Symptomatic Porphyria

PART I. THE PATHOLOGY OF THE LIVER IN HUMAN SYMPTOMATIC PORPHYRIA

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SUMMARY

A study has been made of 7 alcoholic and 2 non-alcoholic patients with symptomatic porphyria. Siderosis (mild or absent in most cases), focal fatty change and lipofuscin pigmentation were noted on light microscopy while ultrastructural study revealed that focal cellular lysis was unexpectedly common. Unidentified cytoplasmic inclusions (possibly porphyrins) were present in 3 cases. It was concluded that increased synthesis and storage of porphyrins in the liver could lead to some cytoplasmic damage, but that alcoholic excess probably accounts for the majority of the hepatocellular abnormalities.

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Human symptomatic porphyria is commonly encountered in South Africa.[1,2] Sufferers present with fragility, pigmentation and blistering of sun-exposed skin. There is frequently a history of alcoholic abuse. Unlike inherited forms of porphyria, a positive family history is exceptional and acute attacks do not occur.

The disease is characterised biochemically by a typical pattern of haem precursor excretion and accumulation. Urine porphyrin excretion is considerably elevated, with uroporphyrin predominating. The faecal porphyrin is only moderately elevated, if at all. There is evidence that the biochemical abnormalities may be the result of increased activity of the mitochondrial enzyme ALA synthetase.[3]

Hepatic accumulation of porphyrin is invariable,[4] and in most cases clinical and biochemical evidence of chronic parenchymatous liver disease can be detected.[5] Liver biopsy studies have demonstrated the existence of various forms of liver damage in a high percentage of cases.[5,6] These lesions include siderosis, focal fatty change and focal cell necrosis. Portal fibrosis and cirrhosis may ultimately develop in as many as 70 - 80% of cases, although liver cell carcinoma is rare. On the available evidence, it has been suggested that alcohol and/or iron overload in the presence of undernutrition may be factors responsible for the liver damage in some cases. The present study was initiated to explore these possibilities more closely by examining the ultrastructure of the liver.

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Part I of this series describes the light and electron microscopic changes in the livers of 9 patients with symptomatic porphyria. A preliminary account of this work was presented at an International Symposium on Porphyrin Metabolism and the Porphyrias held in Cape Town in 1970.[7] Part II describes the ultrastructural changes observed in the livers of rats fed the porphyrinogenic agent, hexachlorobenzene. This experimental procedure has been shown to result in a disorder in the rats which has some biochemical similarity to the human disease.

PATIENTS AND METHODS

The patients studied were 8 males and 1 female who presented with the typical skin lesions of porphyria. The relevant clinical details and biochemical methods[8-10] and results are summarised in Table I. Fragments of liver tissue were obtained from the subjects by percutaneous liver biopsy and were divided into three portions, one of which was fixed in formalin for conventional histology. Sections were stained with haematoxylin and eosin, osmium tetroxide, Perls's iron stain, Schmorl's stain and with the Ziehl-Neelsen and PAS techniques. An unfixed portion was examined under ultraviolet light for autofluorescence. For ultrastructural studies, one portion was fixed by immersion in half-strength Karnovsky fixative in 0,1M phosphate buffer[11] followed by washing in buffer and postfixation in 2% OsO_4 in 0,1M phosphate. The remaining tissue was fixed in 2% OsO_4 in phosphate buffer. After dehydration with alcohol and embedding in Epon, sections were cut and stained with uranium and lead salts and examined in a Siemens Elmiskop 1A at 80 kV. In 1 patient (case 9) fresh liver tissue was not available for ultrastructural study. A portion of the liver biopsy embedded in wax was treated with toluene to remove the wax and it was then processed for electron microscopy by routine methods.

Controls: Biopsy specimens of 2 patients who were under investigation for suspected liver disease, but who were found to have normal liver function tests and normal livers on histological examination, were used as controls.

