# 326. *Catalytic Properties of the Phthalocyanines. Part II. Oxidase Properties.*

By A. H. Cook.

Pigments of the iron phthalocyanine type have been shown to be distinguished by their ability to function as oxygen carriers. Typical oxidations, *e.g.*, of hydrogen iodide, unsaturated compounds, and aromatic chromogens were observed qualitatively, and the catalysed oxidation of benzaldehyde was studied quantitatively, in presence of both free pigments and deposits on inorganic carriers. The results are discussed from the theoretical and the biochemical aspect.

As indicated in Part I, the unique nature of the metallic phthalocyanines and their formal relationship with the porphyrin group made it of interest to investigate how far the oxidative properties of iron (and possibly of other metals) under mild conditions, as modified by association with the porphyrin structure, are shared by the analogous phthalocyanine complexes.

The important rôle of iron in biological oxidative processes (Ando, *Biochem. J. Japan*, 1928, **9**, 187, 201) and other (*e.g.*, proteolytic) actions (Michaelis and Stern, *Biochem. Z.*, 1931, **240**, 192; Maschmann and Helmert, *Z. physiol. Chem.*, 1935, **231**, 51), as well as the occurrence of iron-porphyrin complexes as the prosthetic groups of oxidative enzymes (Bertho, *Chem.-Ztg.*, 1935, **59**, 953), makes information on the dependence of the catalytic activity of iron on the nature of its chemical combination particularly desirable.

Oxidations effected by oxygen in presence of inorganic iron compounds have been studied by many workers, both from a biochemical standpoint (*e.g.*, Neuberg, *Biochem. Z.*, 1908, **13**, 305; Baudisch, *Chem. Rev.*, 1934, **15**, 1; Hill, *J. Biol. Chem.*, 1931, **92**, 471, etc.), and with the object of clarifying the mechanism of such reactions (see below). The early observations of Robinson (*Biochem. J.*, 1924, **18**, 255) on the catalytic effect of hæmin, methæmoglobin, and hæmoglobin on the autoxidation of linseed oil, and of Harrison (*Biochem. J.*, 1924, **18**, 1009; cf. Warburg and Negelein, *Biochem. Z.*, 1928, **200**, 414) on the autoxidation of cysteine were extended to the catalytic oxidation of many unsaturated compounds by Kuhn and Meyer (*Z. physiol. Chem.*, 1929, **185**, 193), who established the heavy-metal catalytic nature of at least part of the autoxidation of benzaldehyde and the ability of hæmin to function as a catalyst (*Naturwiss.*, 1928, **16**, 1028; *J. Biol. Chem.*, 1933, **103**, 25).

Bi- and ter-valent iron phthalocyanines (I and II), iron octatetrazaporphine (III) (see Part I, p. 1762), and tó a more limited extent a water-soluble monosulphonated iron phthalocyanine, are now shown to be outstanding in their ability to catalyse oxidations by molecular oxygen under mild conditions. Neither free phthalocyanine nor any of 28 other metal phthalocyanines examined under the most favourable conditions (oxidation of benzaldehyde) exhibited any considerable catalytic property.

Among inorganic compounds, potassium iodide is easily oxidised under the influence of the above iron complexes, although it was only possible to examine the water-soluble iron phthalocyanine in homogeneous aqueous solution. When exposed to air, potassium iodide solution (10 c.c.) with added starch became coloured a rich blue within 30 mins.

after addition of 0·1 mg. of the sulphonated catalyst, although in buffered or unbuffered solutions the effect was small and was accompanied by disappearance of the pigment by self-induced autoxidation (see below).

A number of unsaturated aliphatic compounds (*e.g.*, oleic acid) in benzene or aqueous solution or suspension, when shaken in air or oxygen with a little of one of the above amorphous pigments, showed a marked uptake of oxygen, accompanied by oxidation of the phthalocyanine as shown by the appearance of a brown coloration in place of the blue-green. The uptake of gas for each molecule of pigment (observed under arbitrary but standardised conditions) was greatest, but relatively slowest, in the case of iron octaphenyltetrazaporphin, intermediate with the phthalocyanine, and small but rapid with the sulphonated iron phthalocyanine.

