CHAPTER III

RESULTS

3.1 AFP and histological grading

Histological classification of carcinoma of the liver was based on the histological findings, and the patients in the present study were divided into 2 groups.

Group 1. Primary carcinoma of the liver: Hepatocellular carcinoma or liver cell carcinoma.

Group 2. Metastatic carcinoma of the liver: Adenocarcinoma.

3.1.1 Degree of differentiation and grading of hepatocellular carcinomas:

Criteria of gradings depend upon the degree of anaplasia of the cells. The histological gradings after Edmondson (58,59) were carried out by studying:

1. The quantity, granularity and acidophilic quality of the cytoplasm.

2. The size and degree of hyperchromatism of the nuclei.

3. The proportion of cell occupied by the nucleus compared to the amount of cytoplasm.

4. The cohesive quality of tumour cells.

5. The evidence of cell function

6. The histological architecture.

The 4 grades of hepatocellular carcinoma are as follow:

Grade I, well differentiated carcinoma: This is not seen as the sole types in any specimen of carcinoma studied, but occurred only locally in predominantly grade II carcinoma. The areas of grade I carcinoma closely simulate normal liver cells.

Grade II, moderately differentiated carcinoma: The cells show a marked resemblance to normal hepatic cells. The nuclei is somewhat larger and more hyperchromatic than normal, but the cytoplasm is abundant, granular and acidophilic. The cell borders are often sharp and clearcut. Acini are frequent, their lumina varying in size from tiny canaliculi to large thyroid like spaces. The lumina are often filled with bile, otherwise they contain a small amount of protein precipitate, as shown in Figure 5,6, page 44,45.

Grade III, poorly differentiated carcinoma: The nuclei are usually larger and more hyperchromatic than those of grade II. These nuclei occupy a relatively greater proportion of the cells. The cytoplasm is less abundant, granular and acidophilic. Sometimes there is segregation of the granular cytoplasmic material toward the end of the cell bordering a lumen that usually do not contain bile. Some break-up or distortion of the usual trabecular pattern is present. More single cells are seen in the intravascular growths. Bile and acini formation are noted less frequently. Tumour giant cells are most numerous, as shown in Figure 7,8, page 46,47.

Grade IV, undifferentiated carcinoma: The nuclei is intensely hyperchromatic and occupy the greater part of the cell. The cytoplasm

is rather scanty and may or may not contain acidophilic granules. Trabeculae are usually difficult to find. The intravenous growths may be devoid of any definite pattern, may produce the intrasinusoidal type of seeding often seen in metastatic carcinoma. Bile is extremely rare, as shown in Figure 9, page 48.

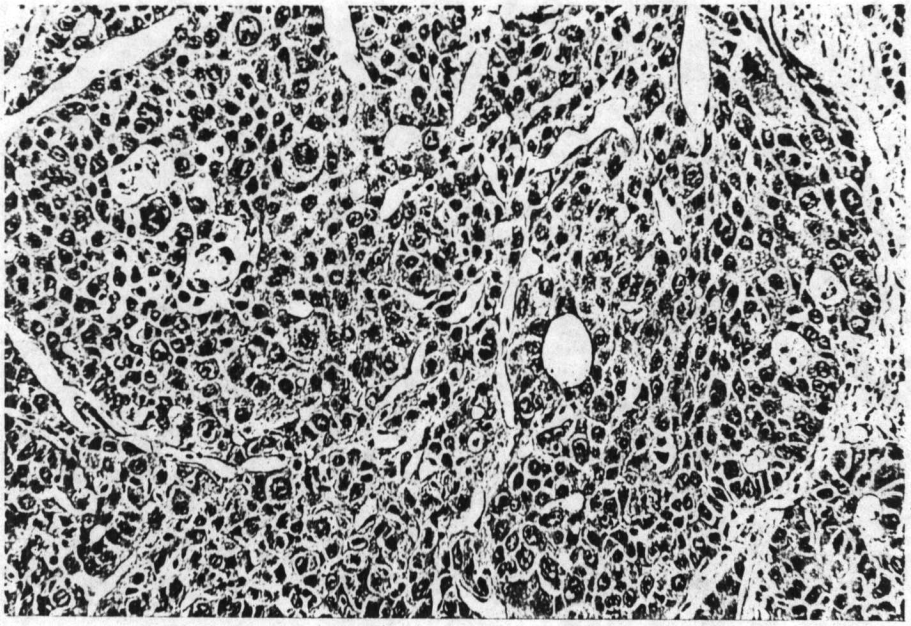

Fig. 5 Histological section of grade II, moderately differentiated carcinoma, beginning of necrosis in some area. (H & E X 100)

