DRUGS AND PORPHYRIN METABOLISM

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INTRODUCTION

Porphyrins are tetrapyrrolic pigments which occur free or in combination in various natural circumstances. They unite readily with metals, and an iron-porphyrin complex is the prosthetic group of haemoglobin and the cytochromes. Small amounts of various porphyrins occur free in mammalian tissues and excreta. These porphyrins are probably formed mostly during the synthesis of the haemoproteins, but they may sometimes occur in other unknown or unrecognised situations. Increased amounts of porphyrin are found, particularly in the urine, in various diseases and under the influence of some drugs and poisons. Large increases occur in the group of metabolic abnormalities known as the porphyrias. Smaller increases are associated with augmented or abnormal

haemopoiesis, and with some disorders of the liver and of the central nervous system. The increments produced by drugs are usually not very large. The drugs concerned include some metals, some hypnotics and some aromatic amines. Knowledge about porphyrin metabolism has increased greatly in the last few years, particularly by the application of chromatographic and isotopic labelling techniques, and this review is concerned particularly with correlating this knowledge with the information available about drugs and porphyrin metabolism.

CHEMISTRY OF PORPHYRINS

Structure of Porphyrins

The chemistry of the porphyrins has been reviewed extensively (55, 102) and only a brief account follows. The porphyrins are red or purple pigments which are most easily detected in the free state by their bright red fluorescence in ultraviolet light. The porphyrin molecule consists of four pyrrole rings joined by methene bridges, and the biologically important porphyrins differ from each other in the substituents on the free β-carbon atoms of the pyrrole rings (Fig. 1).

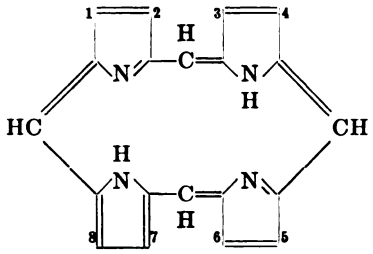

FIG. 1. Porphin, the basic porphyrin nucleus

Each pyrrole carries one methyl radicle (or an acetic acid radicle in uroporphyrin) and one other group, which may be propionic acid or an ethyl or vinyl radicle (Table I). When the second group is the same on all four pyrroles, four stereoisomers are theoretically possible (I, 1:3:5:7-; II, 1:4:5:8-; III, 1:3:5:8-; and IV, 2:3:5:8-tetramethyl, with the remaining sites occupied by the alternative groups) but only types I and III are known to occur naturally. Conversion of type I to type III porphyrins, or the reverse, involves exchanging the substituents on one pyrrole ring without interfering with the others, and it is generally assumed that this is unlikely to occur in the body. On the other hand, conversion of uroporphyrin to coproporphyrin of the same type involves only decarboxylation and can easily take place naturally. When there are three substituents, as in protoporphyrin (tetramethyldivinylporphindipropionic acid), more isomer types are possible, but only the type known as type IX (which can be derived from uroporphyrin III or coproporphyrin III by decarboxylation and oxidation) is important. Protoporphyrin IX is widely distributed, combined with iron, in haemoglobin and the haem enzymes, and it and some other dicarboxylic porphyrins (mesoporphyrin and deuteroporphyrin), also of type IX,

TABLE I

Varieties of Porphyrins

NAME	COMPOSITION	NO. OF —COOH GROUPS
Uroporphyrin I	Porphin-1:3:5:7-tetraacetic acid-2:4:6:8-tetrapropionic acid	8
Uroporphyrin III	Porphin-1:3:5:8-tetraacetic acid-2:4:6:7-tetrapropionic acid	8
Coproporphyrin I	1:3:5:7-tetramethylporphin-2:4:6:8-tetrapropionic acid	4
Coproporphyrin III	1:3:5:8-tetramethylporphin-2:4:6:7-tetrapropionic acid	4
Mesoporphyrin IX	2:4-diethyl-1:3:5:8-tetramethylporphin-6:7-dipropionic acid	2
Protoporphyrin IX	1:3:5:8-tetramethyl-2:4-divinylporphin-6:7-dipropionic acid	2
Haematoporphyrin IX	2:4-dihydroxyethyl-1:3:5:8-tetramethylporphin-6:7-dipropionic acid	2

are found free in some biological material. It was formerly believed that haematoporphyrin, which is obtained by treating haem with sulphuric acid, occurred naturally, but the identity of the porphyrin of haem is established beyond doubt as protoporphyrin and there is no evidence that haematoporphyrin ever occurs in animals. Its absorption spectrum closely resembles that of coproporphyrin and reputed appearances of haematoporphyrin in urine and other materials were probably due to the presence of coproporphyrin. Unfortunately the terms haematoporphyrin and haematoporphyrinuria have become embedded in textbooks of medicine and physiology and still occasionally and inaccurately persist.

Extraction and Identification

The porphyrins are amphoteric substances, readily extracted from organic solvents by strong acids or alkalies and passing into organic solvents from neutral or faintly acid solutions. The uroporphyrins, with most carboxyl groups, are least soluble in organic solvents, though uroporphyrin, anyway of type III, can be extracted from urine with ethyl acetate if the pH is carefully controlled. The uroporphyrins are usually extracted by adsorption on talc, or on precipitates of phosphates or other convenient media. The porphyrins with fewer carboxyl groups are usually extracted with mixtures of ether and acetic acid and are purified by partition between ether and hydrochloric acid, or chromatographically. When free porphyrins are heated, they decompose without melting, and it is usual to form the methyl ester and determine its melting point in order to identify a particular specimen. Solutions of porphyrins can be estimated either by means of their intense red fluorescence in ultraviolet light, or by their light absorption, which is greatest at a point near 400 mμ, the exact position depending on the particular porphyrin and on the solvent. For identification of a particular porphyrin, its chemical behaviour, its absorption spectra in organic solvents and

in acid and alkali, the melting point of the methyl ester and the chromatographic properties of the free porphyrin and its methyl ester are all useful. Chromatographic methods have been developed considerably in recent years (70, 72, 122) and their value has been much increased by their accurate calibration with known porphyrins. In particular it has been shown that the order of elution of the methyl esters and the R_f of the free porphyrins on partition on paper between lutidine and water both depend on the number of carboxyl groups in the porphyrin. The second method is easier to apply for purposes of identification as known standard porphyrins can be run on the same strip of paper as the unknown. The method has shown the presence in biological materials of intermediate porphyrins which behave as though they contained three, five, six or seven carboxyl groups. Full supporting evidence for the number of carboxyl groups in these hitherto unrecognized porphyrins is not always available and their detailed chemical structure has yet to be worked out, but their separate identity is clearly established and they must be considered in any interpretation of the metabolism of porphyrins.

