CHAPTER IV

RESULTS

4.1 Purification of monoclonal antibodies (MAbs)

In expecting to have the most efficient and convenient method for large number of MAbs, preparing pure MAbs by the ammonium sulfate precipitation was compared with the affinity chromatography.

4.1.1 Ammonium sulfate precipitation The volume of starting ascites was all started with 1 ml. The protein concentration of each ascites ranged between 25.18-46.96 mg. The percentage of IgG after precipitation when compared to the protein that determined from ascites at start were around 25 % (mean). The proportion of IgG were paralelly to the total IgG at start. The MAbs number #54 and #58 showed relatively higher immunoglobulin. Nine out of ten samples had the concentration of IgG ranged between 5.01-15.35 mg per 1 ml while the #54 had the highest concentration of 15.35 mg IgG, as shown in Table 6.

Table 6. Concentration of IgG and the proportion to the IgG in ascites after the ammonium sulfate precipitation.

Ascites	starting IgG protein (mg)	IgG after precipitation (mg)	% IgG/starting protein
#43	25.18	5.01	19.90
#44	33.69	7.24	21.49
NS-1	29.04	6.38	21.97
#40	31.34	7.52	23.99
#36	29.92	7.46	24.93
#16	38.7	9.76	25.27
#27	36.84	9.35	25.38
#20	28.24	10.18	26.62
#58	35.72	10.68	29.90
#54	46.96	15.35	32.69

: 1 ml of each MAbs in ascites form were all precipitated with ammonium sulfate precipitation at 4 c. The starting IgG protein and precipitate IgG concentration were calculated by the following formula:

- IgG concentration = $\dfrac{\text{absorbance at 280 nm}}{\text{extinction coefficient of IgG at 280 nm (13.6)}} \times 10$ mg/ml

The % IgG recovery from starting protein was calculated by this formula:

-% IgG/starting protein = $\dfrac{\text{concentration of IgG (mg)}}{\text{starting IgG concentration (mg)}} \times 100$ %

4.1.2 Affinity chromatography   The 3 ml of ascites which the starting protein ranged between 82.4-198 mg after passed through the affinity purification, the IgG2a gained around 6.4% (mean) IgG2a to starting IgG concentration in ascites. The details was shown in Table 7. According to these raw data it seem to

suggest that immunoglobulin G of affinity purification was only one third of the ammonium sulfate precipitation.

Table 7. The concentration of IgG2a and the percentage proportion to the starting IgG in ascites after affinity chromatography.

Ascites	starting IgG protein (mg)	IgG2a after purification (mg)	% IgG2a/starting protein
NS-1	150	3.06	2.04
#58	158.94	4.12	2.59
#44	122.5	3.52	2.87
#40	123.75	5.78	4.67
#54	82.4	4.36	5.29
#75	126.7	7.5	5.92
#43	105.6	8.76	8.3
#27	198	16.8	8.48
#36	101.46	8.96	8.83
#16	40	3.78	9.45
#20	182.35	22.95	12.59

: The IgG2a was purified by affinity chromatography; protein A-sepharose column chromatography. The starting IgG protein of ascites and IgG2a concentration were calculated by the formula as previously described below Table 6.

For example of affinity chromatography purified pattern, MAb#27 purification was chosen to shown the result after loading the ascites #27 to the column in Figure 11. The first peak of pass-through protein was unbound fraction containing IgM and albumin at 1-17 fractionation. The second peak represent a false peak which absence of protein (may be some debris that can absorped the OD280) at

53-77 fractionation. The IgG2a was collected at fraction number 78-87 by 0.1 M CB, pH 4.5 elution.

One disadvantage of ammonium sulfate precipitation of antibodies is that the resulting antibodies will not be pure. They will be contaminated with others high-molecular-weight proteins. The percentage of precipitated IgG per starting IgG was also higher than the affinity chromatography purification because of the contamination. Eventhough the affinity chromatography purification took a long process but it resulted a more purity of IgG2a. According to these reasons the chromatography was selected to purify the MAbs.
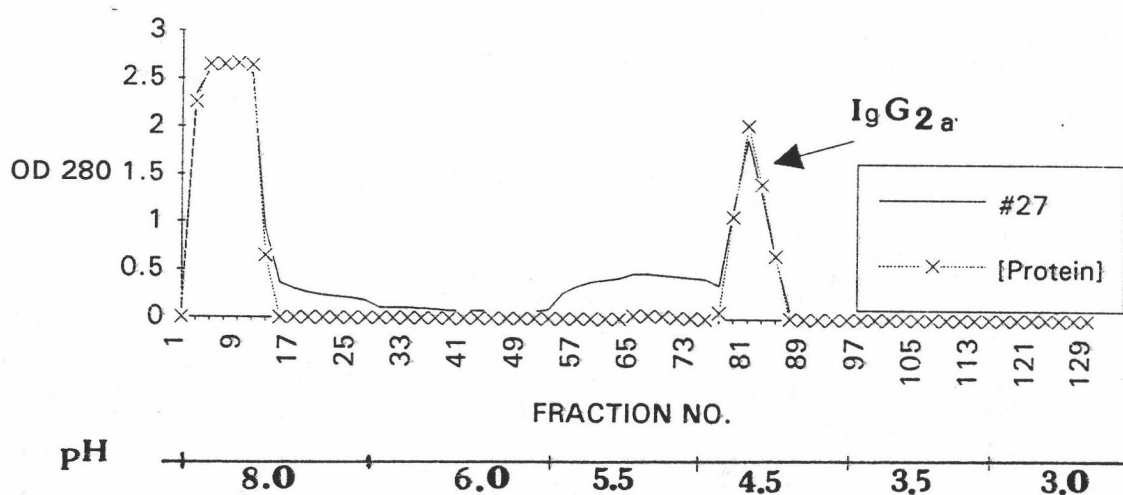
Figure 11 Affinity purification of anti-hepatoma MAb#27 using protein A-sepharose column. From 3 ml of ascites contained 81 mg proteins was found pure IgG2a 6.09 mg after purification. With flow rate of 0.5 ml/min, the IgG2a which was eluted with 0.1 M was collected at fraction number 78-87 CB, pH 4.5.

4.2 The saturated concentration of anti-hepatoma MAbs

Firstly, the method ELISA was used to find out the saturated concentration and reconfirmed by the indirected fluorescent flowcytometry method.

4.2.1 First selection, ELISA method: Nine of anti-hepatoma MAbs were selected for this immunoreactivity screening. For example the anti-hepatoma MAb#27 was shown in Figure 12. The saturated concentration of MAb #27 was 0.312 µg per 2×10^4 cells. There was seven of nine MAbs reached the saturated concentration below than 1 microgram per 2×10^4 cell in this series. The MAb #58 is that anti-alphafetoprotein needed higher concentration the same as MAbs #44. According to this result anti-hepatoma MAb#27 was selected as model MAb for further studied. The saturated concentration of all the anti-hepatoma MAbs were described in Table 8.
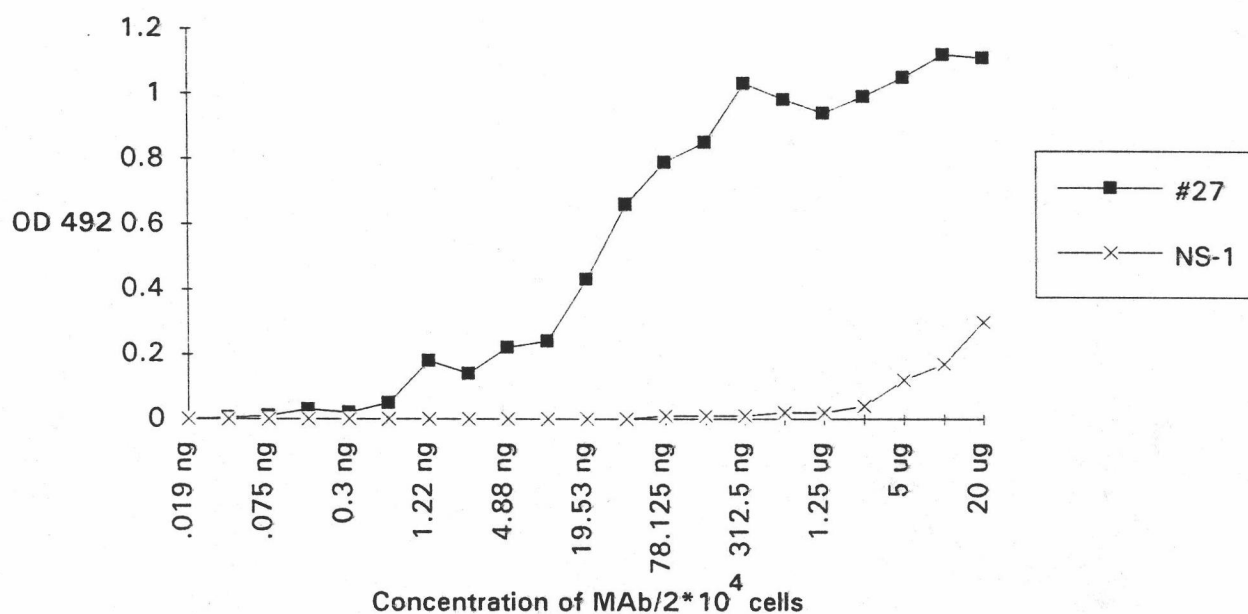
Figure 12. Reactivity of anti-hepatoma MAb #27 on HCC-S102 cell lines. The IgG2a #27 was diluted in serial dilution in 0.5% BSA that the starting concentration was 20 µg. The MAb was incubated on 96 well plate which HCC-S102, 2×10^4 cells per well were priorly fixed at 4 c for 12 hrs. The non-specific NS-1 was used as negative control.

Table 8. The saturated concentration of anti-hepatoma MAbs.

Anti-hepatoma MAbs	Saturated concentration (μg per 1×10^4 cell)
#20	0.078
#27	0.157
#36	0.157
#16	0.313
#54	0.313
#75	0.625
#43	0.625
#58	1.25
#44	2.5

The saturated concentration of MAbs can devided into three groups. The MAbs #20, #27, #36, #16, and #54 showed lowest concentration in reaching the saturation at one ten of 1 microgram. The #75 and #43 were in the middle range but still are in good high affinity while the #58 and #44 showed higher saturated concentration at dose over 1 μg per 1×10^4 cells.

4.2.2 Indirect fluorescent flowcytometry method

The major study of this thesis is to use the excess concentration of MAbs in studying the tumoricidal effect to the hepatocellular carcinoma cell lines. The exact saturated concentration was the most of one important factor. For the confirmation, the saturated concentration which was screened by the ELISA were again tested with this indirect fluorescent flowcytometry method. (Figure 13).
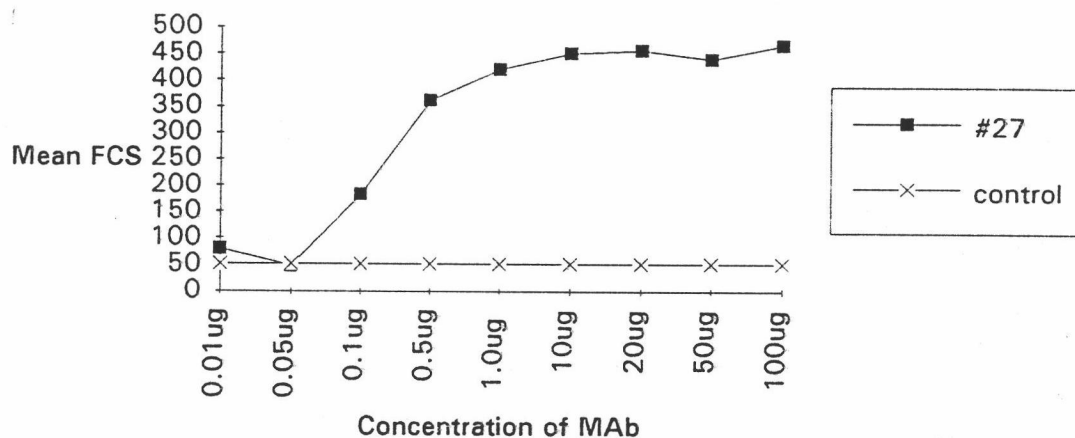
Figure 13. Reactivity of anti-hepatoma MAb #27 on HCC-S102 cell lines. The saturated concentration of MAb #27 was started from 0.5 to 1 μg per 1 $\times 10^5$ cells.

The result closed to the former ELISA method as had the saturated concentration of MAbs between 0.5 to 1 μg per 1×10^5 cells. Thus, the ELISA method is more simplest method, later on the reconfirmation by indirected fluorescent flowcytometry method was not performed.

4.3 The tumoricidal effect of anti-hepatoma MAbs present as the cell viability and cell growth inhibition

4.3.1 Selection of the best MAbs which have tumoricidal effect

In 1988, Fukuda reported that MAbs S1 and 225.28s (IgG2a) alone had 9-10% tumoricidal effect on Hc-4 hepatoma cell line after treated 3 days and the dead cells did not increase after 72 hrs of incubation. For comparison this investigation took the same concentration by using 5 µg per 1×10^4 cells. This concentration was also similar to the studied of Varunee, 1994 (Varunee, in press) followed the immunofluorescence method which used HCC-S102 and anti-hepatoma #27 to find out the saturated binding. The incubation time was divided into three different estimatings, the 24, 48 and 72 hrs after the treatment.

The anti-hepatoma MAbs of this series formerly were classified into three groups according to the antigens it recognized, for this study two of them were selected out. One was those recognized ODA, the anti-ODA, were #16, #20, #27, #43, #44, #58, #75, and the others was anti-TAA MAbs, the #36 and #54 (see Table 5). The hepatoma cell line was HCC-S102. The NS-1, non specific MAb and the non-treated cell were both used as negative control.

The effective %viability was those the %viability was lower than 80% when compared to the control (untreated cell). The result of this study was shown in Figure 14.
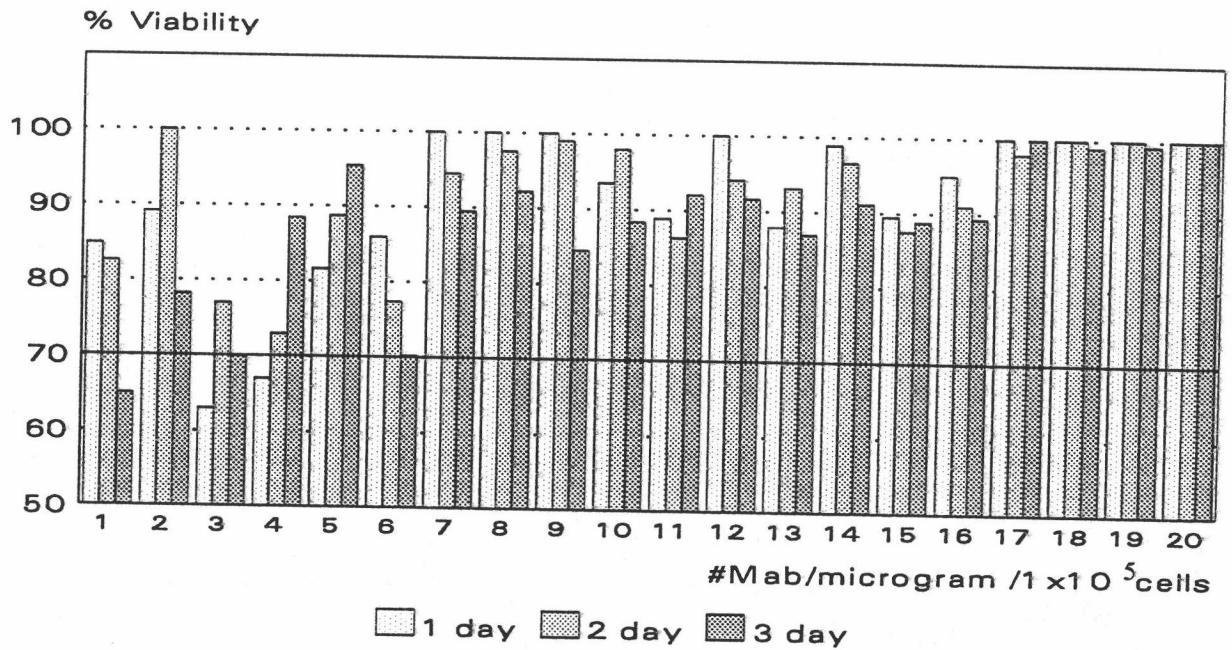
Figure 14. The tumoricidal effect of anti-hepatoma MAbs on HCC-S102 cell line
was showed in the percentage of viability when compared with
control (untreated cell) on day 1 to day 3.

immunoreactivity with both MAbs was used as negative control cell. The results were shown in Figure 15-24.

