CHAPTER IV

RESULTS

4.1 Selection of IFN-sensitive hepatoma cell line

The hepatoma cell line, S102, HepG2 and R12 were tested for their *in vitro* sensitivity to IFN-α. Normal fibroblast was used to test the sensitivity to IFN-α as non related negative control. The effect of IFN-α on growth of three hepatoma cell lines and fibroblast cell line were shown in figures 2, 3, 4, and 5, respectively. As shown in Figure 2, the effect of IFN-α on the growth of S102 cells caused significant suppression of cell growth. According to statistic analysis, even 200 IU/ml of IFN-α was significantly suppressed growth of cells compared with untreated control ($p<0.05$). On the other hand, HepG2 and R12 hepatoma cell lines were not significantly supressed by IFN-α either 500 IU/ml or 1000 IU/ml (Figure 3 and 4). Similar result was observed on fibroblast cell line (Figure 5), which was used as insensitive control. The percent cell growth inhibition of IFN-α at 1000 IU/ml was massively down to the degree of below 10% cell growth inhibition (Table 7). From the experiment, S102 hepatoma cell line composed of the most IFN-sensitive cells, which was good to be chosen as a model for further study of the effects

of IFN-α on the hepatoma cells.

4.2 Selection of IFN-α concentration

Three different concentration of IFN-α was tested: 200, 500 and 1,000 IU/ml (0 IU/ml, IFN free, as control). One thousand IU/ml of IFN-α was the most potent cytocidal effect with remaining of 3% viable cells on day 8 and showing 100% cell growth inhibition. S102 hepatoma cells were treated with 200 IU/ml and 500 IU/ml, and the antitumor effect showed 78% and 100% cell growth inhibition, respectively (Figure 6). Although 500 IU/ml of IFN-α gave equal %CGI to 1,000 IU/ml of IFN-α, the percent viability of IFN-α at 500 IU/ml was about 30%, which was higher than 1,000 IU/ml of IFN. Thus, IFN at concentration of 1,000 IU/ml was selected to study the process of lethal effects of IFN-α on S102 hepatoma cells and its surface antigen.

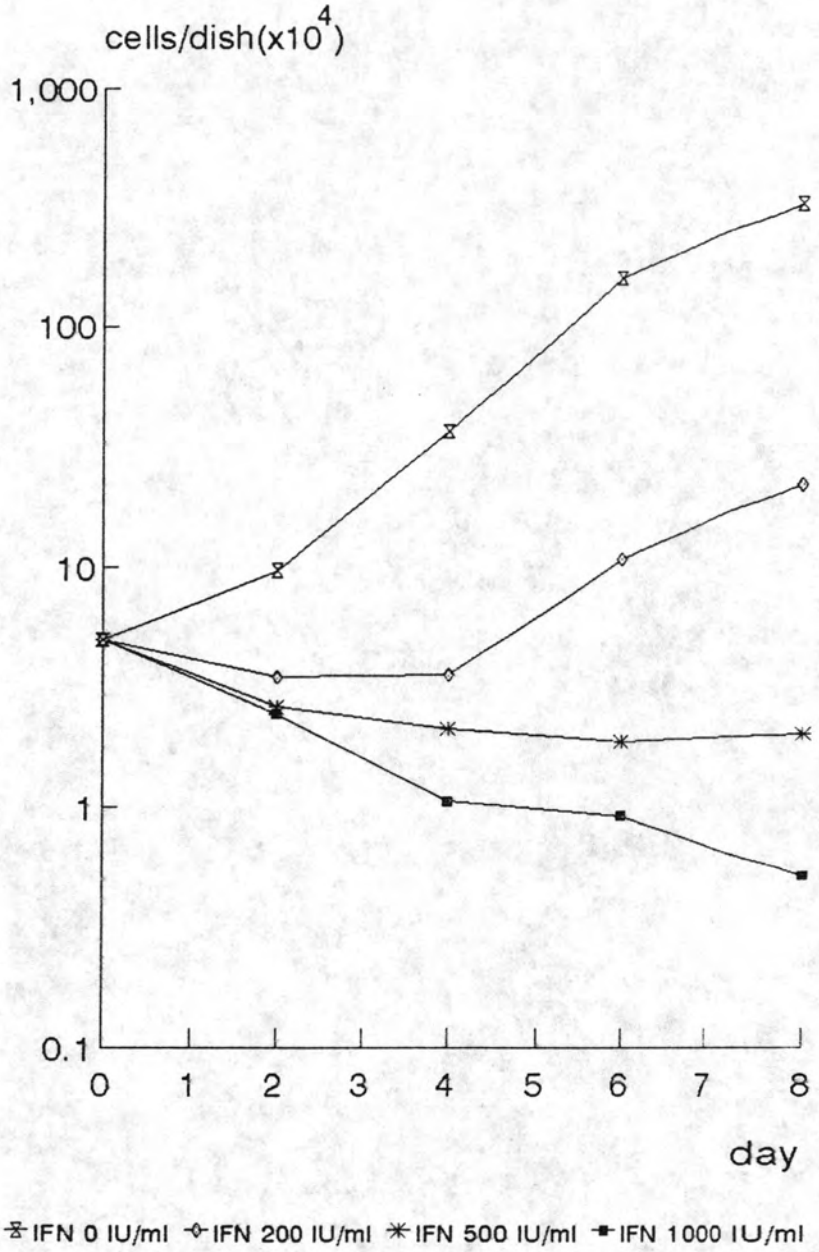

Figure 2 **Effect of IFN-α on the growth of S102.** The concentration of IFN-α were 200, 500 and 1,000 IU/ml per $5x10^4$ cells in 30 mm-tissue culture dish. Each sample represented the mean of triplicate tested. The concentration of 1,000 IU/ml of IFN-α per $5x10^4$ cells showed most effective result.

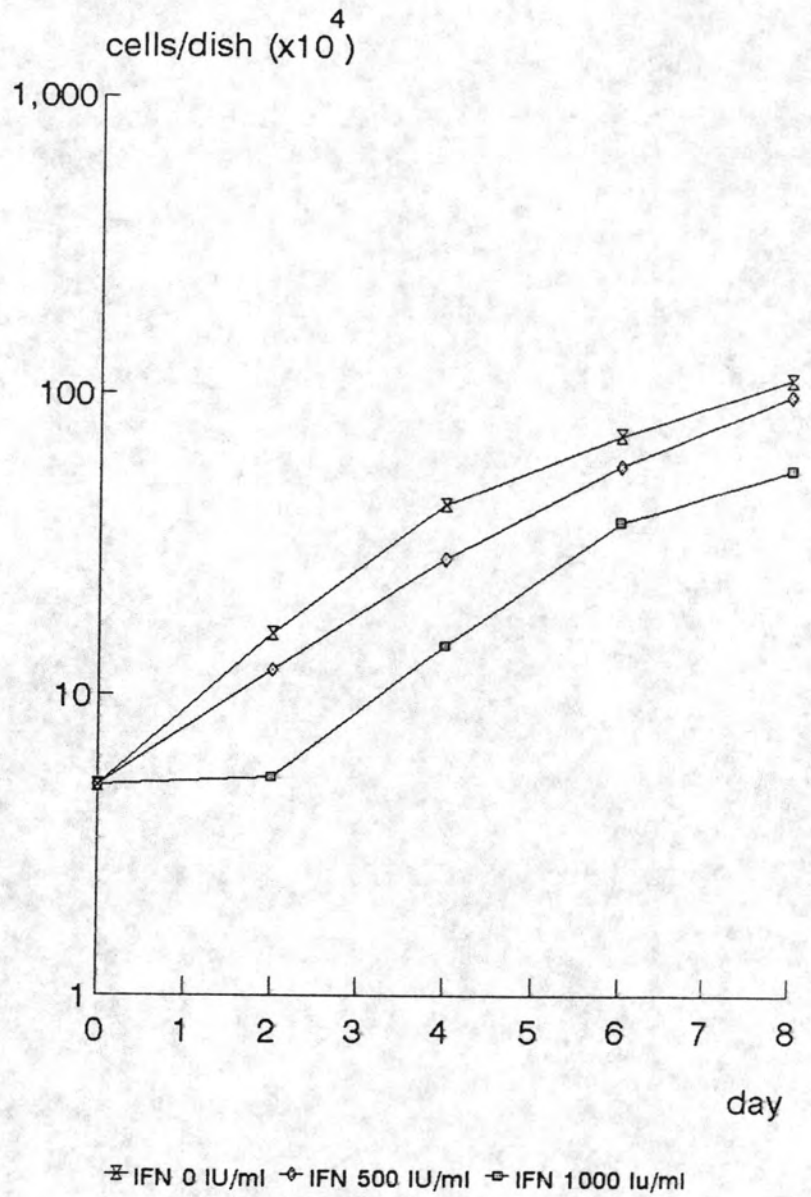

Figure 3. Effect of IFN-α on the growth of HepG2

$5x10^4$ cells in triplicate sample were incubated with IFN-α at concentration of 500 and 1,000 IU/ml in 30 mm-tissue culture dish. There was no significant growth inhibition in this cell line after 8-day observation.

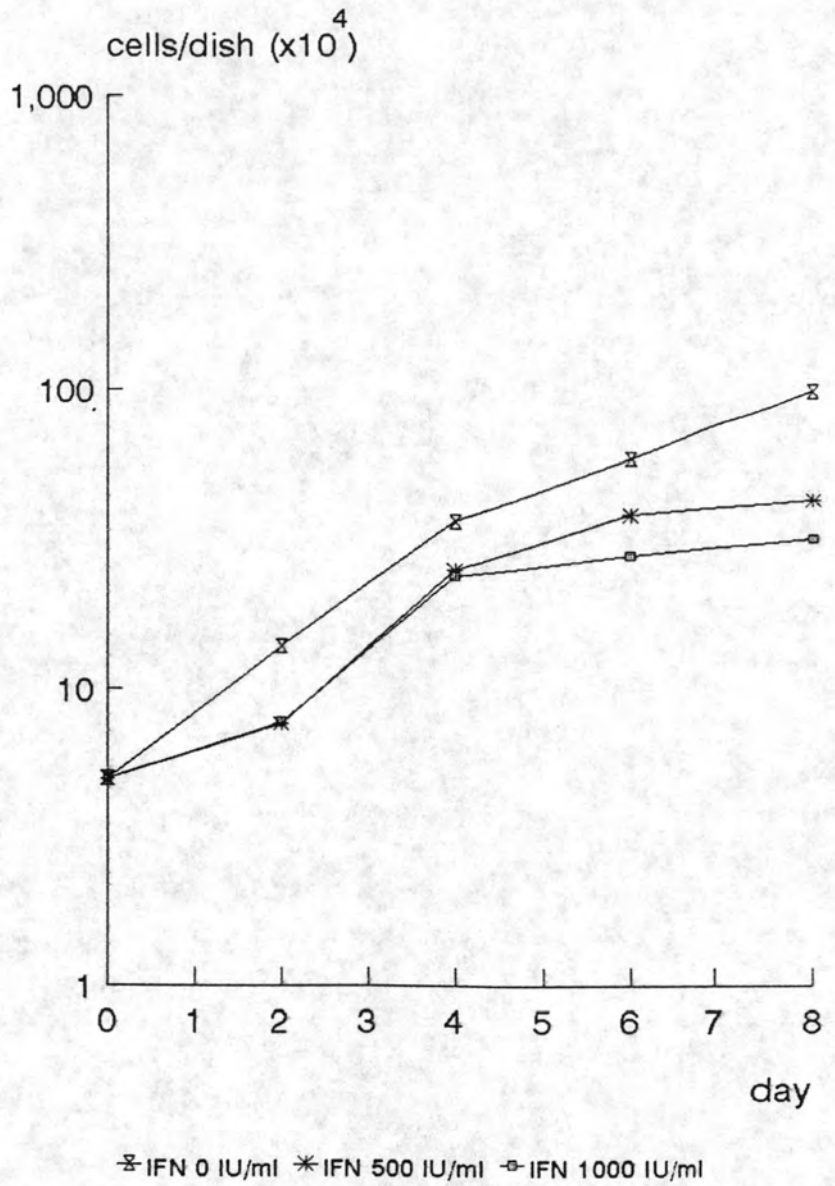

Figure 4. **Effect of IFN-α on the growth of R12**

$5x10^4$ cells in triplicate were incubated in the medium with the concentration of 500 IU/ml and 1,000 IU/ml of IFN-α in 30 mm-tissue culture dish. There was no significant growth inhibition with this cell line after 8-day observation.

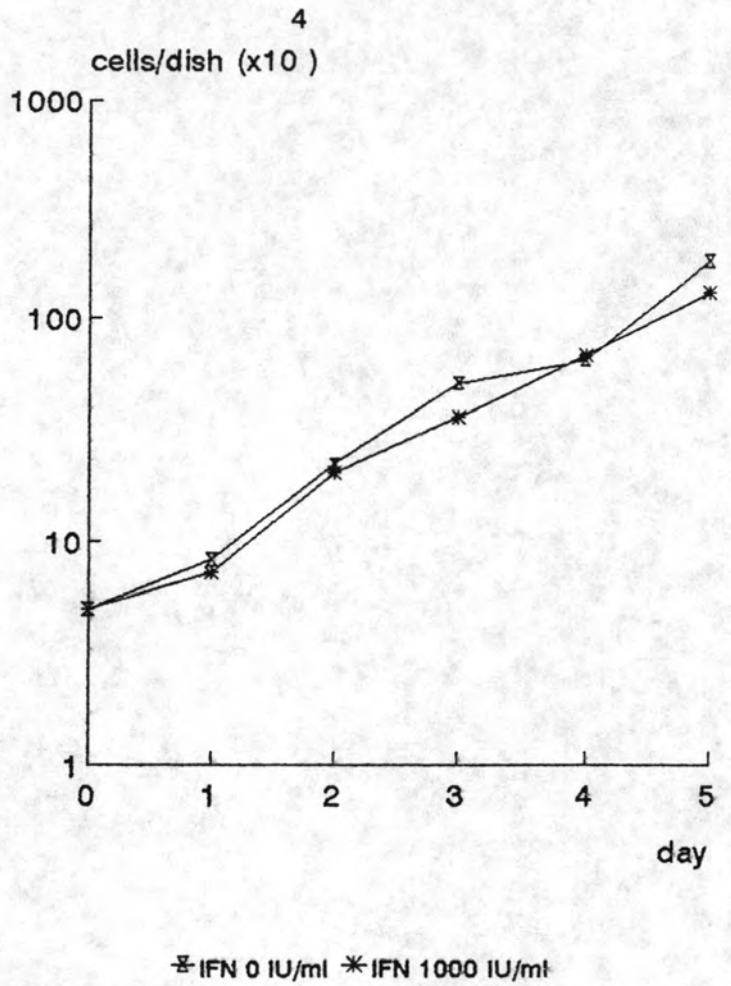

Figure 5. Effect of IFN-α on the growth of L929, the fibroblast cell line. $5x10^4$ cells in triplicate were incubated for five days in the medium with or without IFN at 1000 IU/ml in 30-mm tissue cultured dish. There was no significant growth inhibition in this cell line.