Isomer Analysis of Porphyrins

Methods for distinguishing the isomeric type of a particular porphyrin, or the proportion of different isomers in a mixture, are generally unsatisfactory. The distinction is important, because there is no reason to believe that the naturally occurring types I and III are interconvertible, and the type of a particular porphyrin is some indication of its origin. When the crystalline methyl ester is available, its melting point is a guide to its identity, but not to its purity. Melting point curves for mixtures of type I and III isomers have been published for coproporphyrin (88) and for uroporphyrin (125) and show that up to ten or fifteen per cent admixture of the opposite type to that mainly present has little or no effect on the melting point. (Small amounts of uroporphyrin III have more effect on the melting point of uroporphyrin I.) The extent to which the isomers separate on crystallization or recrystallization is uncertain, and with uroporphyrin it is clear that stable mixtures or loose molecular compounds are formed, of which the melting points are not changed by repeated recrystallisation (96). Melting point determinations by themselves are therefore of limited value in determining the original composition of an isomeric mixture.

An early claim that coproporphyrins I and III could be differentiated by their pH-fluorescence curves (50) has not been substantiated on repetition with well purified materials (88). A separation of these esters by chromatography on alumina and elution of the type III isomer with 35% acetone (195) has not been confirmed (80, 88) and has since been reported to give uniform results only with previously crystallized coproporphyrin (150). The lower solubility of the type I isomer in 30% acetone has since been used by Watson's group in a fluorescence quenching method, and much clinical material has been analysed in this way (149, 150, 190). Results using this method appear not to have been reported from other laboratories, and difficulties have been found in reproducing the technique (48).

Paper chromatographic methods of separating the coproporphyrins (25) and the uroporphyrins (45) now appear the most promising procedures though further experience of their use and limitations is desirable. The application of infra-red spectroscopy also has possibilities, as there are distinct differences in the absorption of different position isomers in this range (48).

Porphyrin Precursors

In some circumstances, particularly in urine, some or all of the porphyrin which can be extracted is derived from a precursor which changes readily to the free porphyrin during extraction, or in the time between the secretion of the urine and its analysis. The best known precursor is found in pathological urines containing uroporphyrin. It is characterized by giving a red colour with Ehrlich's reagent, (not extractable with $CHCl_3$, in contrast to the reaction of urobilinogen) (196). This material, named porphobilinogen by Waldenström and Vahlquist (178), has been the source of much controversy in the last ten years, which appears to be adequately disposed of by Westall's (203) crystallization of the material and Cookson, Rimington and Kennard's (29, 30) identification of its structure (Fig. 2). Four molecules of this substance combine readily and non-enzymically to yield uroporphyrin, mainly of type III, and under appropriate enzymic treatment it is converted also to copro- and protoporphyrins (46).

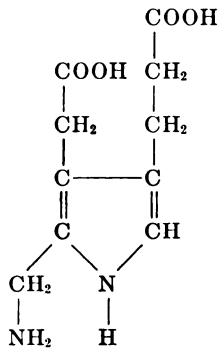

FIG. 2. Porphobilinogen

Some at least of the coproporphyrin found in the urine, normally or pathologically, is also excreted as a precursor. This observation, made originally in 1896 (139), has received only fitful attention until recently, and appears to be regarded mainly as a possible source of inaccuracy in coproporphyrin estimations due to the overlooking of unconverted precursor (110, 113, 186, 192). However, it is possible that the precursor is more important physiologically, and indeed there are difficulties in believing that the kidney can excrete coproporphyrin at all. Large quantities of coproporphyrin have sometimes been found in the urine without any detectable porphyrin in the plasma (197); and coproporphyrin injected intravenously is largely recoverable from the bile without any appearing in the urine (35, 173, 174, 194). (Earlier contrary observations (54, 77) are

reported with insufficient detail to be convincing.) These observations suggest strongly that the so-called "pre-formed" coproporphyrin is also derived from precursor but too rapidly to be detected in its original state.

The identity of the precursor of coproporphyrin is not known. Its conversion appears to be accelerated by light, ultraviolet radiation, mild oxidizing agents (127, 192) and possibly simply by acidification (42). In some cases the precursor studied is probably a metal complex of coproporphyrin (65, 89): in view of the ease with which porphyrins form metal complexes such a complex could form secondarily after development of coproporphyrin from some other precursor. Watson *et al.* (192), on the basis of their observations with oxidising agents, incline to regard the chromogen as reduced or tetrahydro-coproporphyrin. Alternatively, by analogy with porphobilinogen, the possibility of a monopyrrolic precursor must be considered. This last hypothesis has the attraction that when monopyrroles condense to form porphyrins, mixtures of types are known to occur in somewhat variable proportions (30, 106, 156), and if this were true in the present case it would possibly account for the discrepant observations which have been made on the normal urinary isomer type (*vide infra*).

There is some evidence that porphyrins in the bile, faeces (192) and plasma (98) are also present partly if not entirely as precursors, but very little detailed work has been published. Until means are found for preventing the precursor turning to free porphyrin it is difficult to assess how important precursors of this sort are likely to be in the physiology of the porphyrins.

OCCURRENCE AND METABOLISM OF PORPHYRINS IN MAMMALS

Occurrence in Urine

Considerably more attention has been paid to the porphyrins of urine than to those elsewhere in the body. This is probably due to the ease with which urine can be obtained rather than to the particular importance of the material excreted in this way. The urinary porphyrin in normal humans, as usually extracted and estimated, is mostly a mixture of coproporphyrins of types I and III. Type I is generally believed to predominate, as it was crystallized by Fink and Hoerburger (51) and by Watson (182) and by Grotepass (76). However, all these authors recognised the presence of type III coproporphyrin, which crystallizes less readily, in their extracts, and Grotepass, who worked with much the largest quantity of material, crystallized almost as much of the type III porphyrin (87 mg.) as of the type I (96 mg.). The melting point of the type I crystals obtained by Watson was, moreover, very low (228° instead of 248–252° for the pure synthetic ester) and from Jope and O'Brien's data for melting points of mixtures of coproporphyrins I and III (88) the crystals examined could by themselves have consisted of fifty per cent type III, apart from the additional type III which failed to crystallise. In uncrystallized material from normal individuals, Watson *et al.* (189) generally found, by the fluorescence quenching method (149), a preponderance of type I, but in one group of subjects type III was consistently present in excess. Type III also predominated in a small series studied by Comfort, Moore and Weatherall (26) using the paper chromatographic method of

Chu *et al.* (25), so it seems possible that the "normal" isomer type is more variable than is usually recognised.