RESULTS

Light Microscopy

Alcoholic patients (7 cases): The unfixed tissue exhibited a strong, reddish fluorescence when irradiated by ultraviolet light, indicating abnormal accumulation of por-

TABLE I. CLINICAL DETAILS, BIOCHEMICAL METHODS AND RESULTS

Case	Sex	Age	Alcohol abuse*	Liver disease†	Urinary haem precursors: Uroporphyrin (μg/L)	Urinary haem precursors: Coproporphyrin (μg/L)	Urinary haem precursors: ALA (mg/L)	Urinary haem precursors: PBG (mg/L)	Faecal porphyrins: Copro (μg/g dry wt)	Faecal porphyrins: Proto (μg/g dry wt)
1	M	31	+	+	1 364	133	2,7	4,3	41	43
2	M	55	+	+	1 954	256	6,0	2,2	57	27
3	M	49	+	+	4 004	769	1,9	2,5	80	12
4	M	30	0	+	4 238	461	2,8	1,2	59	70
5	M	69	+	+	3 000	310	2,1	1,4	98	22
6	M	43	+	+	3 545	538	4,1	1,4	132	103
7	M	52	+	+	4 868	175	4,4	1,4	151	83
8	M	64	+	+	884	114	2,3	0,6	102	102
9	F	37	0	+	2 320	387	—	—	55	89

* Based on patient's own assessment of his drinking habits.

† Liver disease judged to be present on the basis of 2 or more of the following criteria: clearly palpable, firm hepatomegaly, raised SGOT, abnormal flocculation tests; elevated serum bilirubin, raised serum globulin, bromsulphthalein retention or abnormal liver biopsy.

The following methods were used without modification: urinary ALA and PBG — Mauzerall and Granick;[8] urinary uroporphyrin and coproporphyrin — Rimington and Sveinsson;[9] faecal coproporphyrin and protoporphyrin — Holti et al.[10]

phyrins. In 2 patients (cases 6 and 8) the parenchymal cells contained a light and diffuse deposition of haemosiderin granules, whereas in the remaining 5, the amount of haemosiderin was minimal or absent. Very little iron was present in Kupffer cells. Focal fatty change was occasionally noted, but individual cell necrosis or fibrosis was not seen. Four of the biopsy specimens contained prominent yellowish-brown lipofuscin granules in the cytoplasm of groups of liver cells, sometimes in close association with areas of fatty change (Fig. 1). In ultraviolet light they fluoresced with the typical yellowish-brown colour of lipofuscin. Perls's reaction was mostly negative but some granules were weakly positive. Some of the granules reacted positively with the PAS and Ziehl-Neelsen stains but many granules appeared to be unreactive. Schmorl's reaction was positive in all cases. The granules usually were dense black after exposure to osmium tetroxide but some were of a dark greenish colour.

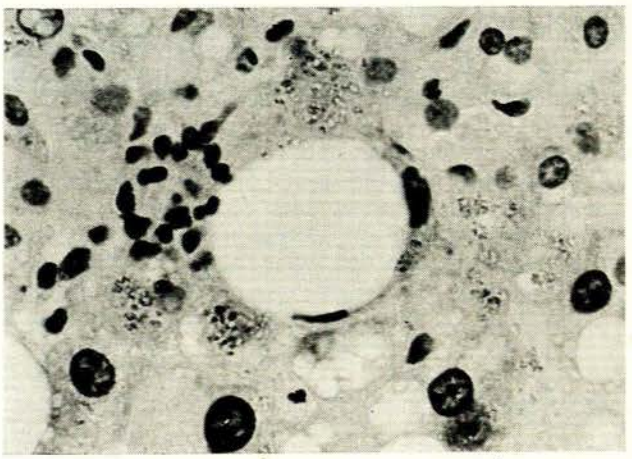

Fig. 1. Focal fatty change. Conspicuous lipofuscin granules are visible in some cells.

Non-alcoholic patients (2 cases): The liver of 1 patient (case 9) showed a significant degree of siderosis, with the iron predominating in the liver cells. Abundant lipofuscin was also noted in the parenchymal cells and this was positive with all the histochemical tests. The other biopsy was normal.

Electron Microscopy

Control normal livers: The liver cells in both cases resembled those described in a recent publication.[12] Haemosiderin granules and autophagic vacuoles were seen in both specimens only after a prolonged search.

Alcoholic patients: The commonest abnormalities included those already noted on light microscopy, i.e. siderosis, focal fatty change and lipofuscin pigmentation. In addition, focal cellular lysis of varying grades of severity was found in all biopsy specimens, while unidentified cytoplasmic inclusions were observed in 3 cases.

While increased numbers of haemosiderin granules were found in all livers, their numbers varied widely and they were seldom conspicuous in a given cell. The iron was mostly present in peribiliary lysosomes but free ferritin particles were occasionally observed in mitochondria and the cytoplasmic ground substance in unstained sections. Moderate amounts of lipid were present in some cells.

