REFERENCES

- Adolph, E. F. (1943). *Physiological Regulations*, 1st ed. p. 266. Lancaster, Pa.: Jacques Cattell Press.
- Alpert, L. K. (1941). *Johns Hopk. Hosp. Bull.* **68**, 522.
- Boothby, W. M., Sandiford, I., Sandiford, K. & Slosse, J. (1925). *Trans. Ass. Amer. Phys.* **40**, 195.
- Davidson, C. S., Wilcke, H. L. & Reiner, P. J. (1946). *J. Lab. clin. Med.* **31**, 721.
- Dean, F. R. A. & McCance, R. A. (1948). *J. Physiol.* **107**, 182.
- Dicker, S. E. (1946). *Brit. J. Pharmacol.* **1**, 194.
- Dicker, S. E. (1948a). *J. Physiol.* **107**, 8.
- Dicker, S. E. (1948b). *Biochem. J.* **43**, 444.
- Dicker, S. E. (1949a). *J. Physiol.* **108**, 197.
- Dicker, S. E. (1949b). *Biochem. J.* **44**, 274.
- Dicker, S. E. & Heller, H. (1945). *J. Physiol.* **103**, 449.
- Dicker, S. E., Heller, H. & Hewer, T. F. (1946). *Brit. J. exp. Path.* **27**, 158.
- Dudley, H. W. (1920). *J. Pharmacol.* **83**, 11.
- Eggleton, M. G., Pappenheimer, J. R. & Winton, F. R. (1940). *J. Physiol.* **98**, 336.
- Fisher, R. A. (1944). *Statistical Methods for Research Workers*, 9th ed. London: Oliver and Boyd.
- Fisher, R. A. & Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research*, 2nd ed. London: Oliver and Boyd.
- Folin, O. (1905). *Amer. J. Physiol.* **13**, 66.
- Gollan, F. (1948). *J. clin. Invest.* **27**, 352.
- Govaerts, P. & Lequime, J. (1942). *Bull. Acad. Méd. Belg.* **7**, 260.
- Harris, G. W. (1948). *J. Physiol.* **107**, 430.
- Hawk, P. B. & Bergeim, O. (1942). *Practical Physiological Chemistry*, 11th ed. p. 888. London: Churchill.
- Heller, H. (1947). *J. Physiol.* **106**, 245.
- Heller, H. & Urban, F. F. (1935). *J. Physiol.* **85**, 502.
- Keys, S., Taylor, H. L., Mickelsen, O. & Henschel, A. (1946). *Science*, **103**, 559.
- Leslie, S. H. & Ralli, E. P. (1947). *Endocrinology*, **41**, 1.
- McCance, R. A. & Wilkinson, E. (1947). *J. Physiol.* **106**, 256.
- McCance, R. A. & Young, W. F. (1941). *J. Physiol.* **99**, 265.
- Mainland, D. (1938). *The Treatment of Clinical and Laboratory Data*, 1st ed. London: Oliver and Boyd.
- Ralli, E. P., Robson, J. S., Clarke, D. & Hoagland, C. L. (1945). *J. clin. Invest.* **24**, 316.
- Smith, H. W., Goldring, W. & Chasis, H. (1938). *J. clin. Invest.* **17**, 263.
- Stare, F. J. (1945). *Nutr. Rev.* **3**, 225.
- Young, W. F. & McCance, R. A. (1942). *Arch. Dis. Childh.* **17**, 65.

Pigments Derived from Tryptophan: (1) Urorosein, (2) Tryptochrome

BY W. R. FEARON AND W. A. BOGGUST

Department of Biochemistry and Department of Chemistry, Trinity College, Dublin

(Received 2 May 1949)

Pigments obtained from tryptophan include representatives of two classes: (1) indigoids, formed by the action of halogens or other oxidizers on free tryptophan; and (2) pigments of the Hopkins-Cole type, formed from free or peptide-linked tryptophan by condensation with aldehydes, and subsequent oxidation. The recently described pigment, tryptochrome (Fearon, 1948) appears to belong to the first class and as indole-3-aldehyde is an oxidation product of tryptophan (Hopkins & Cole, 1903; Ellinger, 1906), a study was made of its chromogenic properties to see if it was an intermediate in the formation of tryptochrome.

