there was no band at 340 m μ . Results are

fluorescence when examined under Wood's light. Solutions containing the substances at 0.5M concentration were also irradiated in presence of dissolved oxygen, and under these conditions no product absorbing at 340 m μ . appeared in any case.

The absorption curves of DPN after irradiation in presence of 0.5M formic acid at pH 7.8 (oxygen-free) were different in type from any of the others, in that they showed an unusually large increase in optical density in the region 290–300 m μ .

Role of hydrogen atoms in the reduction of DPN⁺

It was thought of interest to examine the effect of free hydrogen atoms on DPN⁺. Experiments were performed with a palladium diffusion apparatus of the type used by Mee & Sutton in their study of the effect of hydrogen atoms on catalase (Sutton, 1952). With a current of 1A it was first shown that 3 ml. of 0.3 mM methylene blue could be about 70 % decolorized in 1 hr. It was

Table 1. *Production of a reduced form of DPN by irradiation with X-rays in presence of various substances*

Substance	Whether an absorption band at 340 m μ . is produced by irradiating DPN ⁺ in a 0.5M solution of the substance	Whether an absorption band at 340 m μ . is produced by irradiating DPN ⁺ in a 0.01M solution of the substance
Methanol	Doubtful	Yes
Ethanol	Yes	Doubtful
<i>n</i> -Propanol	Yes	Yes
<i>iso</i> Propanol	Doubtful	Yes
<i>n</i> -Butanol	Yes	Doubtful
<i>iso</i> Butanol	Yes	Doubtful
Allyl alcohol	Yes	Yes
Ethylene glycol	No	Doubtful
Pinacol	Doubtful	No
Glucose	No	No
Lactic acid	No	Yes
β -Hydroxybutyric acid	Yes	Yes
Malic acid	No	Doubtful
Formic acid	Doubtful	Yes
Acetic acid	No	Doubtful
Succinic acid	No	No
Maleic acid	No	No
Benzoic acid	No	No
Glycine	Doubtful	Yes

given in Table 1. In every case where an absorption band was noted at 340 m μ ., air-free aqueous solutions of the substance were irradiated without DPN⁺, to ensure that the changes were not due to the substance itself. Also a test with lactic dehydrogenase was performed. Except where the substance was lactic acid at 0.01M concentration, it was always found that the product was not DPNH. The test was not successful with lactic acid because the enzyme was found unable to oxidize added DPNH. The reason for this is not known. None of the irradiated solutions containing the product exhibited more than a trace of visible

further shown that, with a stream of air bubbling through the solution, 3 ml. of mM ferrous ammonium sulphate solution in 0.1N-H₂SO₄ was 65 % oxidized in 1 hr., whereas with no current flowing a control showed only 6 % oxidation. It was found that the ultraviolet absorption spectra of 3 ml. portions of 0.3 mM DPN⁺ at pH 7.8, 10.2 or 13.0 were unaffected after treatment for 1 hr. at 1A (oxygen-free).

The yield of molecular hydrogen in oxygen-free solutions irradiated with a dose of 31 000 r has been determined. Two 100 ml. lots of 0.27 mM DPN⁺ containing 0.5M ethanol in 0.01M sodium pyro-

phosphate-HCl buffer at pH 7.8 were irradiated. The amounts of gas present in the two lots were 0.18 and 0.21 ml. (corrected to s.t.p.). On combustion with 0.29 and 0.25 ml. oxygen respectively the total volumes became 0.20 and 0.22 ml. respectively. The amounts of hydrogen are given by two-thirds of the decrease in total volume on combustion, and are 0.18 and 0.16 ml., respectively. The second value is lower than the volume of gas in the irradiated solution, but the result is probably within the experimental error. The yield for the production of molecular hydrogen (G) is calculated from the mean of all four volumes to be 3.2 molecules produced/100 ev absorbed. In similar experiments with no DPN^+ present, ethanol solutions gave 0.17 and 0.21 ml. gas, and after combustion with 0.29 and 0.25 ml. oxygen the residual volumes became 0.24 and 0.22 ml. respectively. From these results $G=2.8$. This experiment shows that the yield of hydrogen in irradiated aqueous ethanol is almost independent of the presence of DPN^+ .