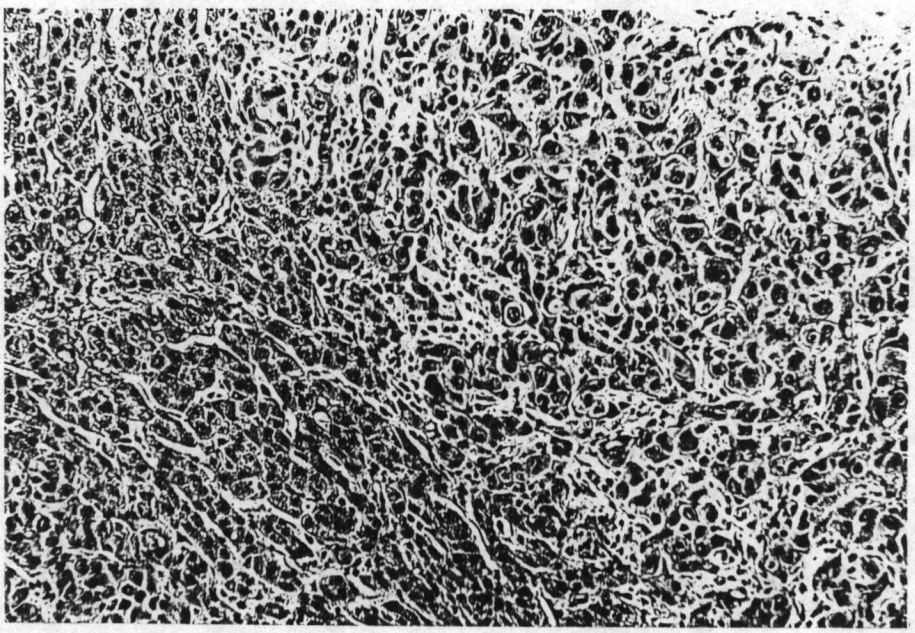

Fig. 6 Histological section of grade II, moderately differentiated carcinoma, compared to the normal hepatic cells. (lower left) (H & E X 100)

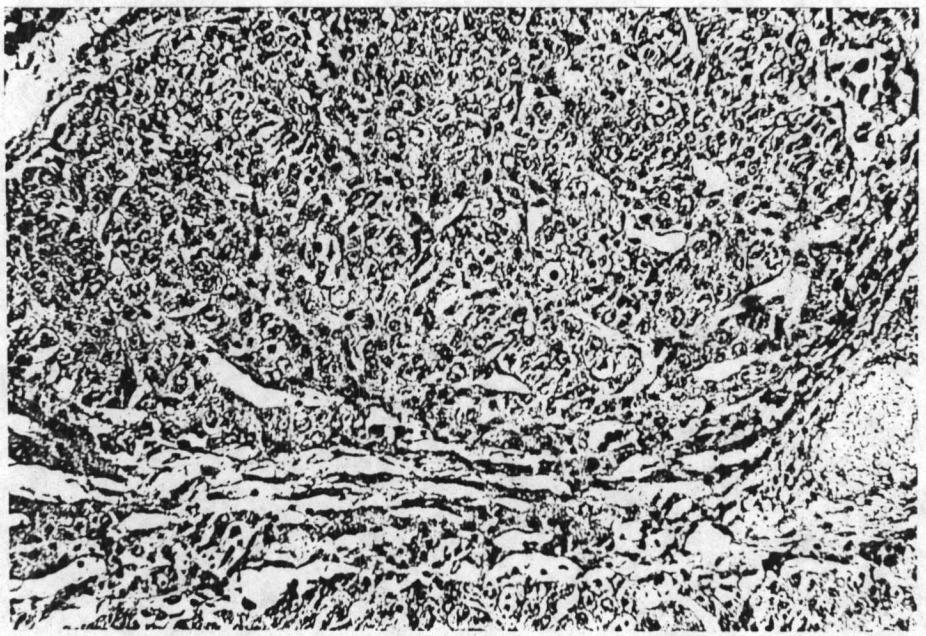

Fig. 7 Histological section of grade III, poorly differentiated carcinoma, the tumour cells were irregular and large in size.(H & E X 100)