In about half the specimens, the liver cells contained unusually large and numerous lysosomal dense bodies of the lipofuscin type which corresponded to the pigment granules noted on light microscopy (Timme,[7] Fig. 5). Although they could be found throughout the cell, a preferential location close to the nucleus was often evident. Some contained ferritin particles. In addition to these bodies, many liver cells also contained 'multivacuolated' bodies, three to four times the diameter of mitochondria (Fig. 2). They were usually lined by a single or double limiting membrane, but the inner vacuoles were not bound by defined membranes. Less commonly, these

multivacuolated bodies were completely devoid of any outer limiting membrane.

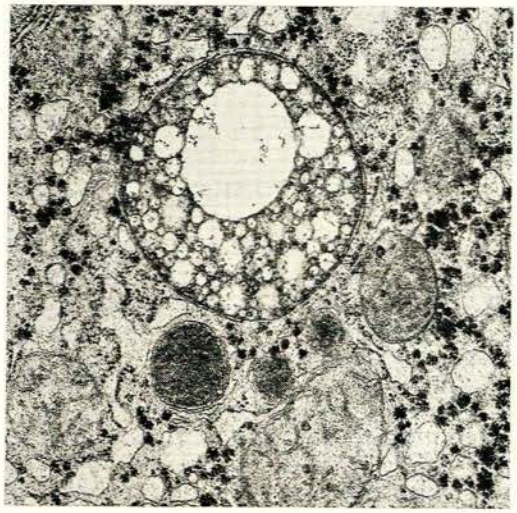

Fig. 2. Multivacuolated body (ceroid) surrounded by a double limiting membrane (× 24 000).

Focal cellular lysis was a notable feature of 3 biopsies (cases 1, 7 and 8), but was also detected in the remaining cases with an increased frequency, compared with the controls. In the most severely affected areas the cytoplasm was filled with many irregularly-shaped cavities (Fig. 3). The latter were partly or completely membrane-lined and contained osmiophilic debris or fragmented membranes, and probably represented early autophagic vacuoles. In these severely damaged cells mitochondria were sparse, and those which were present frequently showed degenerative changes, e.g. swelling of the matrix and membrane rupture; these features strengthened the impression that mitochondrial degeneration had contributed to the process of focal cellular lysis. Despite these abnormalities, the nuclear chromatin, glycogen content and plasma membranes were undisturbed, and the cells still appeared to be viable. In a few cells, however, chromatin clumping and severe vesiculation of the rough endoplasmic reticulum suggested that they were probably in an early necrotic stage.

Mitochondria in the comparatively unaffected cells characteristically contained rather prominent intramitochondrial granules, some of which were ring-shaped. Other signs of cellular damage encountered included multimembranous fingerprint bodies (Fig. 4).

Three specimens (cases 5 - 7) contained cytoplasmic inclusions which were confined to the hepatocytes. Some of these have been illustrated in the earlier paper.[5] They were of widely varying morphology but the majority were elongated structures with pointed or bulbous ends and with sharply-defined linear electron-lucent zones internally (Fig. 5). They occurred anywhere in the cytoplasm and showed no constant relation to any normal organelles. Some of the inclusions were associated with areas of focal cellular lysis.

Alcoholic hyaline and 'crystalline' mitochondrial inclusions, of the type associated with alcoholism, were absent.[13] In 1 case there was a slight increase in the smooth endoplasmic reticulum. Microbodies and the Golgi apparatus