Polyphenols and aromatic chromogens generally undergo ready oxidation in air in presence of iron pigments of the phthalocyanine type; *e.g.*, solutions of pyrogallol were rapidly oxidised, and again the behaviour of the sulphonated compound was noteworthy (see below).

As polyphenolase models are usually non-specific and active as indophenolase models (*e.g.*, complex cobalt salts, K. and Y. Shibata, " Katalytische Wirkungen der Metallkomplexverbindungen," 1936, p. 10), it was to be expected that the sulphonated pigment (which alone was examined, since the necessary use of pyridine when testing other pigments invalidated the result) would be active in this respect also: weakly alkaline solutions containing equivalent quantities of *p*-phenylenediamine and α-naphthol developed the indophenol colour within 5 mins. in presence of this pigment, air in solution functioning as an oxidising agent.

The self-induced autoxidation of these pigments was more closely studied with reference to the sulphonated catalyst, as the phenomena were more readily demonstrated in this case. Buffered quinol solutions containing this catalyst and freely exposed to air became oxidised to a limited extent, the pigment disappearing; *e.g.*, 0·2 mg. of catalyst in 4 c.c. of very dilute alcohol at $p_H$ 4·6 (acetate) is stable indefinitely alone, but fades to an almost imperceptible green solution within 1 min. on addition of 7 mg. of quinol, and simultaneously, a measurable amount of benzoquinone, approximately equivalent to the quantity of catalyst employed, appears:

| Units of catalyst used | 1 | 2 | 3 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| Benzoquinone formed | 0·85 | 1·35 | 2·1 | 2·7 | 3·5 | 4·3 | 5·15 |

(The units are arbitrary; see Experimental.)

Variation of $p_H$ (<7·0) and temperature had only a slight effect. Each molecule of pigment induces the oxidation of approximately 3 molecules of quinol while itself undergoing oxidation. The disappearance of the phthalocyanine cannot be explained as a simple oxidation by benzoquinone, for the pigment is only very slowly affected by benzoquinone solutions many times as concentrated as would be encountered in the above partly oxidised solutions. Moreover, this mutual autoxidation at room temperature also takes place when the quinol is replaced by other compounds normally susceptible to heavy-metal catalysed oxidations, *viz.*, pyrogallol, benzaldehyde, *p*-phenylenediamine, hydrazobenzene, benzidine, α-naphthol, etc., but not by such compounds as formaldehyde, phenol, or tartaric acid. Neither the water-soluble catalyst nor any of the other pigments had any appreciable effect on the autoxidation of aliphatic aldehydes (formaldehyde, acetaldehyde, acraldehyde); these seemed to exert a poisoning action, so that even autoxidation of benzaldehyde failed to take place in their presence. It is noteworthy that the ability of hæmin to catalyse the oxidation of aromatic aldehydes does not extend to aldehydes such as methylglyoxal (Euler and Ahlström, *Z. physiol. Chem.*, 1931, **200**, 233), and some iron-porphyrins may function as anti-catalysts (Dufraisse and Horclois, *Compt. rend.*, 1930, **191**, 1126). However, that the disappearance of pigment when catalysing the oxidation of other organic compounds is due to oxidative degradation induced by the catalysts, is probable from the following observations (established for the water-soluble catalyst but probably true also for the others): (1) Even in presence of the above autoxidisable compounds, the phthalocyanine is stable in complete absence of air or oxygen. (2) In no case was it possible to restore the colour by

mild oxidation or reduction; aqueous solutions of sulphonated iron phthalocyanine may be decolorised by reducing agents (hydrogen sulphide, sodium hyposulphite) but are reoxidised on exposure to air, so it is unlikely that " redox " behaviour plays any essential part in these catalyses. (3) The effect is certainly a heavy-metal catalysis, as it is inhibited by typical poisons (potassium cyanide or thiocyanate, mercuric chloride, potassium iodide). The powerful inhibitory action of potassium cyanide is due to the formation of a complex, as is indicated by the change in colour (blue to green) and in absorption spectrum, the bands being as follows, and (i) being stronger than (ii) in both cases :

Sulphonated iron phthalocyanine in $H_2O$ : (i) 6900 A.; (ii) 6320 A.
" " aqueous KCN : (i) 6685 A.; (ii) 6005 A.

