CHAPTER III

RESULTS

Results of Study 1

In the first series of experiments, the morphological features, plasma membrane integrity, and metabolic integrity of freshly isolated rat hepatocytes were determined.

The morphology of freshly isolated rat hepatocytes were shown in Figs. 4-7. Under the light microscope (Figs. 4-5), cells were well separated but two or three tightly apposed cells may be frequent (Fig 4A, 4B). Cells appeared to be mainly mononucleated even if some of the binucleated cells were presented (4A). Most of the cells were intact and retained their well defined outline and roundish shape (Figs. 4B, 5A) which were distinguished from a small portion of the flattened groundglass-looking damaged cells with stained nucleus (Fig. 5B).

Under the transmission electron microscope (Figs. 6-7), isolated hepatocytes presented a well-preserved structure and organelles. They exhibited a continuous villous plasma membrane (Fig. 6, MV) with round nucleus and clearly visible nucleolus (Fig. 6, N). The mitochondria were rich and uniformly distributed inside the cytoplasm (Figs. 6, 7A, M). The rough endoplasmic reticulum was composed of parallel running cisternae (Figs. 6, 7A, RER). Glycogen area appeared uniformly gray (Fig. 6, Gl). No vacuoles were presented.

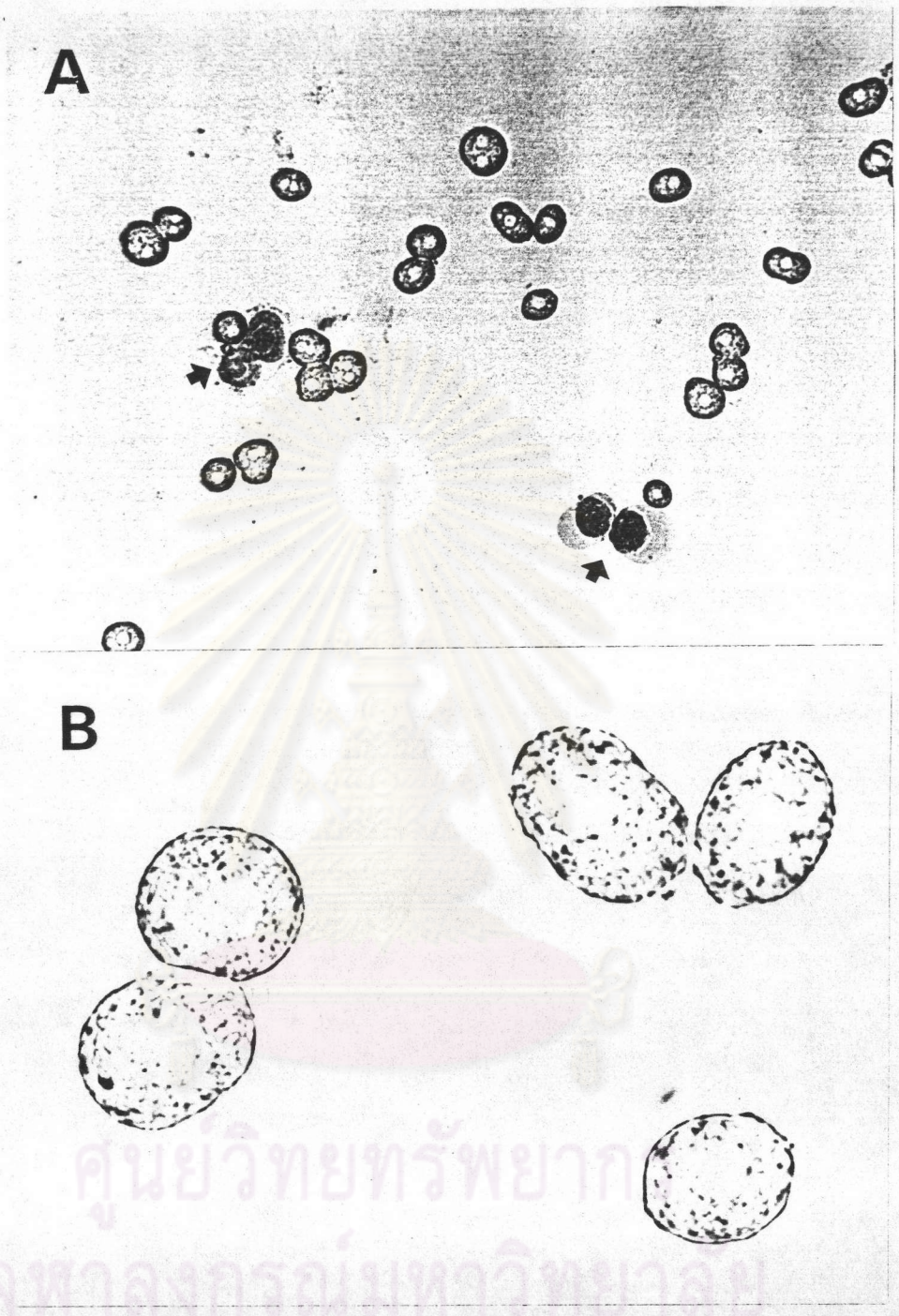

Fig 4. Photomicrograph of suspension of freshly isolated rat hepatocytes in modified Krebs-Hensleit physiological solution. (A) Cell suspension containing mainly intact cells with a few damaged cells (arrows) (x 40). (B) Intact single ll and doublets (x 100).