The appropriate concentration of MAbs on HCC-S102 and HepG2 cell were compared by the %viability at the first. The results represented the average of duplicated samples two time and were calculated into %viability and %cell growth inhibition. The HCC-S102 and HepG2 cell lines were treated with MAbs #27 and #43. The %viability of treated HCC-S102 cell which was treated with MAb #27 was shown in Figure 15.

The concentration that had %viability lower than 70 % was defined as effective, interesting concentration and were selected for further study. %cell growth inhibition was included in the concept of evaluation.

The effective concentration and incubation time of MAb #27 for tumoricidal effect were seen from the concentration of 3 μg per 1×10^4 cells on day 3. The 5 μg, 10 μg, and 20 μg were one day earierly occured (at day 2). The others concentration (0.1, 1.5, 3 μg) were not effective even after the media changed on day 4. The tumoricidal effect in comparison %viability with %CGI of MAb #27 on HCC-S102 cell was shown in Figure 16.

The anti-hepatoma MAb #27 at 3 μg gave the best tumoricidal effect on day 3. Its %viability was 65% and the %cell growth inhibition was about 25 %. The anti-hepatoma MAb #27 at 5 μg showed 62-65 %viability on day 2 and 3 and its %cell growth inhibition was 65%. Interestingly, eventhough the %viability was not different, the %CGI of 5 μg pronounced the more effective after 3 days treatment. According to this reason the appropriate concentration and incubation time of MAb #27 were considered to be the 5 μg per 1×10^4 HCC-S102 cells treating for 3 days long which conditions were used for preparing the specimens which were used for study the destructive process on EM level.
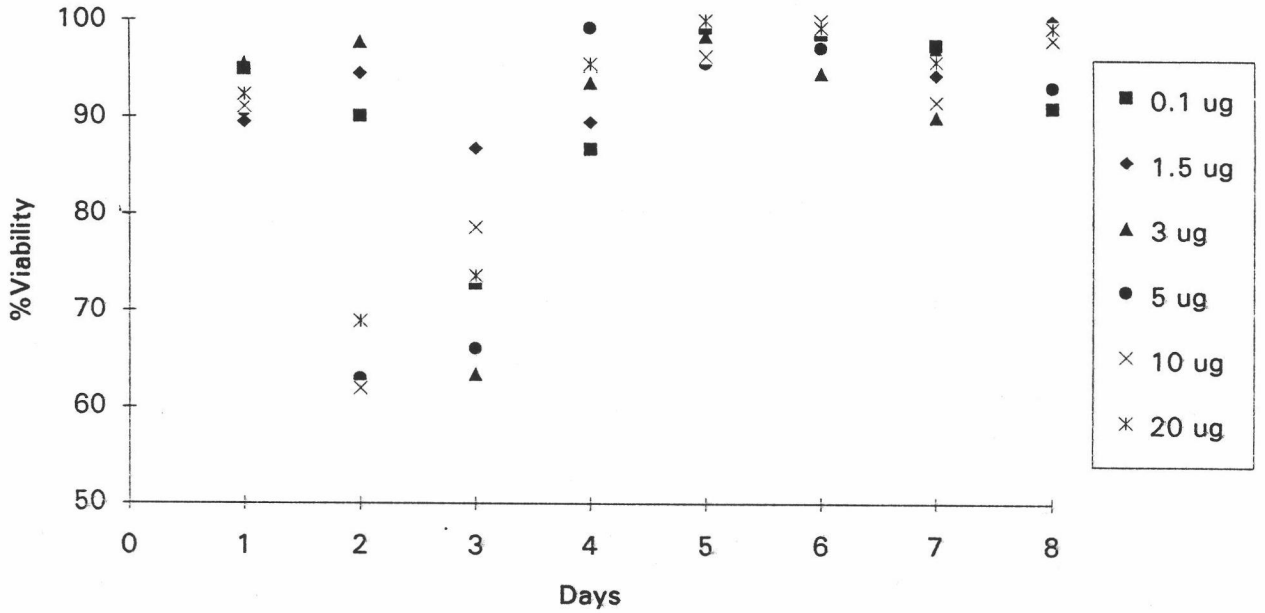
Figure 15. The tumoricidal effect of anti-hepatoma MAb #27 with the various concentration per 1×10^4 cells on HCC-S102 cell for 1 to 8 day was represented by %viability when compared with the control (untreated cell). The media was changed on day 4.
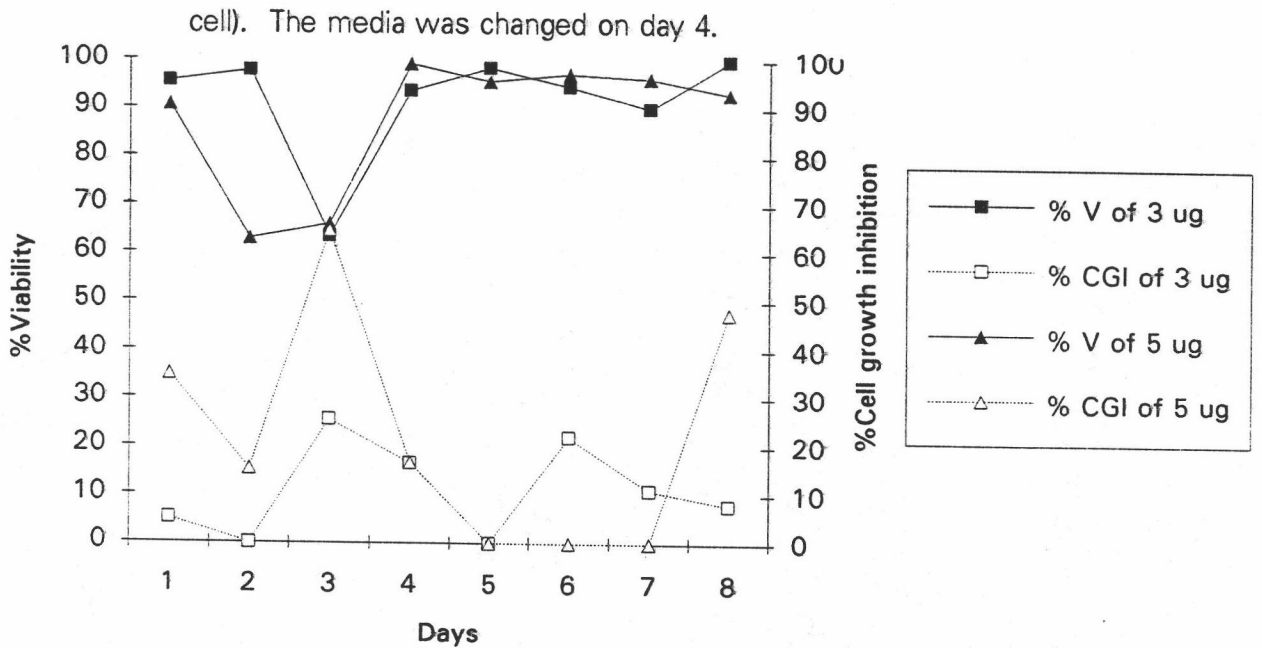

Figure 16. The tumoricidal effect of anti-hepatoma MAb #27 with various concentration per 1×10^4 cells on HCC-S102 cell for 1 to 8 days was represented by %viability and %cell growth inhibition when compared with control (untreated cell). The media was changed on day 4.

The %viability of treated HCC-S102 cell with anti-hepatoma MAb #43 was shown in Figure 17.

The effective concentration and incubation time for tumoricidal effect of MAb#43 on HCC-S102 was 5 µg per 1×10^4 cells seeing on day 1. The 10 and 20µ g showing on day 1 and 2. But the most effective incubation occured on day 4. However, the %viability seem to increase after day 4. The rests were not effective at all. This MAb #43 needed higher concentration and incubation time in alternative words it is the lately destroyed MAb (#27 had best tumoricidal effect on day 3). The concentration at 20 µg was much higher to be used in the experiment which the starting cell for preparing the EM specimen has to be high as over 2×10^6 cells for a sample, so it was not selected as a major concentration. The relation of %viability to the %cell growth inhibition of anti-hepatoma MAb#43 at concentration 5 and 10 µg were summarized in Figure 18.

The anti-hepatoma MAb #43 at 5 µg had the best tumoricidal effect on day 1 as the %viability was 65 %. Relatively increased to the 70-80% on day 2-4 and after 4 days it stayed at 80%. The %CGI was highest at 66% on day 2 and stayed at over 50%CGI for 2 days before fell down on day 4 after the lowest %viability on day 1. It seem to increased again on day 8. The anti-hepatoma MAb #43 at 10 µg had the best tumoricidal effect on day 4. The %viability was 40% while the %CGI was about 70%. This is the case that the data showed a clear complete confirmation to each other of the %viability and %CGI. The unrelated response was discussed in the part of discussion. The %CGI was highest at 72% on day 4 which had lowest %viability. Infact that the concentration at 10 µg was more effective and used more time this concentration was one fold higher than 5 µg. Its high dose was not appropriate for preparation of EM specimen that required more than 2×10^6 cells. So the MAb #43 was used as the control of anti-hepatoma MAb #27 more than to estimated its exact tumoricidal effect in studying the destructive
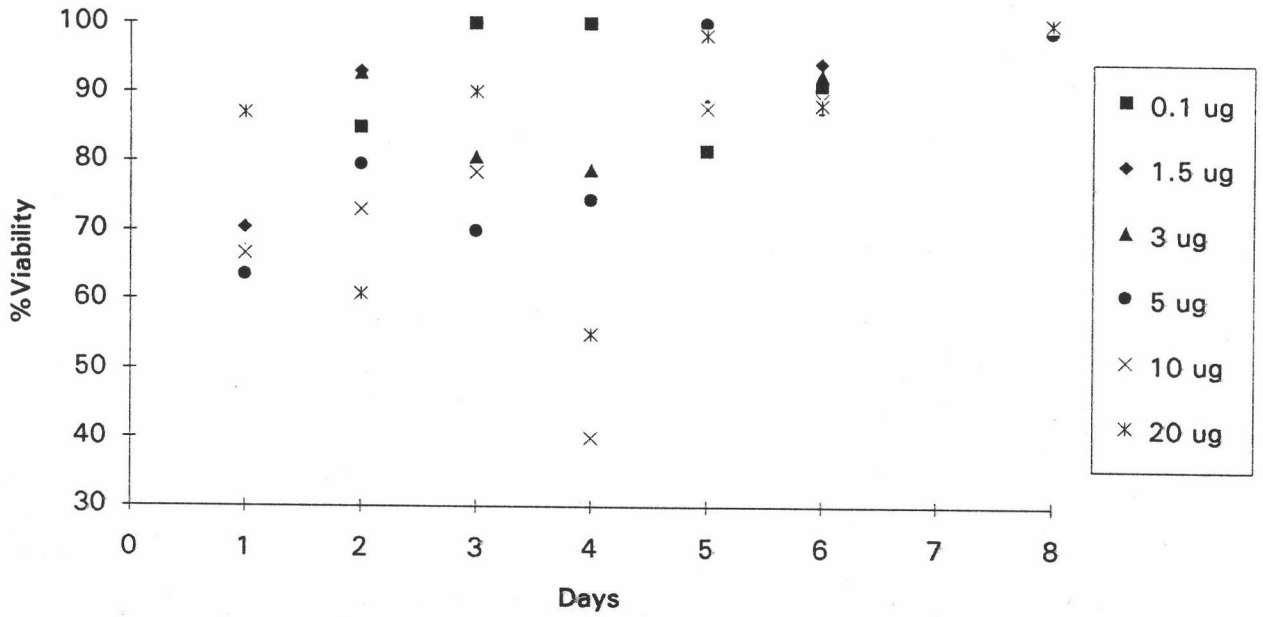
Figure 17. The tumoricidal effect of anti-hepatoma MAb #43 with the various

