Light-Microscopic Demonstration of Drug-Induced Myelin Bodies in the Liver of Rats

Ultrastructural changes in liver cells upon administration of various drugs to animals have been described by several workers. Among the effects observed was the formation of myelin bodies (= multilamellated bodies, MB) in the cytoplasm of hepatocytes and other liver cells. Histochemical evidence indicates that MB are derived from lysosomes and contain mainly phospholipids[1]. The drugs eliciting this phenomenon include chloroquine[1], triparanol[2,3], erythromycin[4], clindamycin[4], the diazafluoranthene derivative AC-3579[5] and several others[3,6]. The presence of MB in the liver, in all these cases, was demonstrated with electron microscopy.

Bodies or organelles which consist of shells or membranes in concentrical or eccentrical arrangement present the typical Maltese cross birefringence of sphaerulites in polarized light, i.e. a black cross (corresponding to the two planes of polarization) which separates 4 bright quadrants on a dark background. As judged from ultrastructural findings, drug-induced MB possess such a structure and should therefore be expected to present Maltese cross birefringence between crossed polarizers. Indeed, using polarization microscopy, cytoplasmic inclusions with the optical properties of sphaerulites were easily detectable in fresh liver tissue after treatment with MB-inducing drugs.

Experimental. The present study is concerned with the effects of triparanol, chloroquine, and mepacrine in the liver. The third compound has not been described so far as eliciting MB formation in the liver by electron microscopy. Of particular interest is the strong fluorescence of mepacrine[7], which permits its intracellular localization with fluorescence microscopy.

Male albino rats weighing about 100 g, from the randomized outbred Füllinsdorf stock, received 2 to 4 varying daily doses by stomach tube of the drugs suspended in 5% gum arabic; control rats obtained the suspending solution alone. 24 h after the last application, the rats were killed, some small pieces of liver tissue squashed between slide and cover slip and such preparations inspected between crossed polarizers. For fluorescence microscopy, BG 12 (Schott) was used as a primary filter, and a Zeiss filter 53 as secondary barrier filter.

Results and discussion. Squashed liver fragments from control rats possessed only isotropic inclusions in their hepatocytes. In rats treated with one of the 3 drugs, the cytoplasm of hepatocytes and other liver cells contained in addition birefringent inclusions which presented Maltese cross configurations (Figure 1). The doses of the drugs required to obtain this effect are listed in the Table which also presents a semiquantitative evaluation (0 =

[1] R. Abraham, R. Hendy and P. Grasso, Expl. molec. Path. *9*, 212 (1968).
[2] Z. Hruban, H. Swift and A. Slesers, Lab. Invest. *14*, 1652 (1965).
[3] Z. Hruban, A. Slesers and E. Hopkins, Lab. Invest, *27*, 62 (1972).
[4] J. E. Gray, A. Purmalis, B. Purmalis and J. Mathews, Toxic. appl. Pharmac. *19*, 217 (1971).
[5] O. Thys, J. Hildebrand, Y. Gerin and P. J. Jacques, Lab. Invest. *28*, 70 (1973).
[6] H. Lüllmann, R. Lüllmann-Rauch and O. Wassermann, Dt. med. Wschr. *98*, 1616 (1973).
[7] A. C. Allison and M. R. Young, Life Sci. *3*, 1407 (1964).