UROROSEIN

In presence of acids, indole-3-aldehyde spontaneously changes into the red pigment, urorosein (Ellinger & Flamand, 1909; Homer, 1913, 1915). Urorosein, first obtained by the action of an acid and an oxidizer on urine, is distinguished from other red pigments of urinary origin by: (a) its insolubility in chloroform, (b) its reversible bleaching by alkalis,

and (c) the spectrum of its solution in amyl alcohol, which shows bands at 550–530 and 500–480 m μ . (Maillard, 1913; Homer, 1915). The natural precursor in urine is either 3-indolylacetic acid (Herter, 1908) or the glycine conjugate, *N*-(3-indole-acetyl)-glycine (Ewins & Laidlaw, 1913; Homer, 1913). As yet, no structural formula for urorosein has found general acceptance. Those suggested are: nitroso-indolylacetic acid (Herter, 1908), a triindolyl-methane derivative (Ellinger & Flamand, 1909), and indolylindolylidene (König, 1911). According to Riesser (quoted by Ellinger, 1913), urorosein is an oxidation product of the leuco base,

formed by condensation between 1 molecule of indole-3-aldehyde and 2 molecules of 3-indolylacetic acid. The chemistry of the urinary indigoids, up to 1912, has been reviewed by Maillard (1913), but, as Rimington, Holiday & Jope (1946) observe, there has been much confusion regarding the nomenclature and identity of the red pigments obtainable

from urine. It is now established that indirubin (indigo red) and urorosein are independent compounds, each arising from its own special chromogens. From acidified autoclaved urines, Rimington *et al.* (1946) isolated indirubin in crystalline form, characterizable by its absorption spectrum. Solutions of 3-indolylacetic acid, similarly treated, yielded no indirubin. For urorosein production, 3-indolylacetic acid requires an acidic condensing medium and an oxidizer. Ordinary concentrated hydrochloric acid usually can provide both factors. Rimington *et al.* have found that a solution of 3-indolylacetic acid in 20% (w/v) hydrochloric acid turns red on standing, and acquires the absorption band with maximum at about 530 m μ ., characteristic of urorosein salts. We have observed that a similar solution in pure 20% (w/v) sulphuric acid, protected from the atmosphere, does not generate urorosein.

In contrast, urorosein formation from indole-3-aldehyde in acid solution requires no oxidizer, and can take place spontaneously even in warm, glacial acetic acid. However, in presence of concentrated mineral acids, indole-3-aldehyde, like other aromatic aldehydes, can condense with substituted indoles to produce chromogens and pigments of the Hopkins-Cole type, depending on the oxidizer present (Rydon, 1948). A reaction of this nature may have been responsible for the triindolymethane pigment obtained by Ellinger & Flamand (1909).

EXPERIMENTAL

Preparation of urorosein

(1) *From indole-3-aldehyde.* Recrystallized indole-3-aldehyde (0.1 g.) in 35% (w/v) H₂SO₄ (80 ml.) was heated at 60° in a water bath, for 45 min. The mixture rapidly turned red and a dark red crystalline precipitate eventually separated. After remaining for some hours at room temperature, the precipitate was collected, washed free from excess of acid, and recrystallized from hot glacial acetic acid. Urorosein formed dark purple needles, with a greenish lustre. Yield was 60 mg. m.p., approx. 231° (decomp.). Thus obtained, the pigment appears to be the mixed sulphate of a hitherto unrecorded base, C₁₈H₁₀N₂. (Found: C, 55.8; H, 4.6; N, 7.4; SO₄, 33.6. (C₁₈H₁₂N₂SO₄)₃·H₂SO₄ requires C, 56.1; H, 3.4; N, 7.2; SO₄, 33.2%.) When a mixture of equal parts of saturated aqueous picric acid and urorosein sulphate in glacial acetic acid was kept at room temperature for several days, a *picrate* separated in red, mossy fronds, m.p., approx. 120° (decomp.). (Found: N, 14.5. C₁₈H₁₀N₂·C₆H₃O₇N₃ requires N, 14.49%.)