Table 7 Comparison the antitumor effect of IFN-α on three hepatoma cell lines, S102, HepG2, R12

Cell lines	% Cell growth inhibition	
	IFN-α 500 IU/ml	IFN-α 1000 IU/ml
S102	100	100
HepG2	38	74
R12	36	38
L929	ND*	0

% Cell growth inhibition was evaluated on day 4.

ND* = not detect.

control: normal fibroblast cell line, L929

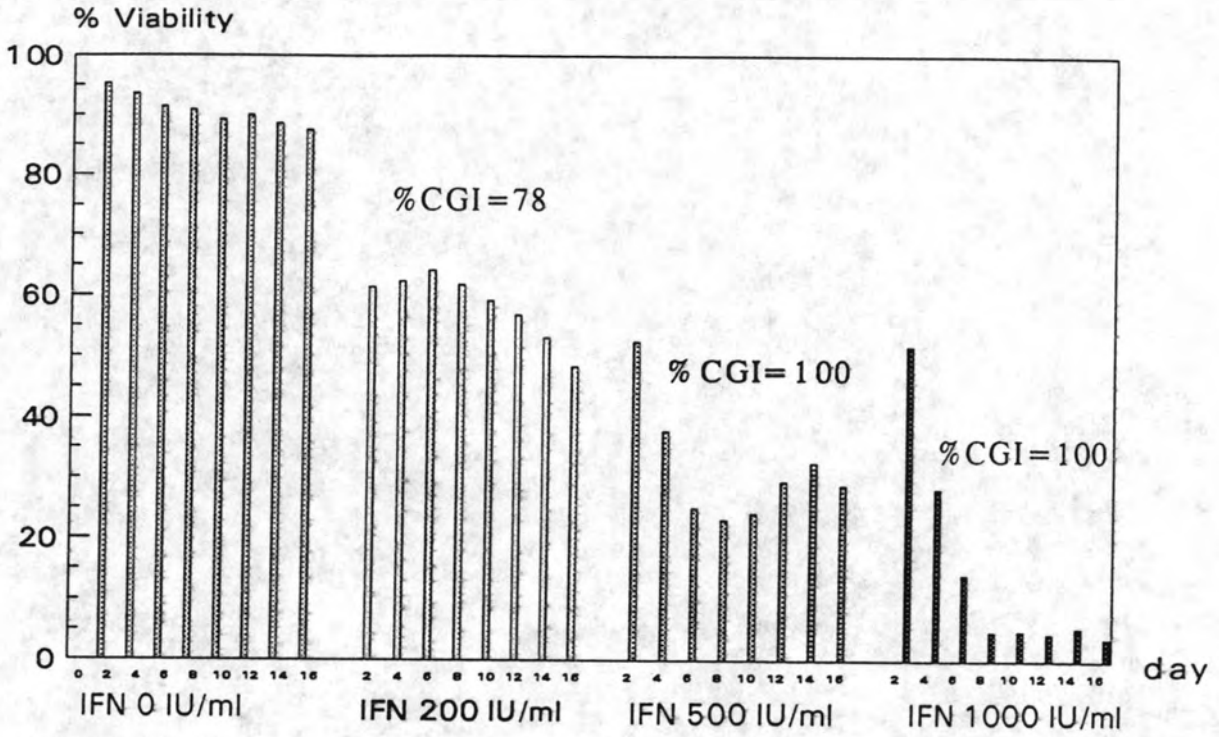

Figure 6 Effect of IFN-α on S102 hepatoma cells, represented by the viability. % Viability was calculated by formula as described in Materials and Methods.

4.3 Selection of suitable fixative

Three different fixatives, 1% glutaraldehyde, 2.5% glutaraldehyde and 2% paraformaldehyde were found to be adequate fixative in preserving the antigen on the plasma membrane. All fixatives did not give any damage to the cell surface Ag. Some Ag were stained in form of fine granule while most of them stained diffusely on surface membrane. PLP which expected to be the best fixative for immunoelectron microscopic study showed negative staining. NS-1 Ab in ascitic form was used as negative MAb. The details were summarized in Table 8. One percent of glutaraldehyde was selected as the fixative for further immunoelectron microscopic study because of its property. Glutaraldehyde in lower concentration had superior cross linking properties, which made an excellent fixative for preservation of ultrastuctures and antigens (Polak and Varndell, 1987).

4.4 The appropriate concentration of anti-hepatoma monoclonal antibody #27 related to the incubation time and temperature

In this experiment, 1% glutaraldehyde was selected for fixation. The temperature during incubation was divided into two groups, the 37°C and 4°C. Along to the temperature, the 37°C incubation were tested with 2 and 3 hours incubation. For the 4°C, the incubation time was

overnight. Parallely to the above study, the concentrations of Anti-hep MAb #27 were varied all togather at 5 μg, 20 μg and 40 μg. The results were summarized in Figure 7.

Antibody stained better with S102 cells than $HepG_2$ cells. Incubation time at 4°C overnight resulted in high expression of antigen. For S102 cell line, 20 μg and 40 μg of anti-hep MAb at 4°C overnight stained cells as high as 50 and 60% respectively which there was no significant difference. According to this results, the concentration of anti-hep MAb selected in preparing specimens for immuno-electron microscopy experiment was 20 μg per $1x10^5$ of S102 cells with 4°C incubation overnight.

Table 8 The fixative for preserving the surface antigen of hepatoma cell lines

Fixatives	2.5% glu		1% glu		2% para		2% PLP	
Antibodies	#27	NS-1	#27	NS-1	#27	NS-1	#27	NS-1
Hepatoma cell line								
S102 cell	+	-	+	-	+	-	-	-
HepG2 cell	+	-	+	-	+	-	-	-

(-) represent negative staining

(+) represent positive staining

glu represent glutaraldehyde

para represent paraformaldehyde

PLP represent Periodic-Lysine-Paraformaldehyde

#27 represent hepatoma specific Mab

NS-1 represent negative control Ab.

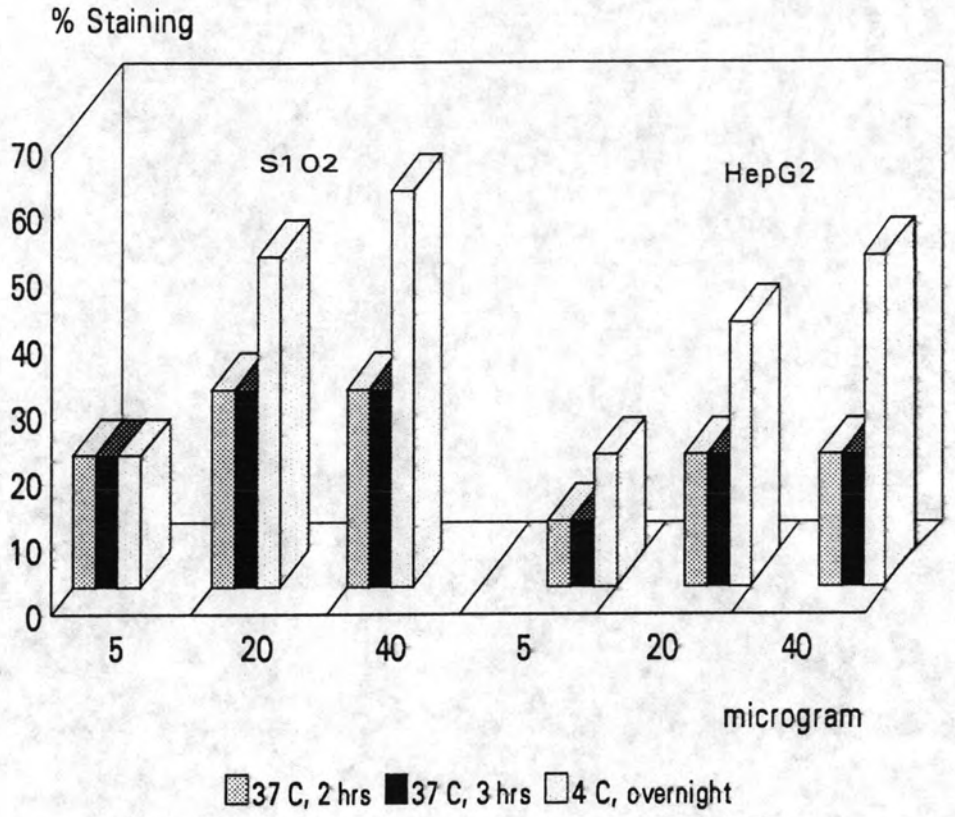

Figure 7 The efficacy in identifying the cell surface antigen studied the relationship of concentration of anti-hepatoma #27 to the incubation time and temperature.

4.5 The appropriate dilution of protein-A conjugated gold particles

Fixed cells before the reaction of immunogold staining gave negative staining with no matter the 1:10 or 1:50 dilution was used. In contrast, the anitgen localization of those fixed with 1% glutaraldehyde at 4°C for 1 hour after gold staining, all perfectly showed localization on surfaces of cells. The 1:10 dilution gave more gold particles than 1:50 dilution. The number of gold particles at 1:10 dilution was among 25-40 particles, while the 1:50 dilution provided less than 5 particles.

From this result, the procedure in preparing specimens was as followed: the antigen localization was done using anti-hep MAb#27 at concentration of 200 μg per $1x10^6$ of viable hepatoma cells and incubated at 4°C overnight. After incubation with the primary Ab, the protein-A conjugated gold particle at 1:10 dilution was added and again incubated at 4 °C for 6 hours. The cells were fixed in 1% glutaraldehyde after all the immunoreaction was over. Then the specimen was passed through the general preparation for transmission electron microscopic examination.

4.6 Effect of IFN-α on the hepatoma cell

4.6.1 Antitumor and reversibility effect

S102 hepatoma cells which were treated with 1,000 IU/ml of IFN-α, showed two different growth appearances: the floating cells and the adherent cells. In order to evaluate the reversibility, all floating cells from each day of IFN treatment were checked by reculturing in supplemented media for 7 days. The results showed that all these floating cells did not adhere at the floor of culture plate and no viable cells were found. The reversible effect was checked with the adherent cells (S102 hepatoma cell is a monolayer cell) on 2, 4, 6 days after IFN release. The result was summarized in Table 9.

The longer treatment showed more significant in decreasing the viability. Eight days continuously treatment showed only 3.08% viable cell. The reversible effect was also compared the degree of viablilty on day 2, 4, 6 after IFN-free medium incubation. Almost all treatment showed 100% cell growth inhibition (%CGI), which suggested the complete suppression of cell growth. However, the cell multiplication of those treated for 2, 4 and 6 days had reversibility with increasing by time out no significant difference from control. Interestingly, 8-day treated group, the cells decreased in 3% viability and

showed reversible effect with 100% CGI and the lower cell multiplication than other treatment (Figure 8).

These IFN resisted cells which derived on day 8, were separated into two groups to investigate the reversibility effect. One was released from IFN-α completely and continuously cultured for 20 days. Another was maintained continuously with IFN. The continuous treatment with IFN showed constant in number of cell with % viability about 3% and the CGI is still 100% with no multiplication of cells. In contrast, the reversible effect of IFN-resisted cells cultured in IFN free medium (IFN -), showed the reversibility. The cell multiplication showed moving-up slightly increasing but significantly different from control. Although there was increasing in cell multiplication of IFN-resisted cells, it was shown that reversible effect of IFN resisted cell did not reach to the number of the control culture.

Table 9 The effect of IFN-α on S102 hepatoma cell in various incubation time of IFN treatment.

IFN treatment (day)	Viability (%)	%CGI day after IFN release							Cell multiplication day after IFN release						
		2	4	6	8	12	16	20	2	4	6	8	12	16	20
Control	93	0	0	0					0.64	1.92	2.21				
2	46.23	100	100	98.5					0.44	1.66	1.24				
4	22.46	100	100	99.3					0.53	1.41	1.54				
6	11.5	100	100	99.5					0.35	1.54	1.2				
8	3.08	100	100	100					0.26	0.98	1.28				
3.08%V	IFN (-)		100		100	100	100	99.8		0.92		1.53	1.42	2.10	2.09
	IFN (+)		100		100	100	100	100		0		0	0	0	0
										%V(3.0)		(3.8)	(3.4)	(3.5)	(3.2)
control			0		0	0	0	0		1.95		2.20	2.1	2.60	2.32

% CGI= % cell growth inhibition.

Cell multiplication was calculated by using formula:

$$\log N = \log N_0 + n \log 2$$

where N = cell munber after treatment

N_0 = initial cell number

n = cell multiplication.

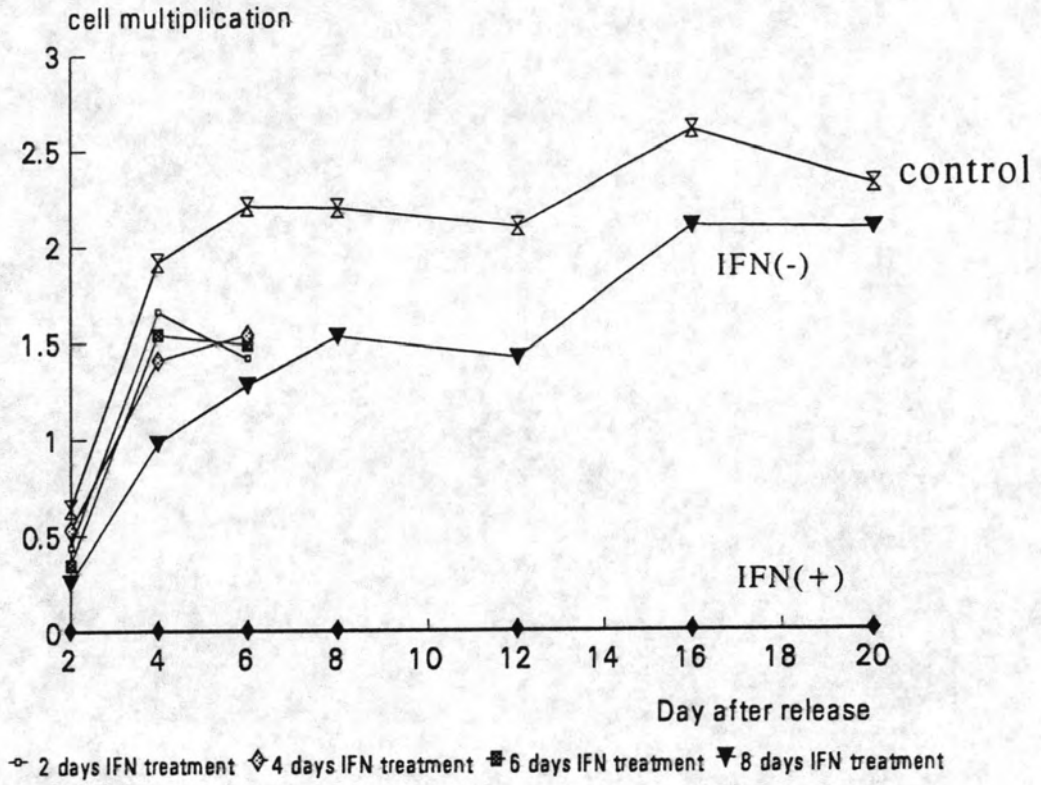

Figure 8 The cell multiplication patterns of various incubation times of IFN treatment.