It is remarkable that reduction by aqueous sodium hyposulphite is also inhibited by cyanide.

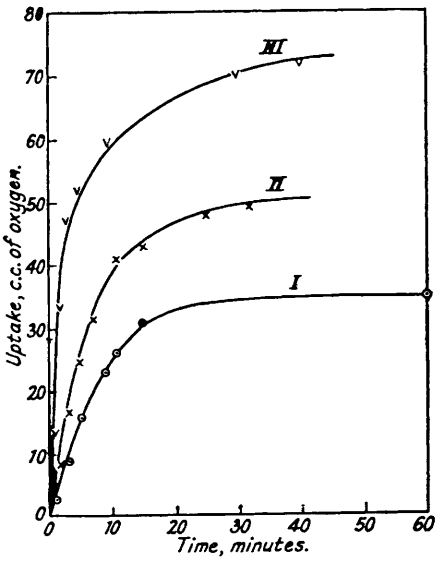
*Autoxidation of Benzaldehyde.*—As the result of many investigations, this autoxidation now appears to be due to a number of normally concurrent catalytic factors; among the more important are photochemical effects (Backström, *Z. physikal. Chem.*, 1934, *B*, **25**, 99), oxidation inhibitors (Meyer, *J. Biol. Chem.*, 1933, **103**, 25), and heavy-metal catalysts (Kuhn and Meyer, *Naturwiss.*, 1928, **16**, 1028). The present communication records some aspects of the ability of synthetic iron pigments of the phthalocyanine group to catalyse the autoxidation of benzaldehyde.

When an aqueous suspension of pure benzaldehyde containing a little amorphous iron phthalocyanine was shaken in an atmosphere of oxygen, the blue-green colour quickly disappeared and was replaced by a pale yellow-brown colour such as was observed by Wieland and Richter when working with simple iron catalysts (*Annalen*, 1932, **495**, 284). This disappearance of the phthalocyanine was accompanied by an uptake of oxygen which was many times that necessary for the degradation of the pigment, and was due to the catalytic oxidation of benzaldehyde to benzoic acid. Similar effects were observed when chloroiron phthalocyanine, iron octaphenyltetrazaporphin, or its salts were employed, but not when any other phthalocyanine pigment not containing iron was added. The course of oxidation was followed by measuring the uptake of oxygen, and the influence of some of the physical variables studied.

Most of the following experiments were carried out in absence of organic solvent. Aqueous acetone or alcohol exerted no influence on the oxidation, but unless rigorously purified solvents were used, catalysis was frequently completely lacking—an effect, presumably, of inhibitors which, as Meyer (*loc. cit.*) has shown, can obscure the effect of many equivalents of a positive catalyst. In no case was catalysis observed when pyridine was employed. Fig. 1 shows that there is no appreciable induction period, and that, under these admittedly arbitrary conditions at least, the oxygen uptake is proportional to the time for the earlier part of the oxidation (approx. 75% of the whole reaction) but falls abruptly before all the benzaldehyde is oxidised. It seems that this is due to a complexity of positive and negative factors, including disappearance of the phthalocyanine as such, and formation of active intermediate complexes and of autoxidation inhibitors. The fact that the phthalocyanine reaction product is far more active than the inorganic iron content might suggest rather adds to the difficulty of examining the behaviour of the pre-formed phthalocyanine alone. That benzoic acid, or possibly perbenzoic acid, acts as an inhibitor is probable from the observation that buffering slightly on the acid side of neutrality resulted in smoother uptake–time curves, and in slightly alkaline solution ($p_H$ 8·0) no inactivation was observed (Fig. 2). A curious feature of the catalyses, which is to be explained by the necessarily complicated conditions of experiment (all were, initially at least, systems in which two liquids and a solid were shaken in a gas), is the relative independence over the range studied of the rate of reaction of quantity of catalyst employed. For instance, the rate at which 1·00 g. of benzaldehyde was oxidised under the standard conditions employed was only slightly increased when the quantity of catalyst (iron phthalocyanine) was increased from 5 to 20 mg. (cf. Fig. 3). The view that the physical conditions were responsible for this apparent anomaly is strengthened by the fact that results of physically similar experiments by Robinson (*Biochem. J.*, 1924, **18**, 255) on the

