In this presentation we present data on the existence of both extracellular and intracellular stages of the parasite which results in a new proposal for the life cycle of the parasite. Up to now the formation of daughter cells in thick-walled organisms is supposed to be the only way of multiplication (1). The present study shows that in rats treated with cortisone acetate the formation of daughter cells also takes place in thin-walled pneumocysts (2).

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CYTOCHEMICAL LOCALIZATION OF CATALASE, URATE OXIDASE AND ALCOHOL OXIDASE IN RAT AND FISH LIVER MICROBODIES

Microbodies in liver cells of many vertebrate species contain a crystalline or amorphous dense core. The presence of a crystalline core has been associated with the presence of urate oxidase. On biochemical grounds it has been suggested that the cores of rat hepatocyte microbodies are associated with other peroxisomal enzymes (WATANABE et al., 1977). This possibility was investigated by cytochemical methods in rat and fish (Gasterosteus aculeatus) hepatocytes.

Catalase activity was localized by the DAB method. The distribution of urate oxidase and alcohol oxidase activity, with sodium urate and methanol as substrates, was studied by the DAB method and, more directly, by the cerium method for H_2O_2 detection, developed by BRIGGS et al. (1975). The latter method proved to be effective for the submicroscopical localization of H_2O_2-producing oxidase (VEENHUIS and WENDELAAR BONGA, 1977).

The crystalline cores of rat microbodies and the non-crystalline cores of the fish microbodies were intensely stained by incubation for urate oxidase and alcohol oxidase activity. Some reaction product was also found after the catalase procedure. In the matrix of the microbodies moderately dense deposits were found after incubation for urate oxidase, while a faint reaction was observed after the procedure for alcohol oxidase. The matrix exhibited high catalase activity.

It is concluded that urate oxidase may occur in crystalline as well as non-crystalline cores. Other peroxisomal enzymes are associated with the cores, like alcohol oxidase and, possibly, catalase. The presence of alcohol oxidase in microbodies of vertebrate liver cells was unknown up till now.

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ON THE VISUALIZATION OF LIPOSOMES IN FIBRIN MATRICES AND IN RAT LIVER DURING ENDOCYTOSIS BY KUPFFER CELLS

In a previous study we used horseradish peroxidase entrapped in liposomes (lecithin, cholesterol, phosphatidic acid, 7:2:1 molar ratio) as a tracer enzyme to visualize the cellular distribution, endocytotic mechanisms, and intracellular localization of a liposome-entrapped protein (1). The enzyme was easily demonstrated cytochemically, but the liposomal membranes were not visible in the same material. Although negative staining gave an impression of the diameter of the liposomes, it was considered that realistic counting and measurement could not be performed on these preparations.

During an investigation on the applicability of methods giving special contrasting, such as tricómplex staining, we found that the addition of Ca^{++} and $Fe(CN)_6{}^{4-}$-ions to both glutaraldehyde and osmium fixatives induced membrane-contrast in liposomes (2).

Liposomes were embedded in a fibrin matrix for determination of the diameter. 4.6 ml of 1.3% bovine fibrinogen was mixed with 1.4 ml liposome suspension; 0.3 ml thrombin (20 NIH/ml) was added, and clotting occurred at room temperature after 30 min. Small discs were punched out and fixed in 1.5% glutaraldehyde containing 0.01 M $CaCl_2$ and 0.01 M $K_4Fe(CN)_6$ and postfixed in 1% osmium containing the same additives. After dehydration in alcohol, the fibrin samples were embedded in Epon for ultrathin sectioning.

For the visualization of i.v. injected liposomes in situ, rat livers were per-