4.6.2 Evaluating the cytological changes caused by IFN-α treatment

The growth inhibitory effect of IFN-α on S102 hepatoma cells, the response was emphasized on time and morphological changes. The IFN-α treated hepatoma cells showed two different responses.

1. The IFN-α most sensitive cells, immediately died; immediate effect: These cells died within 6 hours, after treatment with IFN-α. All these cells were floated and about 90% carried trypan blue exclusion. The nuclei of these floating cells turned to a fragment. The cytoplasm showed the appearance of coagglulative necrosis. The rest, which showed viable cells, had potential in gathering togehter as clumping. The cells of this group were increased in size up to 2-3 times larger than control. The nucleus was pyknotic and the cytoplasm showed vacuolization. The 12-hour IFN-α treated cells were complete dead cells. Ninety-nine percent of cells had no nucleus and some small, round eosinophilic particles, suspicious of inclusion body, which localized near nucleus were seen.

2. The IFN-α slow effect cells, slow died; prolong effect: The cells of this group took 8 days to be dried. Cellular changes of these cells were examined at 6,12, 18 hours. At 18 hours after treatment, there were no signifi-

cant changes except the size, which were relatively about twice larger than control. At twenty-four hour after treatment, the IFN-α treated cells were much larger in size as 1-2 times of control. The nucleocytoplasmic ratio (N:C) was turned upside down from 2:1 to 1:2. The number of cells which contained eosinophilic particle in cytoplasm at 24 hours was counted about 10-20% of examined cells. On day 3 after treatment, nuclear changes were irregular shape, pyknosis and segmented nucleus. On day 4 after treatment, cells which carried segmented nucleus and eosinophilic particles were more prominent, 20-30% of examined cell. On day 5 after treatment, most of IFN-α treated cells were ruptured and had no nucleus. The area which have seen the eosinophilic particle were full with the small, pinklish splinter particle, in turn.

4.6.3 The killing process of IFN-α to the S102 hepatoma cell and the influence to the cell surface antigen

The appearance of control hepatoma cell (untreated cell) was shown in Figure 9. The electron micrograph of a control cell at the same age of culture showed that plasma membrane was well preserved with long microvilli. The cytoplasm contained small amount of organelles. Mitochondria were pleomorphic with double membrane and diminished cristae (Figure 10). There was moderate rough endoplasmic reticulum (RER) with flattened cisternae while smooth endoplasmic reticulum (SER) which showed tubular form, was scanty (figure 11). Golgi apparatuses and electron dense vesicle-like lysosomes were rarely seen. Free ribosomes and glycogens were rarely observed in cytoplasm. The nuclei were large, irregular with cytoplasmic invagination. The nucleocytoplasmic ratio was about 2:1. The nuclear envelopes were well preserved with parallel double membrane and slight heterochromatin aggregation. Nuclear chromatin aggregated into 1-2 clumping was commonly observed. The rounded nucleoli was rarely seen. The surface antigen in control cells resulted in gold labelling ranging from 34-62 particles. The pattern of gold labelling was distributed on cell surfaces with occasional in cluster particles of gold (Figure 12).

When S102 hepatoma cells were treated with 1,000 IU/ml of IFN-α for 3 hours, considerable changes in morphology were observed. Most of cellular appearances remained similar to that of control. The number of surface antigen was not different from control (33-61 particles). After 6 hours IFN treatment, the mitochondria were clearly seen swollen (Figure 13) but with normal appearance in some cells. Other organelles were similar to control. Interestingly, there were non-membrane bounded structure with heterogenous electron translucence in cytoplasm (Figure 14). The antigenic alteration caused by IFN-α treatment was not different from control resulting in gold labelling ranged 33-70 particles with the same pattern as control.

Following culture with IFN-α for 1 day, the majority of cells displayed ultrastructural changes. The plasma membrane remained well preserved. The organelles including mitochondria, RER, SER were also changed in number and structure. Swollen mitochondria were also seen (figure 15) as well as increased in number of RER and SER with normal structure (Figure 16). Free ribosomes and glycogen were commomly seen. The nuclear envelopes were well preserved with variation of chromatin pattern. Mostly, chromatin aggregation into clumping pattern was frequently seen as similar to control. In contrast, thicken chromatin

aggregation attaching nuclear envelope was also commonly seen (Figure 17). The heterogenous electron translucent non-membrane bounded structures were larger than that of treatment for 6 hours (Figure 18). Interestingly, the antigenic alteration caused by IFN treatment was increased in number of gold labelling ranging from 48-77 particles with the same distributing pattern as control, however, it was not significantly increased compared with control.

Three days after treatment, indented nuclei with some fragmented nuclei were commonly seen (Figure 19,20). The changes of nuclei displayed with scattered chromatin. The heterogenous electron translucent non-membrane bounded structures remained in cytoplasm with smaller size than that of one day IFN treatment but increased in number of these granules (Figure 21,22). In addition, glycogens were frequently seen in cytoplasm with some cluster (Figure 23, 24). Interestingly, the antigenic surface alteration caused IFN treatment was significantly increased in gold labelling ranging from 82-105 particles, frequently in cluster of gold particles distributed on cell surfaces (Figure 25,26). Four days after treatment, the changes of nuclear envelope displayed with ruffle outer membrane. Cytoplasmic organelles remained changes as those in three days treatment (Figure 27,28) but cytoplasmic granules containing an electron dense matrix were commonly seen.

These granules were alike autophagosomes (figure 29-31). In addition, the changes of nuclei displayed with scattered chromatin was commonly seen (Figure 32). In contrast, heterogenous electron translucent non-membrane bounded structures did not found in this peroid of treatment. The antigenic surface alterations caused by IFN-α treatment were not different from control resulting in gold labelling ranged from 51-71 particles.

Additional culture was maintained for 8 days to analyse the ultrastructural changes of resistant cells to IFN. These cells were well preserved and displayed invaginated nuclei with scattered heterochromatin (figure 33). Cytoplasmic organelles including mitochondria were swollen. RERs were moderately seen with dilatation (Figure 34) but SERs were predominant and hypertrophic (Figure 35). Golgi apparatuses were moderately seen with golgi-derived vesicles around themselves (Figure 36,37). In addition, electron dense vesicle-like lysosome was frequently seen. Gold labelling ranged from 44-72 particles with similar in number and pattern to control.

In other experiment, $HepG_2$ hepatoma cells were evaluated of the morphological and antigenic alteration caused by IFN-α treatment. In the untreated cells displayed indented nuclei with clumped heterochromatin (Figure 38).

Nucleocytoplasmic ratio was about 2:1. Cytoplasmic organelles had moderate amount including mitochondria with pleomorphic shape (Figure 39). RERs were moderately seen associated with mitochondria while SER was scanty (Figure 39). There was scanty golgi apparatus and electron dense vesicle-like lysosome. Free ribosomes and glycogen were rarely seen. Gold labelling of $HepG_2$ untreated cells yielded lower than of S102 untreated cells with only 4-10 particles around surface of cells. Following culture with 1,000 IU/ml of IFN-α for 3 days, the majority of cells displayed changes in cytoplasmic organelles. Mitochondria were irregular and swollen (Figure 40,41) while RER and SER remained similar to that of untreated cells. In addition, glycogen particles were frequently seen. The antigenic alteration caused by IFN-α treatment, showed increased in number of gold labelling ranging from 6-15 particles with distributed around surface of cells.

In addtion, the experiment was performed to confirmed the effects of IFN-α on morphological changes of normal fibroblasts as control cells. The ultrastructure of untreated cells displayed indented nuclei with clumped heterochromatin and large rounded nucleoli. Nucleocytoplasmic ratio was less than that of two hepatoma cells with 1:2-3. Cytoplasmic organelles had numerous amount including mitochondria with pleomorphic shape. RERs were

prominently seen while SERs were scanty. There was scanty of golgi apparatus and glycogen particles. Free ribosomes and electron dense vesicle-like lysosomes were commonly seen (Figure 42,43). After IFN treatment for 3 days, there was no significant changes in cytoplasmic organelles and nuclei. These cells remained similar to untreated cells as mentioned above (Figure 44,45).

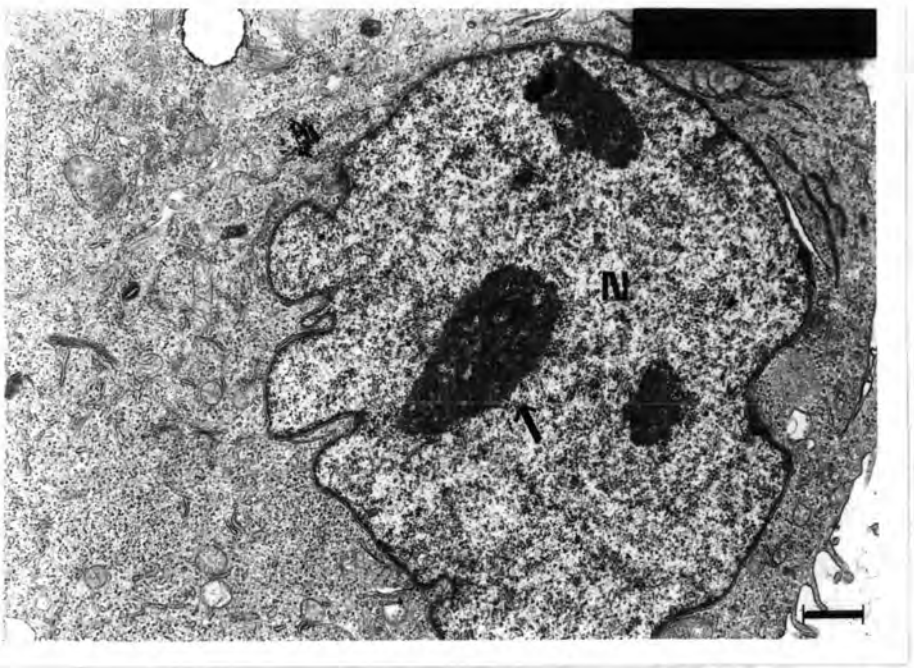

Figure 9 S102 hepatoma cell (untreated cell) shows chromatin aggregation in clumping pattern (arrow). (Bar =1 μm ; x7,750)

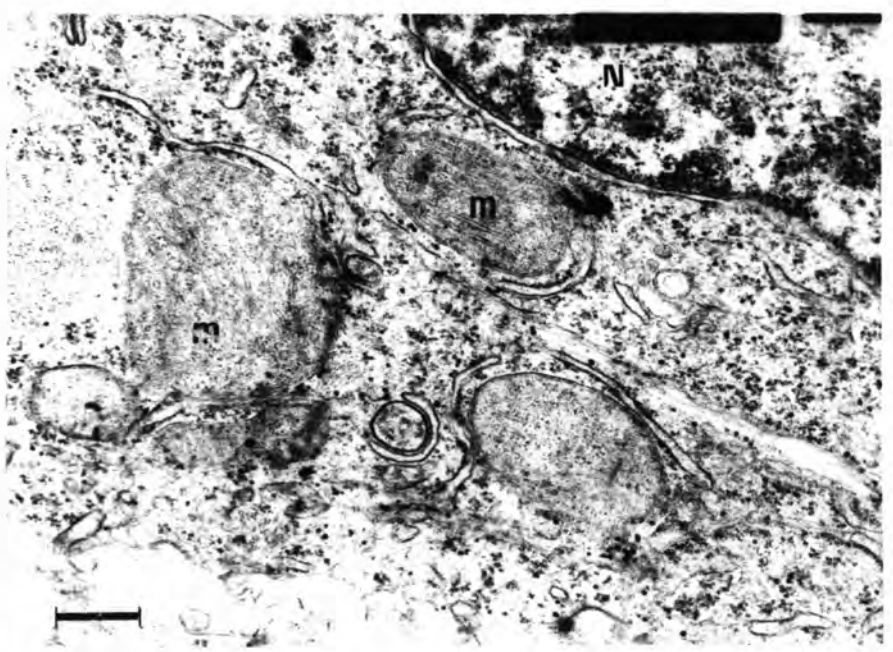

Figure 10 S102 hepatoma cell (untreated cell) shows pleomorphic mitochondria (m). (Bar = 0.3 μm ; x30,800)

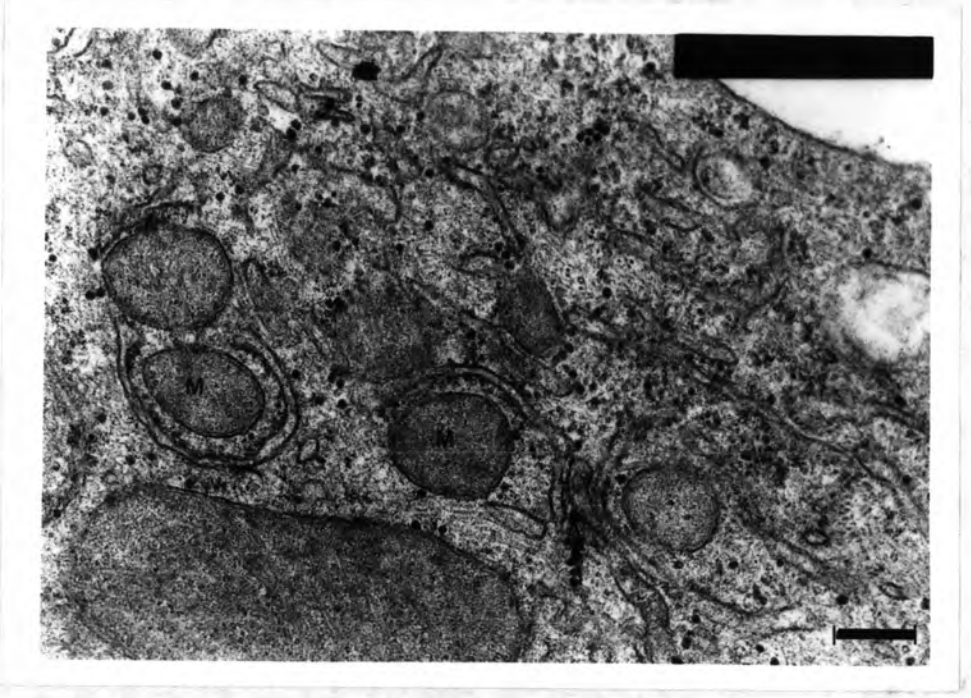

Figure 11 S102 hepatoma cell (untreated cell) shows normal mitochondria (M). (Bar = 0.2 μm ; x45,000)