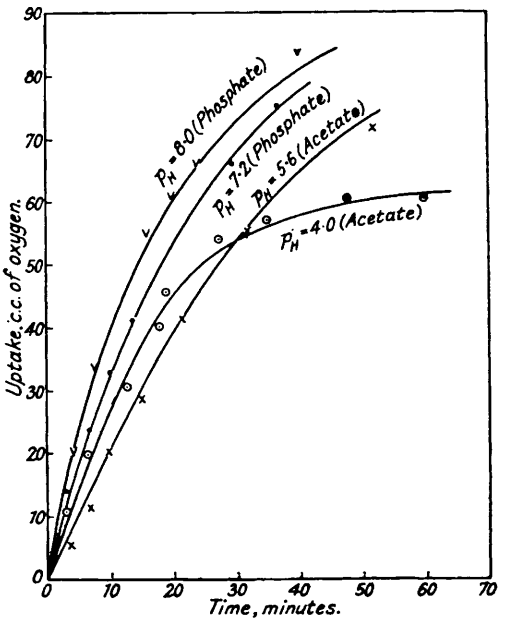
autoxidation of linseed oil in presence of hæmoglobin showed a lack of proportionality between quantity of catalyst and reaction rate. Again, although the phthalocyanine colour vanished at $p_H$ 8·0, the rate of oxidation remained much above that which would be accounted for by the iron content of the pigment, and it is not impossible that a stable intermediate continues catalysis after the phthalocyanine as such has been destroyed. The isolation of any intermediate would be difficult, and it would seem that if one is produced, it cannot be any loose oxygenation product of iron phthalocyanine, for in no case could the pigment be regenerated by reducing agents. The initial stages of catalysis are accompanied by a relative change in the strengths (not the positions) of the spectral absorption bands of the phthalocyanine (dissolved in pyridine), but this phase is only transient and is not recorded on the oxygen-uptake curve. The production of a particularly

FIG. 1.




*Oxidation of* 1·00 *g. of* Ph·CHO:
I. *In* 10 *c.c. of* $COMe_2$ + 12·5 *c.c. of* $H_2O$ + 10 *mg. of* FePc.
II. *In* 12·5 *c.c. of* $COMe_2$ + 12·5 *c.c. of* $H_2O$ + 5 *mg.* FePc *on* 1 *g. of* $BaSO_4$.
III. *In* 25 *c.c. of* $H_2O$ + 10 *mg. of* FePc.

FIG. 2.



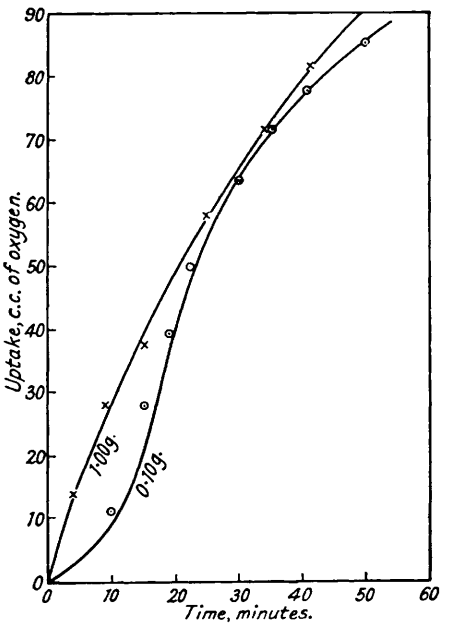

*Oxidation of* 1·00 *g. of* Ph·CHO *suspended in* 50 *c.c. of buffer solution in presence of* 2 *mg. of iron octaphenylporphyrazine deposited in* 200 *mg. of* $BaSO_4$.