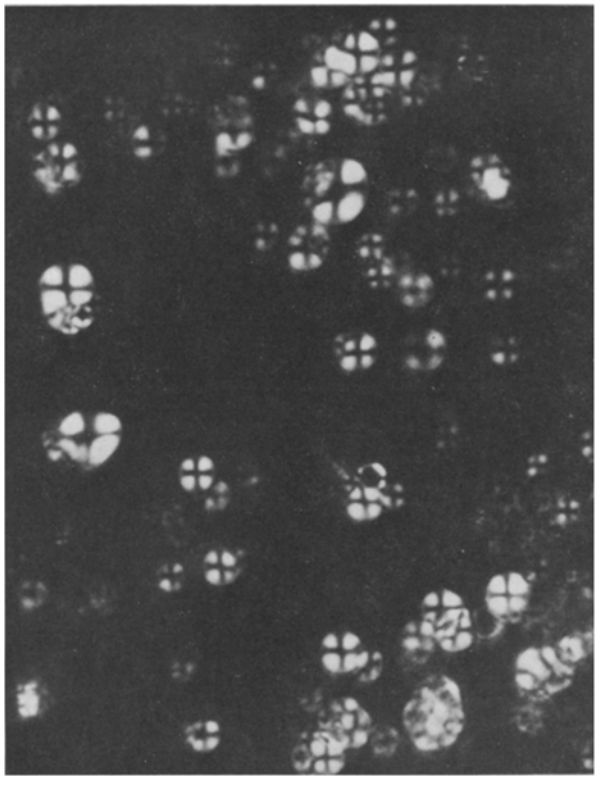

Fig. 1. Part of the cytoplasm of squashed rat hepatocytes showing birefringent inclusions with simple or composite Maltese cross. Mepacrine 4 × 400 mg/kg. Polarized light. × 2300.

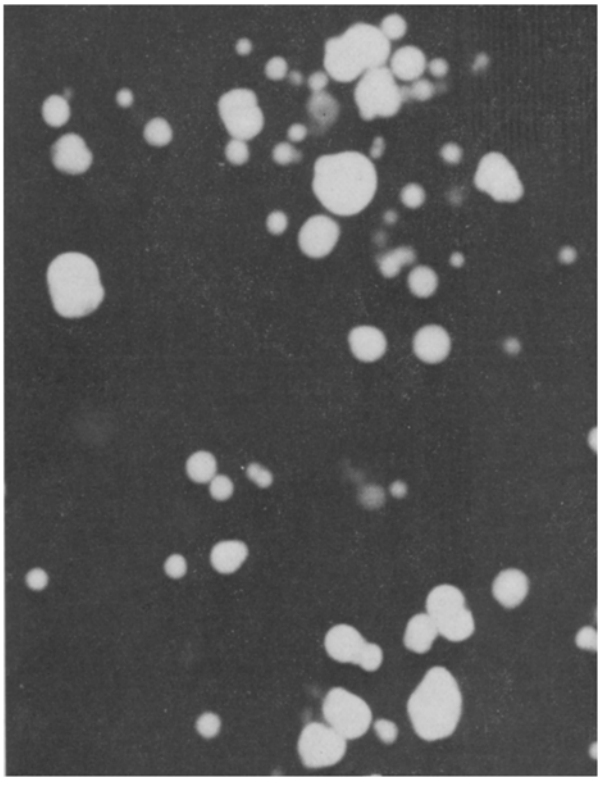

Fig. 2. Same as Figure 1 with fluorescence microscopy. The birefringent inclusions are strongly fluorescent. Some inclusions are out of focus and therefore not visible. × 2300.

negative; 4 = maximum effect) of the amount of Maltese cross inclusions. The Table demonstrates a clear dose-effect relationship; at maximum manifestation, virtually each cell contained extremely large amounts of birefringent bodies in its cytoplasm. These presented either a clear-cut simple Maltese cross, or were of composite structure with the Maltese cross distorted to various degrees (Figure 1). The latter apparently represent multicentric MB which were frequently observed with electron microscopy[1-3,5]. In particularly clear cases, a limiting membrane surrounding the birefringent inclusions was discernible.

Two lines of evidence indicated that these birefringent inclusions represent MB and contain large amounts of phospholipids: 1. After standing for several hours, tube-like extrusions emerged from the inclusions of fresh tissue preparations which, in suitable cases, eventually gained the extracellular space from injured cells and formed tortuous birefringent 'myelin tubes' showing slow movements. Their formation strongly suggests that MB inclusions contain large amounts of polar lipids. 2. In small pieces of liver stained in an alcoholic solution of Sudan black B and embedded in glycerine jelly, the inclusions were light- to dark-blue stained, showed bronze-coloured birefringence with Maltese cross, and dichroism in linearly polarized light. These optical features have been found to be typical for phospholipids[8]. Birefringent buds or tubes with the same optical properties may emerge from such fragments.

Of particular interest are the MB induced by mepacrine, which has a marked affinity to lysosomes[7]. It was found that the Maltese cross inclusions presented the typical green fluorescence of mepacrine (Figure 2). Chloroquine-induced MB were also fluorescent. These facts support the view that MB are secondary lysosomes, and also suggest that the inducing drug is accumulated in these bodies.

