CHAPTER IV

DISCUSSION AND CONCLUSION

Discussion of Study 1

The objective of any cell isolation techniques is to produce as many intact cells as possible. The quality of a technique used, can therefore be expressed by the total number of intact cells obtained, taking into consideration both the quality of the cell suspension and the total cell yields. By using the collagenase perfusion technique, the possibility of getting over than 90% intact cells was achieved (Seglen, 1976 a). The morphological features, permeability of the cell membrane and metabolic activity were used as the criterion to verify the quality of isolated hepatocyte preparations (Baur, 1975). The intact cells should have a complete normal cell structure and organelles as shown in organized tissue. Cells with intact cell membrane should exclude dye such as trypan blue, retain intracellular K^+ and cytoplasmic enzymes (e.g. lactate dehydrogenase, ALT), and metabolic activities (Seglen, 1976; Jeejeebhoy and Phillips, 1976).

In the first series of experiments, viability of parenchymal cells isolated from rat liver by the enzymatic technique was verified. A yield of 2-4 g. cell wet weight from 5 to 7 g. liver (200-250 g. rats) was routinely obtained with TB exclusion index of greater than 90%.

For morphological features, nearly all of the isolated cells retained their normal cell structure and organelles under light and electron microscope (Figs. 4-7). The electron micrographs of isolated rat hepatocytes were comparable to those reported by other investigators (Seglen, 1976; Autuori et al., 1979; Wanson et al., 1979).

The cell membrane of the obtained hepatocytes was also intact as reflected by a high percentage of TB exclusion index, the normal initial values of intracellular K^+ and ALT release (Gotts chall et al., 1983; Stacey and Priestly, 1978).

In addition the metabolic capability of the hepatocytes as measured by gluconeogenesis from different substrates (L-alanine, Sod. pyruvate, L(+)-lactic acid, and β-D-fructose) (table 1) were compared nicely with the results obtained by other investigators (Seglen, 1976; Cornell and Filkins, 1974). The selected substrate concentration, the incubation time and the fasting period were based on the finding of Cornell and Filkins (1974) that 10 mM substrates and 60 to 120 min-incubation yielded the maximal gluconeogenic rates in the isolated hepatocytes and fasting of rats for 24 hours or more produced a marked stimulation of gluconeogenesis.

The activity of microsomal N-dealkylation of aminopyrine was also demonstrated in isolated rat hepatocytes. The result was similar to that found in other investigations (Stewart et. al., 1978).

Both biochemical and histological results demonstrated that freshly isolated hepatocytes obtained are structurally and metabolically intact.

Discussion of Study 2

It is known that approximately half of the administered cadmium dose is accumulated initially in the liver (Friberg et al, 1974). Cadmium was mainly taken up by hepatic parenchymal cells (Cain and Skilleter, 1980). Acute liver damage has been reported in vivo with the alterations in both hepatic functions and morphology (Hoffmans et al, 1975).

In the present study, cadmium affects both cell membrane integrity and metabolic integrity of isolated rat hepatocytes. Presentation of cadmium in the incubation medium resulted in a dose-dependent injury to cell membrane of hepatocytes as shown by the decrease in % TB exclusion (Fig. 8) and intracellular K^+ (Fig. 10), and the increase in ALT leakage (Fig. 9). This suggests that cadmium may have the direct toxic effect on cell membrane. The change of influx permeability examined by the TB exclusion test could not detect the initial damage of cell membrane that was detected by the lost of intracellular K^+ and ALT (Figs. 8-10). Thus the leakage of intracellular K^+ and cytoplasmic enzyme may be the sensitive indices for testing the cytotoxic effects of many hepatotoxins.

The metabolic integrity of hepatocytes, as measured by gluconeogenesis from lactate was also affected by cadmium. Gluconeogenesis from lactate has been reported to be a quick test for intactness of isolated hepatocytes (Letko, 1978). In this study, gluconeogenesis was susceptible to a smaller cadmium dose (1 μM) than the cell membrane integrity (Fig 10). A likely explanation for the relationship between cadmium dose and gluconeogenic rate, is that damaged cells lost their capability to exhibit normal carbohydrate metabolism

at the high cadmium dose, and with the sublethal dose hepatic gluconeogenesis may be inhibited in the intact cells.

The morphology of isolated rat hepatocytes after 30 minutes exposed to 5 μM cadmium was shown in Fig 12. Intracellular vacuoles and fat droplets were observed. This finding was in agreement with alteration produced by cadmium in vivo. Numerous alterations to the parenchymal cells of experimental animals have been detected following acute intoxication with cadmium (Hoffmann et al, 1975; Meiss et al, 1982). The most significant alteration is the toxic fatty infiltration of the hepatic parenchymal cells, which is exhibited following both oral and subcutaneous administration. These findings also occur with alcohol abuse (Gall and Mostofi, 1973), and insecticide intoxication (Kimbrough et al, 1972). The vacuoles observed in the present investigation were also described in wide ranging cases of intoxication (Robenek et al, 1980 a, b). These cytoplasmic vacuoles were attributable to phagosomes, which were probably autophagosomes. The autophagic phenomenon is a normal property of hepatic cells which appears to be increased in many sublethal types of liver injury (Shelburne, 1973), including injury by some heavy metals (Hoffmann et al, 1974). Many other ultrastructure alterations reported to cause by cadmium were the alteration of mitochondria, proliferation of the RER and SER, etc. (Hoffmann et al, 1975; Meiss et al, 1982).

All of the cytotoxic effects of cadmium found in the present study was dose-dependent and was similar in nature to the effects seen in intact animals and primary culture of rat hepatocytes (Dudley et al, 1982; Santone et al, 1982). This suggests that cadmium is a hepatotoxin and suspension of freshly isolated rat hepatocytes may

be used as a model system to evaluate the hepatotoxicity of other substances. A variety of machanisms have been proposed to explain cadmium induced liver injury including increased lipid peroxidation (Stacey et al, 1980), altered calcium flux across the cell membrane (Farber, 1979), and direct cytotoxicity to cellular organelles with concomittant disruption of essential biochemical process (Fowler, 1978). Changes in oxidative phosphorylation have also been reported to occur in vivo (Jacobs and Jacob, 1956; Sporn, 1970). So for, the actual mechanism of cadmium hepatotoxicity is still unclear, and needed to define by further investigations.

Conclusion

The structural and metabolic integrity of parenchymal cells isolated from rat liver by this enzymatic techique was verified by a normal morphological features under light and electron microscopes, a high percentage of TB exclusion index, a low degree of soluble enzyme released into the incubation medium, a high intracellular K^+ content, the ability to synthesize glucose from differrent substrates and the active microsomal aminopyrine demethylase activity.

For study on the effects of $CdCl_2$ on freshly isolated rat hepatocytes, there was the evidence of direct toxic effect to both membrane and metabolic integrity in a dose-dependent manner. The toxic effect could be detected at the very low concentrations used (1 and 5 μM) after 30 min-incubation.