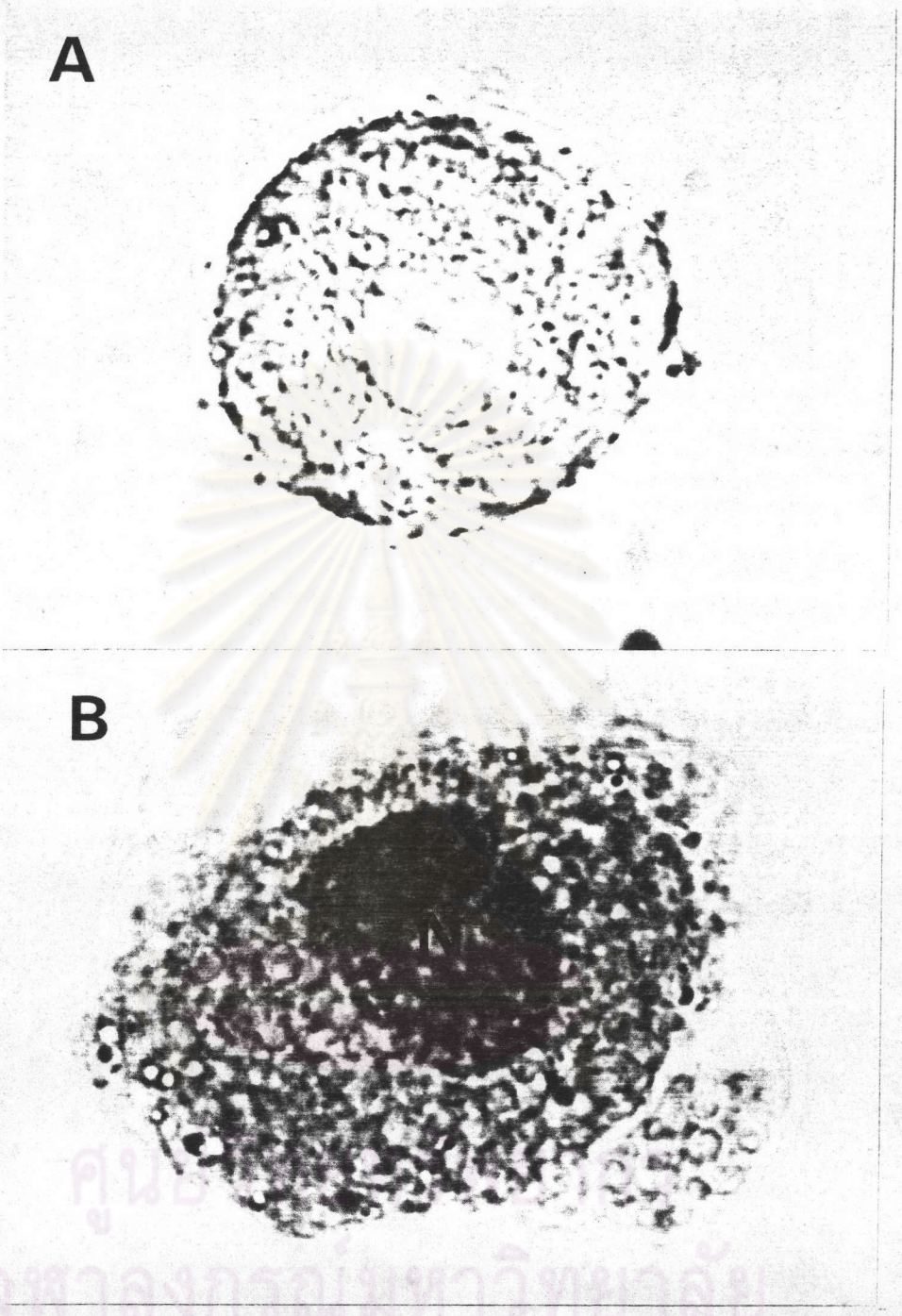

Fig 5. Photomicrograph of freshly isolated rat hepatocytes. (A) Intact cell with round appearance and unstained nucleus (x 1,000). (B) Dead cell with stain nucleus (trypan blue) (x 1,000).

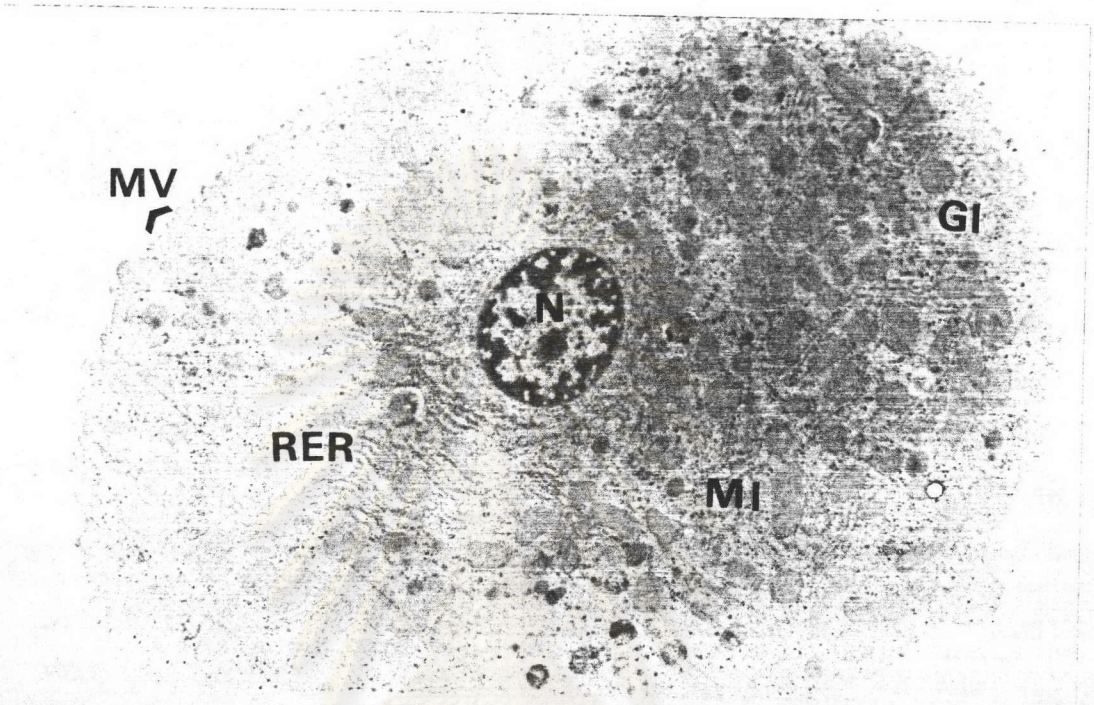

Fig 6. Transmission electron micrograph of isolated rat hepatocyte, presenting a well preserved ultrastructure. Notice the numerous microvilli (MV.) on the continuous cell membrane, nucleus (N), mitochondria (MI), rough endoplasmic reticulum(RER), and glycogen areas (GL) (x 3,000).