Minute amounts of other porphyrins have been found by paper chromatography of extracts of human urine (28, 122). They have not been fully characterized, but appear to include uroporphyrin and other porphyrins with more than four carboxyl groups. The amounts are so small that they would not have been detected by the less sensitive methods used by Grotepass (76) or Brachvogel (12), who reported the absence of uroporphyrin in normal urine. This discovery is of great interest alongside recent observations which suggest that uroporphyrin is an intermediary in the synthesis of less highly carboxylated porphyrins and of haem (46, 47, 120, 141, 186). Its pathological occurrence is more comprehensible if it is due to an arrest of normal metabolism rather than due to a diversion involving the abnormal carboxylation of proto- or coproporphyrin, as was formerly believed by Fischer.

The urinary porphyrins have received less attention in species other than man. Coproporphyrin I has been extracted and crystallized as the methyl ester from the urine of dogs (100): the extent to which coproporphyrin III or other porphyrins may be present has not been established. In the rabbit coproporphyrin III predominates (36, method not stated; 153, fluorescence quenching method), and small amounts of tri- and penta- or hexa-carboxylic porphyrin are present (27, 201). Uroporphyrin has also been reported, but the (fluorescence) method employed was not fully described and appears to be of questionable specificity (151). Coproporphyrin III predominates also in rat urine and again uroporphyrin has been reported, by a method of unvalidated specificity (21). The fox squirrel, *Sciurus niger*, has a quite different porphyrin metabolism: its urine contains large amounts of uroporphyrin and some tri- and pentacarboxylic porphyrin and resembles that of a patient with porphyria rather than that of other normal mammals (123, 168).

Occurrence in Bile and Faeces

There are more porphyrins in the faeces than in the urine and they have been less fully investigated. Various dicarboxylic porphyrins are present and are probably partly or entirely produced by micro-organisms in the alimentary canal. Coproporphyrin I has been reliably demonstrated (crystals, melting point and mixed melting point) in normal subjects (34, 183) but coproporphyrin III and uroporphyrin apparently have not been found in normal faeces. Watson (183) also did not find any coproporphyrin I in the faeces in patients with complete obstruction of the common bile duct, even when occult blood was present in the samples analysed. It therefore appears unlikely that it is formed by bacterial action, and its biliary origin is supported by various observations of its presence in normal bile (102). Coproporphyrin and other porphyrins have been observed in meconium (123).

Occurrence in Blood and Tissues

Porphyrins have been detected in various tissues normally and pathologically. The amounts in the plasma are normally very small or undetectable, and have

not been reliably identified (102 for refs.): there is some indication that larger amounts may be formed from a precursor (98). Erythrocytes contain moderate amounts of protoporphyrin IX (2–20 μg/100 ml.) (9, 19, 71, 148) and smaller amounts of coproporphyrin, mainly III (152, 185). The supposed correlation of increased blood protoporphyrin with reticulocytosis (187) has not been substantiated (188) and there is a closer correlation between reticulocytosis and increased erythrocyte coproporphyrin (185). Porphyrins are present in the bone marrow (146, 159). Schmid, Schwartz and Watson (146) give concentrations of about 100 μg/100 g. for protoporphyrin and 2–8 μg/100 g. for coproporphyrin in rabbits. Uroporphyrin is also present, though normally in very small amounts (141). The liver contains protoporphyrin IX (132), and the brain coproporphyrin III (2–5 μg/100 g.) (24). Substantial amounts of porphyrin are present in the Harderian glands of mice and rats, especially in cancer susceptible strains (162). Protoporphyrin is predominant (107, 161, 166, 167) and traces of coproporphyrin have also been reported (107). Red tears, which owe their colour to porphyrin, are secreted by the glands under the influence of muscarinic drugs (58) and a method of assaying anticholinesterases has been based on this observation (17).

Synthesis of Porphyrins in the Body

Various theories have been proposed to account for the formation of porphyrins in the body and particularly for the occurrence of two and only two isomeric types (36, 102, 120, 131, 133, 169). Evidence about the origin of porphyrins has come mostly from isotopic studies by Shemin, London and Rittenberg and by Grinstein and Watson in America, and by Neuberger, Muir and Gray in England (69, 73, 105, 120, 154, 155).

The nitrogen atoms of the porphyrin ring are derived from glycine: the carbons of the methene bridges are derived from the methylene carbons of different glycine molecules: and carbon from acetate and pyruvate is utilized in forming the pyrrole rings. The acetate or pyruvate is probably converted to succinic acid or a related substance, which, on some very recent evidence of Shemin and Russell (155), condenses with glycine to form a compound δ-aminolaevulinic acid (Fig. 3). This substance is utilized preferentially to glycine or succinate in

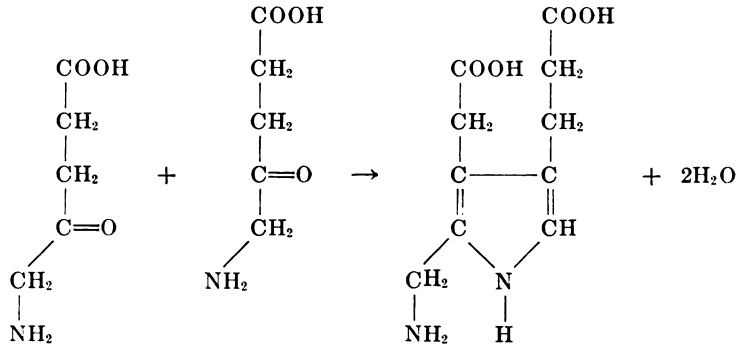

FIG. 3. δ-Aminolaevulinic acid and porphobilinogen

the synthesis of haemin by duck's blood, and its α-carbon atom is much more efficiently incorporated than the corresponding atom of glycine. Condensation of two molecules of δ-aminolaevulinic acid would yield a substituted pyrrole with the same structure as has been proposed for porphobilinogen (29, 30). The independent pieces of evidence are therefore in convincing agreement, and it appears very likely that both δ-aminolaevulinic acid and porphobilinogen are normal intermediaries in the formation of haemoglobin. The concept of the normal occurrence of porphobilinogen in traces too small to be detected presents no difficulty, as it is readily utilized by normal tissue systems to form uro-, copro- and protoporphyrins, and uroporphyrin can be converted to protoporphyrin by bone marrow homogenates (141) and by chicken red cell haemolysates (46). Some coproporphyrin is formed also in the latter circumstances, especially anaerobically. Utilisation of coproporphyrin by cell haemolysates has not been demonstrated, and it is not rapidly metabolized by the whole animal. Its position in the normal anabolic pathway is uncertain.