Prolonged treatment of rats with the drugs used elicited also Maltese cross inclusions in a large number of other organs. This agrees with electron microscopic findings of several authors[2,3,6,9] and further confirms the identity of MB with Maltese cross inclusions. Several reports recently described the drug-induced formation of foam cells in the lungs of laboratory animals[10-12]; ultrastructurally, foam cells are characterized by the occurrence of MB in their cytoplasm[10,12]. The three drugs mentioned and several other compounds eliciting MB in the liver also induced foam cells in the lung of rats[13]. It thus appears that MB formation in various organs represents manifestations of the same generalized cytopathological mechanism which results in a 'drug-induced lipidosis'[6,10]. The findings reported here show that this pathological condition can be visualized very simply and rapidly by light-microscopy.

Semiquantitative evaluation of the induction of MB in the rat liver by the three drugs

1	2				3				4			
Single dose (mg/kg) (dosage group)	Estimation of the incidence of hepatic MB											
	Drug and No. of doses											
	Triparanol (4×)				Chloroquine (4×)				Mepacrine (2×)			
1600					–	–	–	–	–	4	4	4
800	–	–	3	3	–	4	4	4	3	4	4	4
400	–	3	4	4	3	4	4	4	3	4	4	3
200	2	3	4	2	1	2	3	2	2	2	3	3
100	2	2	1	2	2	1	0	0	2	0	0	1
50	1	0	0	1					0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0	0	0

4 doses (triparanol, chloroquine) or 2 doses (mepacrine) were given orally to 4 rats in each dosage group (column 1) at daily intervals. 24 h after the last application, the effect in the liver cells (= frequency of inclusions with Maltese cross birefringence) was evaluated semiquantitatively for each rat (0 = negative; 4 = maximum observed effect) (columns 2, 3, and 4). A dash indicates that the animal died before the experiment was terminated.

Zusammenfassung. Sekundäre Lysosomen mit multilamellärer Myelinkörper-Struktur, die in der Rattenleber nach Behandlung mit verschiedenen Agenzien (z.B. Triparanol, Chloroquin, Mepacrin) induziert werden können, sind aufgrund ihrer Malteserkreuz-Konfiguration im polarisierten Licht lichtmikroskopisch nachweisbar. Mit fluoreszierenden Substanzen (Chloroquin, Mepacrin) zeigen die Malteserkreuz-Einschlüsse im Zytoplasma starke Fluoreszenz, was auf ihre Akkumulation in den Myelinkörpern hinweist. Nach längerer Behandlung treten Myelinkörper auch in zahlreichen andern Organen auf und induzieren unter anderem die Bildung von Schaumzellen in der Lunge.

G. R. STAIGER

Department of Experimental Medicine, F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basel (Switzerland),
6 November 1973.

[8] C. W. M. ADAMS, *Neurohistochemistry* (Elsevier Publishing Co., Amsterdam 1956), p. 51.
[9] W. K. READ and W. W. BAY, Lab. Invest. *24*, 246 (1971).
[10] R. LÜLLMANN-RAUCH, R. REIL, G. H. ROSSEN and K. U. SEILER, Virchows Arch. Abt. B Zellpath. *11*, 167 (1972).
[11] G. MAGNUSSON and O. MAGNUSSON, Beitr. Path. *146*, 79 (1972).
[12] E. THEISS, H. HUMMLER, H. LENGSFELD, G. R. STAIGER and J. P. TRANZER, Schweiz. med. Wschr. *103*, 424 (1973).
[13] G. R. STAIGER, unpublished results.

Enhancement and Deactivation of Some Microsomal Glycosyl Transferases[1]

The activity of uridine diphosphoglucuronyl transferase and numerous other hepatic microsomal enzymes is enhanced by pretreatment of animals with phenobarbital or with other microsomal enzyme inducers[2]. More recently mammalian liver microsomes have been found to contain also uridine diphosphoglucosyl transferase capable of transferring the glucose moiety to such endogenous substrates as estrogens[3], or bilirubin[4-6] or an exogenous substrate such as *p*-nitrophenol[7,8]. Additionally, liver microsomal preparations also contain glycogen synthetase which sediments together with particulate glycogen and glucuronyl transferase[9,10]. Glucosyl transferase activity in microsomes is much lower than that of the latter enzyme[5,7]. In this study