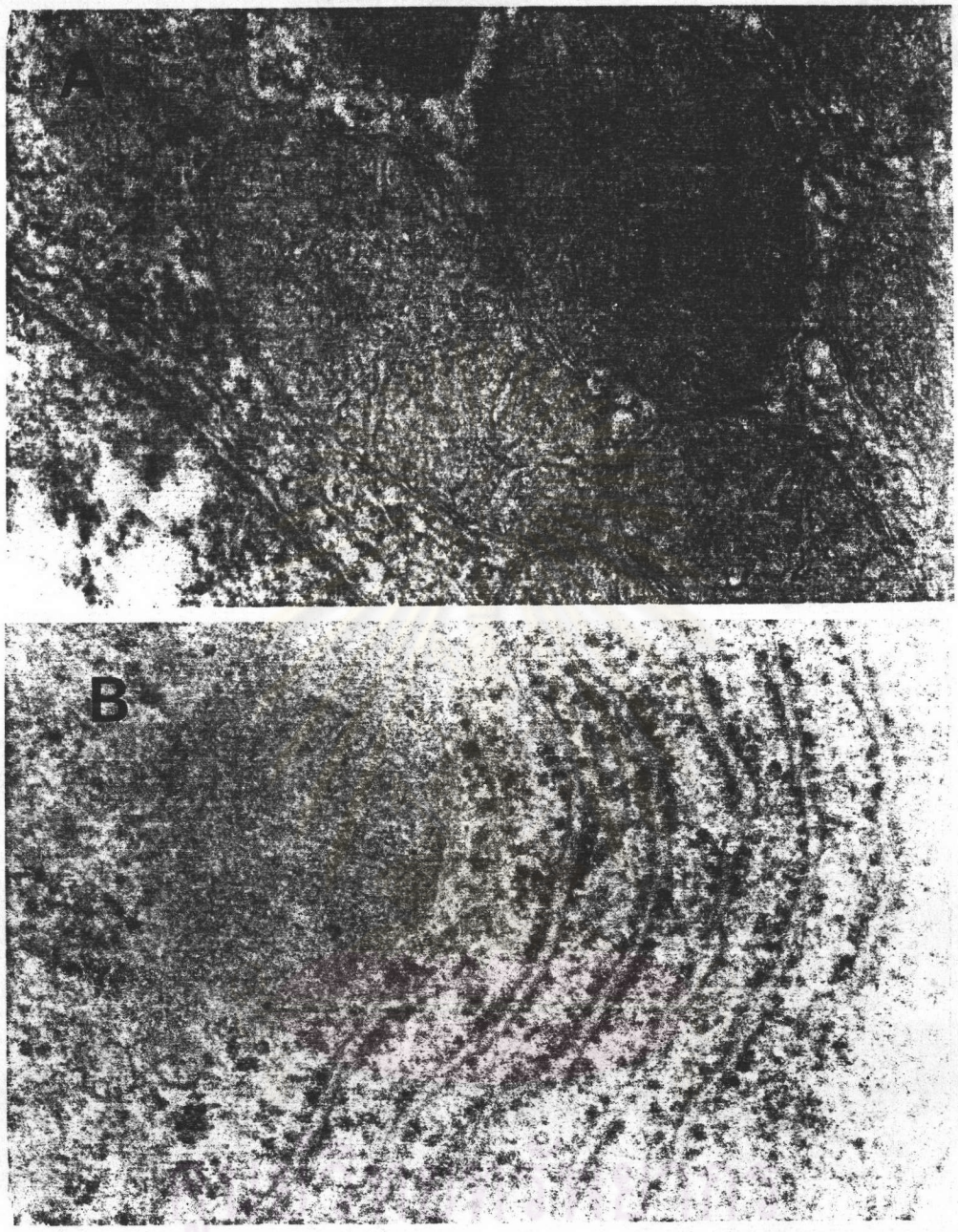

Fig 7. Transmission electron micrograph of isolated rat hepatocytes with mitochondria (A) rough endoplasmic reticulum (B) (x 50,000).

Cell integrity of isolated rat hepatocytes was confirmed by using the parameters of cell membrane integrity from 5 separated experiments. These parameters included the number of hepatocytes that excluded the trypan blue (% TB exclusion index), the intracellular K^+ content, and the cytoplasmic enzyme (ALT) released into the medium before and after 30 and 60 minutes of incubation at 37^oC (Table 1). The % TB exclusion index decreased with time of incubation. Correspondingly, the amount of intracellular K^+ was decreased when the released ALT was increased. There were the statistically changes ($p < 0.05$) in these parameters after 60 minutes of incubation.

The metabolic integrity of isolated cells were demonstrated by gluconeogenesis from 10 mM substrates (L-alanine, sod. pyruvate, L(+)-lactate, and β-D-fructose) (Table 2).

The microsomal drug-metabolizing enzymes in isolated cells were also demonstrated as aminopyrine demethylation rate (Table 2).

Table 1 Cell membrane integrity of freshly isolated rat hepatocytes.