(2) *From 3-methyl-indole (scatole) 2-methyl-indole and 3-indolylacetic acid.* After purification, none of these compounds gave any colour when heated at 60° in the 35% H₂SO₄, unless an oxidizer was added: H₂O₂, HNO₂, Fe⁺⁺⁺, persulphate, perborate, or chloroauric acid. Maximum colour development was achieved by one mol. equiv. of O₂. Under these conditions, red pigments spectroscopically indistinguishable from urorosein sulphate were obtained as

crystalline precipitates. Excess of oxidizers and prolonged heating converted the pigment into isatin. The production of urorosein when 3-methyl-indole or 3-indolylacetic acid is oxidized in acid solution has been recorded also by Homer (1915).

Properties of urorosein. Urorosein salts are soluble in water, ethanol and higher alcohols, forming orange to red solutions, the shade and intensity of which depend on the degree of dissociation of the salt. In strongly acid solutions the colour is a characteristic carmine-rose. The free base is yellow, and can be extracted from slightly alkaline solutions by CHCl₃, in which the red salt does not dissolve. The base is unstable to atmospheric exposure, but dissolves in acids, regenerating the stable red pigment. As commonly prepared in urine, urorosein is an equilibrium mixture of both forms. In concentrated ethanolic solution, urorosein salts show a broad band between the *D* and the *E* lines, which on dilution recedes from the red end of the spectrum, and shows a maximum about 510 m μ . Pure urorosein sulphate in ethanol shows bands at 540–480 m μ . (log ϵ $\approx$ 4.0), 370 m μ . (log ϵ $\approx$ 2.9), 285 m μ . (log ϵ $\approx$ 4.5), and 225 m μ . (log ϵ $\approx$ 5.0). Urorosein base shows a band about 350 m μ . and an intense band at about 510 m μ ., which is prominent in the spectra described by earlier investigators.

DISCUSSION

Urorosein appears to be formed by the direct condensation of 2 molecules of indole-3-aldehyde. No evidence could be obtained that the aldehyde side

Fig. 1. Photometric absorption curves for urorosein, isatin and indirubin.

chain is split off during the process, and the final mixtures were free from likely degradation products, such as formaldehyde, formic acid and carbon dioxide.

The absorption curve (Fig. 1) is of interest on account of its resemblance to that of isatin (Ward, 1923), which shows maximum absorption at 420 m μ . (log ϵ $\approx$ 2.7), 310 m μ . (log ϵ $\approx$ 3.4), and at 245 m μ . (log ϵ $\approx$ 4.2). Urorosein has a similar system, all bands being relatively displaced with slightly in-

creased intensities. The 350 $m\mu$. band of the free base is somewhat masked by the rise of the curve to the intense absorption region at about 510 $m\mu$. The 420 $m\mu$. band of isatin is characteristic of the $\alpha\beta$ -dicarbonyl or similar double-bond system, and is found at about 380 or up to about 400 $m\mu$. in phenazines that contain the *ortho* di- $\text{C}=\text{N}$ - linkages (Bednarczyk & Marchlewski, 1938; Cholewiński & Marchlewski, 1938). From the data available, the

100,000. As a fluorescence test, the sensitivity is at least 1 in 10^6 .

Selectivity. The reaction requires an indole nucleus with an unsubstituted C in the 2-position of the pyrrole ring, and a $-\text{CH}_2\text{CH}(\text{NH}_2)-$ side chain at the 3-position. It is not given by unhydrolysed proteins, by 2-methyltryptophan, by β -3-indolylpropionic acid, or by any of the common amino-acids, other than tryptophan. It will detect tryptophan in protein hydrolysates, and, under favourable conditions, in urine. A red colour, unaccompanied by the

simplest structure that can be assigned to urorosein is indolo (3':2'-2:3) carbazole, which also explains its formation from either 2-methyl- or 3-methylindole.