An old belief that coproporphyrin could be formed by the liver from protoporphyrin was based on evidence (9) which has not been confirmed (193) and in the light of recent developments appears most unlikely. The possible derivation of coproporphyrin from methaemoglobin, which now also appears less likely than when it was suggested, is discussed below.

DISTURBANCES OF PORPHYRIN METABOLISM

It is convenient for present purposes to divide disturbances of porphyrin metabolism into three groups.

1) Abnormalities of unknown cause, probably inborn, particularly affecting porphyrin metabolism, *i.e.* the porphyrias.
2) Acquired abnormalities associated with disease of certain organs, especially the bone marrow and the liver.
3) Abnormalities associated with the administration of drugs.

The term "porphyria" is now generally confined to the first group and the term "porphyrinuria" is applied whenever excess of porphyrin appears in the urine. The distinction between these classes is not absolute, as acute episodes in a patient with porphyria may be due to incidental liver disease, or to the administration of certain drugs; or cirrhosis of the liver causing increased porphyrin excretion may be induced originally by toxic drugs. The magnitude of the porphyrinuria is much greater in porphyria than in the other states.

Porphyria

The porphyrias, diseases in which an abnormal production or failure of utilization of certain porphyrins appears to be primary, are usually divided into three types (22, 60, 78, 177, 186): 1) congenital, photosensitive, erythropoietic; 2) acute, intermittent or toxic; 3) mixed, cutaneous, chronic, hepatic.

Congenital porphyria is rare and hereditary. It appears early in life and is characterized by the presence of porphyrin in the bones, teeth and urine. The

predominant porphyrin is uroporphyrin I, but coproporphyrin I and small amounts of uroporphyrin III are also found in the urine, and recently hepta- and pentacarboxylic porphyrins have also been described (138).

Acute porphyria appears in various forms. The excessive excretion of porphyrin may be symptomless or it may be associated with attacks of abdominal pain, or with paralyses and other nervous signs, or with both. Psychological disturbances are not unusual. The characteristic material in the urine is the so-called Waldenström porphyrin, originally regarded as uroporphyrin III (177) but later claimed to be a mixture of uroporphyrin I and a heptacarboxylic porphyrin (70) or a molecular association of uroporphyrins of types I and III (125, *q.v.* for earlier literature). The faecal porphyrin excretion is also much increased. The urine contains porphobilinogen at least during acute attacks. An hereditary element can sometimes be traced. Precipitating events responsible for the acute attacks are commonly sought, and various drugs have been incriminated from time to time. The evidence is apt to be of the *post hoc, ergo propter hoc* kind; the more convincing observations are discussed below with the appropriate drugs.

In chronic porphyria or porphyria cutanea tarda, photosensitivity occurs, though sometimes late. Abdominal pains and jaundice are not unusual, and a mild course is common. The excreted porphyrins vary considerably, both in type and number of carboxyl groups (109, 202). There is some evidence that acute episodes are associated with a diversion of the excreted porphyrins from the faeces to the urine (67, 109).

Porphyrinuria

The symptomatic porphyrinurias are associated particularly with anaemia, liver disease, infectious diseases and various intoxications. The increments in porphyrin excretion are much smaller than those which occur in the porphyrias and rarely exceed a total output of one to two milligrammes a day. Increments in the faecal excretion of dicarboxylic porphyrins probably occur, but most observations have been concerned with coproporphyrin which may appear in excess in the urine or faeces or both. As the available evidence suggests that very little coproporphyrin, whether of type I (35, 173) or type III (173, 174, 199) is excreted by the kidneys, the urinary coproporphyrin is probably an index of an excess of circulating precursor rather than of coproporphyrin itself. In liver disease increased amounts of either type of coproporphyrin may appear in the urine, and the faecal coproporphyrin is often decreased (104, 118). When the porphyrin is of type I this has been envisaged as due to failure of the liver to perform a normal excretory function. When the urinary porphyrin is of type III, as is commonly the case in alcoholic cirrhosis and sometimes in other kinds of liver disease (14, 165) this explanation does not serve, because coproporphyrin III is not normally found in the bile or faeces. Possibly the liver normally utilizes the coproporphyrin precursor, or possibly it produces some substance which promotes its normal utilization. In any case, increased excretion of coproporphyrin in the urine does not necessarily accompany injury to the liver (68).

Excessive total excretion of coproporphyrin I, partly in the urine and partly

in the faeces, is associated with increased haemopoiesis, for example in pernicious anaemia in remission, and the rate of excretion has been proposed as a measure of the activity of the bone marrow (34, 36, 131). This proposal is based on the assumption that a small but normally constant fraction of the total porphyrin synthesized is of the wrong type for incorporation into haem and that this fraction is then excreted.

Increased coproporphyrin excretion has been observed in various fevers (59), notably poliomyelitis (194), but the cause is unknown. The porphyrinurias due to drugs are discussed below.

DRUGS AFFECTING PORPHYRIN METABOLISM

Abnormalities in the excretion of porphyrins have been observed after the administration of various drugs and poisons. Much of the experimental work is old and the identification of the porphyrins concerned is probably incomplete if not inaccurate. The clinical observations have often been made on patients in whom treatment with drugs followed some illness so that it is difficult to distinguish between the effects of the drugs administered and the effects of the original disease and the individual's innate metabolic pattern. A large number of observations have been considered by Watson and Larson (190) and need not be repeated here. The substances which affect normal porphyrin metabolism can be grouped as: metals, dimercaprol (British Anti-Lewisite, BAL), aryl amines, hypnotics, salicylates, vitamins.

Metals

(*a*) *Lead.* The best known change in porphyrin metabolism in lead poisoning is the appearance of abnormal amounts of coproporphyrin III in the urine. The pigment appears rapidly, within a day or less if lead is administered intravenously (31, 172, 200) or within three days when lead is given by stomach tube (198). The relatively slow onset and gradual increase of porphyrinuria after intraperitoneal or subcutaneous injection of lead salts (153) is to be expected from the very slow absorption of lead from these sites (43, 204). Porphyrinuria is one of the first symptoms of chronic lead poisoning and is at least as sensitive as and possibly more specific for diagnostic purposes than punctate basophilia (99, 111, 180).

The quantity of porphyrin present depends to some extent on the species as well as the dosage of lead; in man and rabbits increments of the order of five to fiftyfold over normal are to be expected (153, 190, 198) while in rats the increments are smaller (128) and in dogs relatively slight (140). As far as the evidence goes, the extent of the porphyrinuria in different species is parallel to the liability to develop anaemia after exposure to lead (3), though in individual members of a species there is little obvious correlation (93, 198).