active iron oxide owing its activity to its colloidal state rather than to any organic association is possible but unlikely in view of experiments (Part I) on the degradation of iron phthalocyanine by hydrogen peroxide. Oxidation of benzaldehyde in presence of these iron pigments is, as are other heavy-metal catalyses, inhibited to a very marked extent by hydrogen cyanide (Fig. 4). Antioxidants (*e.g.*, quinol) also counterbalance the positive effect of the iron pigments.

In view of the enhanced activity and permanence of iron phthalocyanine and similar pigments in decomposing hydrogen peroxide (Part I) when deposited on charcoal, it was of interest to see if these advantages were retained when the same catalyst oxidised benzaldehyde. It is remarkable that such charcoal deposits were quite inactive when either freshly prepared or aged, even when considerable quantities were employed (Fig. 4). Equally surprising was the observation that deposits of normally active (*i.e.*, iron) pigments on barium sulphate, although possessing an activity towards hydrogen peroxide approximately corresponding to that of the pigment content in homogeneous solution (75% pyridine), were also at least as active when catalysing the oxidation of benzaldehyde as when the pigment was employed without a support. Again the quantity of catalyst was not,

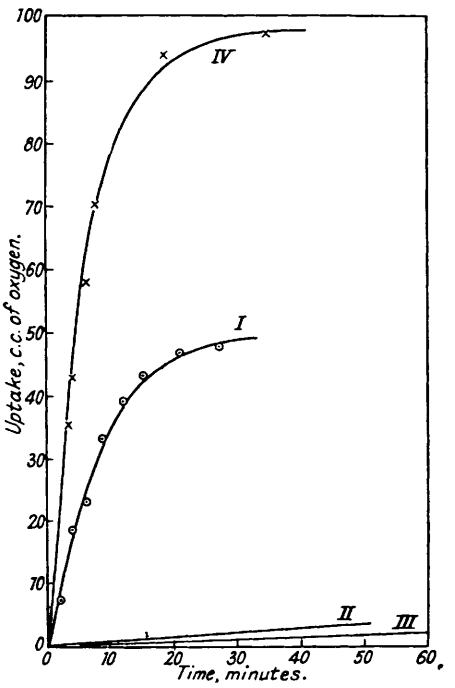
within limits, an important factor deciding the rate of oxidation; quantitie containing as little as 0·25 mg. of iron octaphenyltetrazaporphin (*i.e.*, $10^{-7} \times 2{\cdot}56$M) were sufficient to oxidise 1·0 g. of benzaldehyde completely to benzoic acid in 50 mins. at room temperature. The effect of $p_H$ change on deposits of iron phthalocyanine or octaphenyltetrazaporphin on barium sulphate was quantitatively comparable with the effect on the free pigments, but both pigments possessed a greater stability as deposits, so that an appreciable quantity of pigment could often be recovered from the spent catalyst on extraction with pyridine. In marked contrast to the free pigments, the barium sulphate deposits were insensitive to hydrogen cyanide, and in 1N-hydrogen cyanide solution showed a very

FIG. 3.




*Oxidation of* 1·00 *g. of* Ph·CHO *in* 50 *c.c. of buffer solution* ($p_H$ = 8·0, *phosphate*). *Catalyst: iron octaphenylporphyrazine deposited on* $BaSO_4$ (1 : 100).

FIG. 4.