TRYPTOCHROME

The term 'tryptochrome' is applied provisionally to the highly fluorescent, scarlet pigment obtained when free tryptophan or tryptamine in 70-90% acetic acid is oxidized with iodate. A fluorescent, violet iodinated precursor, 'iodoprotryptochrome' has also been isolated.

EXPERIMENTAL

The tryptochrome reaction

Method. About 1 ml. of a dilute (about 0.1%) aqueous solution of tryptophan is mixed with 5 ml. of aldehyde-free glacial acetic acid and two drops of 1% aqueous KIO_3 . On warming, an eosin-red colour, accompanied by a greenish orange fluorescence, rapidly appears. Colour development continues as the mixture cools, and persists in the solution, even after exposure for a year to air and diffused light. With more concentrated solutions of tryptophan, the colour is violet (iodoprotryptochrome), but becomes red when warmed with additional KIO_3 .

Sensitivity. As a colour test, the reaction will show free tryptophan or tryptamine in dilutions down to 1 in

characteristic fluorescence, is given by a few biological compounds, notably adrenaline, which forms iodoadrenochrome when treated with iodate in acid solution (Richter & Blaschko, 1937). This reaction, however, can take place in acetic acid solutions as dilute as 2%, under which conditions tryptophan does not react.

Comment. The acetic acid must be in considerable excess, otherwise the colour reaction is inhibited by the formation of insoluble iodinated tryptophan derivatives which separate out as a dull brown precipitate. For detecting very small amounts of tryptophan, aldehyde-free glacial acetic acid should be used. The iodate can be replaced by BrO_3^- , Br_2 , or hypochlorite, but these rapidly destroy the pigment. Peroxide, persulphate, perborate, MnO_2 , Fe^{+++} , ferricyanide, ceric acid, and chloro-auric acid alike are ineffective for tryptochrome production, although they can form urorosein from its chromogens.

Isolation of tryptochrome. When the solution in acetic acid is diluted with 4 vol. of water, tryptochrome separates out almost completely, and can be extracted with ether, CHCl_3 , or *iso*-butanol. The extract, washed free from water and dried *in vacuo*, on addition of light petroleum, deposits tryptochrome as an iodine-free, fine amorphous powder, or occasionally, as garnet-red microcrystals. Crystalline material in sufficient quantities for analysis could not be obtained. Analytical results for three reprecipitated samples of amorphous tryptochrome are: C, 69.8, 70.1, 69.9; H, 4.7, 4.6, 4.7; N, 14.0, 13.9, 13.5. Mol. wt. (Rast), approx. 300. $\text{C}_{18}\text{H}_{11}\text{O}_2\text{N}_3$ requires C, 71.7; H, 3.7; N, 14.0%, mol.wt. 301.

Preparation of iodoprotryptochrome

DL-Tryptophan (1 g.) in 96% (v/v) acetic acid (150 ml.) was kept at 60° for 30 min. during the gradual addition, with continuous stirring, of one equiv. (1.05 g.) of KIO₃ in water (25 ml.). After remaining for a further 30 min. at 60°, the mixture was let cool overnight. The dark purple precipitate of *iodoprotryptochrome* (0.5 g.) was collected, washed free from acid, and dried *in vacuo*. Crystallization from hot, glacial acetic acid yielded minute dark violet prisms, with a bright lustre. On heating, they began to shrink at 250°, and decomposed about 300°. Mol.wt. (Rast), approx. 480. (Found: C, 50.3; H, 2.9; N, 12.0; I, 27.0. C₂₀H₁₈O₂N₂I requires C, 51.2; H, 2.9; N, 11.9; I, 27.1%.)