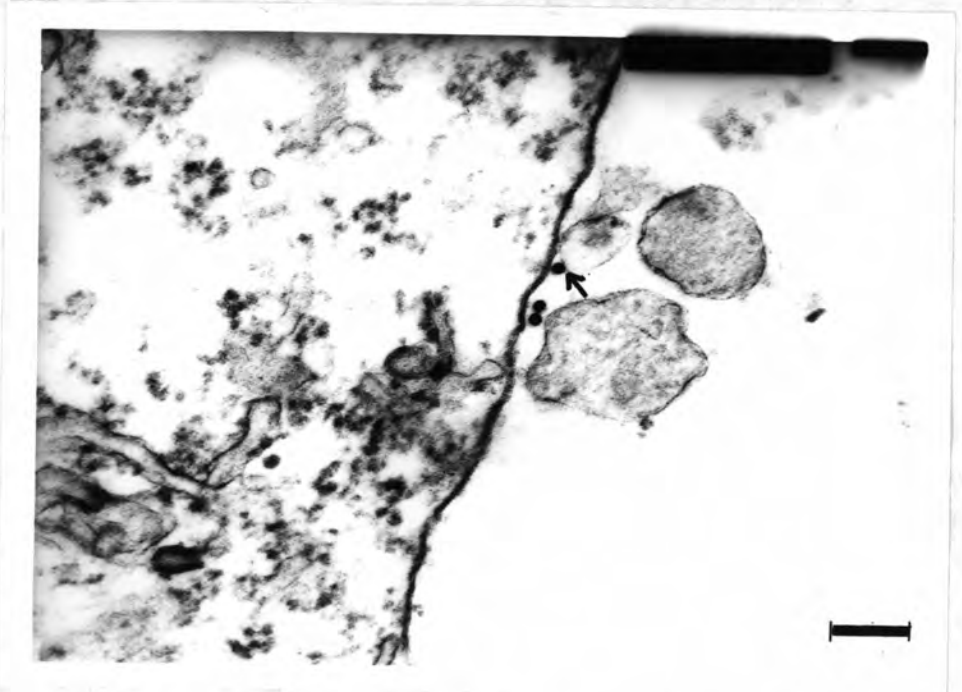

Figure 12 S102 hepatoma cell shows gold labelling (arrow) distributed on a surface of a S102 hepatoma cell. (Bar = 0.1 μm ; x81,200)

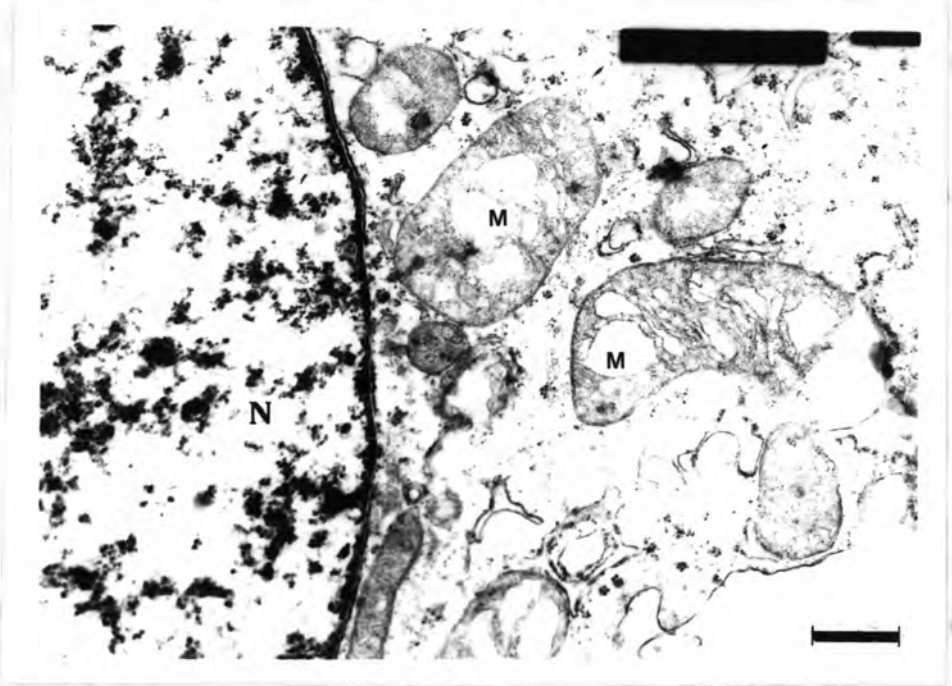

Figure 13 S102 hepatoma cell after 6 hr IFN treatment shows swollen mitochondria (M). (Bar = 0.5 μm ; x19,800)

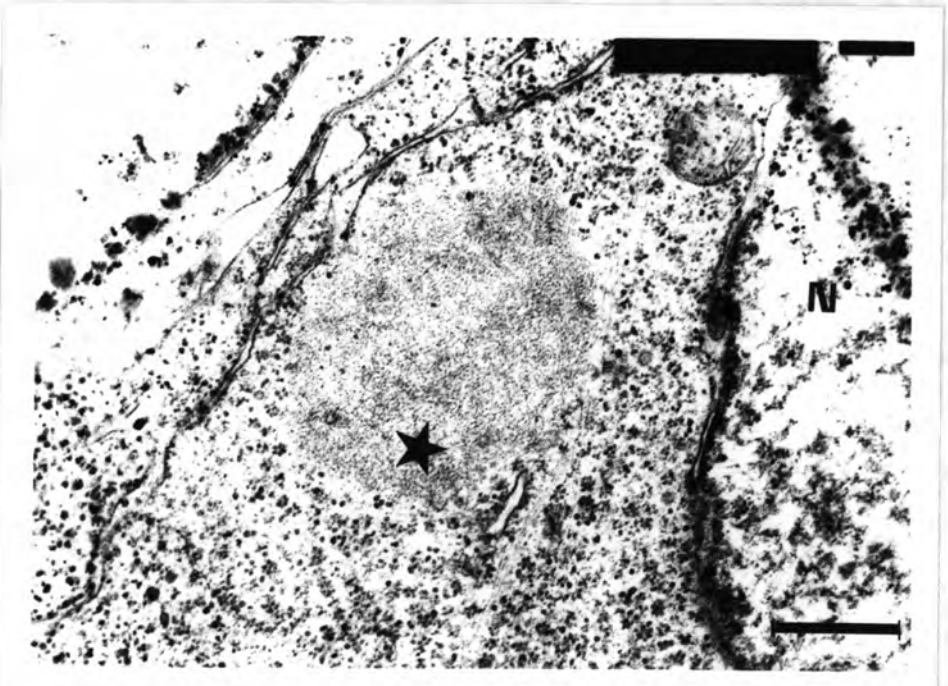

Figure 14 S102 hepatoma cell after 6 hr IFN treatment shows heterogenous electron translucent non-membrane bounded structure (*). (Bar = 0.5 μm ; x30,240)

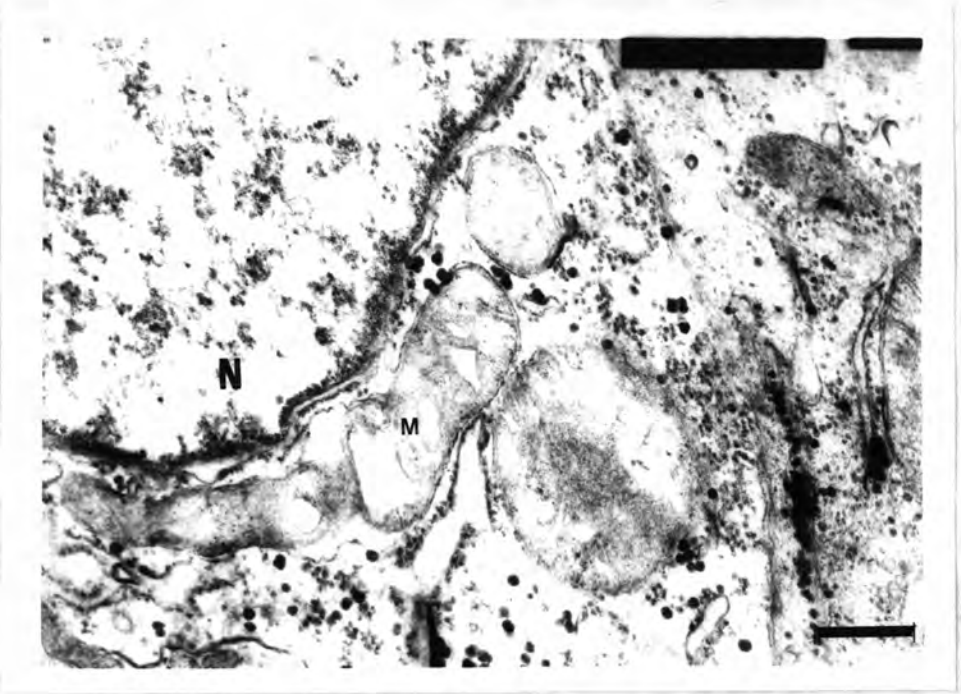

Figure 15 One day IFN treated S102 hepatoma cell shows swollen mitochondria (M). (Bar =0.3 μm ; x38,000)

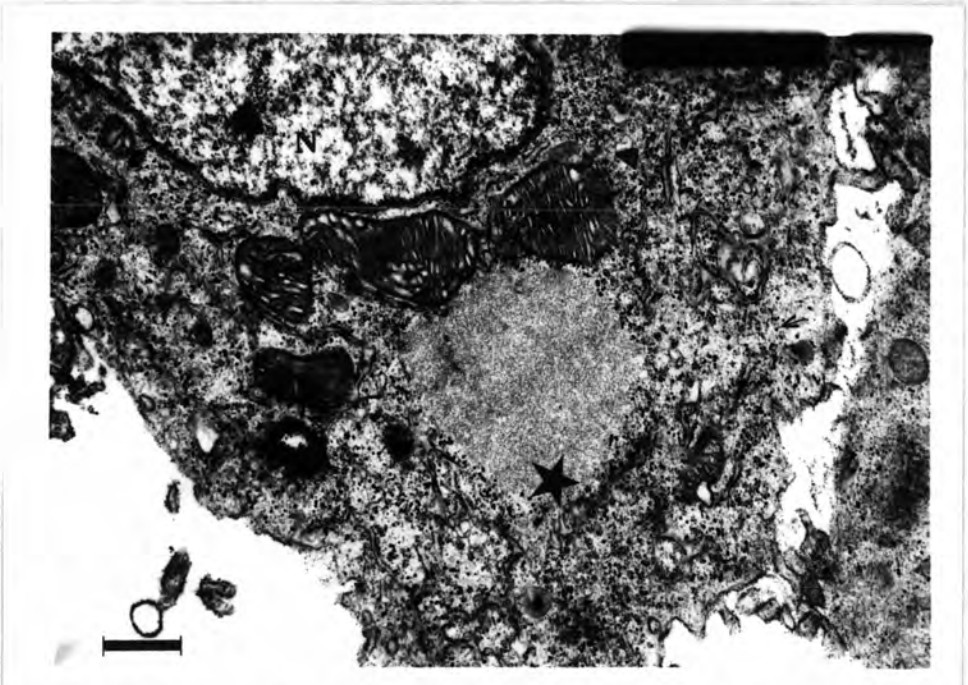

Figure 16 One day IFN treated S102 hepatoma cell shows heterogenous electron translucent non-membrane bounded strucutre (*), swollen mitochondria (M), and moderately increased in RER, ribosome (arrow). (Bar =0.3 μm ; x30,240)

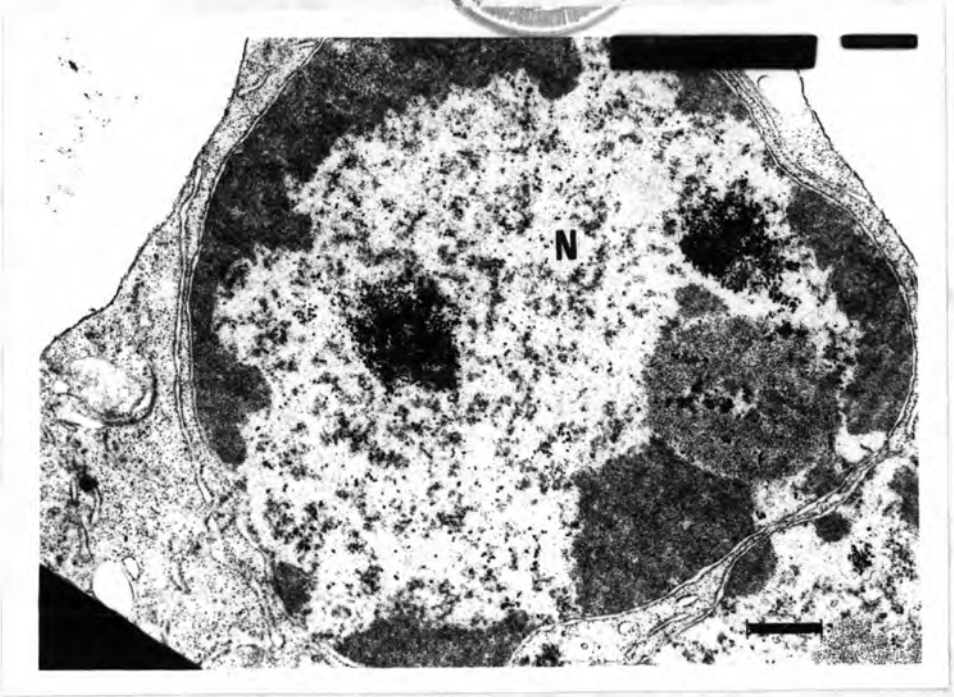

Figure 17 One day IFN treated S102 hepatoma cell shows chromatin aggregation attaching nuclear envelope. (Bar =0.5 μm ; x15,200)

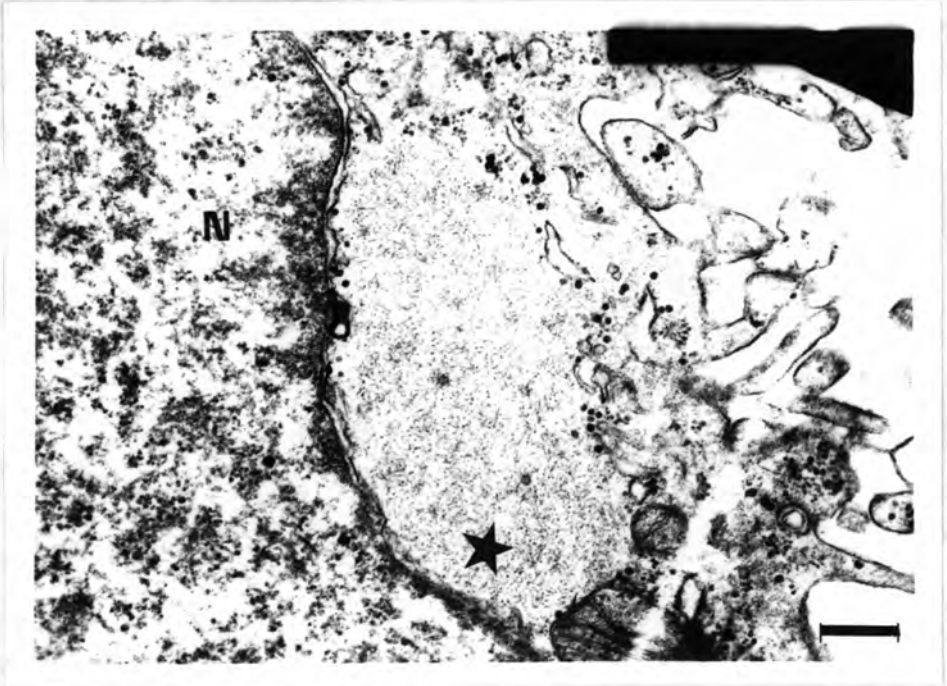

Figure 18 One day IFN treated S102 hepatoma cell shows heterogenous electron translucent non-membrane bounded structure (*). (Bar =0.3 μm ; x30,240)