Reaction of thiol radicals with DPN

Theorell & Bonnichsen (1951) have discovered a compound of liver alcohol dehydrogenase with DPNH which absorbs at 325 $m\mu$., the formation of which appears to be associated with the presence of thiol groups in the protein. They found that simple thiol compounds did not react with DPNH. Experiments were conducted to determine whether free thiol radicals, prepared by irradiating cysteine solutions (Swallow, 1952), would react with DPN^+ or DPNH. An oxygen-free 0.01M sodium pyrophosphate solution was made 0.27 M in DPN^+ and 0.05 M in cysteine and the pH adjusted to 7.8. It was found that no change occurred in the absorption curve on standing for about 1 hr., or on irradiation with an X-ray dose of 32 000 r. When the experiment was repeated, but with DPNH instead of DPN^+ , it was again found that no spectral change took place on standing, and on irradiation with an X-ray dose of 32 000 r; although the absorption band at 340 $m\mu$. decreased in intensity, there was no indication of the formation of a substance absorbing at or near 325 $m\mu$. These results confirm Theorell & Bonnichsen's observation that cysteine does not react with DPN^+ or DPNH. They also indicate that under these conditions thiol radicals do not react with DPN^+ or DPNH to give a compound with an absorption maximum at 325 $m\mu$.

DISCUSSION

In a previous publication (Swallow, 1953) it was shown that irradiation with γ -rays of DPN^+ in oxygen-free aqueous solutions containing ethanol

resulted in the production of a substance absorbing at 340 $m\mu$., taken to be reduced DPN. The results given here show that this substance can also be produced by X-rays, and Barron, Johnson & Cobure (1954) have also reached this conclusion. It should be noted that values of the yield of irradiation product calculated by Barron *et al.* from figures published previously (Swallow, 1953) should be multiplied by four. The agreement between the yields of Barron *et al.* and those obtained by the present author is quite good.

Nature of the irradiation product

The lack of fluorescence of irradiated solutions, and the experiments with lactic and alcohol dehydrogenases appear to prove conclusively that the irradiation product is not identical with enzymically reduced DPN. It is therefore surprising that Barron *et al.* concluded that half the product consisted of DPNH. On the basis of the experiment with alcohol dehydrogenase reported here (which was performed after seeing the paper of Barron *et al.*) it is suggested that their experiment is capable of a different interpretation to the one given. They partly evaporated their irradiated solutions to remove acetaldehyde and some of the ethanol, and then added alcohol dehydrogenase. At $t=0$ min., acetaldehyde was added and measurement made of the rate of decrease in optical density at 340 $m\mu$. It is now suggested that in their experiment the removal of ethanol was not complete, and when alcohol dehydrogenase was added some of the excess of DPN^+ became reduced to DPNH. On adding acetaldehyde the DPNH was reoxidized, leaving only the irradiation product. If we assume the rate of evaporation to have been proportional to the vapour pressure and the mole fraction of each constituent, and that the solution was evaporated to 0.34 of the original volume, then the ethanol concentration in the solution to which enzyme was added would be 0.022 M. Taking Racker's (1950) value for the equilibrium constant of the alcohol-dehydrogenase reaction as 1.15×10^{-11} and assuming that none of the irradiation product is DPNH, it can be shown that at $t=0$ min. the optical density of the solution used by Barron *et al.* should have been 0.72, and that this should have been reduced to 0.41 on adding acetaldehyde. Actually, they found 0.83 reduced to 0.41, in remarkably good agreement.

Although the irradiation product is not identical with DPNH, its other properties show that it cannot be very different, and in particular that it is a reduced form of DPN^+ . Experiments on the irradiation of N' -methylnicotinamide, which is a good model for DPN (Rafter & Colowick, 1954), are in agreement (Stein & Swallow, 1954). It can therefore be stated with confidence that the

irradiation product is a form of DPN reduced at the nicotinamide end of the molecule, but not in the ordinary way at C4 in the pyridine ring.

Reaction mechanism

It is highly probable that when water is irradiated with X- or γ -rays, the main primary act is the formation of free hydrogen atoms and free hydroxyl radicals (Weiss, 1944):

In the presence of a solute capable of being oxidized in two steps, the following general type of reaction may occur (Stein & Swallow, 1954):

If a second solute is present at low concentration, it will be exposed to the action of free hydrogen atoms and radicals of the type AH. Both are possible reducing agents.