concentration per 1×10^4 cells on HCC-S102 cell for 1 to 8 days was

represented by %viability when compared with the control
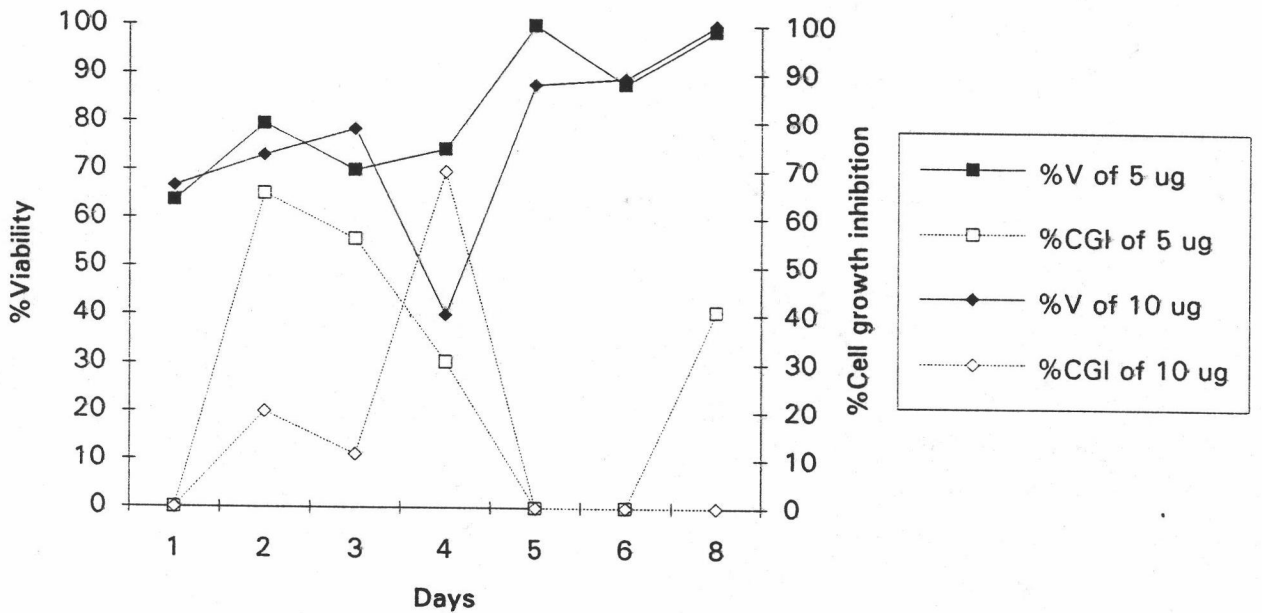
(untreated cell). The media was changed on day 4.
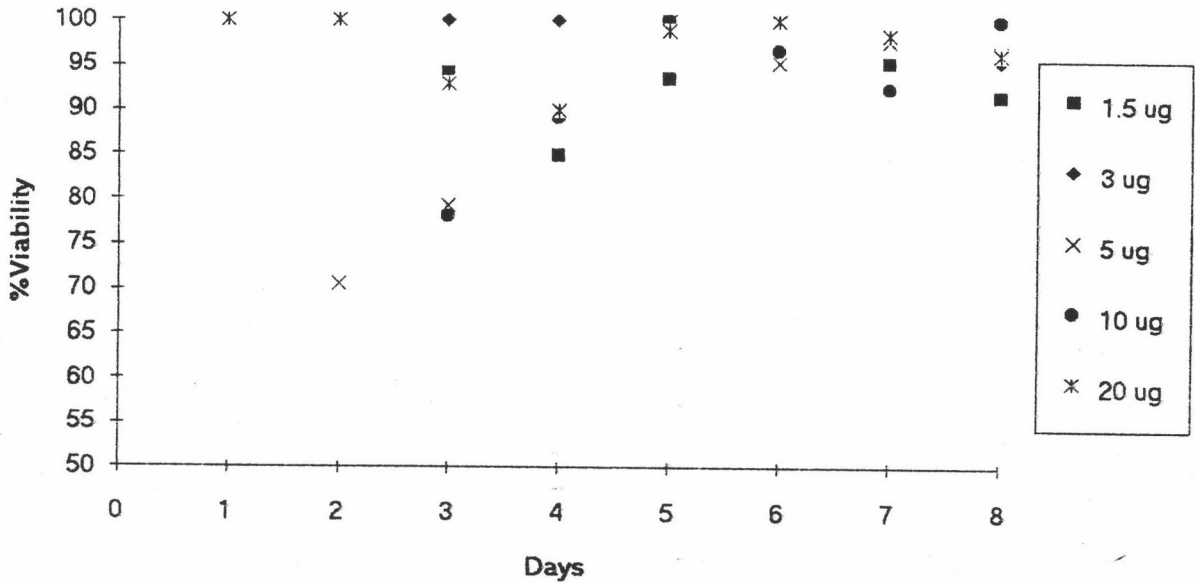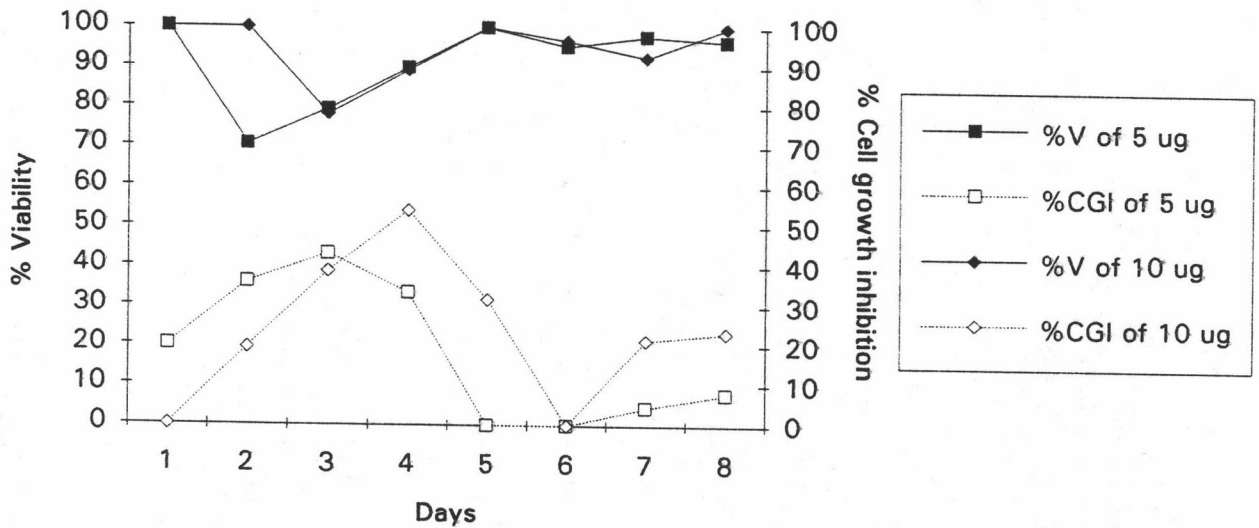

Figure 18. The tumoricidal effect of anti-hepatoma MAb #43 with various

concentration per 1×10^4 cells on HCC-S102 cell for 1 to 8 days was

represented by %viability and %cell growth inhibition when compared

with control (untreated cell). The media was changed on day 4.

process at EM level. The concentration and incubation time were 5 μg per 1×10^4 cells.

In selecting a model hepatocellular carcinoma cell line, HCC cell, the HepG2 were tested the effect on the tumoricidal effect with the same MAbs and condition were estimated. The %viability of MAb #27 on HepG2 was shown in Figure 19.

The concentration and incubation time of MAb #27 on HepG2 cell effective as showed 70%viability for the tumoricidal effect at 5 μg per 1×10^4 cells on day 2. The concentration of 10 μg reviewed 78%viability. After 3 days treatment, the %viability seem to be worsen. The tumoricidal effect of MAb#27 on HepG2 gained only at 5-10 μg per 1×10^4 cells with 2-3 days treatment. This concentration were reconfirmed again, but had compared the %viability to %cell growth inhibition. The results were summerized in Figure 20.

The anti-hepatoma MAb #27 on HepG2 cell at 5 μg had the best tumoricidal effect on day 2 and 3 with 70% and 75% viability, respectively. The %CGI was 40 and 45 on the day 2 and 3, respectively. The %CGI of this concentration quite related to %viability. The anti-hepatoma MAb #27 at 10 μg had the best tumoricidal effect on day 3. The %viability was 74%. The %CGI of this concentration was also related to the %viability. However, the best %CGI showed 55% on day 4 when the %viability was shoot up after the best %viability 1 day.

Figure 19. The tumoricidal effect of anti-hepatoma MAb #27 with the various

concentration per 1×10^4 cells on HepG2 cell for 1 to 8 days was

represented by %viability when compared with control (untreated

cell). The media was changed on day 4.
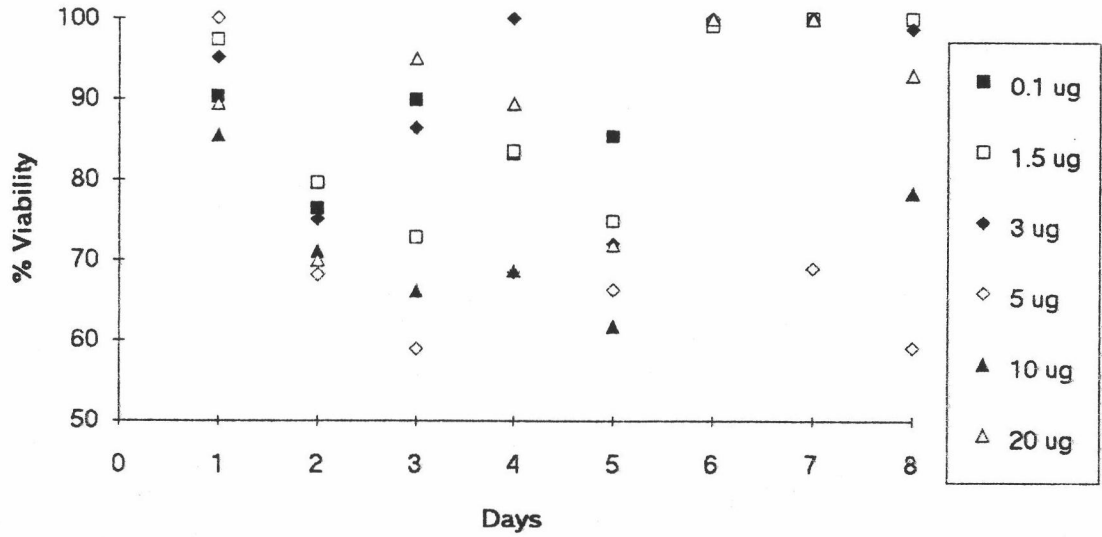

Figure 20. The tumoricidal effect of anti-hepatoma MAb #27 with various

concentration per 1×10^4 cells on HepG2 cell for 1 to 8 days was

represented by %viability and %cell growth inhibition when compared

with control (untreated cell). The media was changed on day 4.

The %viability of MAb #43 on HepG2 was shown in Figure 21. The effective concentration and incubation time of MAb #43 for tumoricidal effect on HepG2 cell was 5 µg from day 2 to day 8. The concentration of 10 µg was effective during day 2-5. The concentration of 20 µg was only showed on day 2. The %viability ranged between 59-70%. The tumoricidal effect of MAb#43 on HepG2 showed prolong effectiveness than MAb #27 on HepG2 cell (see Figure 20). The effect of MAb#43 at 5 and 10 µg were reconfirmed by comparing the %viability to the %CGI as shown in Figure 22.

The anti-hepatoma MAb #43 on HepG2 cell had the best tumoricidal effect at 5 µg and the effect was prolonged type which started from day 2-8. The %viability ranged from 58-70%. The %CGI was lately express when compared to the %viability. The highest %CGI was 60 % on day 5 eventhough the %viability seem to be effective since the day 2. The anti-hepatoma MAb #43 at 10 µg had the same pattern of tumoricidal effect as at 5 µg.

The non-specific NS-1 was used as negative Ab control. The tumoricidal effect of NS-1 on HCC-S102 cell was shown in Figure 23.

Thus the NS-1 did not react with HCC-S102 cell, the present of NS-1 in the study seem to be non-effective. The %viability of treated HCC-S102 cell with NS-1 for 1-8 days was ranged between 80-100% when compared with control (untreated cell). The %CGI was lower than 12%. The concentration of the NS-1 did not effect to the tumoricidal effect, even with high dose.

The pancreatic cancer cell; HS766T was used as negative cell control because it had no reaction with anti-hepatoma MAbs #27 and #43. The tumoricidal effect of MAb#27 on HS766T was shown in Figure 24.

The %viability of treated HS766T cell with MAb#27 for 1-8 days was ranged between 90-100% when compared with control (untreated cell). The %CGI was 0%.

Figure 21. The tumoricidal effect of anti-hepatoma MAb #43 with the various
concentration per 1×10^4 cells on HepG2 cell for 1 to 8 days was
represented by %viability when compared with control (untreated
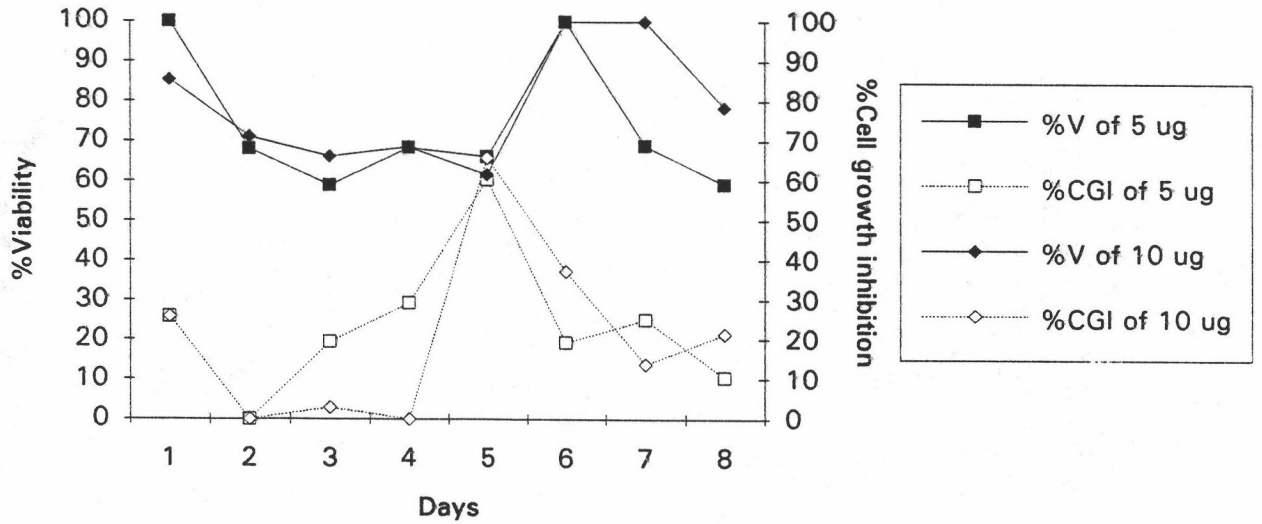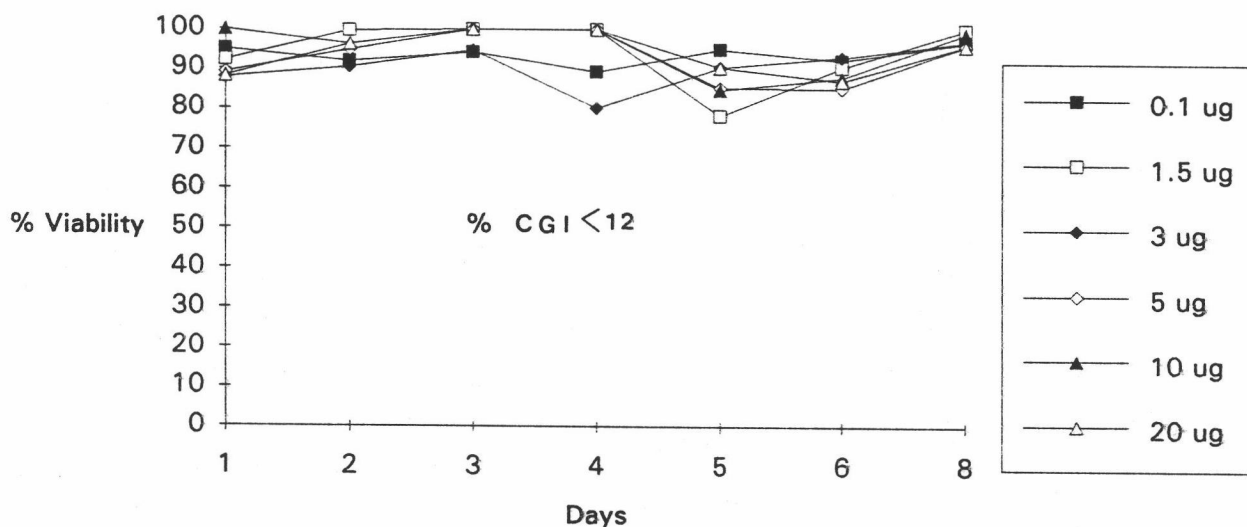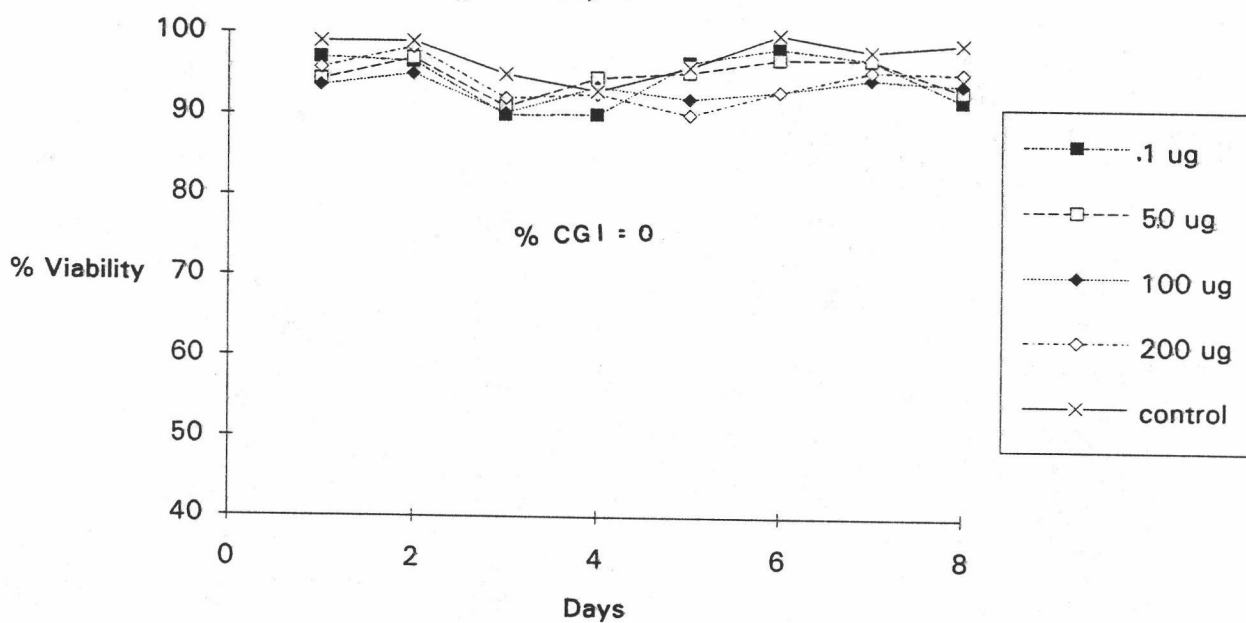cell). The media was changed on day 4.