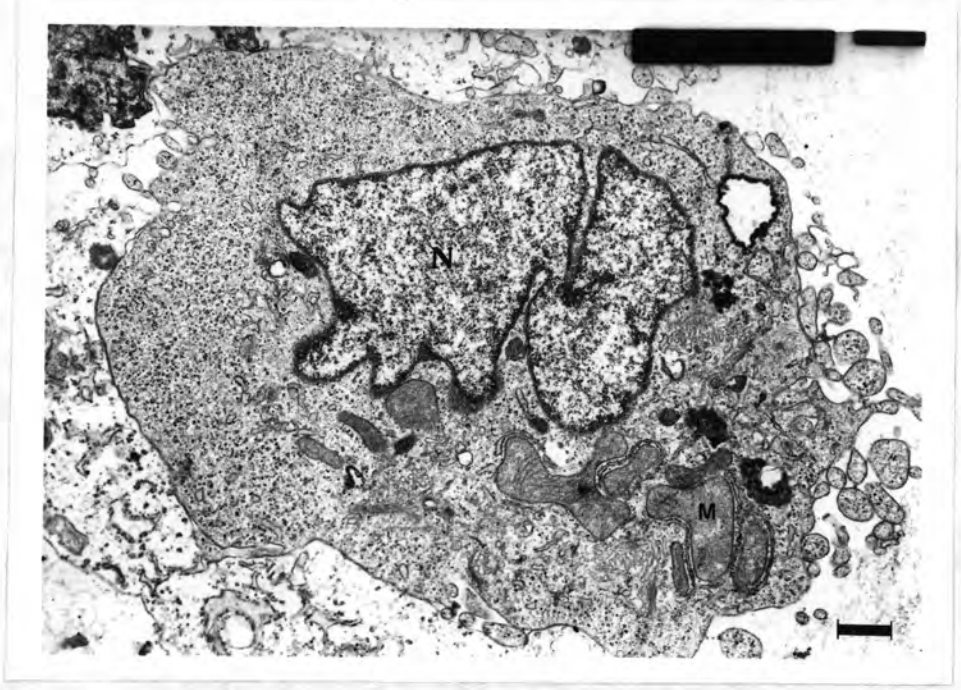

Figure 19 Three days IFN treated S102 hepatoma cell shows segmented nucleus (N) with swollen mitochondria (M).
(Bar = 1 μm ; x7,700)

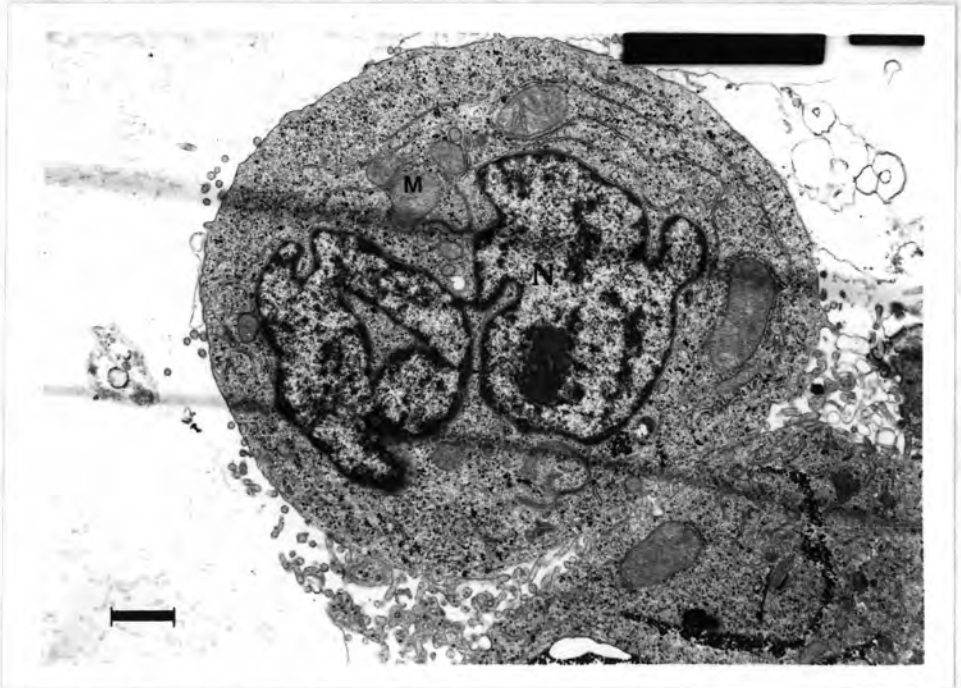

Figure 20 Three days IFN treated S102 hepatoma cell shows segmented nucleus (N) with swollen mitochondria (M).
(Bar = 1 μm ; x7560)

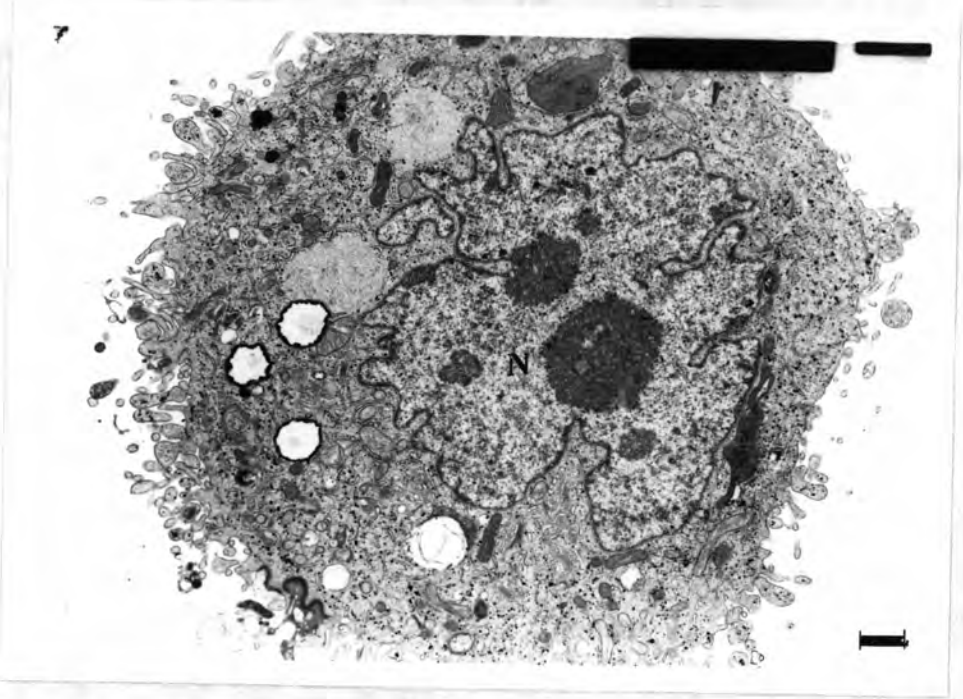

Figure 21 Three days IFN treated S102 hepatoma cell shows heterogenous electron translucent structure (*).

(Bar = 1 μm ; x6,160)

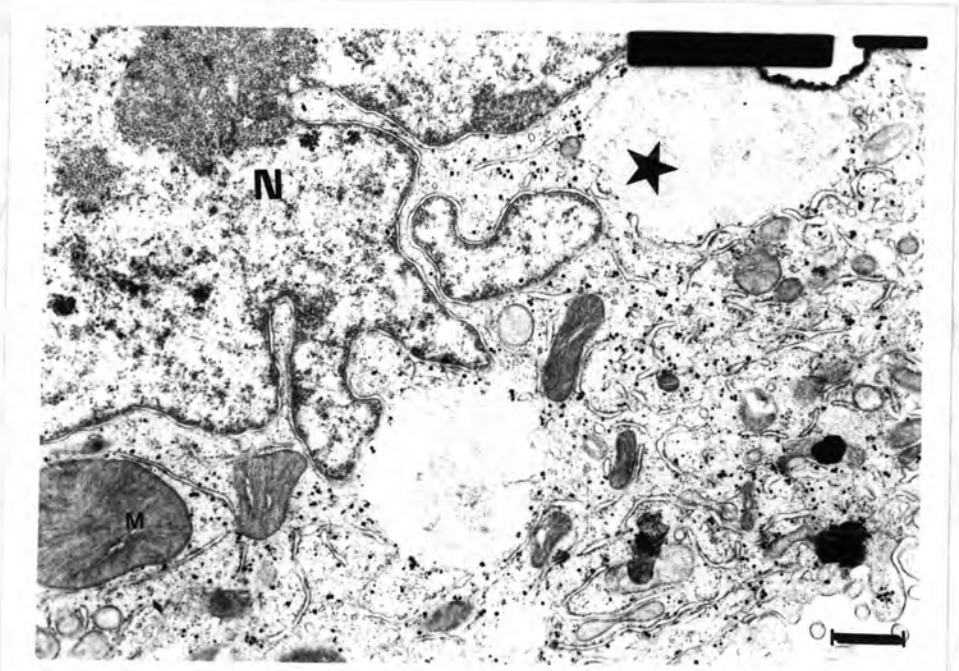

Figure 22 High magnification shows heterogenous electron translucent non-membrane bounded structure (*).

(Bar =1 μm ;x15,400)

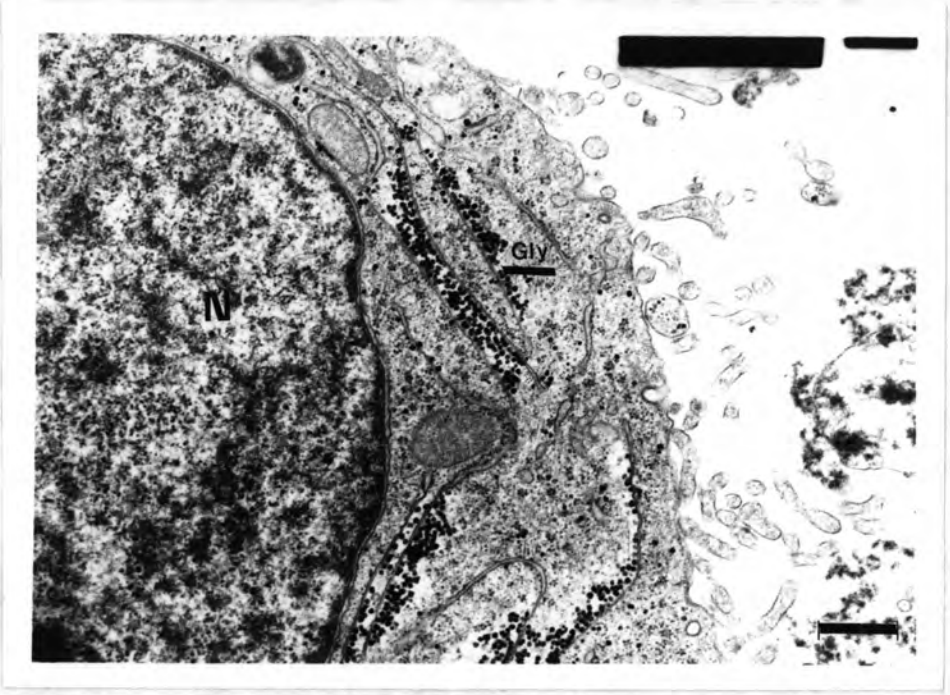

Figure 23 Three days IFN treated S102 hepatoma cell shows increasing in number of glycogen (Gly) in cytoplasm. (Bar =0.5 μm ; x19,400)

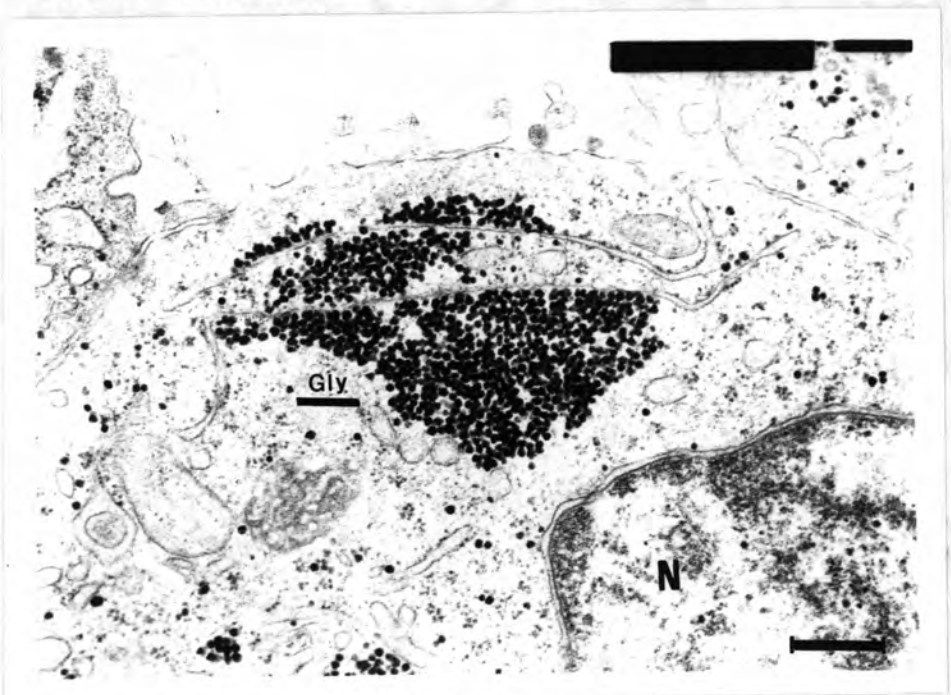

Figure 24 High manification of 3 days IFN treated S102 hepatoma cell shows cluster of glycogen in cytoplasm (Gly). (Bar =0.5 μm ; x22,600)