*Oxidation of* 1·00 *g. of* Ph·CHO.
I. 500 *mg. of* $BaSO_4$–FePc (1 : 100) } 50 *c.c. of* $H_2O$ + 0·25 *c.c. of liquid* HCN.
II. 20 *mg. of* FePc } HCN.
III. 5 *mg. of* FePc *on* 500 *mg. of charcoal* } 50 *c.c. of phosphate buffer,* $p_H$ = 8·0.
IV. 5 *mg. of* FePc *on* 500 *mg. of* $BaSO_4$ } *buffer,* $p_H$ = 8·0.

slightly increased activity. Slight improvement in presence of minimal quantities is not an uncommon observation in work with solid catalysts; *e.g.* Adadurov, Zeitlin, and Orlowa (*Chem. Zentr.*, 1937, i, 3447) find that platinum is less sensitive to poisoning by arsenic when deposited on metal sulphates and oxides. The insensitivity is possibly due in part to competition between carrier and catalyst for the poison which is selectively adsorbed on inactive regions of the carrier surface, but such a theory is obviously inadequate in the present instance; it must be assumed that the quasi-chemical association between the iron atoms and the support renders formation of the inactive cyanide complex more difficult or impossible.

As the conditions of experiment were intentionally maintained constant throughout the series, and precautions were taken to eliminate photochemical action, the effects described above must be ascribed, at least to a predominating extent, to a " heavy-metal catalysis." The positive catalytic effect of many colloidal and finely divided metals,

metal salts, complex salts, etc., on the autoxidation of benzaldehyde has frequently been qualitatively observed, and although iron catalyses have formed the subject of quantitative study by several workers, no single one of the postulated mechanisms will satisfy all the known facts, and indeed, it seems that the final choice of mechanisms will depend on obscure factors. Although it is impracticable to review the many mechanisms proposed to explain iron-catalysed oxidative processes, three broad views are considered in the light of further experiments in Part III (p. 1774).

The variation in catalytic activity on different carriers must clearly be ascribed to a quasi-chemical combination. The catalytic properties of hæmin and of ferric chloride may remain unchanged, or be enhanced or inhibited on adsorption on charcoal or other inorganic carriers (Kuhn and Wassermann, *Ber.*, 1928, **61**, 1550), a quantitative change in one property on adsorption on any one adsorbent being sometimes unaccompanied by a parallel change in the other catalytic properties. The results now reported constitute a further demonstration of the importance of the rôle played by the carrier. The state of the phthalocyanine–carrier catalysts, prepared by precipitating pigment on the support, is possibly more directly comparable with catalysts containing iron "embedded" in charcoal (*e.g.*, those of Warburg and Brefeld, *Biochem. Z.*, 1924, **145**, 461; cf. also Rideal and Wright, J., 1925, **127**, 1347; 1926, 1819, 3182), which have been shown to differ in catalytic behaviour from true adsorbed iron catalysts (Firth and Watson, *Trans. Faraday Soc.*, 1923, **19**, 601). The present observations are, however, also of interest in that they provide synthetic models of the biological system containing a prosthetic group in association with a protein carrier. The activity of enzymes frequently depends largely on a favourable adsorbed state, and a number of natural oxidising enzymes consist essentially of iron–porphyrin complexes associated with proteins (Bertho, *Chem.-Ztg.*, 1935, **59**, 953). The model experiments demonstrate the ability of a carrier to enhance or inhibit normal properties of the prosthetic group, and even to lengthen its working life.

The varying activity of the hæmin enzyme models is thus closely paralleled by the phthalocyanine models, the extraordinary increase in oxygen-transferring power of the iron atom being effected in both cases by the same types of union—a co-ordinative association with four pyrrole nitrogen atoms in a large ring of the porphyrin type.

## Experimental.

*Miscellaneous Substrates.—Iodide.* 10 C.c. of 10% potassium iodide + 5 c.c. of 0·5% starch. The test solution was treated with 1 c.c. of a solution of sulphonated iron phthalocyanine in water (1 c.c. = 0·1 mg.) and became blue after 30 mins. (titration = 0·60 c.c. of 0·001N-sodium thiosulphate) while a blank remained unaffected. The final solution was colourless and showed no spectrum of remaining pigment.

*Polyhydric phenols.* Pyrogallol (50 c.c. of a 1% aqueous solution) absorbed oxygen moderately rapidly in presence of 20 mg. of iron phthalocyanine (control remained blank). After 15 mins. (uptake 43 c.c.) the solution was pale brown.