Some evidence for believing that DPN^+ is reduced by an organic free radical rather than by hydrogen atoms has been given previously (Swallow, 1953). The results obtained with the palladium foil diffusion apparatus indicate that free hydrogen atoms, if prepared under these conditions, although capable of reducing methylene blue, cannot reduce DPN^+ . Most conclusive, however, is the value of the yield of molecular hydrogen in irradiated aqueous solutions of ethanol. The hydrogen may derive from one or more of the following reactions:

If free hydrogen atoms were responsible for the reduction of DPN^+ , then the introduction of DPN^+ into aqueous ethanol must decrease the yield of molecular hydrogen. In fact, however, the yield was found to be if anything slightly increased. It is therefore highly probable that DPN^+ is not reduced by hydrogen atoms, but by free hydroxyethyl radicals, as previously thought.

Mechanism of dehydrogenase action

From the point of view of explaining dehydrogenase action, the important feature of the present work is that DPN^+ has been reduced by free radicals under conditions similar to those prevailing in enzyme action, but the product is inactive. In contrast, when DPN^+ is reduced by sodium dithionite the product is identical with enzymically prepared DPNH, but the reaction is presumably an ionic one (Yarmolinsky & Colowick, 1954; Colowick, 1954; Swallow, 1955). It is difficult to avoid the conclusion that dehydro-

genase action has more in common with the ionic reaction than with the free-radical one.

The role of the thiol group on the enzyme is of special interest with reference to alcohol dehydrogenase action. Mackinnon & Waters (1953) have suggested that the thiol group might form a free thiol radical which might initiate the reaction. However, it is not easy to see how the thiol radical itself could be formed. Also, the experiments with cysteine reported here have failed to show any interaction between cysteine and DPN^+ or DPNH under conditions where free radicals are present. On the other hand, the idea that the thiol group forms a hydrogen bond with the nitrogen atom of the pyridine ring (Kaplan & Ciotti, 1954; Swallow, 1954a) receives some experimental support from Kaplan & Ciotti's (1954) results with *Neurospora* DPNase. V. M. Clark (personal communication) has pointed out that this hypothesis also explains the fact (Theorell & Bonnichsen, 1951) that the alcohol dehydrogenase-DPNH compound absorbs at a shorter wavelength than DPNH itself. That DPNH can exist in an enzyme-substrate compound as a cationic form receives still further support from work by Rafter & Krebs (1954), who have shown that DPNH is unstable in the presence of yeast glyceraldehyde 3-phosphate dehydrogenase, and the product of breakdown has the same ultraviolet absorption spectrum as the product obtained by the addition of dilute acid.

If it is true that alcohol-dehydrogenase action proceeds through an ionic mechanism, then it would be interesting to demonstrate some form of chemical interaction between the substrate and DPN^+ (Burton & Kaplan, 1954). Although this has not yet been found possible with ethanol, the discovery that lactic acid appears to react chemically with DPN^+ (Swallow, 1955) may be equally significant.

SUMMARY

1. Oxygen-free aqueous ethanolic solutions of DPN^+ give a product absorbing at 340 $m\mu$. when irradiated with X-rays.

2. The product has a similar ultraviolet absorption spectrum to DPNH, and the spectrum changes in the same way on longer irradiation or on acidification. The product, like DPNH, will reduce methylene blue.

3. The irradiation product differs from DPNH in that it does not fluoresce, and in that it does not function as a coenzyme for lactic or alcohol dehydrogenase.

4. A number of other substances can be substituted for ethanol, but no product appears when irradiated solutions contain dissolved oxygen.

5. Free hydrogen atoms appear to be unable to reduce DPN^+ . The yield of molecular hydrogen in

ethanolic DPN solutions is the same as in ethanol solutions without DPN.

6. It has not been possible to demonstrate any interaction between free thiol radicals and DPN⁺ or DPNH.

7. The experimental evidence supports an ionic rather than a free-radical mechanism for dehydrogenase action.

The author wishes to express his thanks to Mr C. Reed and Mr A. C. Roberts for technical assistance, to Dr G. Stein for many helpful discussions and to Professor J. S. Mitchell, F.R.S., for his interest.