Incubation time (min)	% TB Exclusion Index	Intracellular K^+ (μmoles/g cells)	Released ALT (IU/ml)
0	94.8 ± 0.5	57.35 ± 0.36	181.5 ± 3.8
30	93.9 ± 0.8	56.14 ± 0.37	210.2 ± 8.1
60	91.8 ± 0.6*	49.85 ± 0.45*	261.9 ±19.6*

Values were expressed as mean ± standard error of 5 separated preparations (n = 5).

Suspensions of isolated hepatocytes were incubated for 30 and 60 minutes at $37^{o}C$.

*Statistically significant at $p < 0.05$ when compared with control (values at zero time incubation).

Table 2 Metabolic capability of freshly isolated rat hepatocytes.

Parameters	mean ± S.E.
Gluconeogenesis[a] (μ moles glucose formed/g cells/hr)	
L-alanine	5.64 ± 0.38
Sod. pyruvate	13.14 ± 0.01
L(+)-lactate	21.00 ± 0.83
β-D-fructose	71.62 ± 6.11
Microsomal aminopyrine demethylase activity[b] (μ moles HCHO formed/g cells/30 min)	1.19 ± 0.11

Values were expressed as mean ± standard error from 5 separated preparations.

[a]Hepatocytes were incubated for 30 minutes at 37°C with 10 mM of gluconeogenic substrates. The gluconeogenic rate with substrates was deducted by the endogenous rate of each experiment.

[b]Hepatocytes were incubated for 30 minutes with 5 μ moles of aminopyrine. The total amount of formaldehyde formed was deducted by the endogenous formation of formaldehyde by the cell blank of each experiment.

Results of Study 2

In the second series of experiments, the effects of cadmium chloride on cell viability and morphological features of freshly isolated rat hepatocytes were investigated.

Effect on cell membrane integrity :-

To determine the effect of $CdCl_2$ (1-100 μM) on cell membrane integrity of hepatocytes, the TB exclusion, the release of ALT and intracellular K^+ were studies. Incubation of hepatocytes with 1-100 μM $CdCl_2$ for 30 minutes resulted in a toxic response as measured by the decrease in % TB exclusion index and intracellular K^+, and the increase in ALT leakage (Figs. 8-10).

Effect of $CdCl_2$ on TB exclusion study was shown in fig. 8. The significant decrease ($p < 0.05$)was observed at 25 μM $CdCl_2$, and almost 100% of cells were stained by trypan blue at 100 μM $CdCl_2$. The results of ALT leakage and intracellular K^+were shown in Figs. 9-10. The significant changes ($p < 0.05$) were observed at 5 μM $CdCl_2$.

Effect on metabolic integrity :-

Metabolic integrity of hepatocytes suspension, as measured by gluconeogenesis from lactate (10 mM), was markedly affected by 30 min-incubation with $CdCl_2$. Even at the lowest cadmium concentration, 1 μM, there was a significant decrease ($p < 0.05$) in gluconeogenic rates(Fig. 11). The reduction of gluconeogenesis was in a dose-dependent manner. It should be noted that the amount of glucose produced by hepatocytes incubated with 100 μM $CdCl_2$ was less than the endogenous glucose produced by cell blank.

