Symptomatic Porphyria

PART II. HEPATIC CHANGES WITH HEXACHLOROBENZENE

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SUMMARY

The administration of hexachlorobenzene (HCB) to rats for periods up to 67 days resulted in the development of gross cytoplasmic vacuolation and the formation of cytoplasmic inclusions. Some of the inclusions consisted of slender tubular structures about 200 Å in diameter. Although it was suspected that the inclusions represented precipitated porphyrins, proof of this could not be obtained. The prior administration of iron to some of the animals before commencing feeding with HCB resulted in a more extensive development of the lesions in the liver, and this could account for the higher levels of excreted porphyrins in the iron-laden animals.

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A number of substances are now known to be able to induce a variety of forms of experimental porphyria in animals.[1] Included in this category are allylisopropylacetamide (AIA), dicarbethoxydihydrocollidine (DDC), griseofulvin and hexachlorobenzene (HCB). In rats and rabbits the latter agent is able to produce a syndrome which resembles porphyria cutanea tarda (symptomatic porphyria) in man.[2,3] It has also been used to explore the effects of iron overload in rats,[4] since it is well recognised that siderosis of the liver is a common feature of human symptomatic porphyria.

This article describes the ultrastructural changes observed in the livers of rats exposed to hexachlorobenzene.

MATERIALS AND METHODS

The animals used in this work were derived from the same groups as those used and described in an earlier communication which dealt primarily with the biochemical and histological aspects of the study and which should be consulted for further details.[4] The rats weighed 150 - 180 g each. One group was made siderotic by intraperitoneal injections of Imferon (5 injections of 10 mg each). The second group received no supplementary iron. Both groups were then fed a normal diet for 2 months before commencing feeding with HCB, given as a 0,3% mixture with rat chow. Urinary porphyrins were measured according to standard techniques. In addition to the histological techniques already described,[4] deparaffinised sections of glutaraldehyde-fixed tissue were examined with the polarising microscope. For electron microscopy, fragments of tissue were immersed in Karnovsky fixative[5] (0,1M cacodylate buffer) and after washing in buffer they were postfixed in 2% OsO_4 in 0,1M phosphate buffer. The tissue was embedded in Araldite. Sections were stained with uranium and lead salts.

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RESULTS

Neurological signs (tremor) were noted soon after commencement of HCB feeding. A rise in urinary porphyrins occurred in both groups, but it was earlier in the siderotic group.

Light Microscopy

In the non-siderotic group, i.e. rats which did not receive prior Imferon injections, the livers contained only traces of stainable iron after HCB feeding. In the siderotic group the iron was initially diffuse in the lobule, but later the centrilobular areas contained only minimal quantities. Red porphyrin fluorescence first appeared in the centrilobular zone in both groups, but in the siderotic group it eventually became widespread throughout the lobule.

Electron Microscopy

Non-siderotic animals: The most obtrusive changes were observed in the cytoplasm of the liver cells, which showed a striking vacuolation. This was largely confined to the centrilobular zones. In the most severely affected cells the cytoplasm had a honeycombed appearance (Fig. 1). The vacuoles were of an exceedingly complex shape, often appearing to engulf small segments of the cytoplasm. Occasionally the larger vacuoles appeared to communicate with each other via a narrow channel and sometimes these could be seen on cross-section. The vacuoles were mostly lined by a single smooth-surfaced membrane, but in areas this may have been deficient. Many of the vacuoles appeared to be devoid of any contents other than some granular debris. Others, however, contained inclusions of the type illustrated in Figs 2 and 3. They consisted of closely packed, slender, tubular structures approximately 200 Å in diameter and arranged in a more or less parallel fashion.