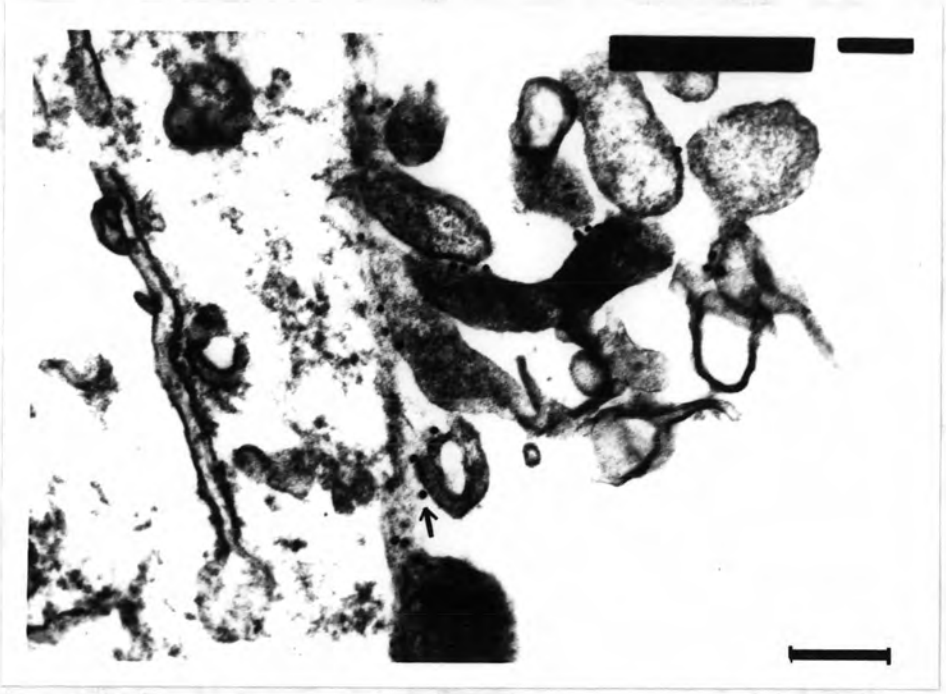

Figure 25 Three days IFN treated S102 hepatoma cell shows increasing in gold labelling (arrow). (Bar =0.2 μm ;x60,480)

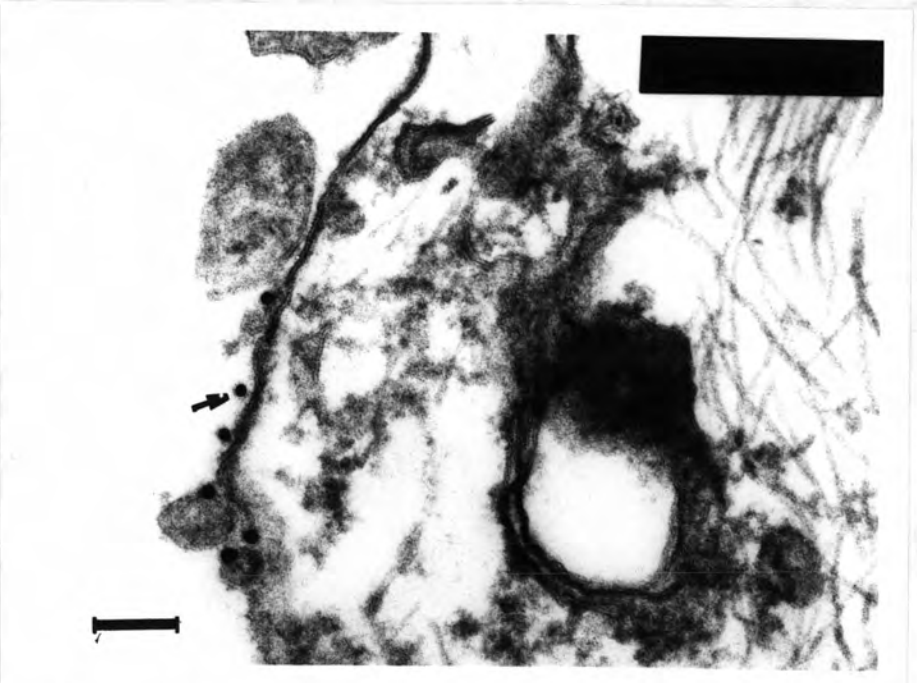

Figure 26 High manification of 3 days IFN treated hepatoma cell shows gold labelling (arrow). (Bar =0.1 μm ;x109,500)

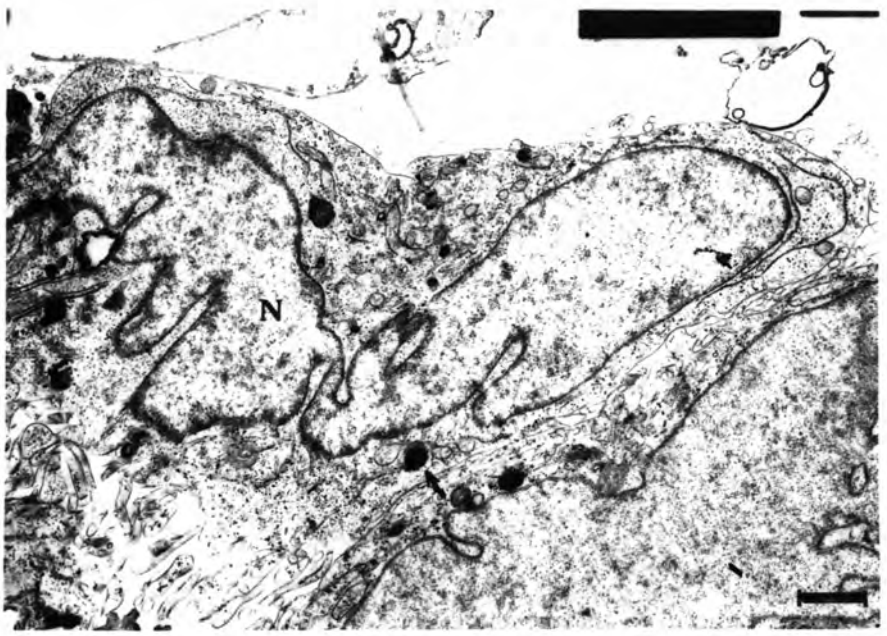

Figure 27 Four days IFN-α treated S102 hepatoma cell shows segmented nucleus (N) with electron dense granules (arrow).
(Bar = 1μm ; x7,560)

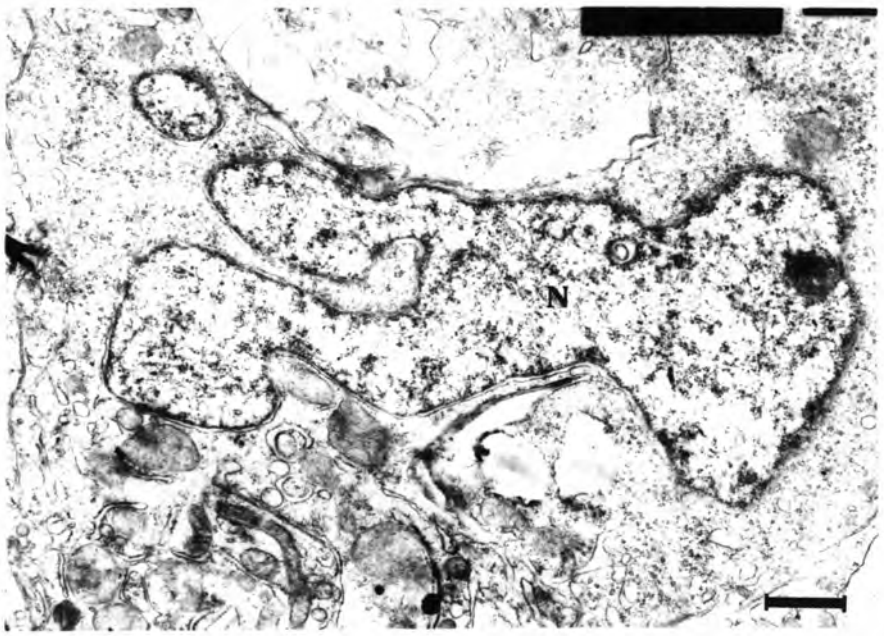

Figure 28 Four days IFN-α treated S102 hepatoma cell shows segmented nucleus (N) with scattered chromatin.
(Bar = 1μm ; x10,800)

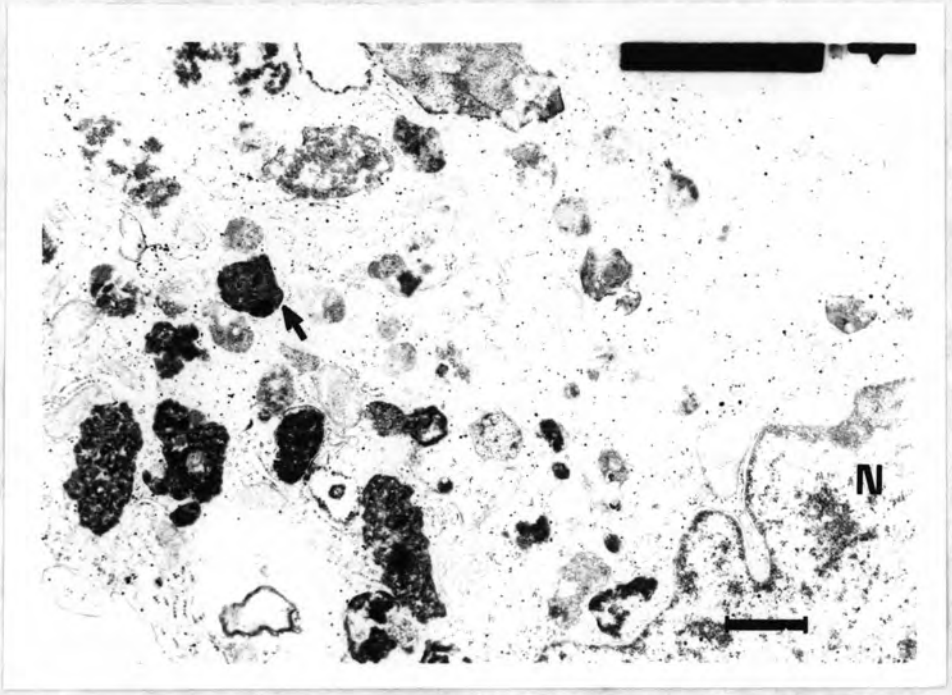

Figure 29 Four days IFN treated S102 hepatoma cell shows electron dense granules in cytoplasm (arrow). (Bar =1 μm ; x10,900)

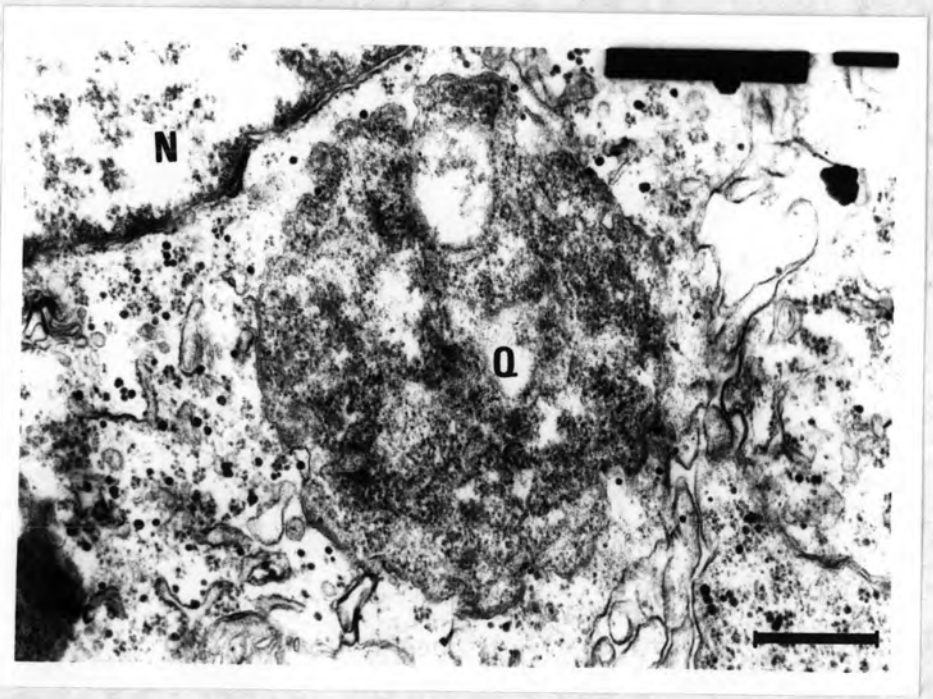

Figure 30 High magnification of electron dense granule (Q). (Bar =0.5 μm ;x30,800)

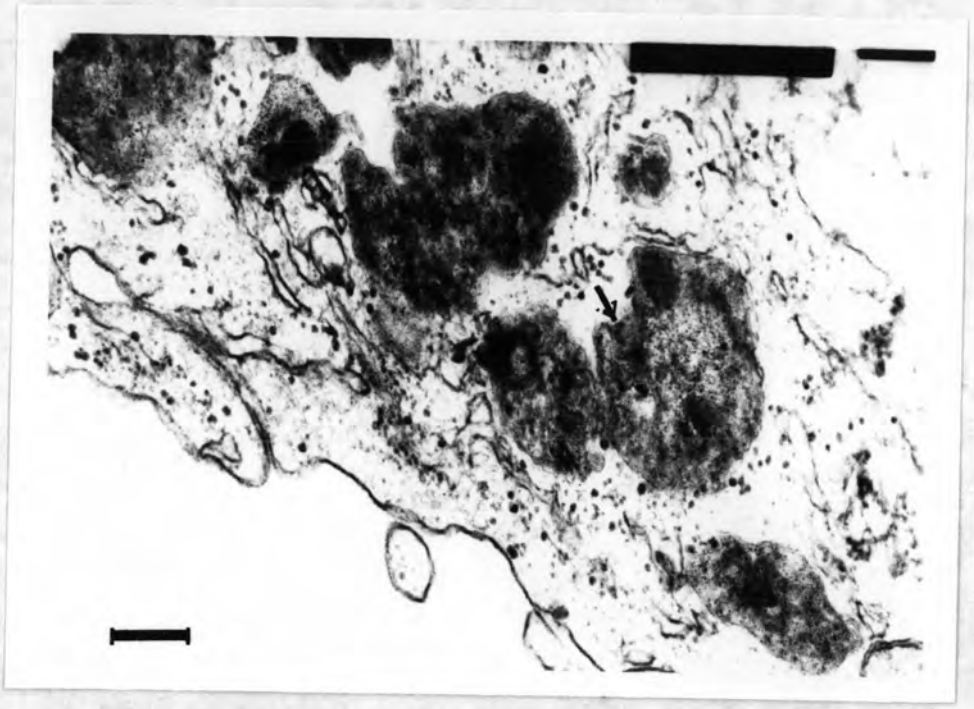

Figure 31 High magnification of electron dense granules (arrow). (Bar =0.3 μm ;x38,500)