The porphyrin has been identified repeatedly by crystallization and measurements of its melting point, absorption spectrum, and fluorescence and chromatographic behaviour (27, 53, 75). Chromatographic methods have shown that smaller quantities of other porphyrins are also present. In four lead workers

Kench, Lane and Varley (94, 95) found a twenty or thirty-fold increase in the urinary excretion of coproporphyrin I as well as larger increases in coproporphyrin III; and in one of their cases an additional unidentified ether-soluble porphyrin was present in about the same quantities as coproporphyrin I. In the urine of lead-poisoned rabbits, Comfort and Weatherall (27) have found a complex mixture of porphyrins. Coproporphyrin III predominated; coproporphyrin I was not found; and the other porphyrins appeared by the methods of Nicholas and Rimington (122, 123) and of Chu, Green and Chu (25) to be tri-, penta- and hexacarboxylic and of two isomer types. It seems probable that at least the coproporphyrin III is excreted as an unidentified precursor (199).

Apart from cases strongly suggestive of one or other type of porphyria occurring perhaps fortuitously or perhaps precipitated by exposure in workers with lead (86), neither porphobilinogen nor uroporphyrin have been reported in the urine in lead poisoning. Small quantities of uroporphyrin may have been overlooked, as most observations made since paper chromatographic methods of detection have become available have been made on ether extracts of urine (27, 94, 95), which would not include uroporphyrin.

The total faecal porphyrins appear to be increased in quantity in lead poisoning but there is little or no increase in coproporphyrin (92, 198) and the coproporphyrin which is present is of type I (175, 183) as in normal subjects. An unidentified porphyrin, "porphyrin x" (methyl ester m.p. 210–215°), has been isolated by Grinstein, Wikoff, de Mello and Watson (73) and tentatively identified by them with the supposed deuteroporphyrin reported by Fischer and Duesberg (53) (ester m.p. 184°) to be present in excess in these circumstances. This may also correspond to a porphyrin of similar origin and ester melting point (187°) obtained by Weatherall (200) which ran as a tetracarboxylic porphyrin on lutidine paper chromatography.

Besides the increased excretion of porphyrins, there is an increase in the concentration of both proto- and coproporphyrin in the erythrocytes (8, 144, 146, 185). Both porphyrins appear to be increased in about the same proportion (185). There is also a large increase in the total porphyrin in the bone marrow, at least in rabbits (40, 103, 144, 146). It has recently been claimed that this increase is due about equally to coproporphyrin III and to uroporphyrin, most surprisingly of type I (144). In view of the controversial position of the uroporphyrins, further observations on this matter are desirable. The cerebral coproporphyrin concentration is not changed in lead poisoned rabbits (24).

Various attempts have been made to modify the effects of lead on porphyrin metabolism, and to study the intoxication in other ways. The most important experiments appear to be those of Grinstein *et al.* (73), who fed N^{15}-labelled glycine to lead-treated rabbits, and recovered coproporphyrin containing N^{15} from the urine and "porphyrin x" also containing N^{15} from the faeces. The largest amounts of labelled nitrogen were present in the earliest samples obtained after its administration, *i.e.*, within the first six days, and therefore it appears that the abnormal amounts of porphyrin appeared as a by-product of some anabolic process, and not from the breakdown of red cells accompanying lead

poisoning. This agrees with earlier observations (10, 92) in which haemoglobin was administered to patients or rabbits with lead poisoning, without any effect on the excretion of porphyrin. It is also consistent with the thesis (131) that in lead poisoning the primary lesion is a failure of haemopoiesis, in which the incorporation of iron in the porphyrin ring is blocked, and there is some accumulation of unutilized porphyrin. However, there appears also to be an earlier metabolic obstruction as coproporphyrin and more highly carboxylated fractions accumulate as well as protoporphyrin. The connection between the porphyrin accumulation and dyshaemopoiesis seems well established, as the bone marrow is the outstanding tissue in which excess of porphyrin has been demonstrated. It must be realised, as Kench *et al.* (93) have pointed out, that in the anaemia of lead poisoning the lack of haem pigment formation in the body is of the order of 50 mg./day, while the excess of excreted porphyrin is at the most a milligramme or so daily. Either there are other factors involved in the anaemia, which is likely enough (3, 39, 44, 108, 158) or the rate of porphyrin synthesis is very closely adjusted to the capacity of the body to utilize the material formed and this adjustment is only slightly upset in lead poisoning.

Other observations on factors influencing the porphyrinuria due to lead are of little importance in the present context because the agents concerned certainly or possibly alter the distribution of lead and so may increase or decrease the quantity of free lead in the circulation or in the bone marrow. The porphyrinuria is increased by the simultaneous or subsequent administration of thorium nitrate, zirconium citrate (153) or dimercaprol (153, 176, 198, 206). These substances all increase the urinary excretion of lead, and it seems likely that their effect on porphyrin metabolism is secondary to mobilization of lead. The action of dimercaprol is discussed further below.

The excretion of coproporphyrin by lead-poisoned animals is increased also by exposure to light (116). Other features of lead poisoning, such as wasting and death, occur more quickly in animals kept in sunlight instead of in the dark. They occur more quickly also when cod liver oil is added to the diet and they have been attributed to increased absorption of lead promoted by vitamin D (128). In de Mello's experiments (116) lead acetate was given subcutaneously or intraperitoneally, and the period of exposure to light was probably too short for significant amounts of irradiated ergosterol to be formed, even if they could have influenced the distribution of lead. Possible changes in lead distribution have not been excluded, but the phenomenon may alternatively have some connection with the action of the dye Rose Bengal, which substantially increases the excretion of coproporphyrin in the urine when the animals treated are also exposed to light (115). This action is discussed below.

(*b*) *Arsenic.* Increased urinary excretion of coproporphyrin, mainly of type III, has been observed after administration of As_2O_3 in rabbits (153) and from time to time after injections of arsphenamine or neoarsphenamine in patients (16, 84, 143). The porphyrin has been identified by crystallization (84); and the isomer type has been determined also by fluorescence quenching methods. When a single dose of arsenic is given to rabbits the excretion of porphyrin is increased

five to tenfold for two or three days and then returns to normal. This rapid and brief effect corresponds to the rapid absorption and distribution of arsenic in the body, and does not suggest a mode of action necessarily different from that of lead. Changes in urinary porphyrins after a single dose of arsenic appear not to have been followed clinically: the reported cases are patients showing chronic toxic effects of arsenical medication. In one of these, administration of dimercaprol increased the urinary coproporphyrin to about double its previous level (143): possible interpretations of this observation are discussed below.