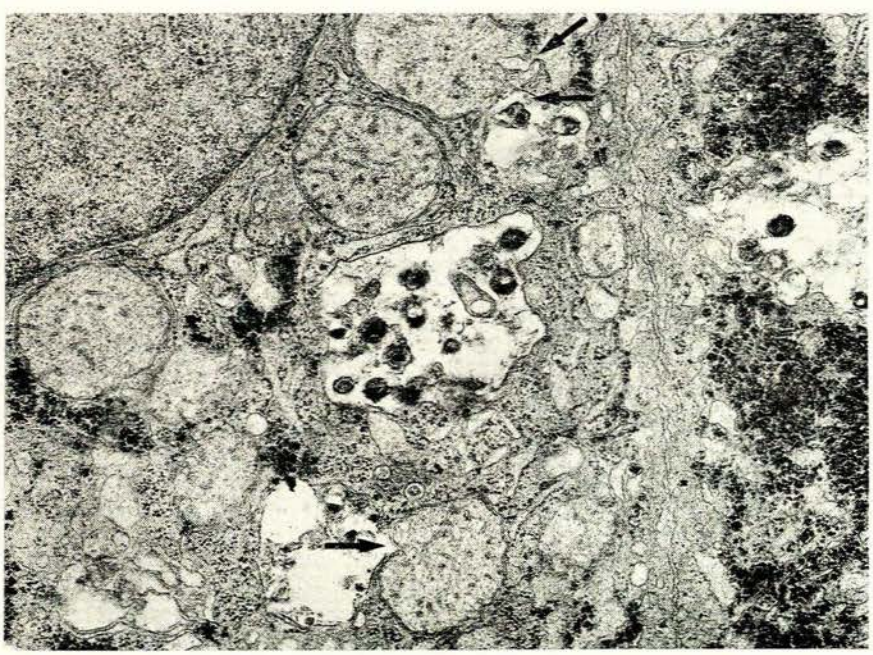

Fig. 3. Autophagic vacuoles. Upper arrows indicate disruption of mitochondrial membranes. In lower half of field, a vacuole herniates into an adjacent mitochondrion (× 30 000).

showed no constant changes. Occasional cells contained increased numbers of multivesicular bodies close to bile canaliculi.

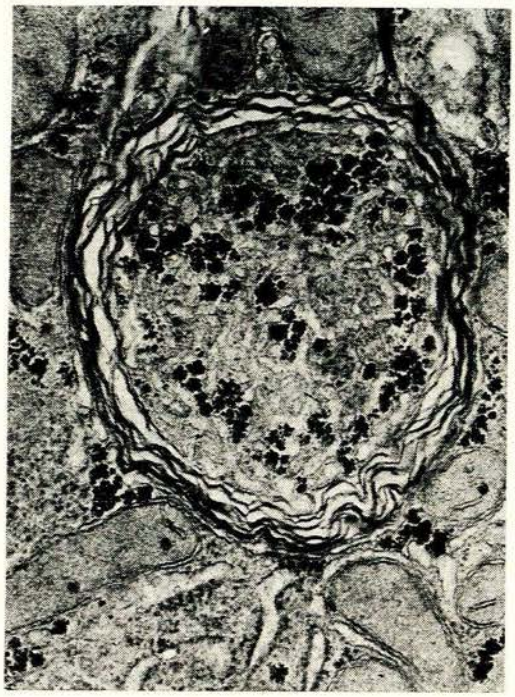

Fig. 4. Ellipsoidal smooth-membraned body (× 25 000).

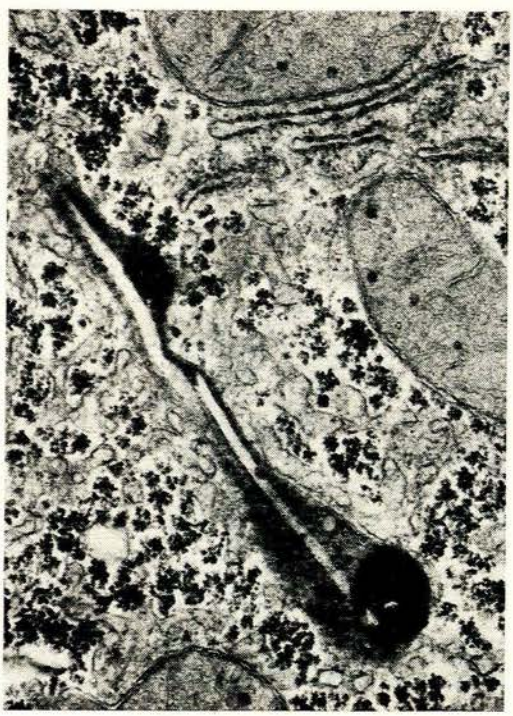

Fig. 5. Inclusion body showing electron-dense and electron-lucent zones (× 28 000).

Non-alcoholic patients: In 1 patient (case 4) the changes were slight, although haemosiderin granules, autophagic vacuoles and lipofuscin granules were judged to be slightly increased, compared with the controls. In the other patient (case 9) detailed ultrastructural study was not possible because of the poor cytological preservation, but numerous large, partially vacuolated lysosomes (lipofuscin) and haemosiderin granules were present, confirming the light microscopic picture.