REFERENCES

- Barron, E. S. G., Johnson, P. & Cobure, A. (1954). *Radiation Research*, **1**, 410.
- Barron, E. S. G. & Levine, S. (1952). *Arch. Biochem. Biophys.* **41**, 175.
- Burton, R. M. & Kaplan, N. O. (1954). *J. biol. Chem.* **211**, 447.
- Colowick, S. P. (1954). In *A Symposium on the Mechanism of Enzyme Action*, ed. McElroy & Glass, p. 353. Baltimore: Johns Hopkins Press.
- Farmer, F. T., Rigg, T. & Weiss, J. (1955). *J. chem. Soc.* p. 582.
- Haas, E. (1936). *Biochem. Z.* **288**, 123.
- Johnson, G. R. A., Scholes, G. & Weiss, J. (1953). *J. chem. Soc.* p. 3091.
- Kaplan, N. O. & Ciotti, M. M. (1954). *J. biol. Chem.* **211**, 431.
- Mackinnon, D. J. & Waters, W. A. (1953). *J. chem. Soc.* p. 323.
- Miller, N. & Wilkinson, J. (1952). *Disc. Faraday Soc.* **12**, 50.
- Racker, E. (1950). *J. biol. Chem.* **184**, 313.
- Rafter, G. W. & Colowick, S. P. (1954). *J. biol. Chem.* **209**, 773.
- Rafter, G. W. & Krebs, E. G. (1954). See Singer, T. P. & Kearney, E. B. (1954). *Advanc. Enzymol.* **15**, 79.
- Stein, G. & Swallow, A. J. (1954). *Nature, Lond.*, **173**, 937.
- Sumner, J. B. & Somers, G. F. (1947). *Chemistry and Methods of Enzymes*, 2nd ed. New York: Academic Press.
- Sutton, H. C. (1952). *Disc. Faraday Soc.* **12**, 281.
- Swallow, A. J. (1952). *J. chem. Soc.* p. 1334.
- Swallow, A. J. (1953). *Biochem. J.* **54**, 253.
- Swallow, A. J. (1954a). Ph.D. Thesis, University of Cambridge.
- Swallow, A. J. (1954b). *Radiation Research*, **1**, 570.
- Swallow, A. J. (1955). *Biochem. J.* **60**, 443.
- Theorell, H. & Bonnichsen, R. A. (1951). *Acta chem. scand.* **5**, 1105.
- Waters, W. A. (1948). *Chemistry of Free Radicals*, 2nd ed. Oxford: Clarendon Press.
- Weiss, J. (1944). *Nature, Lond.*, **153**, 748.
- Yarmolinsky, M. & Colowick, S. P. (1954). *Fed. Proc.* **13**, 327.

The Conversion of Schradan (OMPA) and Parathion into Inhibitors of Cholinesterase by Mammalian Liver

By A. N. DAVISON

Medical Research Council, Unit for Research in Toxicology, Serum Research Institute, Carshalton, Surrey

(Received 8 February 1955)

Schradan [(Me₂N)₂PO.O.PO(NMe₂)₂, OMPA, octamethylpyrophosphoramidate, bis-NNN'N'-tetramethylphosphorodiamidic anhydride] and parathion (OO-diethyl O-p-nitrophenyl phosphorothioate) are weak inhibitors of cholinesterase *in vitro*, but when administered to animals the cholinesterase levels of the blood and tissues drop, and typical signs of acetylcholine poisoning follow (Dubois, Doull & Coon, 1950; Aldridge & Barnes, 1952; Kilby & Gardiner, 1952). It has been shown that in the presence of oxygen parathion is oxidized by mammalian liver slices to paraoxon (OO-diethyl O-p-nitrophenyl phosphate), a highly active inhibitor of cholinesterase (Myers, Mendel, Gersmann & Ketelaar, 1952; Gage, 1953; Metcalf & March, 1953):

Similarly, schradan is converted by mammalian liver slices in the presence of oxygen into the phosphoramidate oxide and other products which are powerful inhibitors of cholinesterase (Dubois *et al.* 1950; Aldridge & Barnes, 1952; Kilby & Gardiner, 1952; Casida, Allen & Stahmann, 1954). This conversion has also been shown to occur in the liver *in vivo* (Cheng, 1951). However, the conversion does not occur in liver suspensions in the presence of oxygen. It therefore seemed interesting to investigate the difference between rat-liver slices and suspensions and to study the nature of the systems involved in the metabolism of parathion and schradan.

Since at 37° the conversion *in vitro* of both organophosphorus compounds is stopped after freezing, heating or dispersing the liver, it is concluded that enzymes probably participate in the process. It seemed possible that on dispersing the liver in buffer either an enzyme or a coenzyme was inactivated. It has now been found that the