(*c*) *Uranium.* The effect of uranium has been studied in some detail by Schwartz and Zagaria (153). Uranium injures the kidney severely, and in lethal or near lethal doses substantially reduced the urinary excretion of coproporphyrin both in normal rabbits and in lead-poisoned rabbits, as well as producing oliguria or anuria. However, smaller doses of uranium increased the amount of ether soluble porphyrin in the urine, though not to more than two or three times the normal level. The type of porphyrin appears not to have been identified.

(*d*) *Mercury.* The evidence that mercury affects porphyrin metabolism is unconvincing. Two reported cases (170, 175) are complicated by other factors and provide no definite evidence on the point. One report of the finding of large amounts of porphyrin in the urine of rats poisoned with mercury lacks any detail about how the porphyrin was recognised (163). On the other hand, Schwartz and Zagaria (153) failed to find any effect from subcutaneous injection of two doses of mercuric chloride in one rabbit. These doses were near the maximum amounts which rabbits normally tolerate, and probably caused renal damage, though this is not stated. The possibility remains that, as with uranium, smaller doses would have produced increased porphyrinuria, and the problem evidently requires further examination.

(*e*) *Other metals.* Thorium and perhaps lanthanum appear to reduce porphyrin excretion, possibly, like uranium, by injuring the kidneys. The effect is milder, and, unlike uranium, thorium increases the porphyria of lead poisoning, probably by mobilising lead (153). Irregular increases in porphyrin excretion have been observed also after beryllium (153). Some increase has been claimed after bismuth, iron, silver and zinc and none after copper nor gold (163), but the experimental details are insufficient to judge the claim. No observations appear to have been made about tin or antimony, in spite of their near chemical relation to highly active elements.

Dimercaprol

It is convenient to consider dimercaprol in this context, because its activity is closely related to the behaviour of metals. Dimercaprol can cause porphyrinuria when it is injected into rabbits in large doses (153, 200). The effect is accompanied by urobilinogenuria, suggesting simultaneous occurrence of liver damage (153). The porphyrinuria is by no means an invariable accompaniment of giving even lethal or nearly lethal doses of dimercaprol. The material excreted appears to be coproporphyrin, on the basis of its HCl number and its absorption spectra (200), but the isomer type appears not to have been established.

Both in arsenic and in lead poisoning dimercaprol commonly increases the excretion of coproporphyrin (143, 153, 198). This effect can be attributed to mobilization of the effective metal from sites where it was stored inactively and its liberation generally or at the site where it affects porphyrin metabolism. Substantial redistribution of lead occurs after dimercaprol (2, 61). Similar redistribution after arsenic has not been described, and seems less likely, as the thiol-arsenite is considerably more stable; however, it is difficult to avoid this explanation in the case of Sands *et al.* (143) where a general exacerbation of the symptoms of arsenical poisoning, and increased excretion of coproporphyrin in the urine, were provoked by dimercaprol.

Possible ways in which dimercaprol might cause porphyrinuria appear not to have been studied or discussed. In view of the irregularity of the phenomenon this is not surprising. At least two mechanisms can easily be conceived. One is that dimercaprol forms a complex with some metal necessary in the synthesis of haem compounds. This is not impossible, as a mild anaemia has been reported in cats treated with dimercaprol (117). It is also possible that dimercaprol mobilizes small quantities of lead which are always present in healthy animals (18) and that this lead is directly responsible for the effect. Both explanations would account for the variability of the phenomenon, the occurrence of which would depend on the amount of the particular metal available or on previous exposure to lead respectively. Other mechanisms, such as injury to the liver, are also possible.

Aryl Amines

Small increments in the excretion of porphyrin have been observed from time to time clinically and experimentally after the administration of sulphanilamide and other aryl amines. Experimentally in white rats, sulphanilamide, prontosil soluble and 4:4′-diaminodiphenylsulphone in large doses increase the urinary ether-soluble porphyrins two- to tenfold (135, 136, 205). The increment is due mainly to coporporphyrin III, identified by the melting point of its methyl ester in each case. Aniline and some other aniline derivatives are equally or more active. Similar observations have been made with acetanilide, amidopyrine, phenazone and phenacetin (13). Rimington and Hemmings (136) found that certain related compounds, including methylacetanilide and *p*-phenylenediamine, were inactive, and pointed out that among the twenty-seven compounds which they studied there was a practically quantitative correlation between the amount of porphyrin in the urine and the degree of methaemoglobinaemia which occurred. This correlation appears to hold good, but the suggestion made at the time that the coproporphyrin which appears in the urine is a breakdown product of methaemoglobin has not received further support. Porphyrinuria has not been described after methaemoglobinaemia due to other causes, and no more details of the postulated conversion of methaemoglobin to coproporphyrin have been elaborated. Additional difficulties in the way of this hypothesis have been elaborated by Lemberg and Legge (102).

Brownlee (13) comments that in addition to coproporphyrin the urine of rats

treated with aryl amines contained material which darkened on exposure to air and light, so that on standing the urine became dark or black, at first on the surface and later spreading downwards. A similar effect has been noted in the urine of lead-treated rabbits (200). The identity of the material is unknown; its appearance in both conditions—both characterized by the presence of excess coproporphyrin III in the urine—suggests that a common mechanism is perhaps involved.

Clinically, increased excretion of porphyrin after administration of sulphanilamide appears to be slight and infrequent and, apart from a possible connection with photosensitisation dermatitis (135), is of little or no practical importance (49). Coproporphyrinuria, presumably produced by a similar mechanism, has been observed in workers with nitrobenzene (134) and may be useful in detecting early stages of excessive industrial exposure.

Phenylhydrazine is of particular interest because it has often been used to produce anaemia experimentally and ensuing changes in porphyrin metabolism have sometimes been attributed to the anaemia without regard for the possible effects of phenylhydrazine directly. It increases the urinary coproporphyrin about threefold in rabbits (151), and causes very substantial increments in erythrocyte coproporphyrin, much more than are observed after anoxia, bleeding or lead (146). The erythrocyte protoporphyrin is not increased (152). Increases in coproporphyrinuria of the same order of magnitude have been observed by Dobriner after administering phenylhydrazine to polycythaemic patients (38) or acetylphenylhydrazine to normal dogs (35). There is not much evidence about the isomer type in any of these observations, and Dobriner's grounds for regarding the porphyrin found in his cases as type I appear to be entirely theoretical. Administration of phenylhydrazine and lead accompanied by exposure to ultraviolet light produces a more profound disturbance of porphyrin metabolism in rabbits, with uroporphyrin and porphobilinogen in the urine as well as coproporphyrin (151).