Properties. Iodoprotryptochrome is freely soluble in strong acids, slightly soluble in ethanol and higher alcohols, and almost insoluble in other common organic solvents and in water. The halogen is loosely bound, and decomposition is readily effected by heating in an inert solvent. Careful addition of H₂O₂, persulphate, or Fe⁺⁺⁺ to a solution of iodoprotryptochrome in hot glacial acetic acid converts it

Fig. 2. Photometric absorption curves for iodoprotryptochrome, tryptochrome and 'indigotindiphenylacetic ester'.

completely into tryptochrome. I₂ is set free, and can be removed by keeping the mixture stirred at 80°. After cooling, the solution is diluted with 4 vol. of water, and the tryptochrome extracted with CHCl₃, and purified as previously described. Tryptochrome is a neutral compound, almost insoluble in water and dilute alkalis, moderately soluble in the common organic solvents, with the exception of light petroleum, and freely soluble in conc. acids. All the solutions show the red colour and fluorescence, which are most marked in acid solvents. Tryptochrome is very unreactive. Prolonged boiling with strong alkali eventually decomposes it, with liberation of indole and 3-methylindole, suggesting that both 2- and 3-substituted indoles form part of the skeleton. It does not liberate CO₂ from bicarbonate solution, indicating that the O atoms are not present as a carboxyl group. Reduction with Zn in acetic acid gives a colourless product that regains its original appearance on aeration. The leuco form gives no reaction for catechol with molybdate, or general phenol reaction with iron, which suggests that tryptochrome is not a quinone. Iodoprotryptochrome in ethanol shows bands at 550, 515, 420, 340, 293 and 254 mμ. Tryptochrome, after repeated purification by chromatography on alumina, when dissolved in CHCl₃ shows bands at 555, 515, 338 and 290 mμ. (Fig. 2).

DISCUSSION

Formation of iodoprotryptochrome or tryptochrome involves 2 molecules of the chromogen, and requires a C₂ side chain carrying an amino group, and an unsubstituted carbon atom in the 2-position of the pyrrole ring. The absorption curves show a close relationship to the indigoids. No evidence for an amino-acid side chain could be obtained. The intense colour and low solubility of the pigments made it difficult to exclude the possibility of a faint positive response to the ninhydrin test, but solution-suspensions in dilute alkali reacted negatively to formaldehyde titration under conditions where the addition of a few mg. of glycine gave an unmistakable positive reaction. The low solubility of both pigments in dilute alkalis appears to exclude the presence of a carboxyl group. Phenylhydrazones could not be obtained, but when iodoprotryptochrome was refluxed in absolute ethanol for some hours with hydroxylamine and calcium carbonate, the violet colour and the fluorescence gradually disappeared and a reddish brown crystalline material separated. This has not yet been identified, but it appears to be either a monoxime or dioxime of tryptochrome. These results suggest that if the tryptochromes contain a ketonic function, it is of the type found in indigotin and indirubin rather than that present in isatin which is much more reactive. The spectra of tryptochrome and iodoprotryptochrome were compared with those of a number of synthetic compounds containing the indole nucleus. Of these, only one, 'indigotindiphenylacetic ester', was found that closely resembled tryptochrome in spectrum, and also in fluorescence and scarlet colour. Its constitution is well established (Posner & Pyl, 1923; Posner & Kemper, 1924). A solution of chromatographically purified 'indigotindiphenylacetic ester' in chloroform gave an absorption curve of the indigoid type (Fig. 2), and showed bands at 552, 515, 370 and 290 mμ. Iodoprotryptochrome can be represented as having a similar nucleus formed from six fused rings. Hence, it is suggested that the pigment is formed by union of two tryptophan units in the α-position, to generate an indigoid system in which both of the original side chains of the amino-acids have contributed to form two additional rings, each carrying a new chromophore system. Tryptochrome appears to be a derivative of protryptochrome arising by oxidation, from which one ring and the chromophore responsible for the 420 mμ. band have been removed, leaving a structure similar to the 'indigotinphenylacetic ester' skeleton. The following constitutions are assigned provisionally to the tryptochrome pigments. The point of attachment of the iodine atom in iodoprotryptochrome is uncertain. Because of its lability, it is unlikely to be

located in one of the benzene rings, and it is assigned to that part of the molecule vulnerable to the attack that leads to the production of tryptochrome.