3·317 G. of quinol were dissolved in 50 c.c. of alcohol and diluted to 1 l. with N/2-acetate buffer ($p_H$ 4·6). 25 C.c. portions of this solution were treated with 5, 10, 15 . . . 50 c.c. of the above aqueous sulphonated iron phthalocyanine solution, the mixtures quickly made up to 75 c.c. with water, and the colour of the pigment allowed to disappear (30 mins.). 10 C.c. of concentrated hydrochloric acid, 5 c.c. of 5% aqueous potassium iodide, and 2 c.c. of 0·5% aqueous starch were added in succession, and the liberated iodine quickly titrated with 0·01N-sodium thiosulphate.

| Catalyst, mg. | 0 | 0·5 | 1·0 | 1·5 | 2·0 | 3·0 | 4·0 | 5·0 |
|---|---|---|---|---|---|---|---|---|
| 0·01N-$Na_2S_2O_3$, c.c. | 0·50 | 1·35 | 1·95 | 2·60 | 3·20 | 4·00 | 4·80 | 5·65 |

The slight effect of change of $p_H$ is shown below (more acid solutions included hydrochloric acid), the conditions being as above, and each determination being carried out with 4·0 mg. of catalyst.

| $p_H$ | 1·6 | 3·6 | 4·6 | 5·0 |
|---|---|---|---|---|
| Titration, c.c. | 4·45 | 4·95 | 4·70 | 5·20 |

Each mol. of sulphonated pigment (*M*, 1056) thus catalyses the oxidation of approx. 3 mols. of quinol while itself undergoing degradation.

*Indophenol formation.* 5 C.c. each of 0·01N-α-naphthol in 50% alcohol, 0·01N-*p*-phenylenediamine in 50% alcohol, and 2·5% aqueous sodium carbonate were treated with 1 c.c. of aqueous sulphonated iron phthalocyanine (0·1 mg.), and developed the typical indophenol colour within 5 mins., the controls becoming pale brown.

*Oxidation of Benzaldehyde.—Materials, apparatus, etc.* The benzaldehyde was a good commercial specimen, redistilled three times at ordinary pressure and finally under reduced pressure in a stream of carbon dioxide in Pyrex apparatus. When a free pigment was employed, it was added as the amorphous powder obtained by precipitating a solution in sulphuric acid (500 mg. pigment in 50 c.c. of concentrated acid) with water, washing the deposit with dilute sodium hydroxide and water, and drying it at 100° for 3 hrs. Although recorded results were generally obtained with alkali-washed products, they were not materially different when this washing was omitted and the catalysts were added as sulphates. When barium sulphate deposits were used, they were prepared by rapidly treating a dilute solution of the pigment in concentrated sulphuric acid with a known weight of barium chloride in aqueous solution (*e.g.*, 20·0 mg. of iron phthalocyanine in 10 c.c. of concentrated sulphuric acid, precipitated in 100 c.c. of water containing 4·166 g. of barium chloride dihydrate, gave a product containing 1 part of pigment "embedded" in 200 parts of barium sulphate). If precipitation is carried out sufficiently rapidly, a homogeneous product is obtained, whereas the pigment tends to separate on the surface of the mother-liquor if slow precipitation is attempted.

Observations were made by weighing out benzaldehyde into a closed flask which, after addition of water, was attached to the volumetric apparatus described in Part I (p. 1768), the catalyst being suspended in the light glass receptacle until equilibrium had been attained, and then shaken into the benzaldehyde suspension. The same flask (100 c.c. round-bottomed Pyrex) was used throughout. All experiments were carried out at room temperature and pressure, but volumes are recorded after correction for pressure of water vapour and conversion to *N.T.P.* All results were obtained by shaking at a constant rate of 130/min. In view of the nature of the unusual number of variable factors and the complicated course of catalysis, the effectiveness of a catalyst is only recorded in graphical form, or as the time necessary to effect a specified degree of oxidation per g. of benzaldehyde. In every case the catalysed oxidations were checked by blank uncatalysed experiments, the negligible rate of uncatalysed oxidation being shown in some of the accompanying graphs.

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