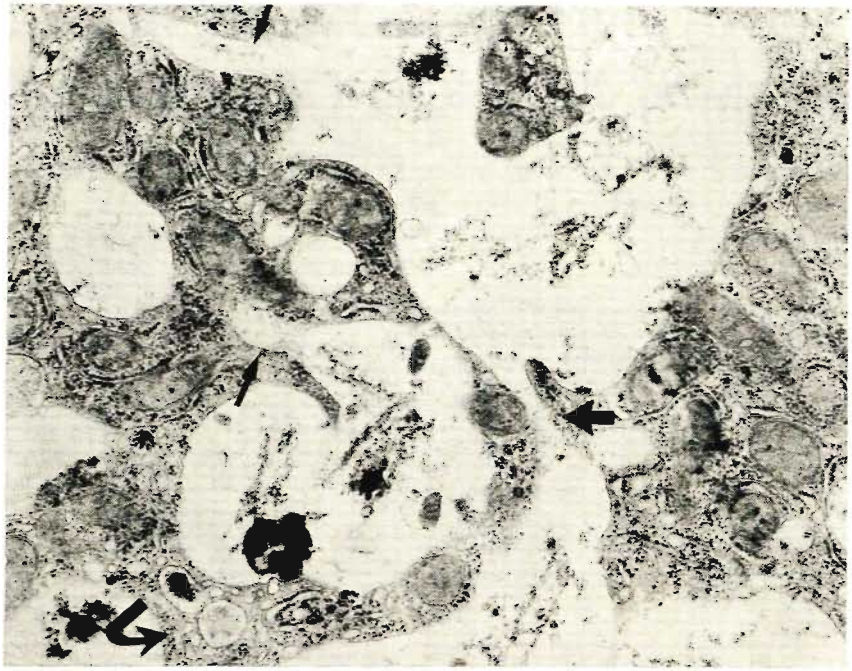

Fig. 1. Centrilobular area, siderotic liver. Cross-cytoplasmic vacuolation has occurred. Broad arrow (right) **points to possible communicating channel. Curved arrow** (below) **indicates multivesicular body. Other arrows point to tubular extensions from the larger vacuoles. Little iron is present in the centrilobular zones (× 25 000).**

Fig. 2. Non-siderotic liver. Tubular inclusions occupy fusiform vacuoles and one is also seen on cross-section (curved arrow). **A Golgi apparatus** (lower left corner) **and 6 multivesicular bodies are also present.**

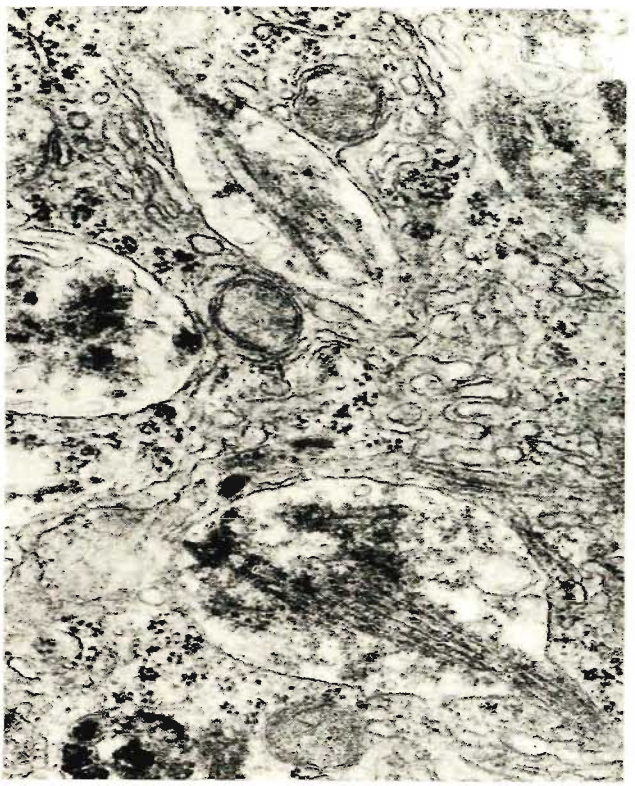

Fig. 3. Non-siderotic liver. Tubular inclusions similar to those in Fig. 2 (× 30 000).