Figure 22. The tumoricidal effect of anti-hepatoma MAb #43 with various
concentration per 1×10^4 cells on HepG2 cell was represented by
%viability and %cell growth inhibition when compared with control
(untreated cell). The media was changed on day 4.

Figure 23. The tumoricidal effect of non-specific NS-1 with the various

concentration per 1×10^4 cells on HCC-S102 cell for 1 to 8 days was

represented by %viability when compared with control (untreated cell).

The media was changed on day 4.

Figure 24. The tumoricidal effect of anti-hepatoma MAb #27 with the various

concentration per 1×10^4 cells on pancreatic cancer; HS766T for 1 to

8 days was represented by %viability when compared with control

(untreated cell). The media was changed on day 4.

4.3.3 In increasing the sensitivity of MAbs by increasing the MAb binding site

Before going on to prepare the specimen for studying the destructive process under the electron microscope, the effect of binding site related to the degree of tumoricidal effect was tried. Usually the silicone was used to prevent the cell adhesion to the culture flask. The monolayer cancer cell is adhesive cell. If the cell was not adhered to the flask, the antigen on cell surface, binding surface, suppose to be increased one fold. According to these reasons this experiment was schedule. The cell culture in silicone coated plate represented the cell that was increase the binding for one fold up to that the adhered site was free while cell in non-silicone coated plate represent the cell carrying general normal binding site. The %viability and %cell growth inhibition were as shown in Figure 25 and 26.

The HCC-S102 treated with anti-hepatoma MAb #27 at 10 μg per 1×10^4 cell in silicone coated plate showed the decrease of %viability down to 60 % on day 1 (20% of cell growth inhibition) while at 5 μg seem to be less in effective. The HCC-S102 in non-silicone coated plate with the same condition also reviewed %viability decreased to 64% on day 3 (45% of cell growth inhibition) see Figure 25, while 10 μg seem to be less effective. In addition, the %viability of all test were ranged between 80-98% after day 3. The result indicated that the silicone coated plate had 5% viability lower than non-silicone coated plate and the cell was destroyed earlier, on day 1 but it used double of concentration together with lower %cell growth inhibition when compared with non-silicone coated plate. So the non-silicone coated plate system was selected.
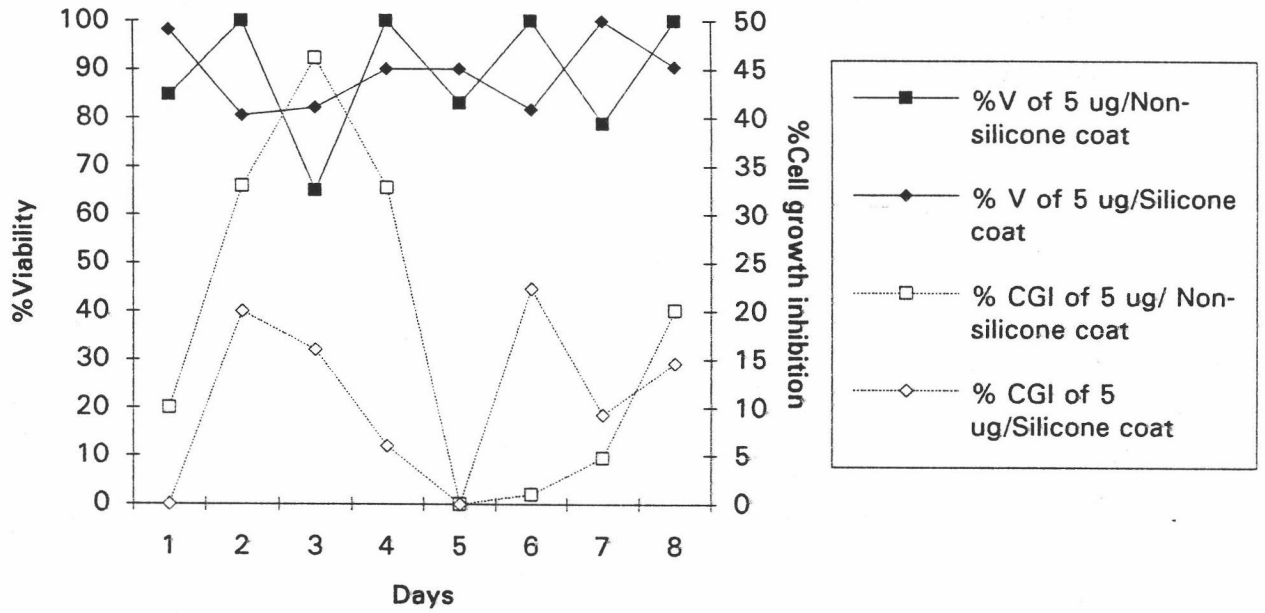
Figure 25. The comparison of tumoricidal effect of anti-hepatoma MAb #27 at 5

μg per 1×10^4 cells for day 1 to day 8 on HCC-S102 cell which was

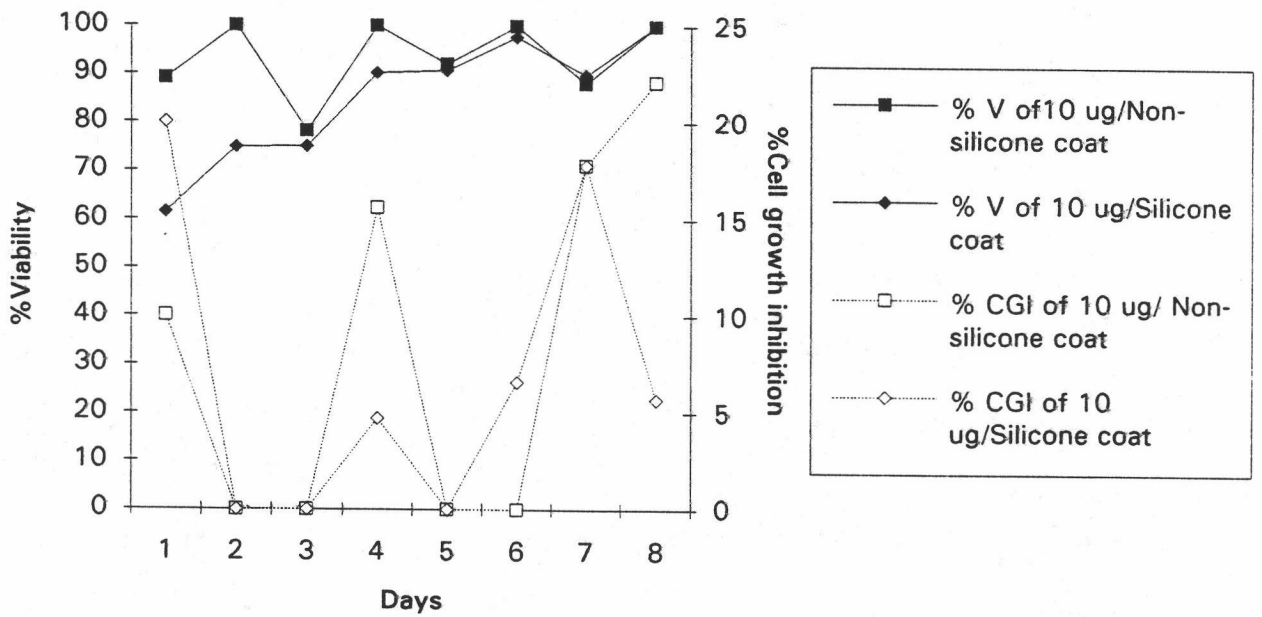cultured in silicone and non-silicone coated plate. The media was

changed on day 4.

Figure 26. The comparison of tumoricidal effect of anti-hepatoma MAb #27 at 10

μg per 1×10^4 cells for day1 to day 8 on HCC-S102 cell which was

cultured in silicone and non-silicone plate. The media was changed

on day 4.

4.4 The destructive process of MAb #27 and #43 to HCC-S102 and HepG2 hepatoma cell lines and the evidence of cell surface antigen.

The untreated HCC-S102 and HepG2 were used as negative controls. The appearance of the two control hepatoma cells (untreated cells) were shown similar by to each other as in Figure 27-30. Electron micrographs of control cell at the same age culture showed well preserved plasma membrane with long microvilli. The cytoplasm contained small amount of organelles. There was moderate rough endoplasmic reticulums (RERs) with flattened cisternae. The mitochondria showed pleomorphism with double membrane and normal size. The smooth endoplasmic reticulums (SERs) was scanty (1-2 SER per cell). The golgi apparatus, and electron dense vesicle-like lysosomes were rarely seen in the cytoplasm. Free ribosomes and glycogen were commonly seen. Nuclei were large, round or oval with some cytoplasmic invagination and mild degree of heterochromatin aggregation. There was about 1 to 2 nucleolus in a nucleus. Nuclear envelopes were well preserved with parallel double membrane. Surface antigens of HCC-S102 localized with anti-hepatoma MAb #27 and gold labelling were found ranging from 20-40 particles per cell. The gold labelling antigens were distributed on cell surface as shown in Figure 31 reviewing no any specific pattern.

From prior study, the anti-hepatoma MAbs #27 and #43 were selected as the best MAbs to study tumoricidal effects on HCC-S102 and HepG2. The appropriate concentration and incubation time of MAb #27 were 5 μg per 1×10^4 cells on the third day while MAb #43 was used with the same condition as for the control (the effective concentration of anti-hepatoma MAb#43 was 10 μg per 1×10^4 cells and the effective incubation was 4 days). Thus the best incubation time was on third day, the harvesting time was started at 3, 6, 12, 24, 48, and 72 hrs after the treatment to following the destructive process of MAb. Decreased in number of RER and shorter microvilli of HCC-S102 were found 3 hrs after the reaction. The number of the others structures were well preserved. HCC-S102 surface antigen

recognized by anti-hepatoma MAb#27, decreased down to nearly half of control at 3 hrs after treatment. This continuously decreased to 5-10 particles within 12 hrs. The gold particles were also found in cytoplasm at 3 hrs after treatment (Figure 32). Interestingly, the antigens disappeared since the first day after treatment. Conclusionally, (summarized in Table 9), the effect of anti-hepatoma MAb #27 displayed the complete disappearance of microvilli (Figure 33). The amount of the rough endoplasmic reticulum (RER) was markedly decreased together with scanty ribosome on its surface (Figure 34). Interestingly, the swollen RERs were generally fill-up with unknown matrix (Figure 35). The mitochondria was also distorted and swollen parallel to the decreasing of the number (Figure 36). The mitochondria often found aggregating on one side of the nucleus or near the cell membrane while in control they also distributed all around the cytoplasm (Figure 37). There were not any effect to the free ribosomes and golgi apparatus. The nuclear envelope displayed no double membrane on some part of membrane. The chromatin aggregation so call the heterochromatin was more frequently seen than the euchromatin (Figure 38-39). Furthermore, vacuoles were markedly seen in cytoplasm (Figure 33 and 40).

In comparison, the controls were set with two conditions. The first used the same concentration of other MAbs, namely anti-hepatoma MAb #43. The second was the condition that used other human hepatoma cell line namely HepG2. HepG2 was treated with the same antibodies and was harvested at the same time.

The effect of anti-hepatoma MAb #43 at 5 µg on 3 to 12 hrs (actually effective dose of anti-hepatoma MAb#43 was 10-20 µg per 1×10^4 cell, see results 4.3) was milder than those seen with anti-hepatoma MAb #27. The plasma membrane was well preserved with long microvilli (Figure 41). The RERs and other organelles at this early period still remained in good shape, except the mitochondria. It was little swelling. On day 1 to 3 , the microvilli were either shorten or rarely seen. The RER was markedly decrease with scanty ribosome on its surface (Figure

42). Most of mitochondria showed swelling and distortion more than what where seen with MAb #27 treatment (Figure 43-44). The vacuole was more pronouncedly seen in the cytoplasm the same as the effect from anti-hepatoma MAb #27. The nuclear envelope was not smoothly paralled (Figure 44- 45). There was no any clear movement on the nucleus. The free ribosomes, glycogen, golgi apparatuses and lysosomes were still preserved.

At early stage of 3-24 hrs, the effect of anti-hepatoma MAb#27 on HCC-HepG2 cells was mostly less. The organelles including mitochondria and RER were remained within normal appearance. Little change could be code on structure and number of RER and mitochondria on the third day. Amount of RER and mitochondria were markedly decreased. Ribosomes showed the same scantiness on RER surface as was recognized with the effect of MAb #27 on HCC-S102 (Figure 46). Mitochondria were swollen. Microvilli were shorten. Nucleolus was markedly increased the size together with some mitochondria. Free ribosomes and glycogen were commonly seen as in the control (Figure 47). Vacuoles were more pronouncedly seen in the cytoplasm the same as on HCC-S102 cell line which were treated with anti-hepatoma MAb #27 and #43.

Plasma membrane, RER and mitochondria of treated HepG2 with MAb #43 for 3-24 hrs remained well preserved with long microvilli as in the control (Figure 48-49). Nucleus showed invagination (Figure 50). After treatment for 3 days, the cells displayed shorter microvilli. There were some vacuoles. Plasma membrane remained well preserved together with the RER, SER, glycogen, free ribosome, golgi apparatus and lysosome (Figure 51-52). The mitochondria were swollen but not decrease in number as was seen with the effect on HCC-S102 (Figure 53). The loose nucleolus was frequenly seen (Figure 54).

Table 9 .