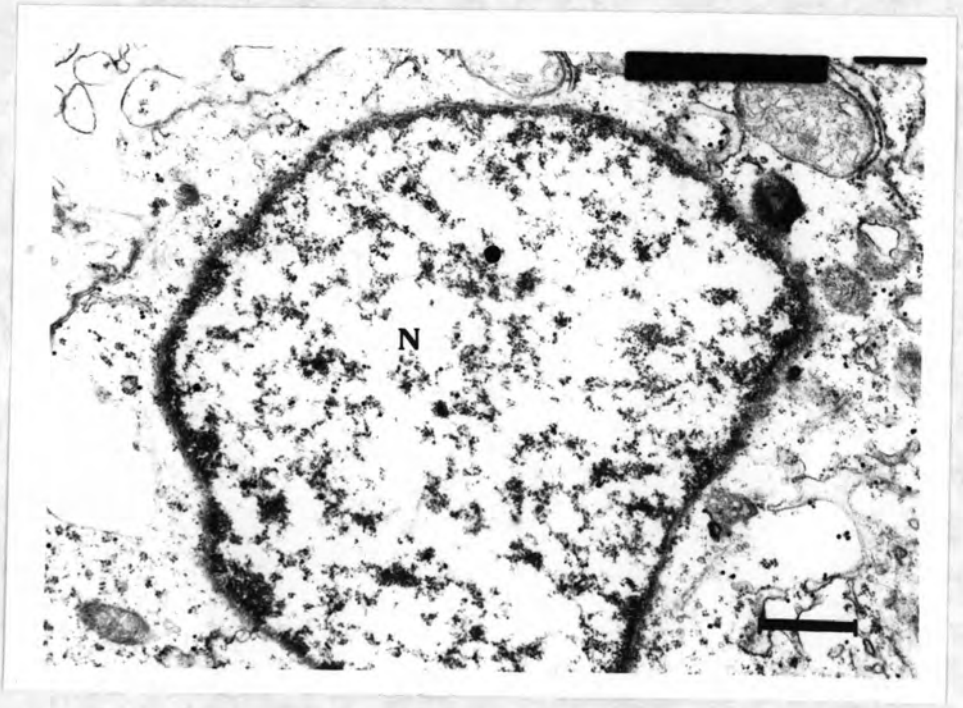

Figure 32 Four days IFN treated S102 hepatoma cell shows scattered chromatin with no clumping (N) and nuclear membrane was not clear. (Bar =0.5 μm; x19,400)

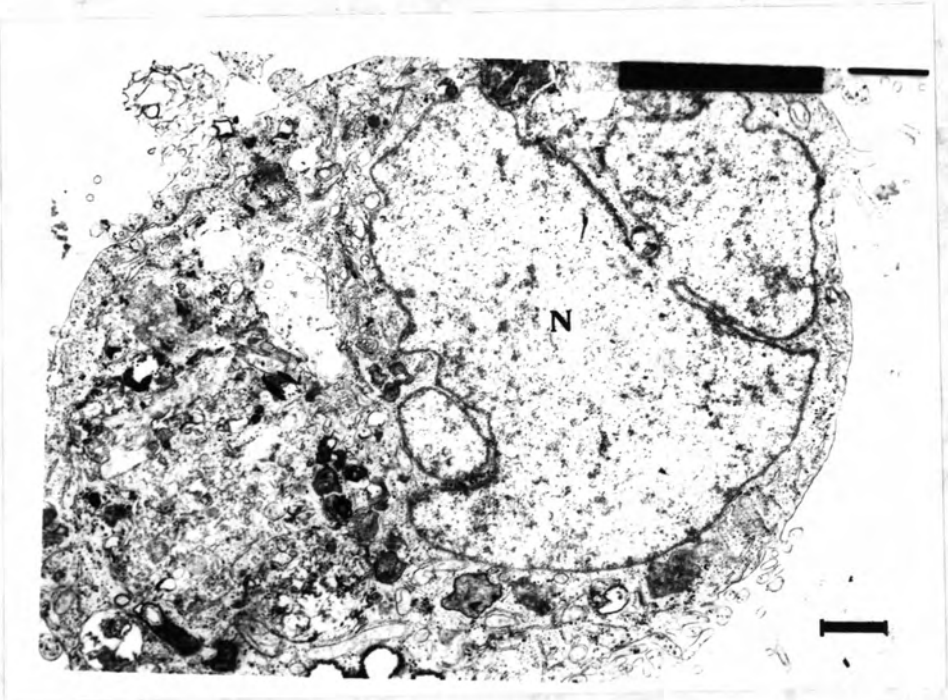

Figure 33 Eight days IFN treated S102 hepatoma cell shows invaginated nucleus with scattered heterochromatin.

(Bar = 1μm ; x7,630)

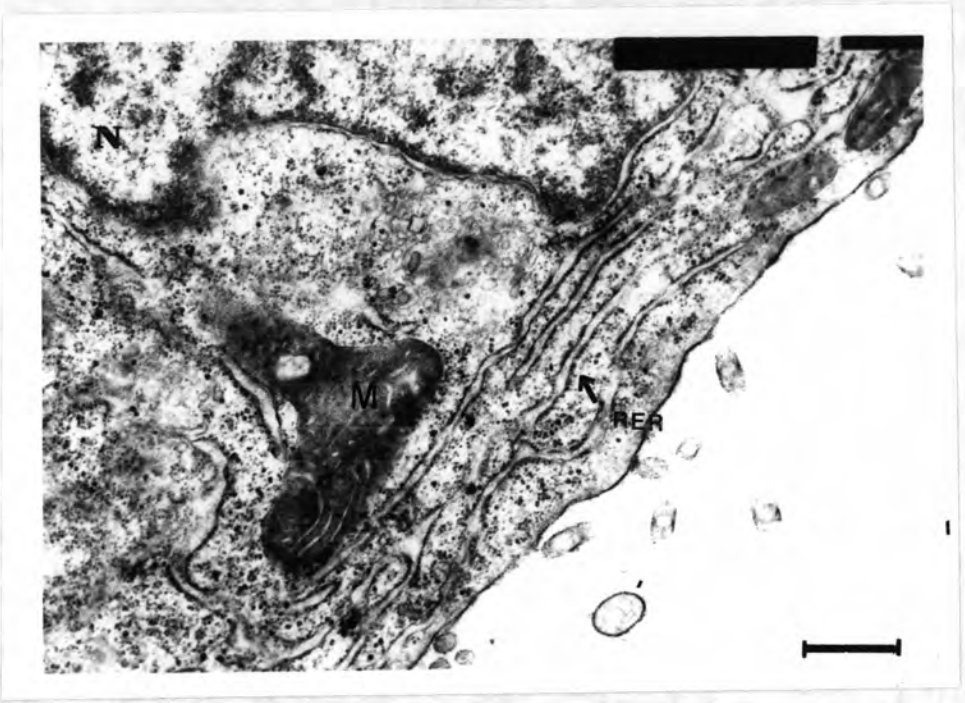

Figure 34 Eight days IFN treated S102 hepatoma cell shows RERs in flattened cisternae with dilatation (arrow) and swollen mitochondria (M). (Bar =0.5 μm ;x23,100)

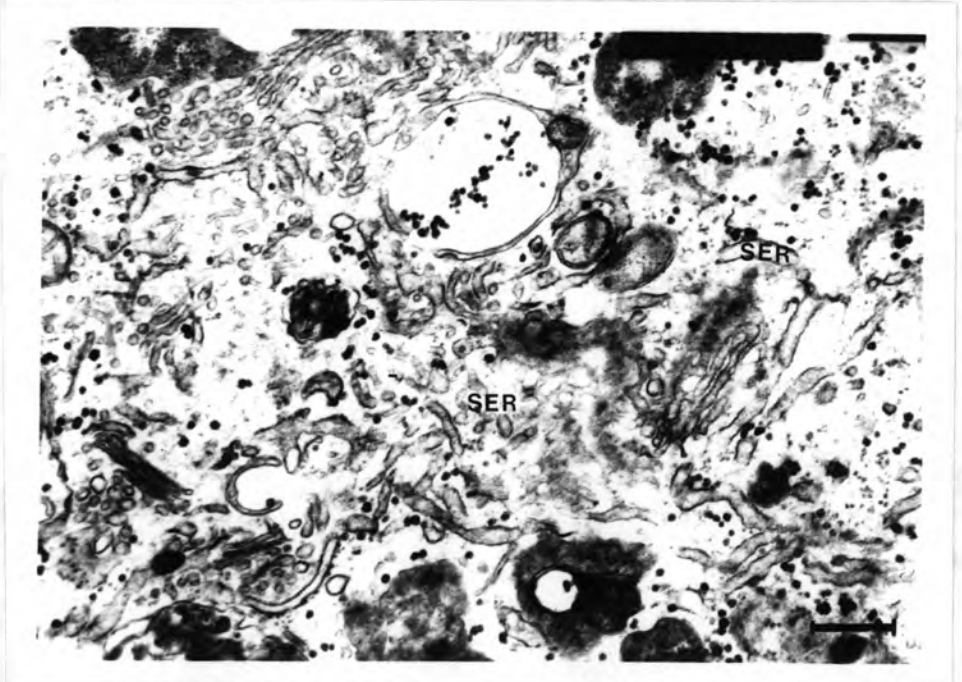

Figure 35 Eight days IFN treated S102 hepatoma cell shows hypertrophic SERs . (Bar =0.3 μm ;x30,800)

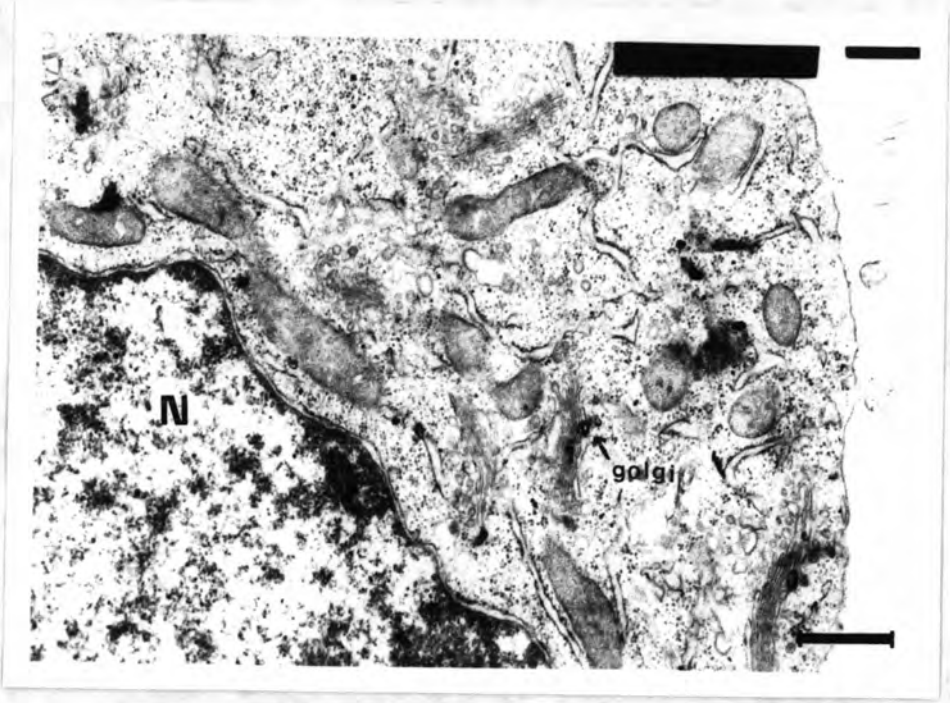

Figure 36 Eight days IFN treated S102 hepatoma cell shows golgi apparatus and its vesicles (arrow). (Bar= 0.5 μm;x19,800)

Figure 37 High magnification of golgi apparatus and its vesicle . (Bar =0.2 μm ;x38,500)

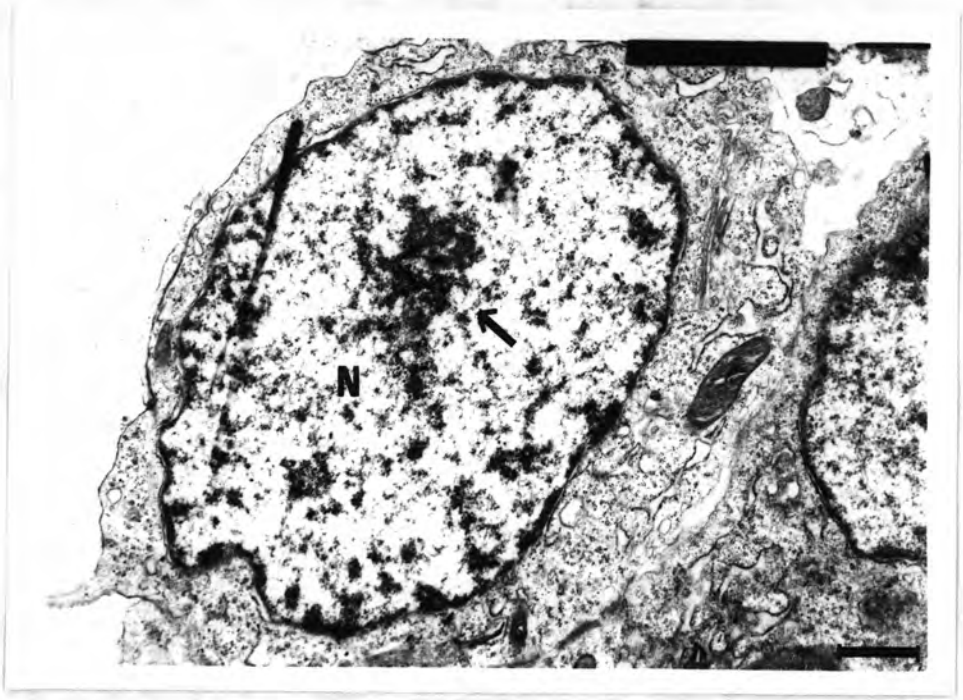

Figure 38 HepG2 hepatoma cell (untreated cell) shows nucleus with clumped chromatin (arrow). (Bar =1 μm ; x11,000)