rapidly, or in excess, a brown precipitate of iodotryptophan or iodotryptochrome will be the result. If the mixture contains more than about 0.1% of tryptophan, the pigment

The tryptophan iodine reaction

While studying the conditions of tryptochrome production, it was observed that free tryptophan gives a colour reaction with iodine in solutions in the region of the isoelectric point, pH 5.9. Outside the range pH 4–6, colour development is insignificant, and in solutions of higher acidity dark brown precipitates of iodinated tryptophans are formed. This may be the reason why the colour reaction appears to have been overlooked, and why earlier workers (Neuberg & Popowski, 1906; Neuberg, 1907) have commented on the inability of iodine to replace chlorine or bromine in the familiar tests. Mahn (1930) also fails to mention the colour reaction in his account of iodotryptophan.

Test. About 3 ml. of the tryptophan solution are mixed with an acetate buffer of pH 4.5, and 1% aqueous or ethanolic I₂ is added carefully drop by drop. The presence of tryptophan or tryptamine is shown by the development of a deep purple colour. Maximum colour development requires only one equiv. of I₂. If the halogen be added

will slowly separate out, and can be extracted readily by CHCl₃. The red and purple pigments obtained by Neuberg & Popowski (1906) contain Cl or Br, but, unexpectedly, the pigment formed by the action of I₂, and provisionally called 'tryptophan purple' is halogen-free, and titration of the residual mixture shows that all the added I₂ required by the reaction has been converted into iodide.

Properties. 'Tryptophan purple' is moderately soluble in ethanol and in glacial acetic acid. The ethanol solution shows bands at 512, 370, 285 and 250 mμ., and the absorption curve indicates a close relationship to uroscorcin and to indirubin. Chromatographic analysis on alumina showed the presence of blue and red contaminants, in addition to the principal violet pigment. The mixture was not further investigated.

SUMMARY

1. The preparation of uroscorcin is described. A study of its optical and other properties indicates that its structure is indolo (3':2'-2:3) carbazole.

2. The production of a fluorescent, red pigment, tryptochrome, is described as a delicate and selective test for free tryptophan and tryptamine.

Table 1. *Absorption maxima of the pigments*

Pigment	Solvent	Wavelength (mμ.)*						
		535	484	—	370	285	225	
Uroscorcin sulphate	Ethanol	—	—	420	—	310	248	
Isatin	CHCl ₃	540	—	—	364	290	242	
Indirubin	CHCl ₃	550	515	420	340	293	254	
Iodoprotryptochrome	Ethanol	555	515	—	338	290	—	
Tryptochrome	CHCl ₃	552	515	—	370	290	—	
'Indigotindiphenylacetic ester'	CHCl ₃	—	512	—	370	285	250	
'Tryptophan purple'	Ethanol	—	—	—	—	—	—	

* Apart from preliminary observations on the visible spectra, all the optical measurements used in constructing the curves were made by Mr E. R. Stuart, by means of the Beckman quartz spectrophotometer. The curves were drawn from experimental points read off the spectrophotometer wavelength scale at 5 mμ. intervals.

3. Tryptochrome and its precursor, iodoprotryptochrome, have been isolated, and provisional formulae are assigned to them.

We are indebted to Mr E. R. Stuart of the Chemical Department of this college for the ultraviolet absorption data and for his help in their interpretation. We offer our thanks to Sir Paul Fildes, F.R.S., for a specimen of pure

2-methyltryptophan, to Prof. Wesley Cocker, for his interest and criticism; to Messrs Glaxo, for the gift of amino-acids, to the Chemical Society, for the award of a Research Grant to one of us (W. A. B.) for purchase of tryptophan. Microanalyses were by Drs G. Weiler and F. B. Strauss, of Oxford. The entire investigation was carried out by the aid of a grant from the Medical Research Council of Ireland.