They lay free in the vacuoles although occasionally they seemed to extend into the adjacent cytoplasm. Their tubular profiles were straight or slightly curved and when visualised on cross-section (Fig. 2) a somewhat angular configuration became apparent. Other large inclusions were also observed, which were not obviously associated with the cytoplasmic vacuoles (Fig. 4). They occurred anywhere in the cytoplasm. They had a tadpole shape with a slender tail. Haemosiderin was abundant, as were linear electron-dense structures. Occasionally one could discern possible remnants of a tubular pattern.

Golgi bodies were commonly observed in the immediate vicinity of the vacuoles and inclusions, while multivesicular bodies were also increased in number. The smooth endoplasmic reticulum (SER) was conspicuously increased in all cells and the glycogen was generally diminished, while the parallel configuration of the cisternae of the rough endoplasmic reticulum (RER) was largely lost. The other organelles did not show any consistent abnormality. Occasional haemosiderin-laden lysosomes were present at random in the cell.

Siderotic animals: The livers of these animals showed a similar type of cytoplasmic vacuolation, but it was more widespread and involved the central and peripheral divisions of the lobule. They, too, contained cytoplasmic inclusions of the types already described, but more commonly they possessed a complex branching structure which seemed to be composed of linear densities encrusted with abundant haemosiderin (Fig. 5). Tubular outlines could not be recognised in these structures. Numerous haemosiderin-laden lysosomes were seen in these livers and free ferritin particles were also present in the cell sap and mitochondria.

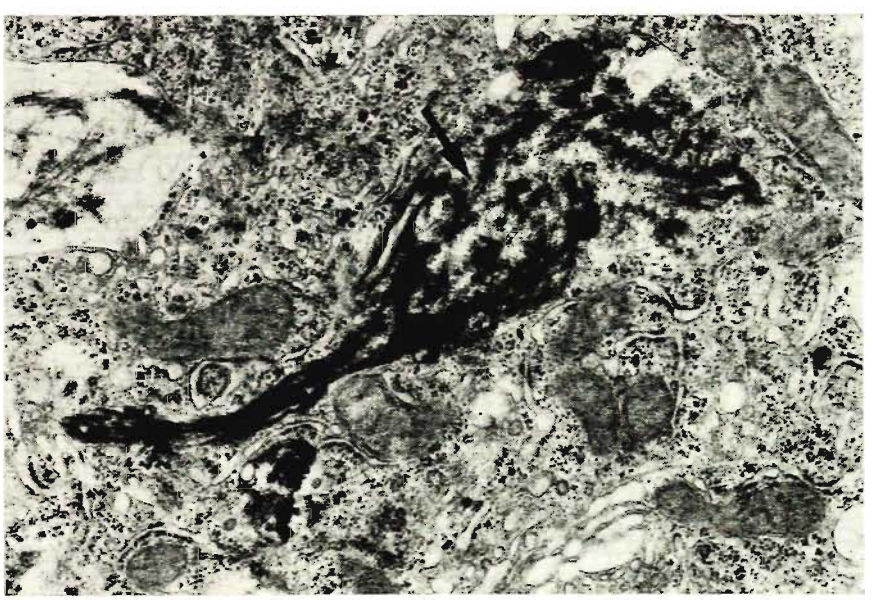

Fig. 4. Non-siderotic liver. Tadpole-shaped inclusion in which the basic underlying tubular structure has largely been obscured by iron. The arrow indicates remnants of a tubular pattern. A Golgi body is again close to the inclusion (× 30 000).

Fig. 5. Siderotic liver. Heavily iron-encrusted inclusion limited by a membrane. A large Golgi complex is immediately adjacent to it (× 25 000).