Effect of Anti-hep-27 and Anti-hep-43 on HCC cell iines , the HCC-S102 and HepG2

The ranking of importance of organelles changes	anti hep-27		anti hep-43	
	HCC-S102	HepG2	HCC-S102	HepG2
Most sensitive organelle: 1) RER :				
decrease in number	strong decrease	milder	after day 3	normal
matrix in between RER	(+)	non	non	non
scanty ribosome	after 3 hrs	after day 3	after day 3	non
SER	non response	non response	non response	non response
2) Microvilli				
shorten	after 3-12 hrs	after day 2-3	after day 3	after 2-3
complete lose	after 24 hrs - day3		after day 3	not significant
3) Vacuole				
large size	after day 3	after day 2-3	non	after day 2-3
small size	(-)		(+)	
accumulation of glycogen	(-)	(-)	(-)	(-)
4) Mitochondria				
decrease in number	after day 1	(-)	after day 3	non
swollen	after day 1	after day 1	after day 3	after day 3(fewer)
5) Nucleus				
invagination	increase prominent (after 24 hrs)	after day 2-3	increase	after day 3
chormatin clumping	after day 1	after day 3	after day 3	after day 2-3
nucleolus	(-)	loose(day 2-3)	(-)	loose(day 2-3)
nuclear membrane	fuse(after day3)	fuse(after day3)	(-)	(-)

(+) :indicates the occerance of a changes in the organelles

(-) :indicates the non occerance of a changes in the organelles
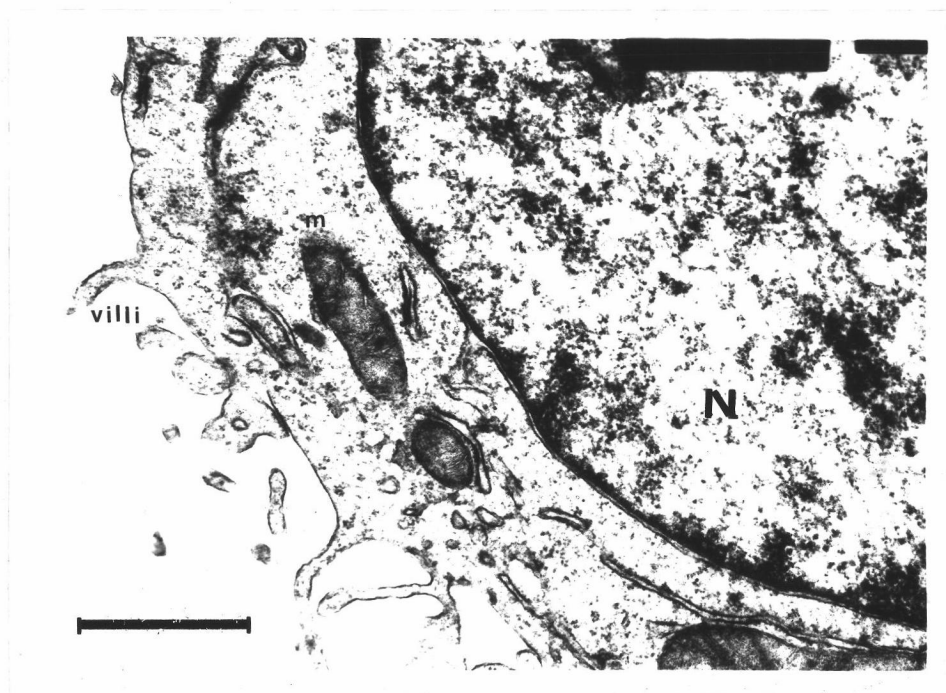
Figure 27 . The general appearance of untreated HCC-S102 shows small amount

of organelles in comparison to normal liver cell. The microvilli and

mitochondria were well preserved. The nucleus had dispersed

heterochromatin. (bar=1μm, ×21,000)
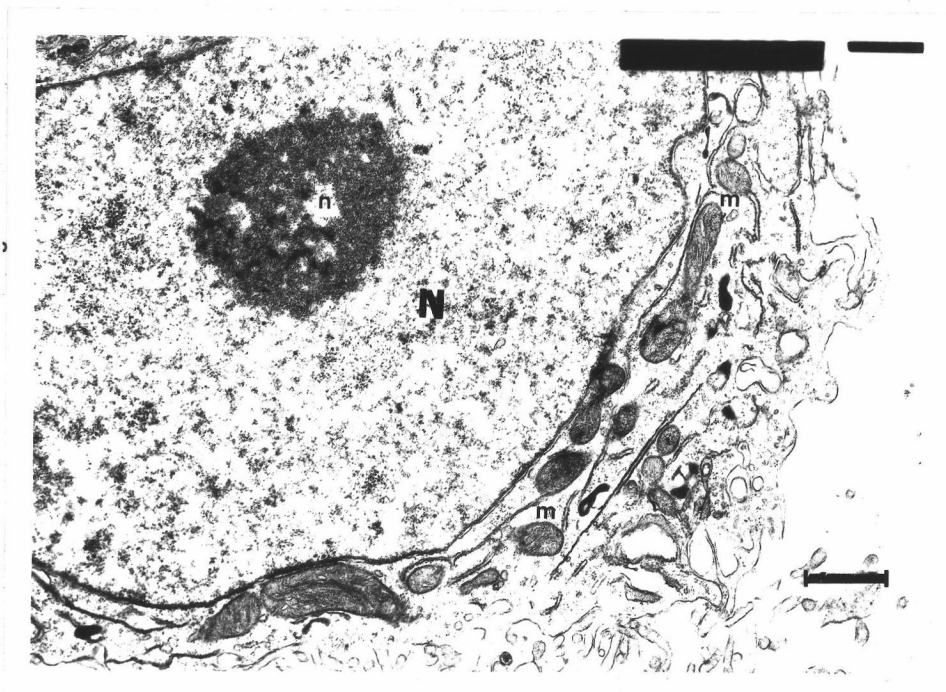
Figure 28. HCC-S102 hepatoma cell often was incubated in 4°c for 6 hrs before

fixation showing the well preserved cell at this hard and long condition.

(bar=1μm,×10,000)
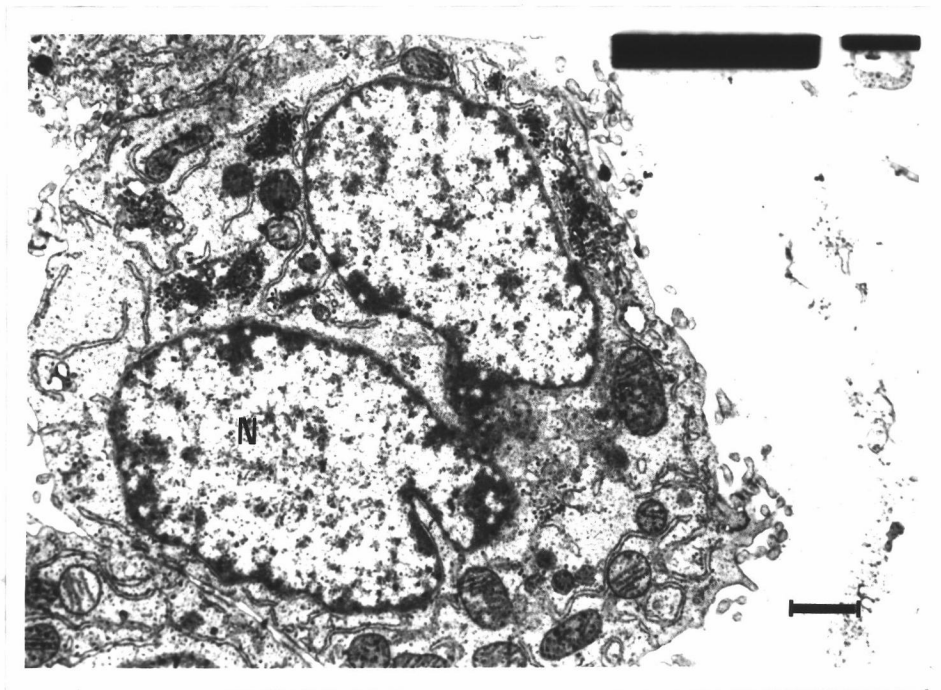
Figure 29. The untreated HepG2 hepatoma cell showing microvilli at their luminal border, numerous well preserve mitochondria and double nuclei. (bar=1 μm, ×7,000)
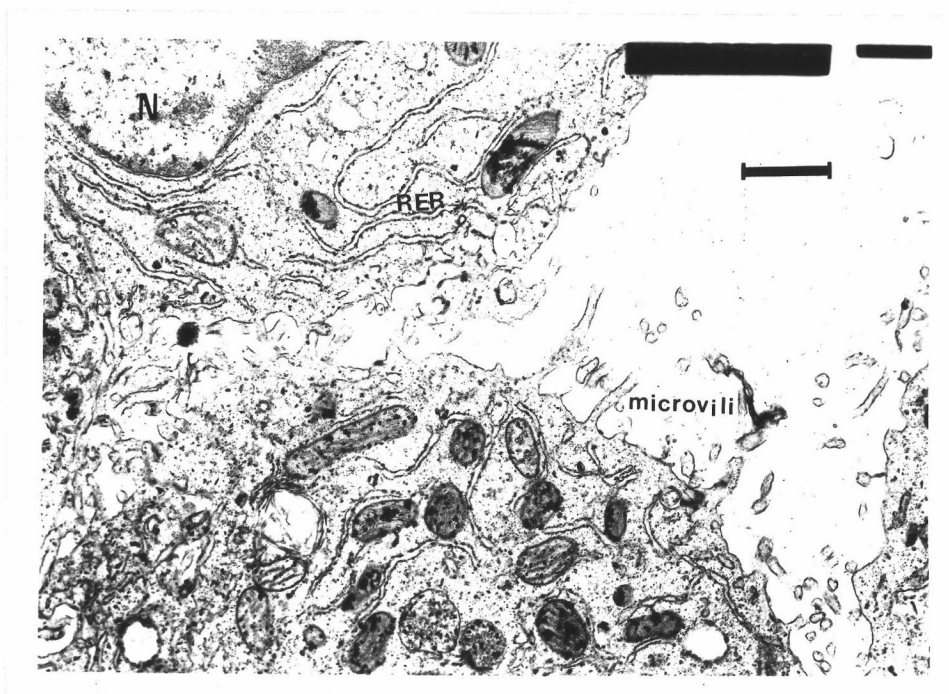
Figure 30. The attachment of untreated HepG2 hepatoma cells. The organelles were well preserved. The microvilli were relatively long. (bar=1μm, × 10,000)
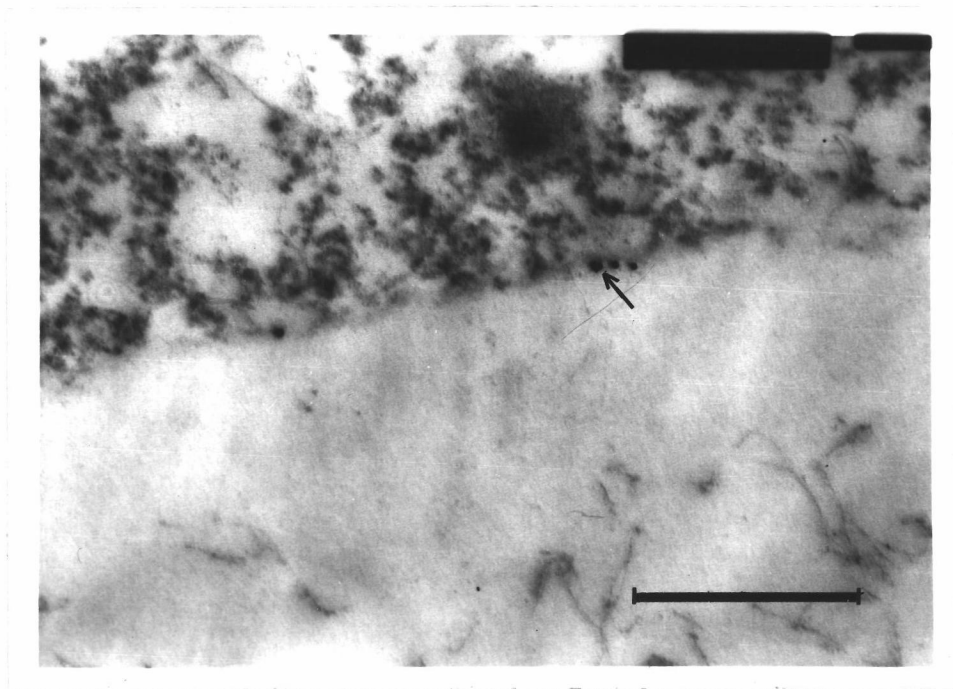
Figure 31. The distribution of gold labelling (arrow) on surface membrane of a HCC-S102 cell localized with anti-hepatoma MAb#27.   The gold particles were found 20-40 particles per cell. (bar=0.5μm, ×56,000)
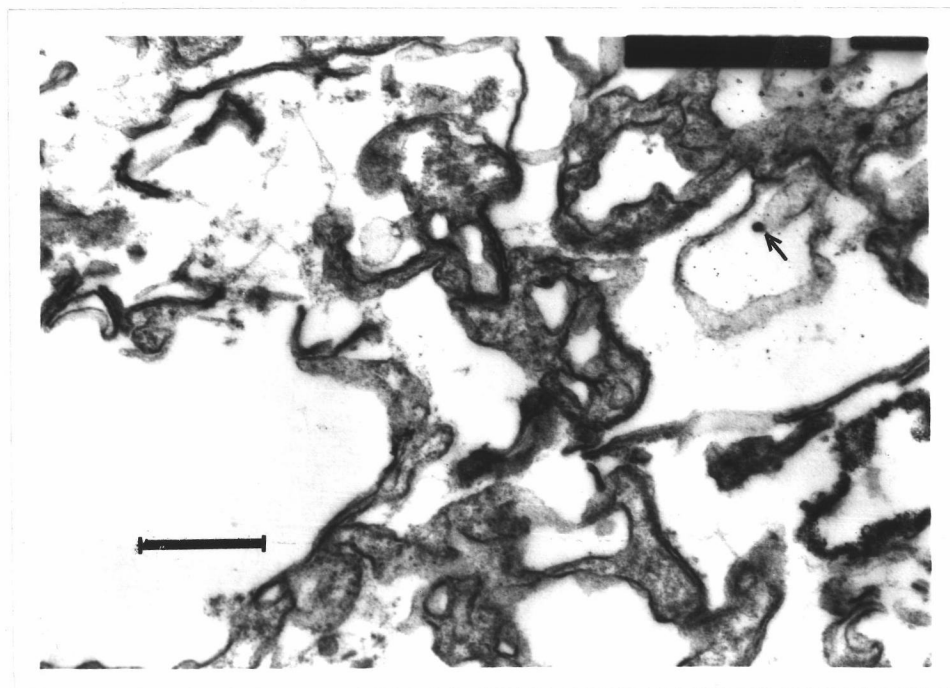
Figure 32. The intracellular gold particle (arrow) in a HCC-S102 cell after treatment with anti hepatoma MAb #27 for 3 hrs. (bar=0.5μm, × 35,000)
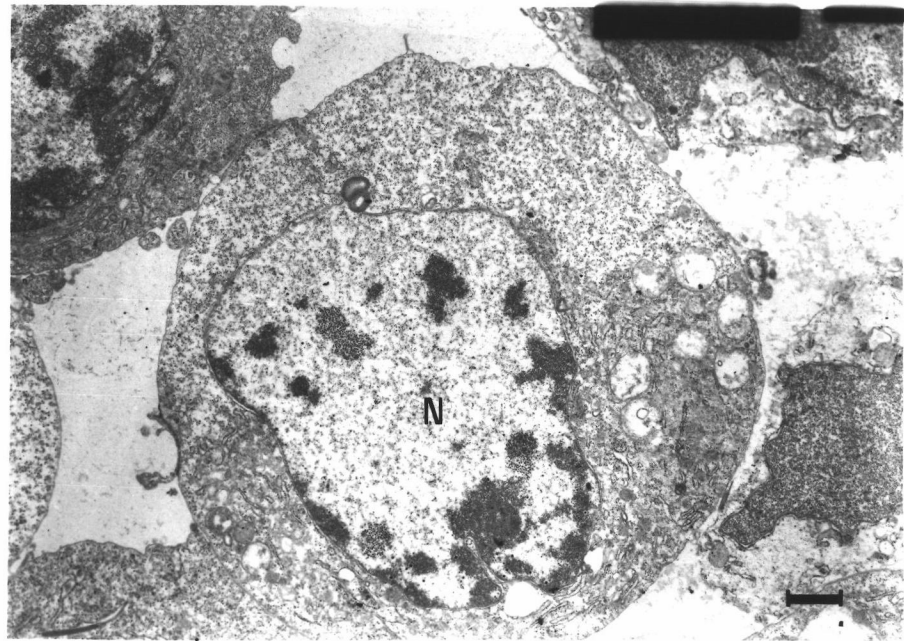
Figure 33. The MAb #27 treated HCC-S102 cell almost lose their microvilli and