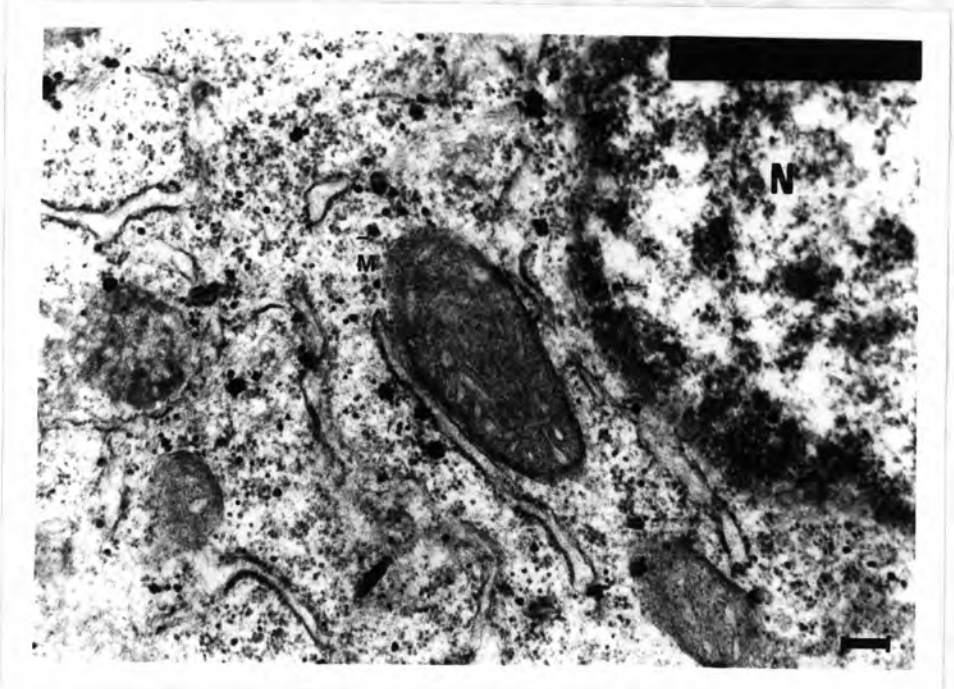

Figure 39 HepG2 hepatoma cell (untreated cell) shows pleomorphic mitochondria (M). (Bar =0.2 μm ; x32,000)

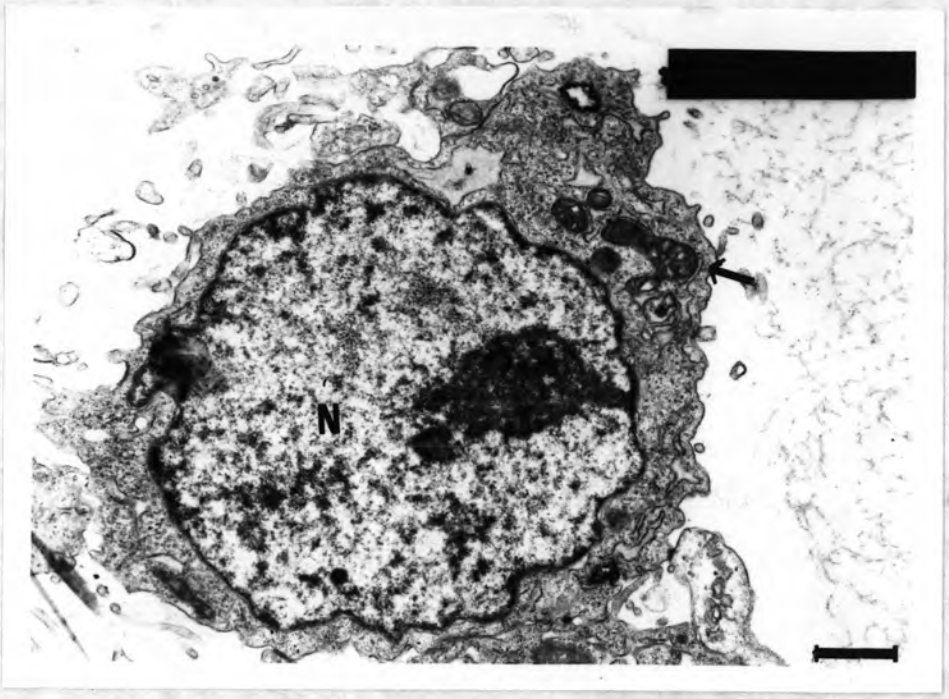

Figure 40 Three days IFN treated HepG2 hepatoma cell shows swollen mitochondria (arrow). (Bar = 1 μm ; x9,600)

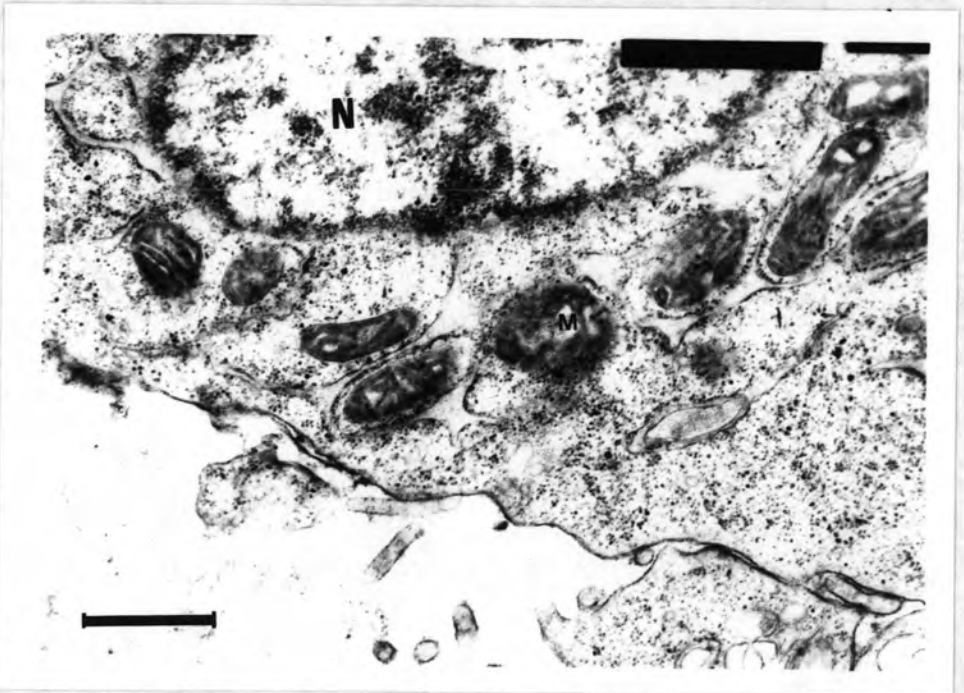

Figure 41 High magnification of three days IFN treated HepG2 hepatoma cell shows swollen mitochondria (M).
(Bar = 0.5 μm ; x32,000)

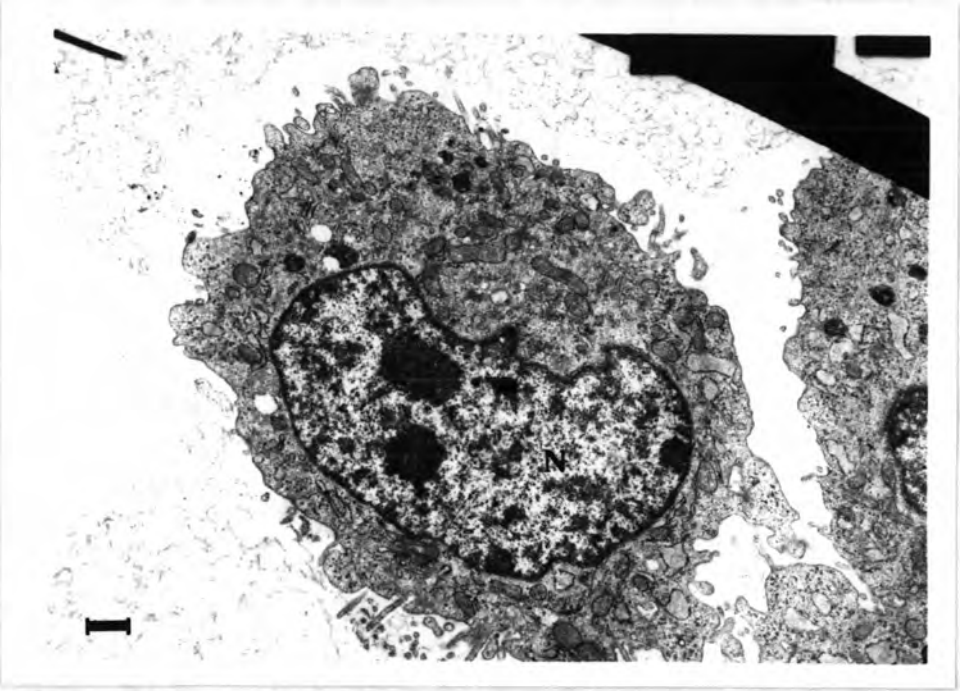

Figure 42 Normal fibroblast cell, L929 shows numerous amount of cytoplasmic organelles. (Bar = 1μm ;x 6,050)

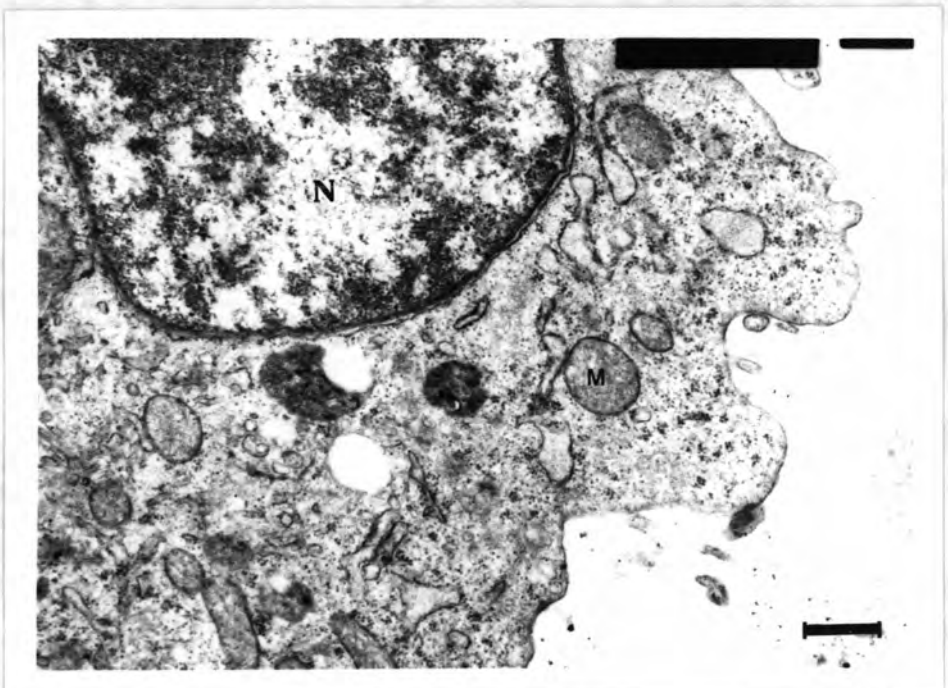

Figure 43 High magnification of normal fibroblast cell, L929 shows mitochondria (M), RER. (Bar =0.5 μm; x19,400)

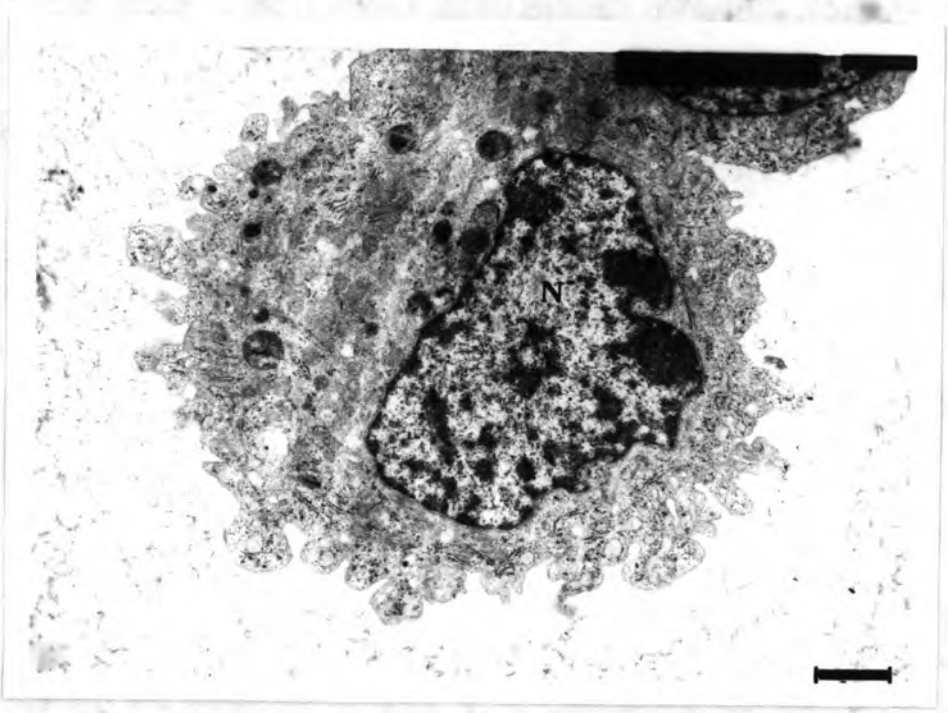

Figure 44 Three days IFN treated L929 fibroblast cells shows cytoplasmic organelles and nucleus with similar to untreated cell. (Bar = 1 μm; x7,560)

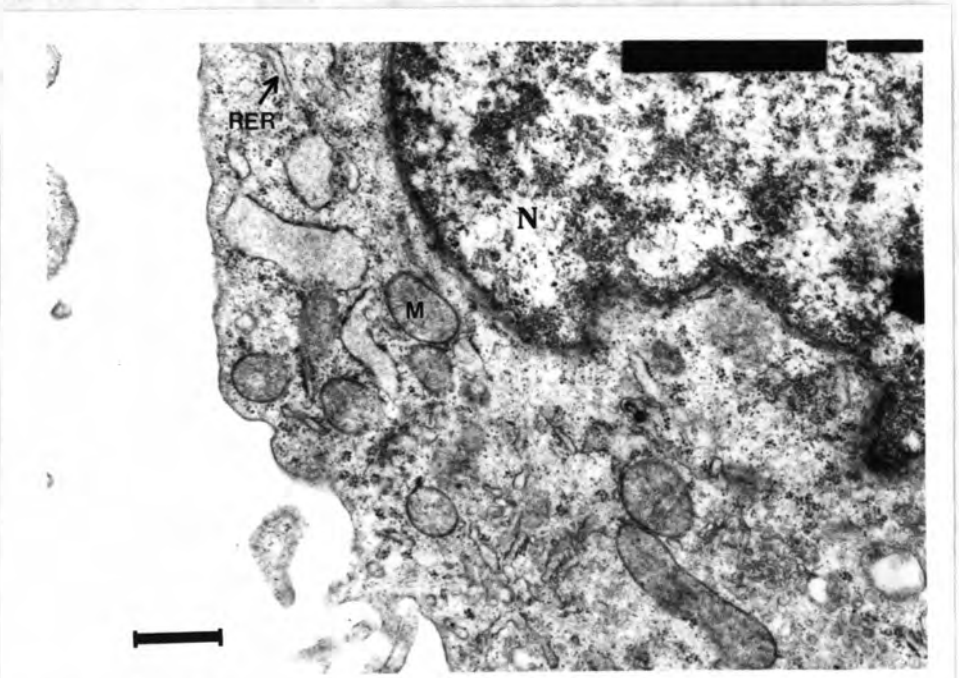

Figure 45 Three days IFN treated L929 fibroblast cell shows mitochondria (M) and RER (arrow) with similar to untreated cell. (Bar =0.5 μm ;x19,400)