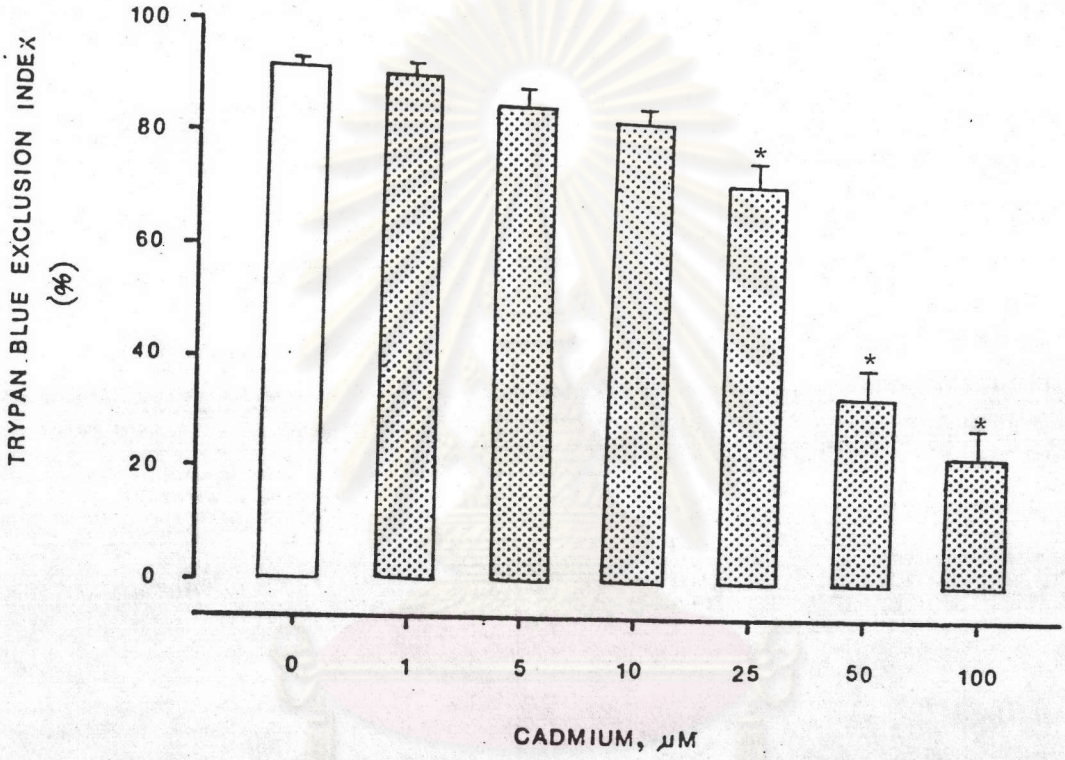

Figure 8 The effect of cadmium chloride on cell membrane integrity.

A. Trypan blue exclusion index

Hepatocytes were incubated with cadmium chloride at the concentration 1-100 μM for 30 minutes. Values represent mean ± S.E. (n = 5).

*Significantly different from control ($p < 0.05$)

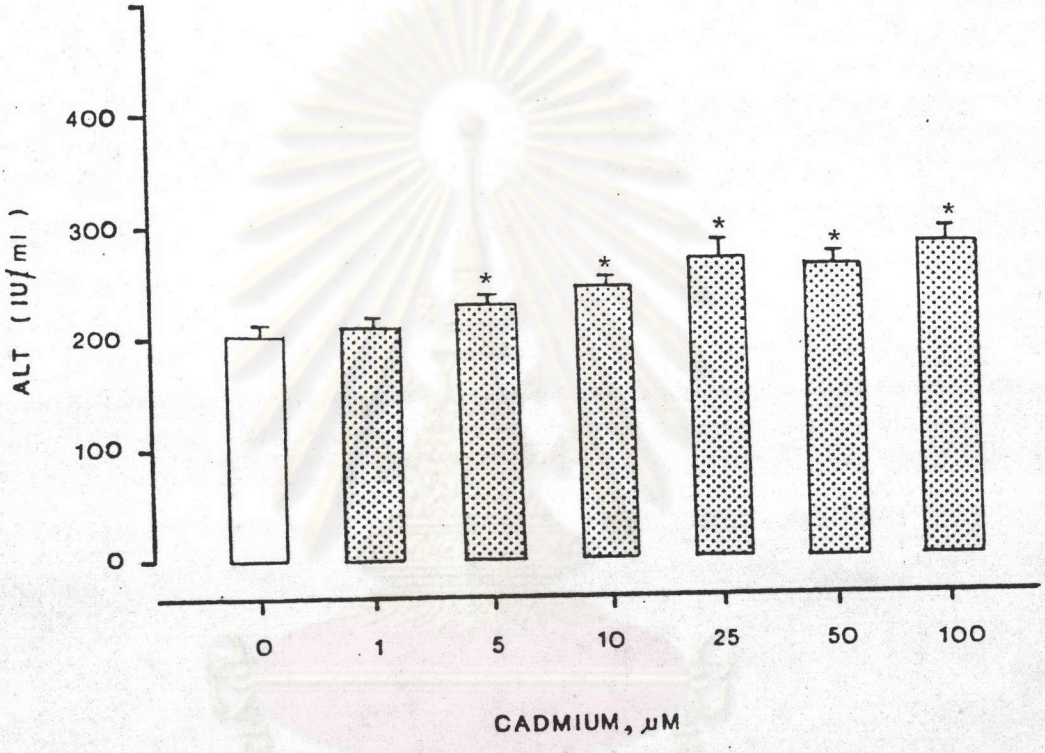

Figure 9 The effect of cadmium chloride on cell membrane integrity.
B. The release of cytoplasmic enzyme (ALT)
Hepatocytes were incubated with cadmium chloride at the concentration of 1-100 μM for 30 minutes. Values represent mean ± S.E. (n = 5).
*Significantly different from control ($p < 0.05$)