showed the vacuole in cytoplasm from the first day and consistently

lose on the third day. (bar=1μm, × 5,600)
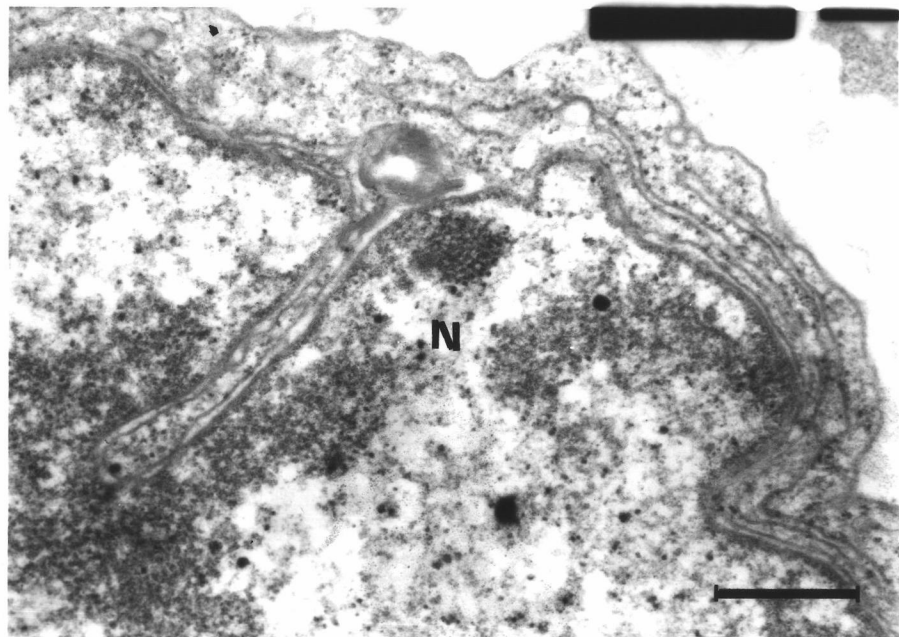
Figure 34. The RER of a HCC-S102 cell with scanty ribosome (arrow) on its surface

