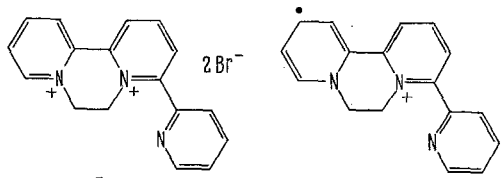
$a:2',1'$-$c$]pyrazinium dibromide (I), which crystallized from aqueous ethanol as yellow-green needles of the monohydrate, mp 290° (dec.) (Found: C, 46.4; H, 3.7; N, 9.4; $C_{17}H_{15}Br_2N_3 \cdot H_2O$ requires C, 46.5; H, 3.9; N, 9.6%). The NMR-spectrum in $D_2O$ consisted of a singlet at $\delta = 5.3$ and a complex multiplet in the range $\delta = 7.7$–9.3 ppm with an area ratio of 4:11. The UV-spectrum showed maxima at $\lambda$ 275 (log $\varepsilon$ 3.80) and 326 nm (4.32).




I II

An aqueous solution of (I) on treatment with zinc dust developed immediately an intense green coloration due to the corresponding radical cation (II). The presence of a high concentration of a stable radical was confirmed by the fact that the intensely coloured solution gave essentially no NMR-spectrum. When the reducing agent was removed and the solution was shaken in air the deep colour discharged. The NMR-spectrum obtained then was identical with that of the original salt indicating, like diquat and paraquat, that the one electron transfer is essentially completely reversible. On polarographic examination in the pH range 1.5 to 8.2, (I) gave a typical symmetrical one-electron reduction wave with a half-wave potential ($E_0$) of $-0.32$ volts independent of pH. A second reduction wave which approximated to the uptake of one electron at a potential of $-0.72$ volts was also present above about pH 6. At lower pH the second wave was less clearly defined and the half-wave potential was pH dependent. This behaviour is reminiscent of that noted recently[7,8] with a number of similar diquaternary salts and is presumably associated with protonation of the radical cation at low pH.

In post-emergent herbicidal tests[3] in the greenhouse, the salt (I) gave a complete kill of 6 plant species when applied at a rate equivalent to 7 lb/acre. In tests at lower application rates on mixed grass flora it was found to be about one-sixth as active as diquat. These results confirm that compactness of the molecule is necessary for outstanding phytotoxic properties. The considerable herbicidal activity obtained from (I), however, suggests that difficulty in reaching the site of biological action of the bipyridylium herbicides is responsible for its reduced activity rather than an inability to participate in the toxic mechanism, although the higher reduction potential of (I) which may not be low enough to exert the fullest effect on the biochemical electron transfer processes with which the bipyridylium herbicides are thought to interfere may be a contributing factor. The salt (I) with its redox potential of $-0.32$ volts provides a useful extension to the range of completely reversible redox indicators of the viologen type which have hitherto been confined[4] to potentials below $-0.35$ volts. KOK, RURAINSKI and OWENS[9] have also recently reported a salt with a redox potential of $-0.32$ volts.

*Zusammenfassung.* 2,2′:6′,2″-Terpyridin gibt mit Aethylendibromid ein diquartäres Salz, das durch Reduktion mit Zink eine Radikalkation liefert. Es handelt sich um ein Redox-System und das Salz besitzt herbizide Wirksamkeit.

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## Rat Hepatocyte Peroxisomes: Ultrastructural Alterations Following Cessation of Chronic Dietary Clofibrate Administration

The purpose of this note is to present our observations of previously undescribed ultrastructural alterations in the matrix of hepatocyte peroxisomes in animals studied 3 weeks following an initial 6-week chronic dietary Clofibrate (CPIB) administration.

Recent studies in our laboratories concerning the response of the rat liver to ethyl-chlorophenoxyisobutyrate (Clofibrate – CPIB) confirm previous reports of hepatomegaly characterized ultrastructurally by a marked increase in cytoplasmic hepatocyte peroxisomes (microbodies)[1–5]. Our findings were noted after 6 and 12 weeks dietary administration of CPIB (0.3% diet) to Sprague-Dawley rats.

In addition, studies were performed to define the ultrastructural features of the hepatocyte 3 and 6 weeks following an initial period of 6 weeks dietary administration of CPIB. The results of these studies indicate reversibility to normal considering absolute and relative liver weight relationships, light microscopy and ultrastructural features at both post-treatment sampling times. No ultrastructural evidence of adverse residual cell damage was noted.

*Abbreviated protocol of experiment.* Eight 150 g Sprague-Dawley (Charles River) rats were assigned to each group. Body weight, food consumption and symptomatology were recorded weekly. Liver specimens were collected from each of the 4 major lobes for both light microscopy (Formalin – H. & E. and Oil Red O) and electron microscopy (glutaraldehyde and osmium fixation – epon araldite embedment). 4 treatment regimens were per-