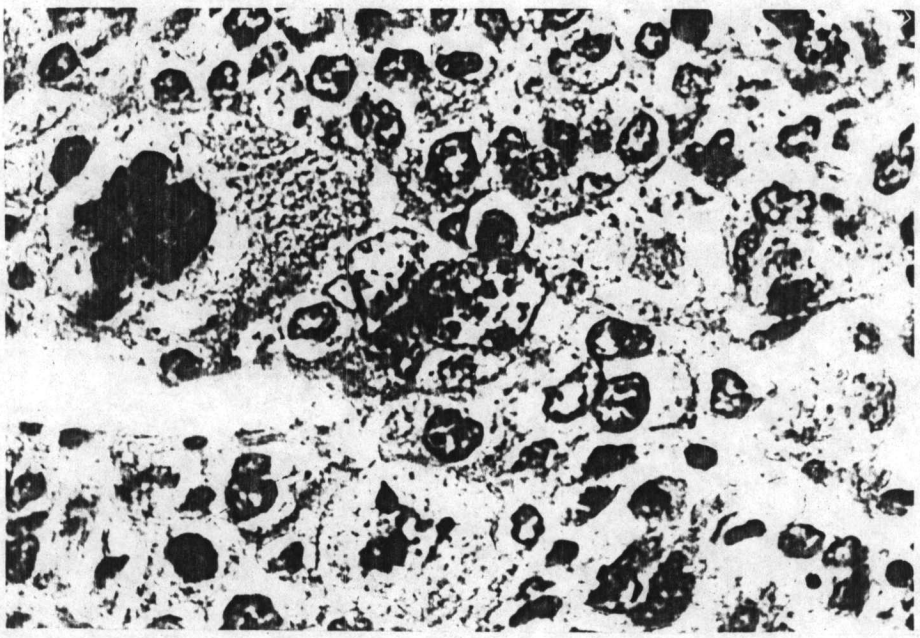

Fig. 8 Multinucleated tumour giant cells cells and mitotic figures were numerous in grade III, poorly differentiated carcinoma. (H & E X 450)

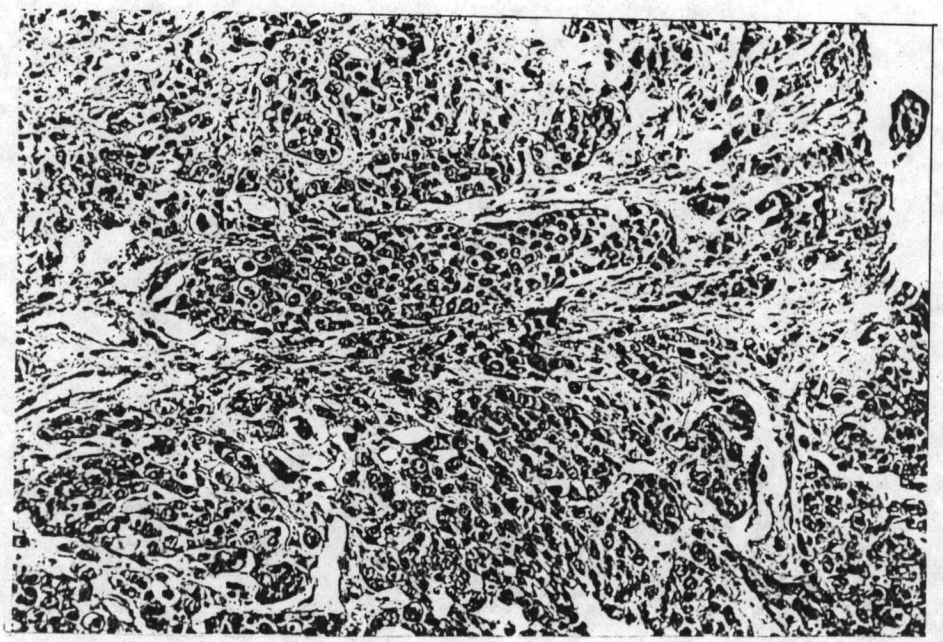

Fig. 9 Light microscopic features of undifferentiated grade IV carcinoma, the tumour cells were grouped and separated by connective tissues. (H & E X 100)