after treatment with anti-hepatoma MAb #27 for 1-3 days.   Generally,

the nucleus was normal. Sometime the invagination of nuclei was seen

as a deep tract but mostly did not showed the hyperactivity. The

chromatin seems to gathered in heterochromatin form which is a sign

suggesting the genetically inactive condition. (bar=0.5μm, × 35,000)
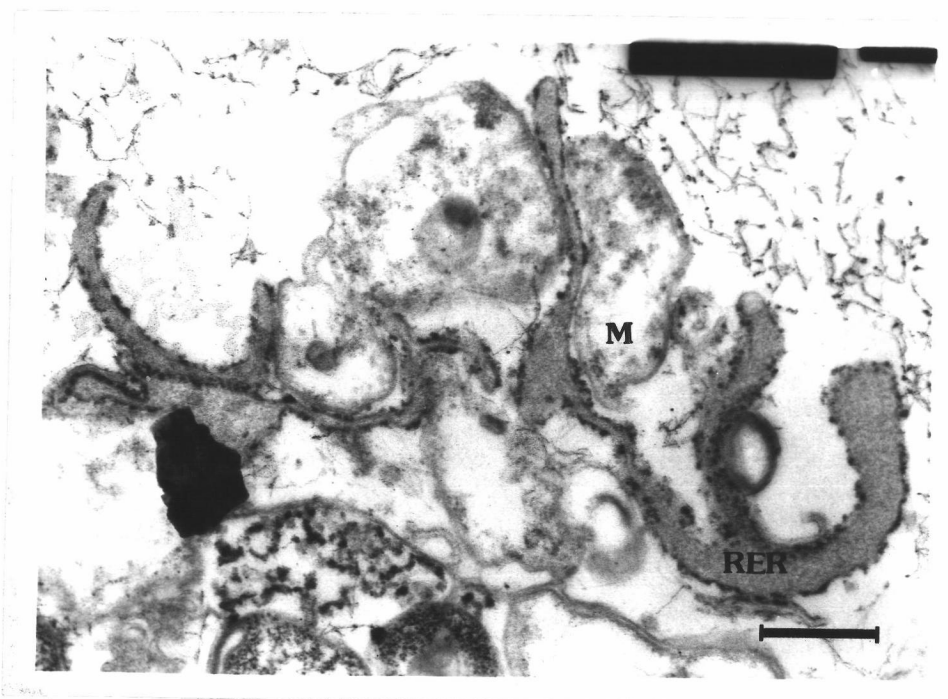
Figure 35. The swollen RER filled up with compact unknown matrix. Usually was seen in HCC-S102 cells after treatment with the anti-hepatoma MAb #27, for 1 to 3 days. (bar=0.5μm, × 28,000)
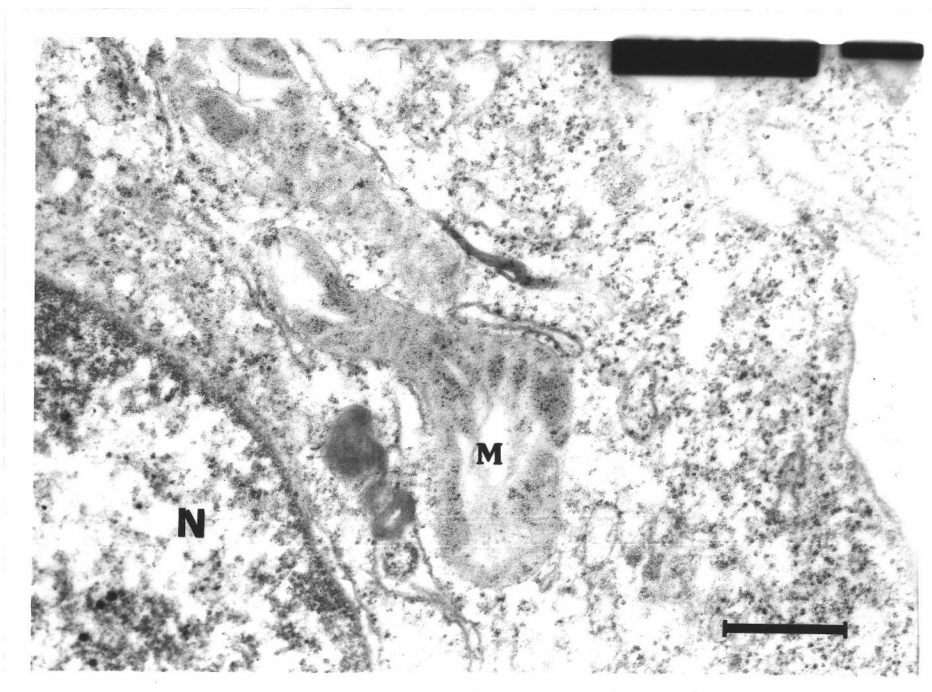
Figure 36. The swollen and distorted mitochondria of a HCC-S102 cell after treatment with anti-hepatoma MAb #27 for 1 to 3 days. (bar=0.5 μm, × 28,000)
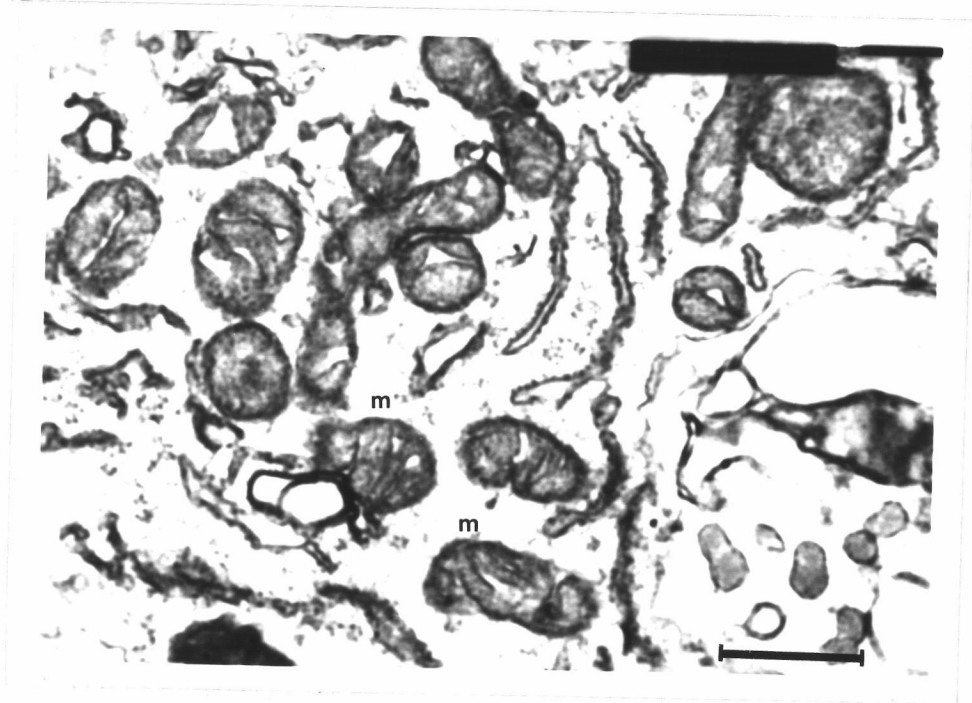
Figure 37 . The mitochondria of anti-hepatoma MAb #27 treated HCC-S102 cell on day 1-3 showed the mitochondria aggregated near the nuclear membrane. The ribosome on RER was scanty with the partial destructive change of RER. (bar=0.5 μm, × 35,000)
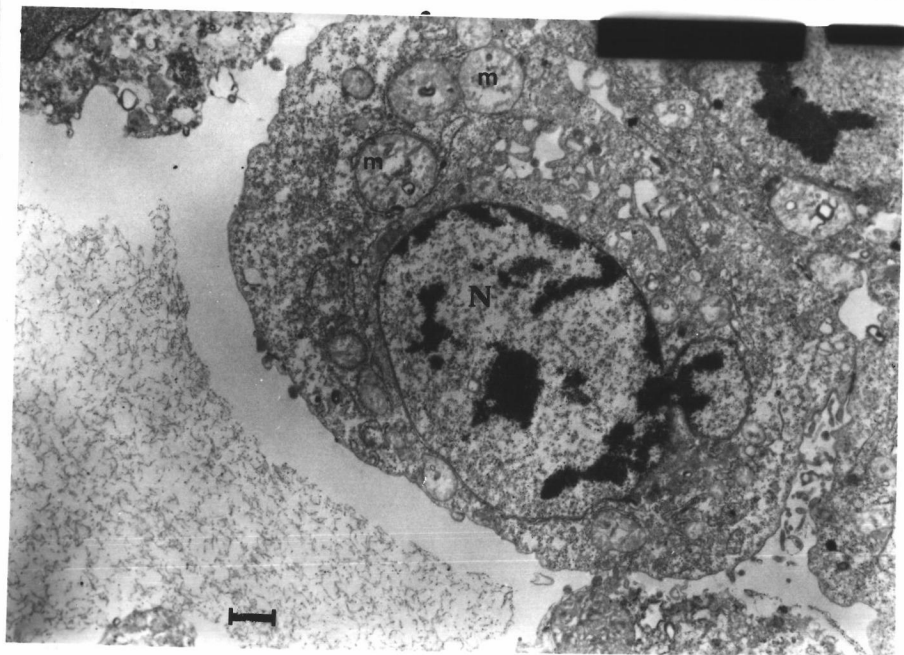
Figure 38. The aggregation of chromatin called heterochromatin in clumping pattern of HCC-S102 cell after treatment with anti-hepatoma MAb #27 for 1-3 days together with the markedly swollen and vacuolar mitochondria. (bar=1 μm, × 4,200)
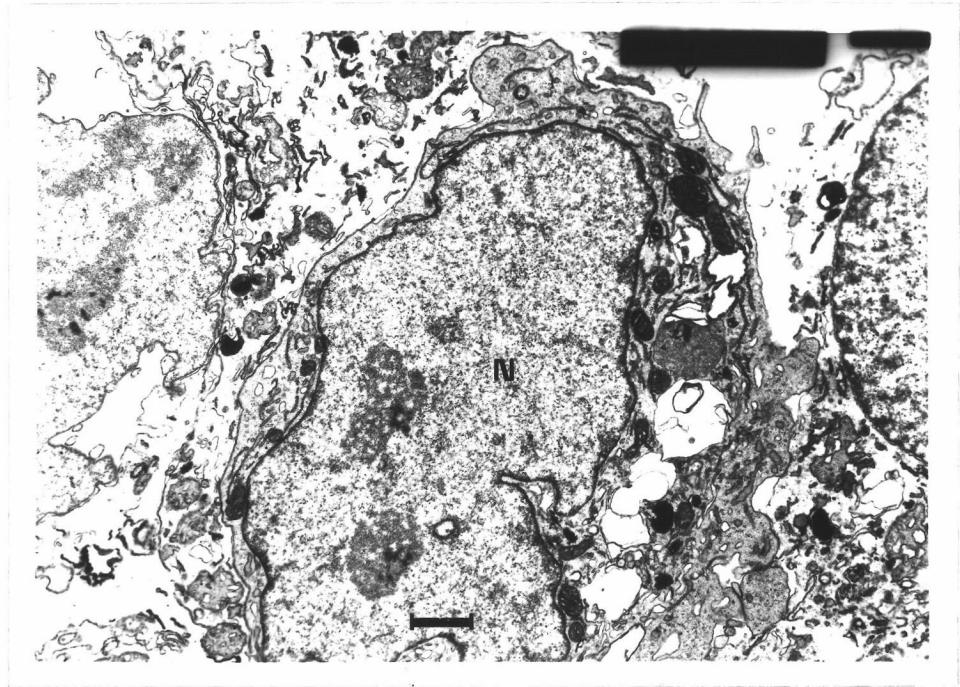
Figure 39. The vacuole in cytoplasm and the big nucleus of an anti-hepatoma MAb
#27 treated HCC-S102 cell showed scattered chromatin on day 1-3.
(bar=1 μm, × 7,000)
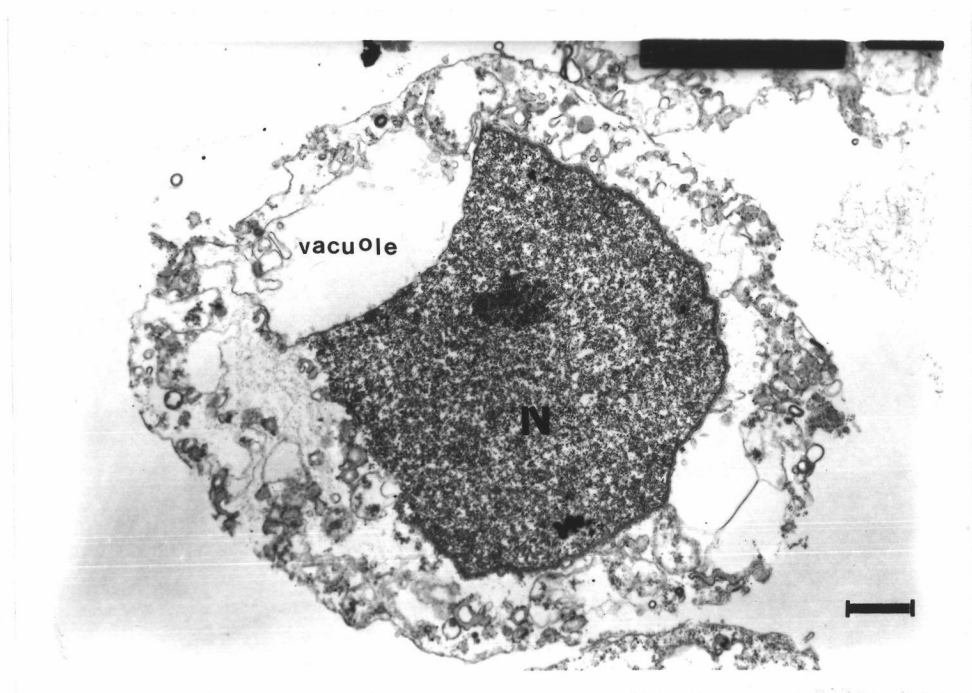
Figure 40. The vacuole in cytoplasm which markedly pushed the nucleus . This
pattern was usually seen in the day 1-3 after CC-S102 cell was
treated with anti-hepatoma MAb #27. (bar=1 μm, × 7,500)
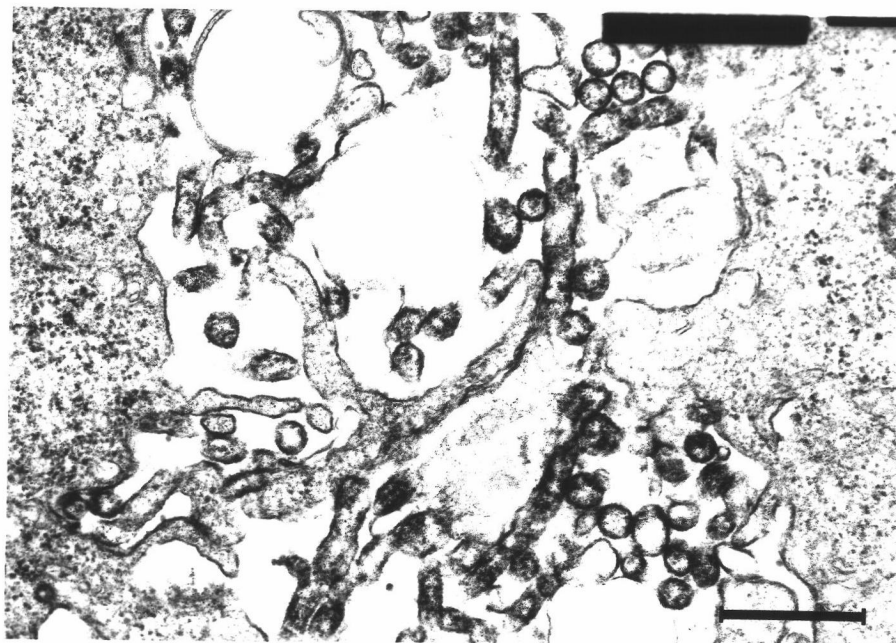
Figure 41. The HCC-S102 cell was treated with anti-hepatoma MAb #43 for 3-12 hrs still showing well preserved plasma membrane and long microvilli. (bar=0.5 μm, × 28,000)
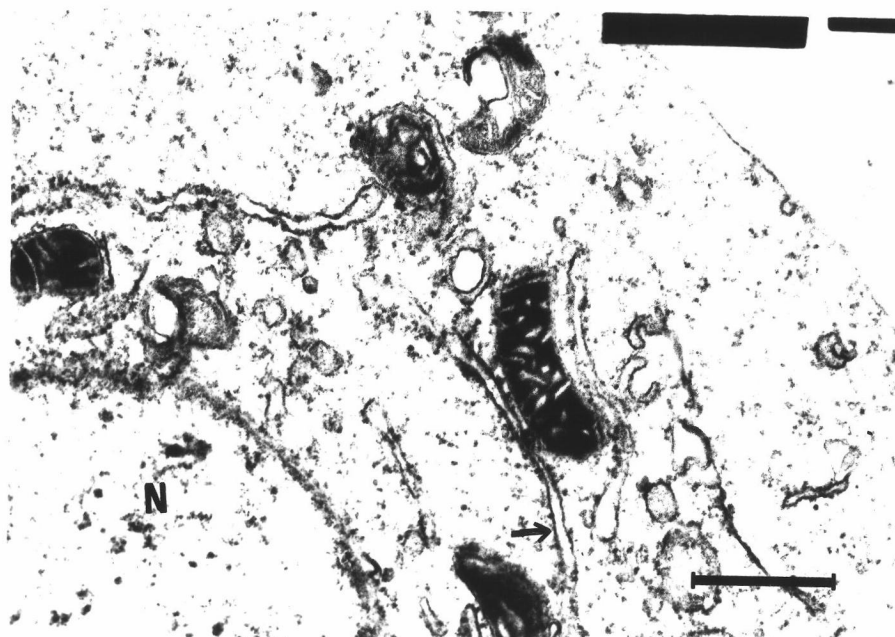
Figure 42. On day 1 to day 3, the anti-hepatoma MAb #43 caused a decreasing ribosome on RER (arrow) in a HCC-S102 cell. The microvilli were absence or rarely seen. (bar=0.5 μm, × 35,000)
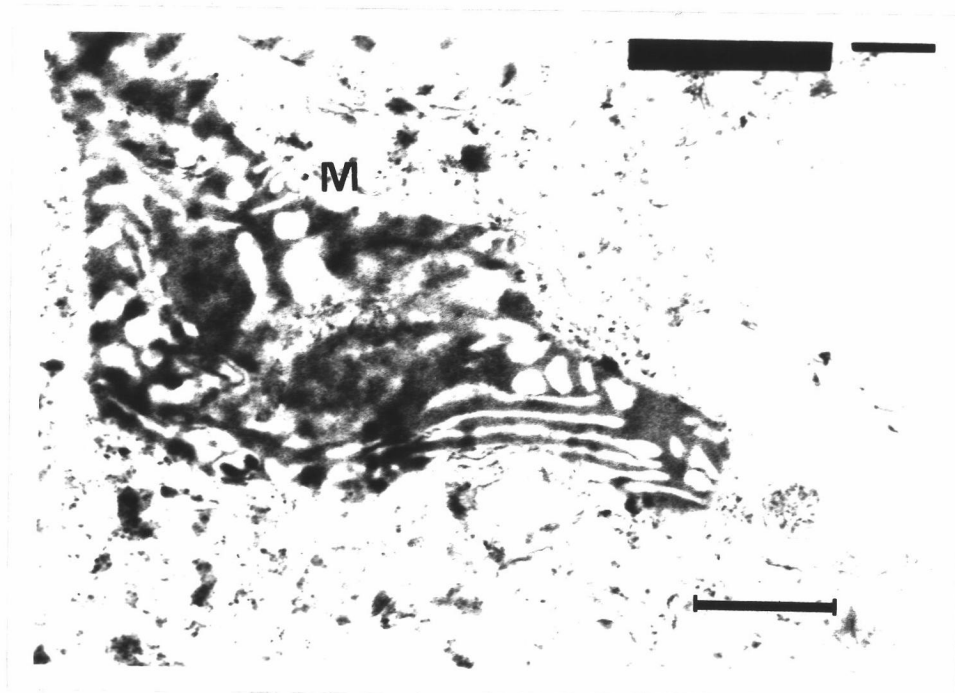
Figure 43. The anti-hepatoma MAb #43 treated HCC-S102 cell showed the swollen and distorted mitochondria on day 1-3 days. (bar=0.5 μm, × 35,000)
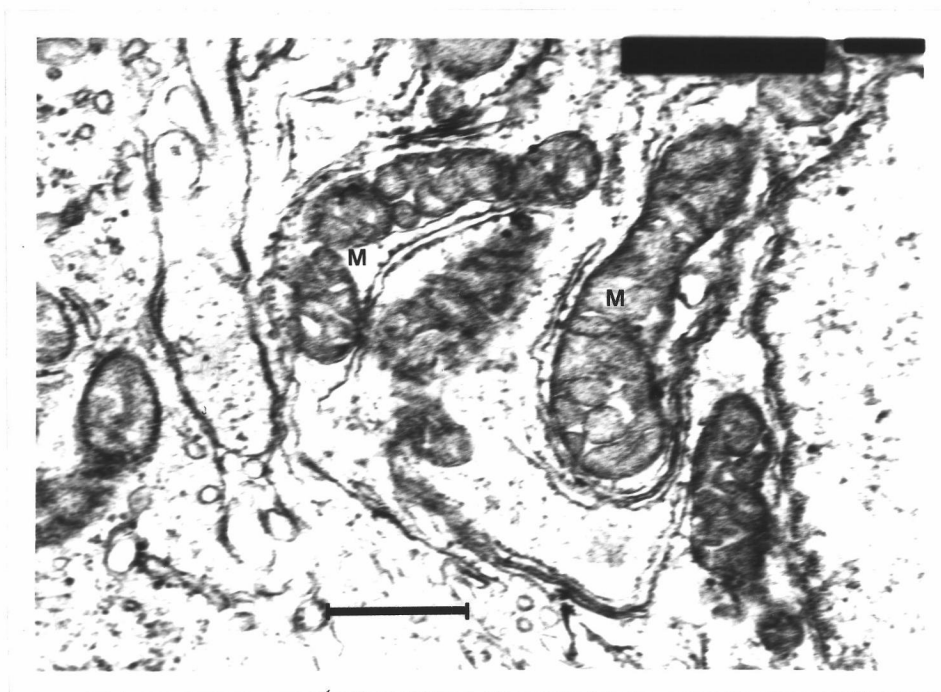
Figure 44. The swolling and distortion of mitochondria of a HCC-S102 cell which was treated with anti-hepatoma MAb #43 on day 1 to day 3. The RER with scanty ribosomes was often seen. (bar=0.5 μm, × 35,000)
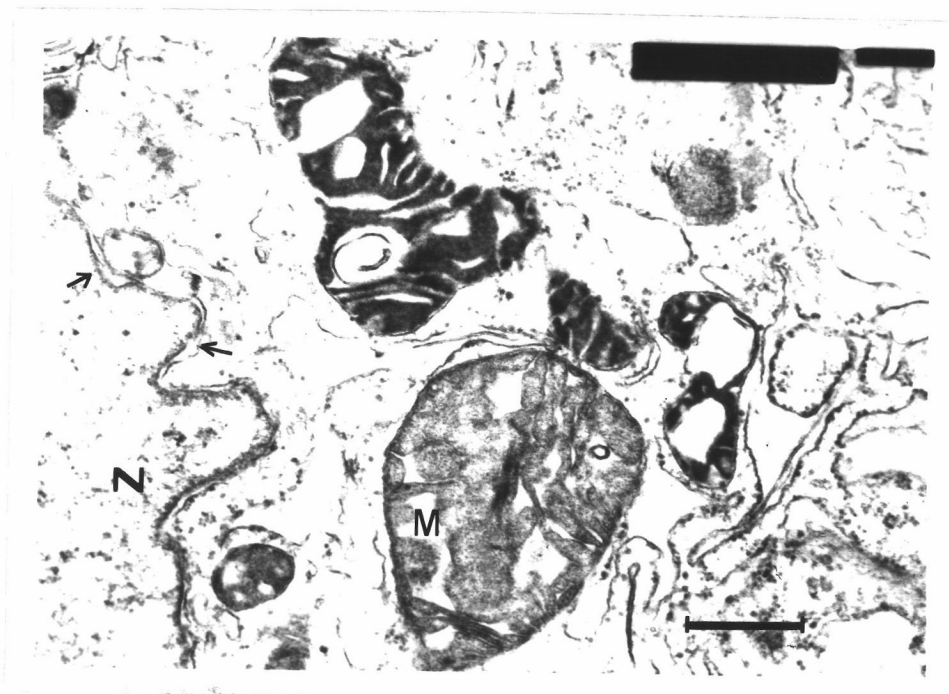
Figure 45. The HCC-S102 cell was treated with anti-hepatoma MAb #43. The nuclear membrane (arrow) was partially adhered at some part. Some was protruded out. The distortion and swolling mitochondria were usually seen on day 1 to day 3. (bar=0.5 μm, × 28,000)
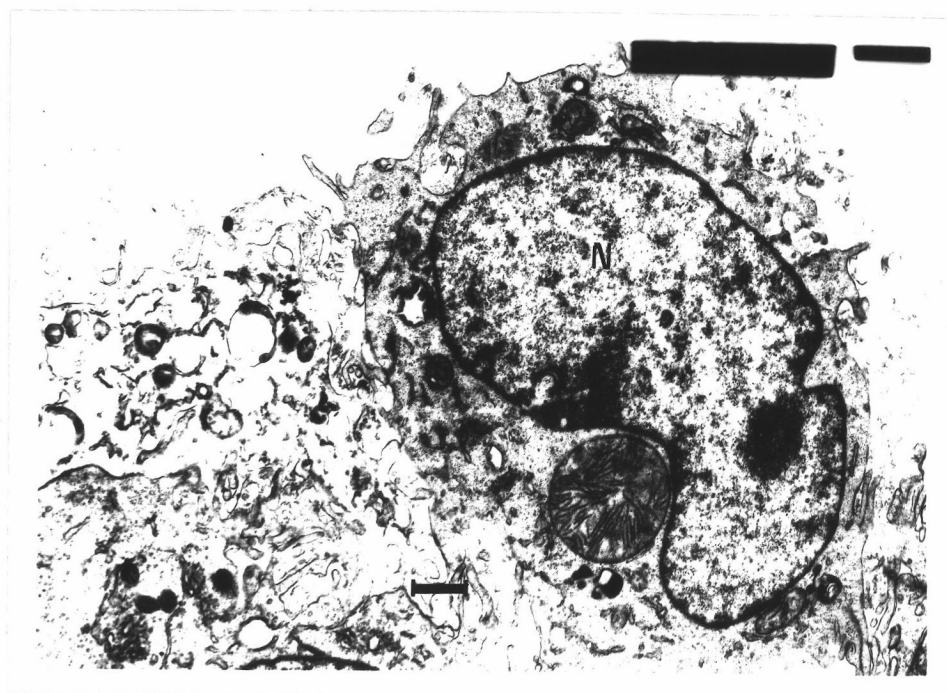
Figure 46. The nucleolus ratio to the cytoplasm was markedly increased. The amount of RER and mitochondria were markedly decreased. Scanty ribosomes were found on day 3 in a HepG2 cell which was treated with anti-hepatoma MAb #27. (bar=1 μm, × 7,000)
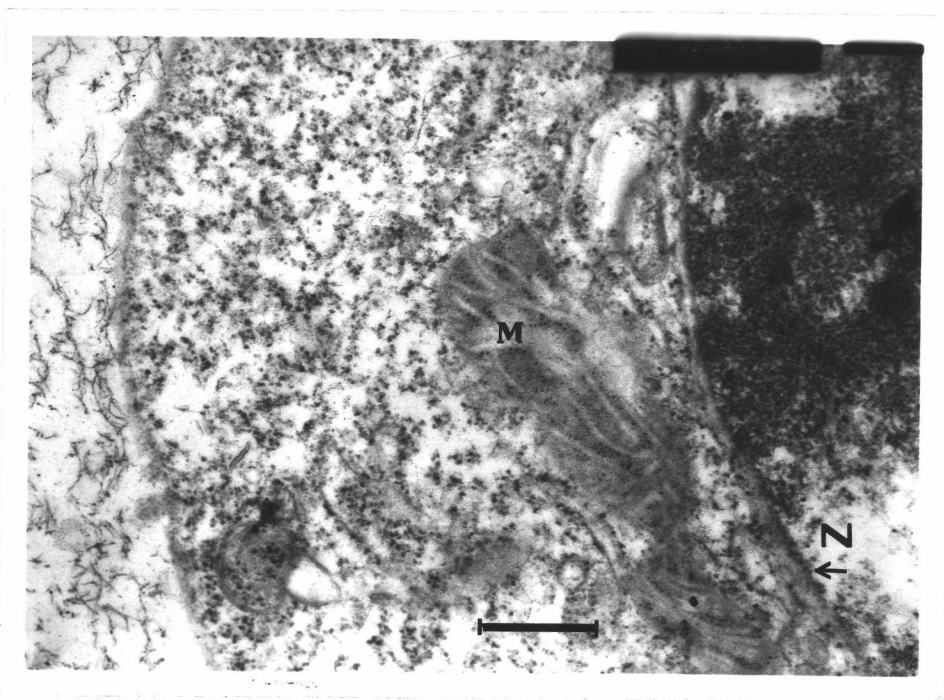
Figure 47. Swollen mitochondria in cytoplasm of a HepG2 on day 3 after treatment of anti-hepatoma MAb #43 was seen in cytoplasm. Free ribosome and glycogen were commonly seen. The nucleus showed massive heterochromatin. The nuclear membrane seem to be fused at some part (arrow). (bar=0.5 μm, × 28,000)
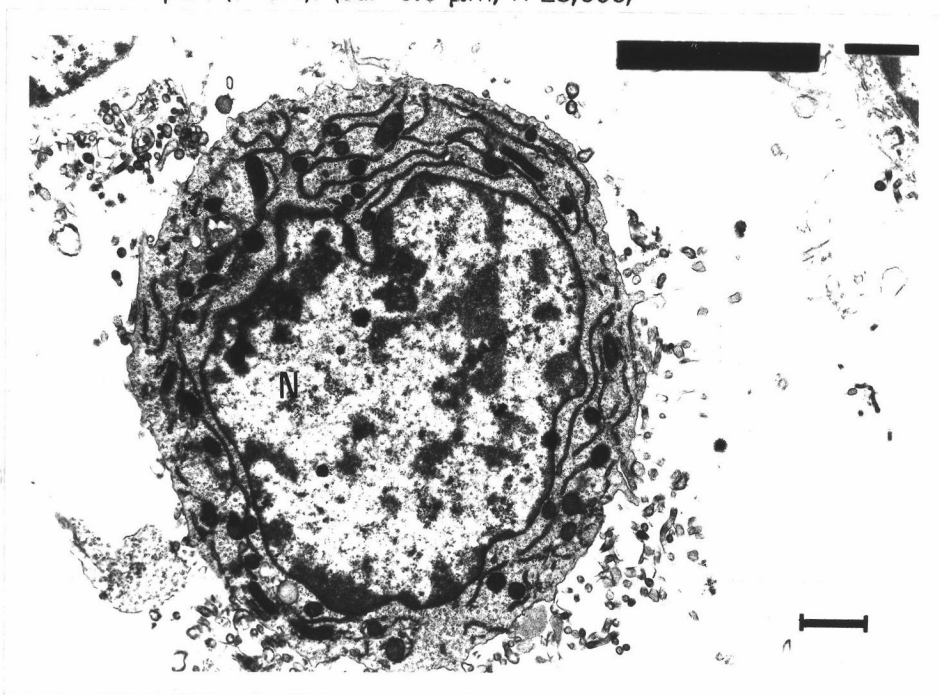
Figure 48. The anti-hepatoma MAb #43 treated HepG2 cell for 3-24 hrs showed the large size of nucleus with evidence of heterochromatin. The microvilli were still within normal length. (bar=1 μm, × 7,000)
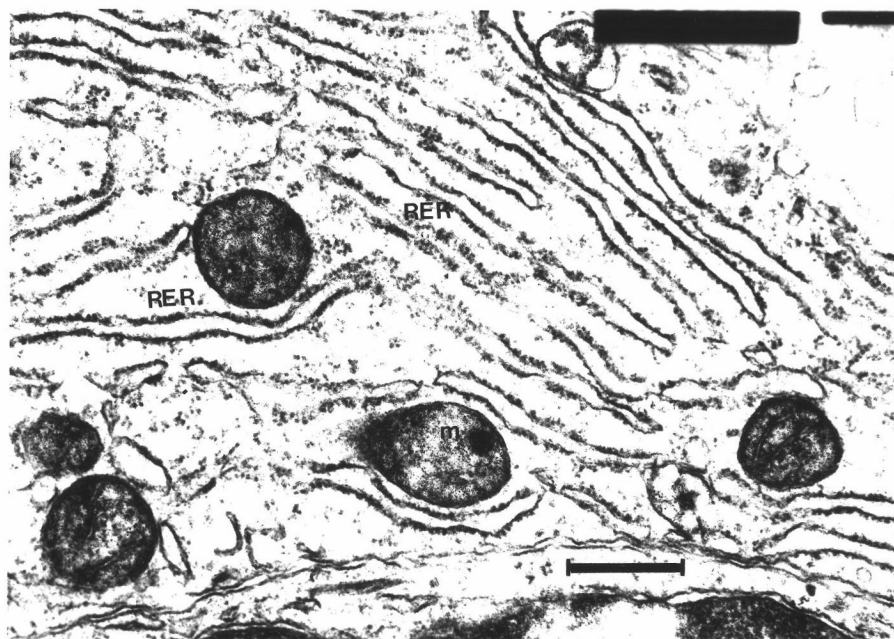
Figure 49. The RER and mitochondria of HepG2 cell which was treated with anti-hepatoma MAb #43 for 3-24 hrs remained well preserved. (bar=0.5 μm, × 28,000)
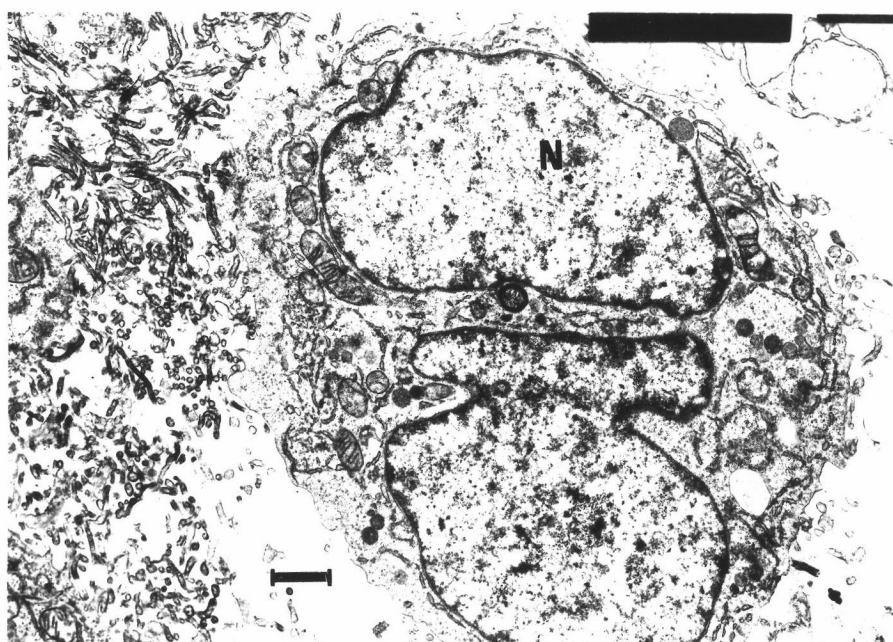