Hypnotics

(*a*) *Sulphonal and Trional.* These are probably the oldest drugs to have a reputation for causing porphyrinuria. Garrod's paper in 1892 (59) contains numerous references to earlier observations in patients, and observations were made on rabbits by Neubauer (119) by 1900. Inadequacies of knowledge and technique at the time prevented identification of the porphyrin or porphyrins concerned, which are generally referred to as haematoporphyrin. Later clinical reports include some cases in which uroporphyrin was isolated from the urine, either of type I (33) or resembling the Waldenström porphyrin (41). It is possible that sulphonal and trional act by precipitating a mild or severe porphyria in constitutionally liable subjects because Waldenström (177) observed that administration of trional to apparently healthy near relatives of patients with congenital porphyria was followed by the appearance of uroporphyrin in the urine. In recent observations on rabbits (101, 179) only small and inconstant increments have been found in the urinary porphyrins, mainly when doses near to lethal amounts

have been administered by stomach tube daily for several days. Insufficient porphyrin for crystallization appears to have been obtained in either of these studies; the available evidence suggested that the material was mainly coproporphyrin. No porphobilinogen was found (179).

(*b*) *Sedormid.* Much more striking changes in porphyrin metabolism have been produced experimentally with the hypnotic "Sedormid" (allyl isopropylacetylurea) (62, 62a, 145) which has also been taken by patients before developing an attack of porphyria (40). Doses of 200–600 mg/kg daily for a few days in rabbits produced porphobilinogen and uroporphyrin in milligramme quantities, smaller amounts of coproporphyrin, and a number of other porphyrins in the esterified material (145). Similar effects are produced by oral administration to rats (21). The excessive production of porphyrin or porphyrin precursors is centred in the liver, where porphobilinogen is sometimes detectable (62, 62a) and the quantity of catalase is diminished (145). Sedormid is well known to produce thrombocytopenia (1), but no changes in the platelet count occurred in Goldberg's rabbits (62, 62a), and neither report suggests any reason to associate the upset porphyrin metabolism with injury to the blood-forming tissues. Goldberg observed that allylisopropylacetamide, which is not hypnotic, is about as potent as Sedormid in its effects on porphyrin metabolism but that propylisopropylacetamide is inactive. Further evidence incriminating the allyl group was obtained when the barbiturates were studied, and is discussed below.

(*c*) *Barbiturates.* As with sulphonal and Sedormid there is a strong clinical impression that barbiturates can produce or precipitate attacks of porphyria. Dobriner and Rhoads (36), reviewing the literature in 1940, found the evidence at most scanty. Experiments on animals have mostly yielded negative results but the range of barbiturates examined has generally been small, and Goldberg (62, 62a) has found that the particular side chain present is of great importance in determining the activity of the drug. Thus allobarbitone (Dial, diallylbarbituric acid) (when administered in large doses for several days) promoted uroporphyrinuria and coproporphyrinuria like Sedormid. Alurate (allyl isopropylbarbituric acid) and quinalbarbitone (Seconal, sodium allyl (1-methylbutyl) barbiturate), each with a single allyl group, had rather less effect, and the other barbiturates examined had either no effect (thiopentone, amylobarbitone or butobarbitone) or produced only slight, though statistically significant, coproporphyrinuria (barbitone, phenobarbitone and pentobarbitone). With much less sensitive methods, also in rabbits, Laubender and Monden (101) obtained practically negative results with isopropylbromallylbarbituric acid (Noctal) and with cyclohexenylethyl barbituric acid (Phanodorm).

(*d*) *Ethyl Alcohol.* There are surprisingly few reports about the effects of single doses of ethyl alcohol on the excretion of porphyrins in normal subjects. Franke (57) and Brugsch (14) reported increased excretion of coproporphyrin after consuming moderate or substantial quantities of alcoholic liquor (90 ml. of cognac or 300–450 ml. of whiskey), but the increments were small and perhaps within the range of normal variation. In chronic alcoholic subjects excessive amounts of coproporphyrin (500–2000 μg per day) have commonly been found,

sometimes of type I and sometimes of type III (14, 165). There is some indication that episodes of acute intoxication are associated with or closely followed by the appearance of type III and that type I appears more persistently in association with cirrhosis of the liver. But the evidence about the isomer type is somewhat conflicting and subject to the usual uncertainties of the experimental methods employed. No studies on experimental animals have been found, and no observations are known to have been made on other aliphatic alcohols.

Salicylates

Irregular increments in the excretion of porphyrin were noted by Brownlee (13) after giving acetylsalicylic acid to rats. The methyl ester of the porphyrin was crystallized and identified as coproporphyrin III. The observation is usually connected with the effects of aromatic amines, which were being studied by Brownlee at the same time, and which also produced coproporphyrin III in the urine, but there appears to be no reason to assume that the mechanism is the same in both cases. The effect of large doses of aspirin or other salicylates in normal man deserves study in connexion with the reported increased excretion of coproporphyrin III in rheumatic fever (59, 90).

Vitamins

Vitamins affect porphyrin metabolism chiefly by influencing haemopoiesis. Vitamin B_{12}, like other effective treatments of pernicious anaemia, increases the erythrocyte coproporphyrin and later protoporphyrin (71, 181, 185). It increases the erythrocyte protoporphyrin also in normal rabbits *in vivo* (6) and increases the rate of synthesis of protoporphyrin by blood obtained from rabbits made anaemic with phenylhydrazine (7). Folic acid has similar effects on the erythrocyte porphyrins (5): the evidence that it also increases the excretion of coproporphyrin in rabbits (5) appears to rest on two observations and deserves further inquiry. Neither vitamin B_{12} nor folic acid has gross effects on the urinary porphyrin excretion in congenital porphyria (66), acute porphyria (63) or experimental lead poisoning (201). Increased formation of haemoglobin by bone marrow cultures with added riboflavin has been shown by Vannotti and Siegrist (171) and it has been claimed that riboflavin diminishes the excretion of porphyrin at least in congenital porphyria (160). Other observations, in congenital (66) and acute (63) porphyria and in porphyria cutanea tarda (202) have not supported this claim. The erythrocyte protoporphyrin declines early in the development of pyridoxine deficiency in pigs, before anaemia and accumulation of iron in the tissues are manifest, and Cartwright and Wintrobe (20) suggest that this vitamin is necessary for the synthesis of protoporphyrin. A supposed excessive excretion of coproporphyrin in the urine in pellagra was claimed (4, 74) on the basis of colorimetric estimations which probably determined mainly urorosein (191). There is much evidence that the urinary coproporphyrin is not increased in pellagra or pellagra-like conditions (114, 126, 137) or in experimental nicotinic acid deficiency in dogs (191) unless there is associated chronic alcoholism (37, 91, 184) and then there does not appear to be definite evidence that porphyrinuria is more severe or more frequent than in non-pellagric alcoholic subjects.