DISCUSSION

Siderosis is an accepted feature of symptomatic porphyria,[5,6] and in alcoholic patients the iron excess is apparently derived from the wines which are commonly consumed.[14] A considerable body of evidence suggests that iron overload may influence the course of the human disease and the induction of porphyrinuria and porphyria-like states in experimental animals.[14-16] However, the present essentially morphological study can neither confirm nor disprove the role of iron in symptomatic porphyria, but two observations are worthy of comment. Firstly, the iron content was moderate in amount in only 2 cases and minimal or absent in the rest and has not approximated the levels employed in experimental studies as assessed histochemically.[16] Secondly, no correlation existed between the degree of siderosis, the extent of other cytoplasmic changes and the biochemical alterations. However, the present observations obviously do not exclude a subtle biochemical influence which cytoplasmic iron may exert on the disorder.

Earlier histological studies of porphyric livers have not described excess lipofuscin pigmentation,[5,6] whereas it was a feature of 5 of the present cases, including 1 who was non-alcoholic. Although the osmiophilic masses were morphologically consistent with a lysosomal origin and fluoresced in the typical manner, the histochemical reactions were often inconsistent. Lipofuscin or ceroid is usually positive with the tests employed.[17] It is believed that the multivacuolated bodies represent earlier stages in the development of the larger dense lysosomes and, as such, probably constitute a form of ceroid (Dr J. W. Grisham, St Louis, USA—personal communication). According to some writers, ceroid represents an early oxidative stage in the formation of the mature lipofuscin.[17] As far as the origin of the lipofuscin and ceroid is concerned, it is possible that the breakdown of cellular membranes derived from the process of focal cellular lysis may be responsible, although evidence of such acute changes is not necessarily found together with the lipofuscin. It has been reported that in alcoholic hepatitis the liver cells may occasionally contain large lysosomes of the type referred to in this article[18] and although a recent ultrastructural study of alcoholic liver disease makes no mention of lipofuscin or ceroid pigmentation,[19] it appears reasonable to assume that in 4 of the 9 cases the ceroid and lipofuscin were probably manifestations of cellular injury of alcoholic origin.

Despite the fact that 7 of the 9 patients were alcoholics, alcoholic hyaline or mitochondrial 'crystalline' inclusions were not observed in any of the livers. A recent study has, however, indicated that neither of these features

is invariable.[19] Instead, the mitochondria may contain prominent granules and show abnormalities of the cristae and focal disruption of their membranes. Focal cellular lysis was also reported. Focal cellular lysis in which autophagic vacuoles contained degenerate mitochondria has previously been described in cases of alcoholic hepatitis[18] and in alcoholic subjects given known test doses of alcohol.[20] If these changes are severe enough, they can obviously explain the occurrence of focal cellular necrosis.[6]

The cytoplasmic inclusions have not thus far been identified and they do not appear to have been described before in human liver disease. They could conceivably be lysosomes of unusual morphology, and if this hypothesis is correct, the included material may be stored porphyrins. Inclusions of a somewhat different kind have been reported in hepatic porphyria in mice,[21] while inclusions of a true crystalline type have been reported in the liver of 1 case of hepatic porphyria.[22] No explanation of their occurrence was given.

Cytoplasmic fingerprint bodies are exceedingly common and non-specific manifestations of liver injury in experimental animals,[23] although they seem to be rare lesions in human liver disease.

The livers of both non-alcoholic patients showed unquestionable ultrastructural abnormalities, although they were non-specific in nature. Siderosis and excessive lipofuscin accumulations were present in both, and marked in one case, while more active changes, e.g. increased focal cellular lysis, were recognised in the other. These findings therefore raise the possibility that porphyria itself may initiate some degree of hepatocellular injury. It is not clear how excess of iron comes to be deposited in these livers. This phenomenon has been reported previously.[6] It is pertinent to note that there is experimental evidence which suggests that in hexachlorobenzene-induced porphyria in rats, the liver may show increased deposits of iron.[14]

On the basis of this study, it is concluded that the parenchymal liver disease of human symptomatic porphyria probably has a dual origin, e.g. excessive porphyrin production and storage and increased alcohol consumption. The precise role of the former still remains to be fully elucidated, but it seems likely that the more acute and severe changes observed in the present series and the ultimate progression of the disease in some cases to a cirrhosis, may largely be attributable to the effects of alcohol.

ADDENDUM

After this article was completed, a further report dealing with the occurrence of cytoplasmic inclusions in the liver in symptomatic porphyria has appeared.[24] In this article evidence is presented that the inclusions contain porphyrins.

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