3.1.2 Adenocarcinoma.

Carcinoma arising from the intrahepatic bile ducts has a simpler structure and fewer of the atypical forms than does liver cell carcinoma or hepatocellular carcinoma. They are obvious adenocarcinomas with a more or less characteristic scirrhous background.

The cell type is columnar, the nucleus is smaller and less variable in size, and the nucleoli less prominent than those in liver cell carcinoma. The cytoplasm is clear to slight granular and rarely as acidophilic as that in the hepatocellular type. Bile is never seen in the acini or cells. Mucous formation is common. It is intracellular or intra-acinar or occasionally it is seen in tissue spaces. The degree of connective tissue proliferation among the bile duct carcinomas varies considerably.

The two additional features may be present, that help to diagnose these tumours apart from liver cell carcinoma.

1. Where they grow into blood vessels, they tend to use the endothelial surface as a basement membrane and line it with a single row of tumour cells, forming tremendous acinar-like structures.

2. The growth may become papillary in arrangement, this may be an outstanding feature. This is indeed a rare occurrence in liver cell carcinoma.

The histological features of metastatic adenocarcinoma of the liver and carcinoma arising from the intrahepatic bile ducts are identical. The degree of differentiation of metastatic adenocarcinoma of the liver in the present study were placed into three categories in the following manner:

Well differentiated-adenocarcinoma with histological and cellular features that closely resemble normal glandular epithelium, as shown in Figure 10, page 50.

Moderately differentiated-adenocarcinoma intermediate between well differentiated and poorly differentiated, as shown in Figure 11, page 51.

Poorly differentiated-adenocarcinoma with histological and cellular features that only barely resemble normal glandular epithelium.

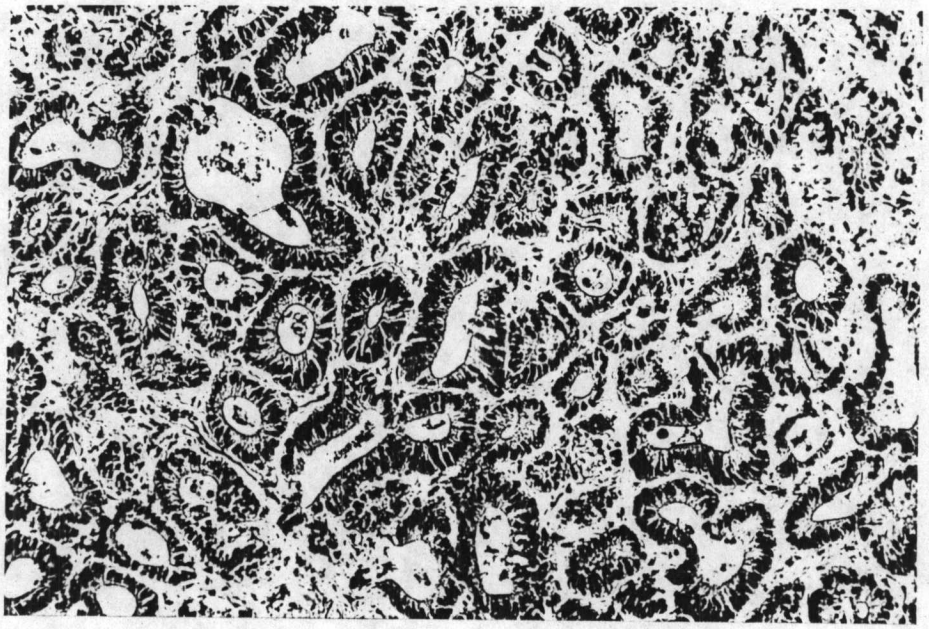

Fig. 10 A photomicrograph of an adenocarcinoma, well differentiated. (H & E X 100)