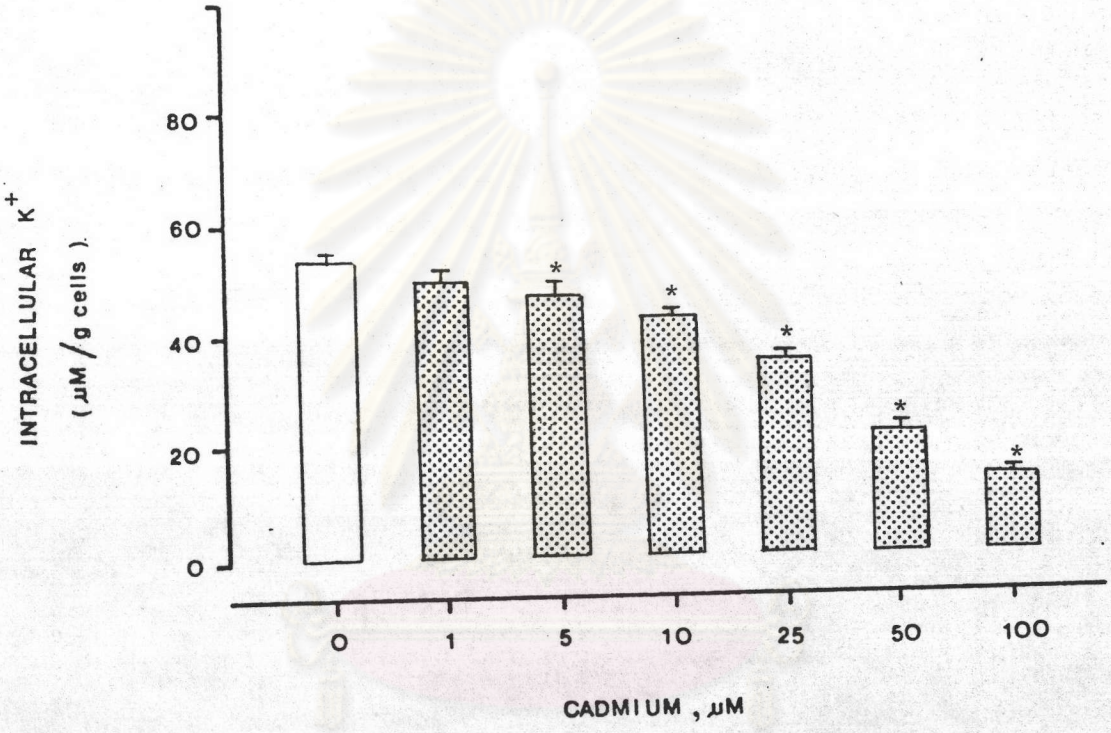

Figure 10 The effect of cadmium chloride on cell membrane integrity. of isolated hepatocytes. C. The release of intracellular K^+ Hepatocytes were incubated with cadmium chloride at the concentration of 1-100 µM for 30 minutes. Values represent mean ± S.E. (n = 5).

*Significantly different from control ($p < 0.05$)