Deficiency of certain vitamins of the B complex promotes the secretion of tears containing protoporphyrin from the Harderian glands (23). Pantothenic acid deficiency has been particularly incriminated, but the phenomenon has been observed also in riboflavin deficient animals and even simply in water deprivation (157). It is not clear whether the red tears appear as a disorder of the secretory mechanism, or whether they reflect a direct disturbance of the porphyrin metabolism of the gland itself.

PHARMACOLOGICAL ACTIONS OF PORPHYRINS

As excessive excretion of porphyrins is often associated with severe symptoms, especially in the porphyrias, it is not surprising that the possible role of the porphyrins themselves in producing symptoms has often been suggested. Especially it has been claimed that porphyrins cause photosensitization and spasm of smooth muscle, or alternatively that they act on vegetative centres in the nervous system and indirectly lead to the colics, constipation and hypertensive attacks. Much of the often-quoted evidence on these points consists only of observed correlation between clinical events and excessive porphyrin excretion, and is too speculative to warrant discussion. Actual experimental evidence is scanty and nearly all unsatisfactory because no criteria of purity are given for the porphyrins used, because traces of pharmacologically active substances likely to be present in materials of animal origin have not been excluded, and because the concentrations of porphyrin used have generally been large compared with the concentrations which are found in blood and tissues. Also haematoporphyrin has been used in most of the experiments, and it does not necessarily have the same actions as physiologically occurring porphyrins.

Porphyrins photosensitize animals to a variable extent so that animals into which certain porphyrins have been injected develop local injury to the skin or generalized and fatal shock unless they are kept in the dark. The relative activity of different porphyrins is assessed variously; on the whole uroporphyrin I, coproporphyrin I and haematoporphyrin appear to be the most noxious (55, 112). Protoporphyrin has not been found to be active (55, 207). Little has been discovered about the mechanism of photosensitization by porphyrins since the subject was reviewed in 1940 (36). On the other hand, the position has been complicated by several recent observations on the effect of light on porphyrin metabolism. Ultraviolet light does not increase the excretion of coproporphyrin in normal animals, but it increases it very substantially in rabbits which have been injected with the photosensitizing dye Rose Bengal (115) or with lead acetate (116). Rose Bengal alone does not provoke porphyrinuria; lead acetate does, but does not photosensitize. The porphyrin was identified by the fluorescence quenching method and by the melting point of its methyl ester as coproporphyrin III in both cases. As ultraviolet and visible light promote the formation of coproporphyrin from its precursors in urine (42, 139, 192) it is tempting to attribute the extra excretion of coproporphyrin to conversion of precursor *in vivo*, but this does not explain why the coproporphyrin is then excreted by the kidney and not as normally in the bile (199). Therapeutic irradiation with I^{131} causes a slight increase in the excretion of coproporphyrin, mainly in the

faeces (97), which is more in keeping with what might be expected from this mechanism.

Various experiments have been devised to show that porphyrins combine readily with proteins, such as fibrinogen (11, 85) and modify their properties, but there is no good evidence that these effects are important *in vivo*.

A supposed action of porphyrin on smooth muscle lacks any good experimental foundation. Reitlinger and Klee (130) observed an inconsistent increase in activity and tone of isolated intestines from rabbits after exposure to copro- or haematoporphyrin, but the concentrations were of the order of milligrammes per 100 ml., and the purity of their materials was not rigorously established. Contraction of intestine treated with haematoporphyrin and then exposed to bright light has been observed; with suitable concentrations no effect occurs if the preparation is kept in the dark (129, 164). Similar observations do not appear to have been made with naturally occurring porphyrins or under more physiological conditions.

Secretion of melanophore hormone, premature ripening of ovarian follicles in young mice, and slight depression of blood sugar and blood calcium in man have been reported after administration of haematoporphyrin or protoporphyrin (81, 82, 83, 87). The statistical significance of some of the changes is questionable. Negative results are less commonly published, but Weatherall (199) observed no effects on the blood pressure, visceral motility and water diuresis after injecting 0.1 mg. coproporphyrin III into normal rabbits, and Goldberg, Paton and Thompson (64) obtained virtually negative results from a more extensive pharmacological analysis.

As patients with porphyria at times excrete large quantities of porphyrins without any untoward symptoms, and rabbits treated with allylisopropylacetamide exhibit no obvious ill effects apart from their gross porphyrinuria, it is difficult to accept the belief that porphyrins have any significant pharmacological actions at all apart from photosensitization.

CONCLUSIONS

One or two ways in which porphyrin metabolism might be disturbed, such as disturbances of the formation and breakdown of myoglobin or of the cytochromes, have not been mentioned, because there is little or no evidence whether drugs affect them. Most of the known disturbances of porphyrin metabolism involve either haemopoiesis or liver function. Lead poisoning is clearly in the first category, poisoning by Sedormid and other substances containing allyl groups in the second. The mild porphyrinuria produced by aromatic amines and nitro-compounds may be due to abnormal haemoglobin breakdown, but seems more likely to be comparable to the effect of lead. The dyshaemopoietic porphyrinurias can be conceived simply as due to blockage at one or more stages of the normal synthesis of iron-protoporphyrin, so that intermediaries or products of diverted metabolism such as coproporphyrin appear in excess. Details about the stage of blockage have still to be worked out. The porphyrinurias associated with changes in the liver are less comprehensible. They may involve abnormal formation or destruction of iron-porphyrin compounds which are

synthesized by a mechanism different from the haemopoietic one, and so susceptible to damage by different agents; or they may arise through some as yet undetermined influence of the liver on haemopoiesis, for example, in synthesizing, excreting or failing to excrete some essential intermediary metabolite. The particular porphyrins which appear in the urine are not diagnostic of the site of the disturbance; porphobilinogen and uroporphyrin appear after Sedormid and after the combination of phenylhydrazine, lead and light and coproporphyrin appears both when the liver is diseased, and after interference with haemopoiesis by lead. The isomeric type is also not diagnostic. There is a connexion between coproporphyrin I excretion and increased haemopoiesis and coproporphyrin III excretion and obstructed haemopoiesis, but with the uroporphyrinurias and in liver disease there is a notable variability in the isomer type in any particular condition. The most promising developments now appear to be in the study of isolated tissue systems or homogenates, in the further investigation of precursors and the conditions under which they form free porphyrins, and in examining in more detail the actions of drugs on the tissue porphyrins as well as on the porphyrins excreted by experimental animals.

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