Figure 50. The 3-24 hrs anti-hepatoma MAb #43 treated HepG2 cell showed the double or segmented nucleus which spreaded over the whole area of the cell. The RER and mitochondria were still well preserved pattern. (bar=1 μm, × 6,200)
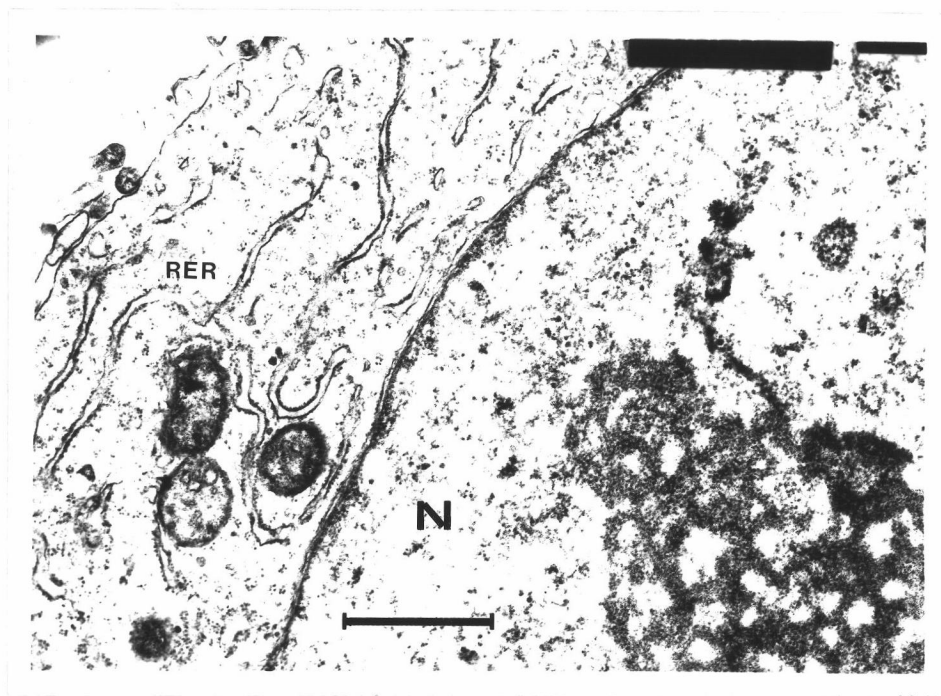
Figure 51. After treatment with anti-hepatoma MAb #43 for 3 days the RER of HepG2 cell, the cristernae of mitochondria were disappeared while the size were still in normal. (bar=1 μm, × 18,000)
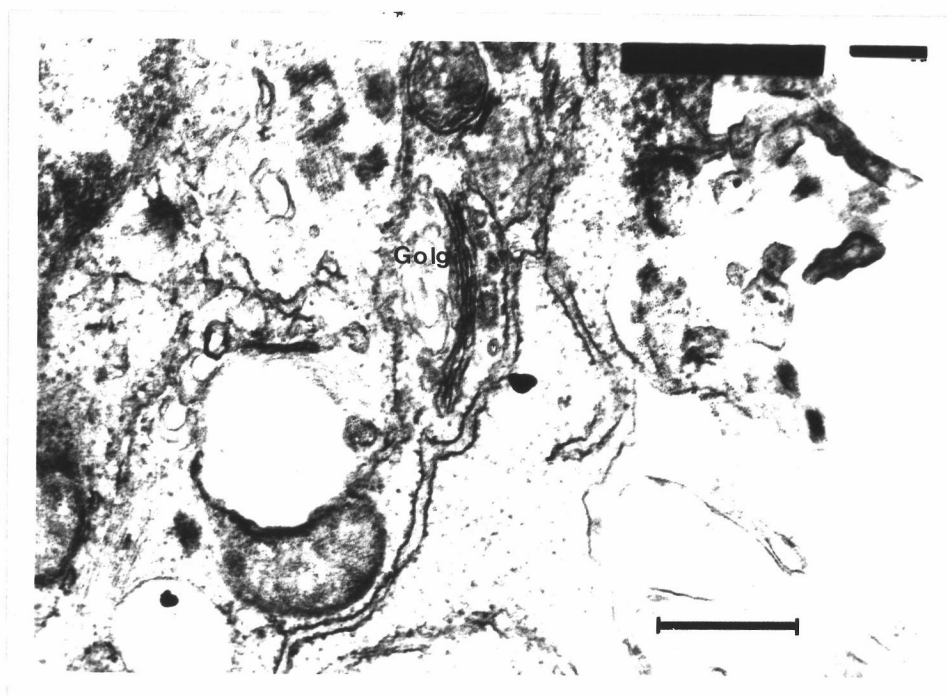

Figure 52. After 3 days treated with anti-hepatoma MAb #43, the HepG2 cell showed some vacuolation. There was no change of the golgi apparatus. The ribosome on surface of RER was normally found. (bar=0.5 μm, × 35,000)
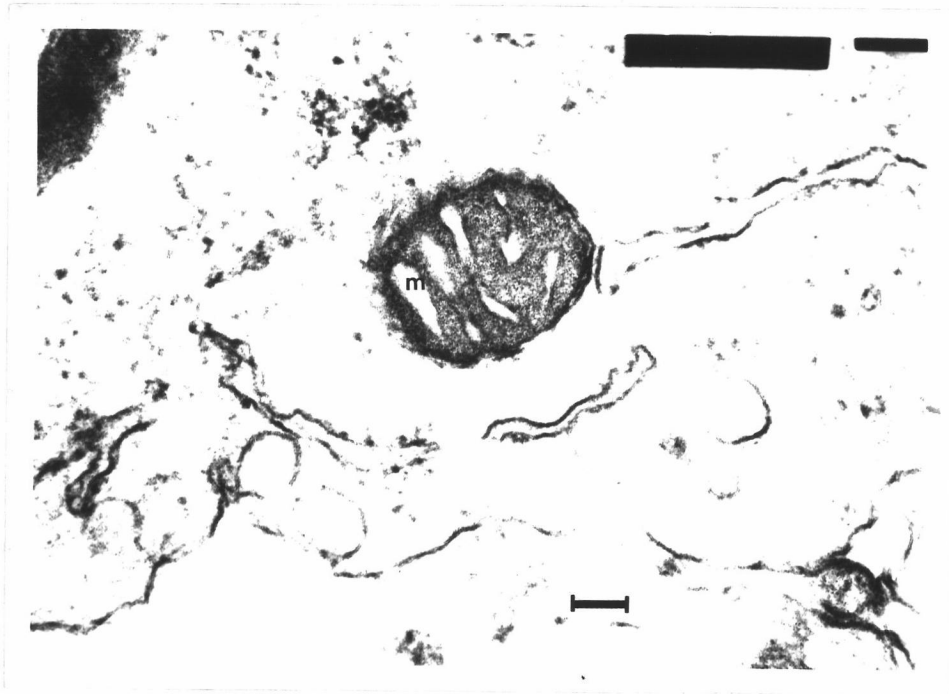
Figure 53. The treated HepG2 cell with anti-hepatoma MAb #43 for 3 days showed slightly swollen of mitochondria while the size was almost within normal limit. (bar=0.1 μm, × 56,000)
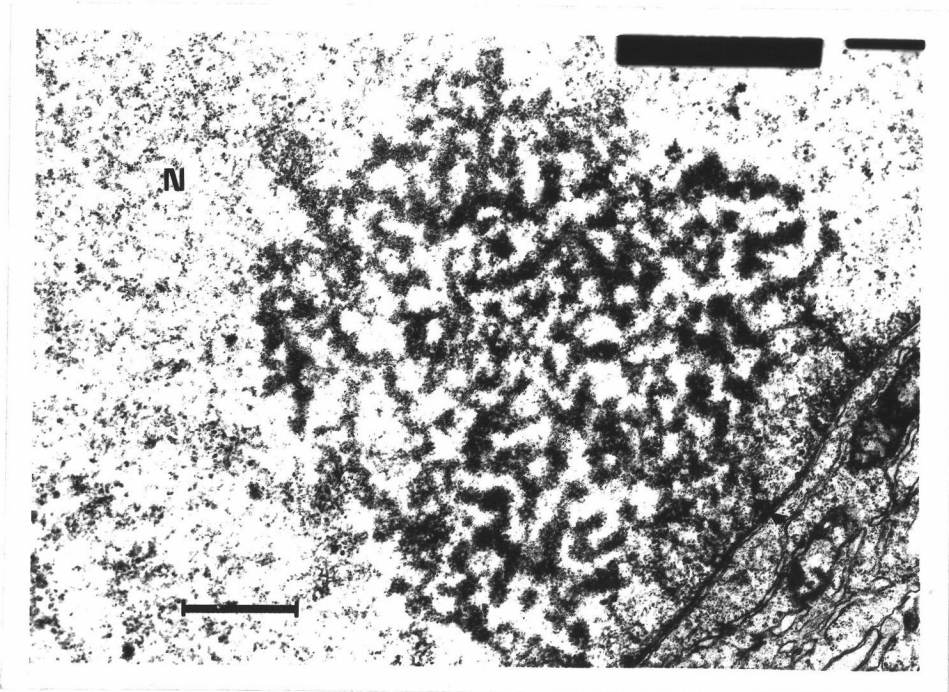

Figure 54. The treated HepG2 cell with anti-hepatoma MAb #43 for 3 days after treatment showed loose nucleolus. The nuclear membrane still remained well preserved. (bar=1 μm, ×14,000)