DISCUSSION

Hexachlorobenzene, a fungicide, was responsible for outbreaks of porphyria cutanea tarda (symptomatic porphyria) in Turkey in 1960.[6,7] In rabbits and rats it produces an increase in the excretion of uro- and coproporphyrin.[2,3] In rats, however, unlike human symptomatic porphyria, there is also a concomitant rise in the levels of porphyrin precursors.[3] The mechanism of action of HCB is not known, but in common with other porphyrinogenic agents it leads to an increase in the levels of ALA synthetase, an important rate-limiting enzyme in the biosynthetic pathway of porphyrins.[1]

Although some of the larger vacuoles in the liver cells may have been isolated structures unconnected with each other, the over-all appearance of the cytoplasm suggested that the vacuoles were part of a labyrinthine complex of intercommunicating spaces. This raised the possibility that the vacuoles arose in a pre-existing structure such as the endoplasmic reticulum. A lack of any histochemical evidence of acid phosphatase activity precluded any statement on the possibility that some of the vacuoles may have represented autophagic vacuoles. By the time the animals were sacrificed, the changes were already advanced, and this made any study of the pathogenesis of the lesions virtually impossible.

The nature of the various inclusions is not definitely known, although it is suspected that they may represent porphyrins (Dr R. Schmid, San Francisco, USA—personal communication). Although it was shown in an earlier article[4] that the liver cells exhibit a red fluorescence when exposed to ultraviolet light, indicating the presence of porphyrins, localisation to a specific part of the cell was not possible. It has also been reported that porphyrin deposits in the Harderian gland of the rat are doubly refractile,[8] but this test was negative when glutaraldehyde-fixed tissue was examined by polarisation optics. It has consequently not been possible to prove the porphyric nature of the inclusions. It is not clear how porphyrins would precipitate in the cytoplasm. It has been stated that porphyrins can form complexes with metallic ions, e.g. Fe^{++},[9] and since there appears to be an increase in haemosiderin even in animals not given Imferon injections, it is possible that porphyrin precipitation, if indeed it does occur, may develop on this basis. It is worth noting that needle-like structures, believed to be composed of porphyrins, have been reported in red blood cells in erythropoietic porphyria, but these were not studied ultrastructurally.[10] A variety of inclusions which were also thought to be porphyrins were demonstrated in the liver of mice exposed to the porphyrinogenic agent DDC, but, once again, proof of the nature of the material was lacking.[11] The deposition of ferritin particles on the inclusions has been observed even in those animals not given Imferon. It is believed that an increase in liver iron may occur with HCB feeding alone and without iron supplementation, but the mechanism of this is not known.[12,13]

It is therefore of some interest that inclusions have been observed in the livers of experimental animals and cases of human symptomatic porphyria, although they did not always resemble each other very closely. Since the completion of Part I of this series, inclusions composed of slender, needle-like or tubular structures having some similarity to those seen in Fig. 2 in this article have been observed in the liver of a patient with symptomatic porphyria.

With reference to the possible influence of iron overload on the changes observed, the only fact which has emerged is that the lesions are more extensive in the iron-loaded group. This could probably account for the higher levels of porphyrin excretion in this group.[4] It is as yet not known if the lesions necessarily appear earlier in the iron-laden animals, although this would be expected to be the case.

It is assumed that the increase in SER is a response to the ingestion of HCB and reflects its metabolic degradation. This association is known to occur in other drugs, e.g. the barbiturates.[14]

The literature contains few references on the histological effects of HCB toxicity.[2,3,12] Centrilobular fluorescence and focal necrosis are among the changes reported. The only ultrastructural studies of the action of porphyrinogenic agents are those which have employed DDC[11] or AIA.[15-17] In the latter instances the changes appear to have been of a non-specific nature and hepatocellular inclusions did not develop. The absence of more significant changes in these experiments may be due to their relatively short duration compared with the present investigation.

The present studies have shown that while some similarity exists between the human disease and the experimental disorder, there are also not inconsiderable differences. It is not known to what extent these may be attributable to the type of animal or dosage used in the present experiments.

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