REFERENCES

- Bednarczyk, W. & Marchlewski, L. (1938). *Biochem. Z.* **300**, 46.
 Cholewiński, J. & Marchlewski, L. (1938). *Biochem. Z.* **300**, 56.
 Ellinger, A. (1906). *Ber. dtsch. chem. Ges.* **39**, 2519.
 Ellinger, A. (1913). *Neubauer-Huppert's Analyse des Harns*, p. 886. Wiesbaden: Kreidels.
 Ellinger, A. & Flamand, C. (1909). *Hoppe-Seyl. Z.* **62**, 276.
 Ewins, A. & Laidlaw, P. P. (1913). *Biochem. J.* **7**, 13.
 Fearon, W. R. (1948). *Nature, Lond.*, **162**, 338.
 Herter, C. A. (1908). *J. biol. Chem.* **4**, 253.
 Homer, A. (1913). *Biochem. J.* **7**, 101.
 Homer, A. (1915). *J. biol. Chem.* **22**, 345.
 Hopkins, F. G. & Cole, S. W. (1903). *J. Physiol.* **29**, 451.
 König, W. (1911). *J. prakt. Chem.* **84**, 194.
 Mahn, H. (1930). *Abderhalden's Biochemisches Handlexicon*, **12**, 716. Berlin: Springer.
 Maillard, L. C. (1913). *Richet's Dictionnaire de Physiologie*, **9**, 217. Paris: Alcan.
 Neuberg, C. (1907). *Biochem. Z.* **6**, 276.
 Neuberg, C. & Popowski, N. (1906). *Biochem. Z.* **2**, 369.
 Posner, T. & Kemper, W. (1924). *Ber. dtsch. chem. Ges.* **57**, 1311.
 Posner, T. & Pyl, G. (1923). *Ber. dtsch. chem. Ges.* **56**, 31.
 Richter, D. & Blaschko, H. (1937). *J. chem. Soc.* p. 601.
 Rimington, C., Holiday, E. R. & Jope, E. M. (1946). *Biochem. J.* **40**, 669.
 Rydon, H. N. (1948). *J. chem. Soc.* p. 705.
 Ward, F. W. (1923). *Biochem. J.* **17**, 891.

The Oxidation of Manganese by Peroxidase Systems

BY R. H. KENTEN AND P. J. G. MANN

Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts

(Received 24 June 1949)

Kenten & Mann (1949) demonstrated the oxidation of manganese by certain root extracts in the presence of added hydrogen peroxide; the oxidizing system consisted of a thermolabile and a thermostable factor, together with hydrogen peroxide. Partially purified peroxidase preparations, in the presence of certain phenolic peroxidase substrates and hydrogen peroxide, were found to oxidize added manganous

stances by peroxidase and hydrogen peroxide can bring about the oxidation of manganese, according to the above scheme which involves the substrate in a cycle of oxidation and reduction. The present work was undertaken to establish the oxidation of manganese by plant peroxidase preparations, in the presence of phenolic substrates and hydrogen peroxide, and to investigate the properties of the system.

ions (Mn^{++}) and it was suggested that the thermolabile and thermostable factors in the root extracts are peroxidase and peroxidase substrate respectively. The hypothesis was advanced that intermediate products of the oxidation of certain phenolic sub-

MATERIALS AND METHODS

Peroxidase preparations of different purity were obtained from horseradish and turnip, while following the method of Keilin & Mann (1937). The 'Purpurogallinzahl' (P.Z.) of these preparations was determined by the method of Keilin & Mann (1937).

Catalase was prepared from horse liver by the dioxane method of Sumner & Dounce (1937). The 'Katalase-fähigkeit' (Kat.f.) of the preparation, as determined by the method of Sumner & Somers (1943), was 20,000.

Hydrogen peroxide (0.05 M). H_2O_2 (2.7 ml. of A.R. 20 vol.) was diluted to 100 ml. A fresh solution was prepared for each experiment, and estimated manometrically with acid MnO_2 . (See Tables 3 and 4.)