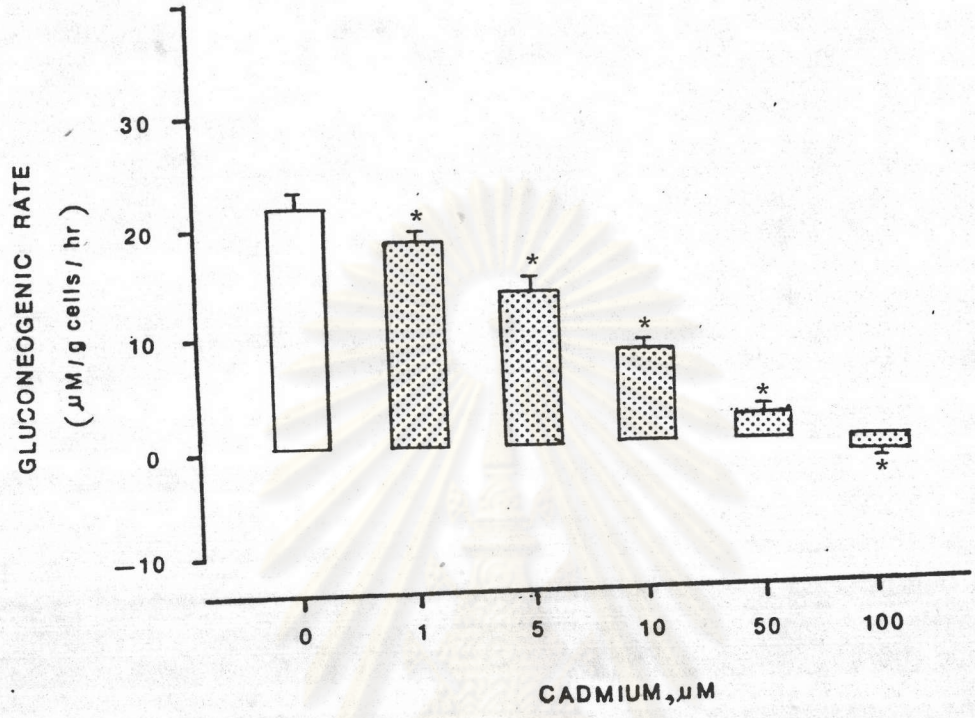

Figure 11 The effect of cadmium chloride on metabolic capability of isolated rat hepatocytes.

Hepatocytes from 24 hours fasting rats were incubated with cadmium concentrations of 1-100 μM for 30 minutes then the gluconeogenesis was determined. Values represent mean ± S.E. (n = 5).

*Significantly different from control ($p < 0.05$)

Effect on morphological features

Incubation of isolated hepatocytes with 5 µM $CdCl_2$ for 30 minutes revealed some remarkable changes under the transmission electron microscope. Fig. 12 showed the electron micrograph of cadmium treated hepatocyte, large-diameter vacuoles were found adjacent to the cell membrane (Fig. 12, V), and fat drouplets were infiltrated in the hepatocytes (Fig. 12, F).

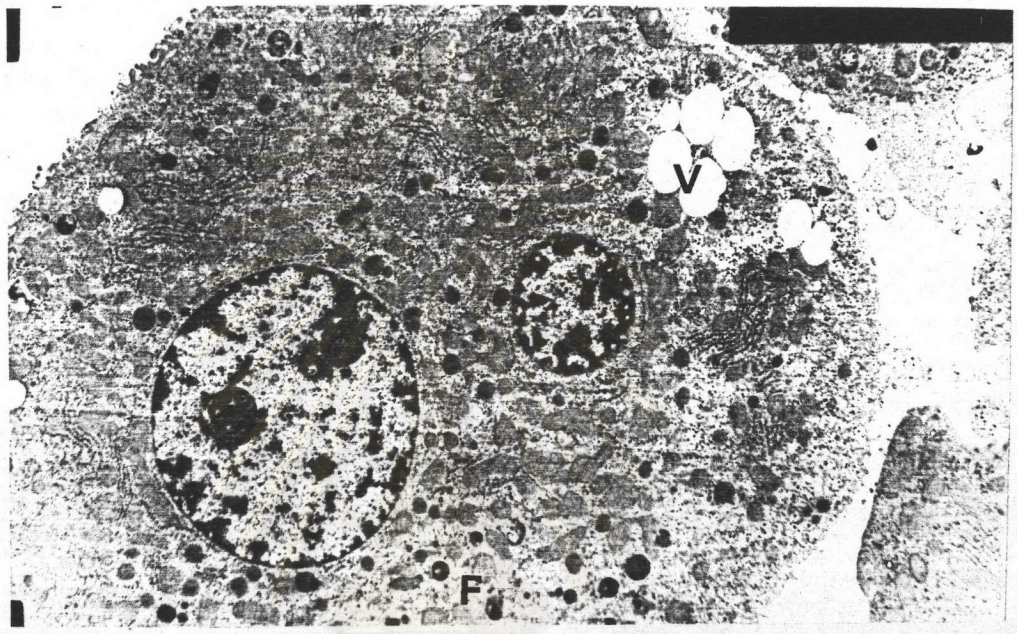

Fig 12. Transmission electron micrograph of cadmium treated hepatocytes present cytoplasmic vacuoles (V) and fat droplets (F) (x 3,000).