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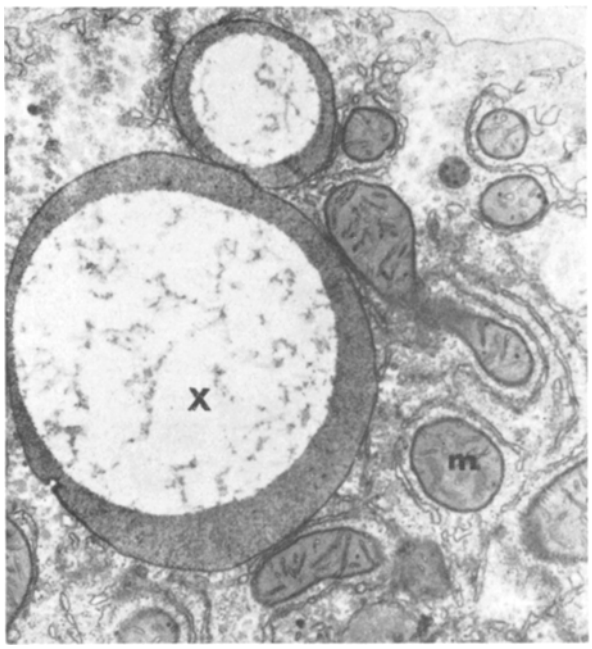


Fig. 1. Numerous altered microbodies noted (x) in which single conspicuous spaces occur in the matrix. Affected microbodies were usually of greater diameter than anticipated. Rat liver × 13,860.

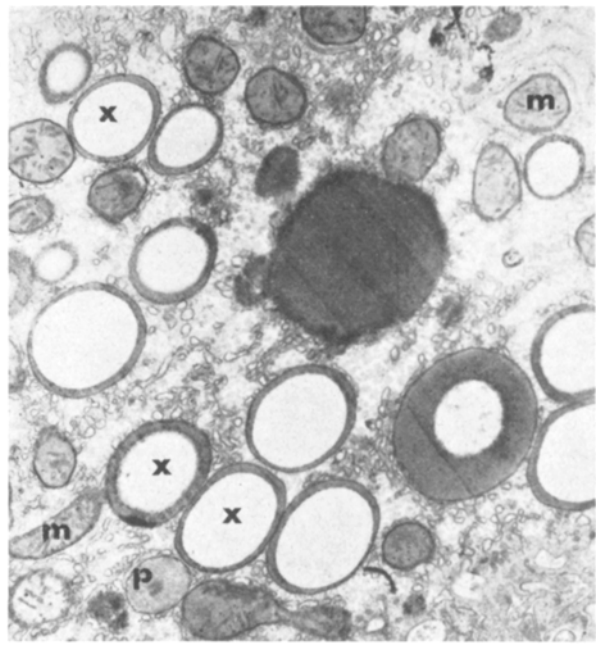


Fig. 2. Vacant spaces within microbodies in which a granular material is present, seemingly arising from the adjacent matrix. Rat liver × 27,950.

formed: (1) 6 weeks CPIB 0.3% of diet; (2) 6 weeks CPIB 0.3% of diet followed by 3 weeks non-medication (group in which peroxizome matrix changes were detected); (3) 6 weeks CPIB 0.3% of diet followed by 6 weeks non-medication; (4) 12 weeks CPIB 0.3% of diet. Similar groups of non-medicated control animals were examined by all parameters for comparison with each experimental regimen.

*Principal observations.* Hepatomegaly (25–30% increase) was the only macroscopic change detected. Histologically the liver cell cytoplasmic content was characterized by an abundance of eosinophilic granular material packing the hepatocytes, which had increased slightly in diameter.

The above observations were absent in specimens studied 3 and 6 weeks following the initial 6-week-administration of CPIB (Regimens 2 and 3).

Electron microscopic findings were those of marked increase in normal appearing hepatocyte peroxisome population after 6 and 12 weeks continuous medication (Regimens 1 and 4). After 3 and 6 weeks withdrawal of CPIB, reversibility of the ultrastructural features noted after 6 weeks medication was the prominent feature. No adverse residual effects were noted (Regimens 2 and 3).

*Altered peroxisome morphology.* The ultrastructural features of the peroxisome matrix alterations are illustrated in Figures 1 and 2. In Figure 1 numerous altered microbodies are noted (x) in which single conspicuous empty spaces occur in the matrix. Close examination reveals a sparce distribution of granular material seemingly derived from the adjacent matrix substance (Figure 2, x).

For dimension reference in both figures either normal peroxisomes (P), or mitochondria (M) are useful. In addition to the matrix changes, there is indication of an increase in size of the altered peroxisome when compared to unaffected microbodies or mitochondria.

Careful review of identically processed specimens from the 4 control groups and the 3 other treatment groups failed to reveal the slightest evidence of similar ultratsructural changes occurring in the hepatocyte microbody matrix substance. This review supports our belief that the alterations herein described are unique to this particular group and represents a true change in the ultrastructural morphology of this organelle. As yet we are not aware of any reports dealing with alterations in microbodies of this nature. While reports of 'abnormal microbodies' do exist, they consist thus far of changes affecting the nucleoid core[1,6].

*Comments.* Demonstration of effect reversibility with no obvious residual ultrastructural effects 3 and 6 weeks following 6-week-medication, represents additional data relevant both to the microbody per se and the effects of CPIB upon the rat liver cytoplasm[7].

From the experiments summarized herein we have no data to further characterize the altered peroxisomes. Thus far these alterations can only be related to the experiment following cessation of treatment with CPIB. While tempting, it is not possible to class these changes as abnormal, regressive or degenerative, nor can a hypothesis concerning their significance be evolved. They are reported as ultrastructural features of peroxisome morphology.

*Zusammenfassung.* Es wird festgestellt, dass 3 Wochen nach Beendigung einer Clofibrate-(CPIB)-Diät an Ratten manche «Hepotocyte Peroxisomen» (Mikrokörper) bisher nicht beschriebene, ultra-organische Veränderungen von unbekannter Bedeutung zeigen, die mit der experimentellen Behandlung in Zusammenhang stehen dürften.

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