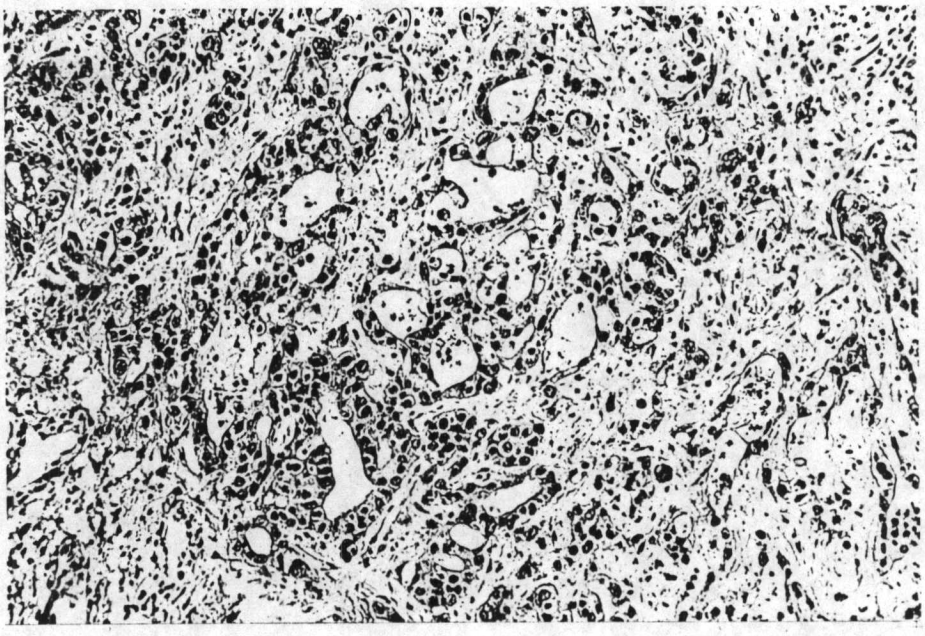

Fig. 11 Histological section of a moderately differentiated adenocarcinoma. (H & E X 100)

A total of twenty-five cases of needle and open surgery biopsy tissues from the patients who underwent hepatic artery ligation or partial hepatectomy were used. Among these there were 14 cases with hepatocellular type, and 11 metastatic adenocarcinomas of the liver. The histological gradings were made by considering the predominant feature of each tumour, based on the criteria described above.

The histological features of the hepatocellular carcinomas were variable. Histological gradings in each case and the numbers of each grade are shown in Table 14, page 53. In each grade, the highest incidence of the degree of cellular differentiation was in grade III. Next was grade II, moderately differentiated hepatocellular carcinoma, of 6 cases. The case of grade IV, undifferentiated hepatocellular carcinoma was small in number.

For metastatic carcinoma of the liver, the moderately differentiated carcinoma outnumbered well differentiated 4.5:1. The results are shown in Table 15, page 53.

Table 16, p, 54,gives the age, sex and race distributions of these 25 patients, whose ages ranged from 19 to 72 years, with a mean age of 47.8 years. The disease occurred predominately in the age group 40 to 59. There were 20 men and 5 women. The tumours occurred in 23 Thais and 2 Chinese.

TABLE 14

Histological classification of hepatocellular carcinoma

Histological grade	No. of cases
Grade I	-
Grade II	6
Grade III	7
Grade IV	1
Total	14

TABLE 15

Histological classification of metastatic carcinoma of the liver

Type	No. of cases
Well differentiated	2
Moderately differentiated	9
Poorly differentiated	-
Total	11

TABLE 16

Age, sex and race distribution

Age (year)	Male		Female		Total
	Thai	Chinese	Thai	Chinese	
10 - 19	1	-	-	-	1
20 - 29	-	-	-	-	-
30 - 39	4	-	1	-	5
40 - 49	7	-	1	-	8
50 - 59	5	-	2	-	7
60 - 69	-	2	1	-	3
70 - 79	1	-	-	-	1
Total	18	2	5	0	25

3.2 Immunofluorescent localization of AFP

The immunofluorescence technique, involving fixation in 95 % ethyl alcohol containing 1 % glacial acetic acid, followed by embedding in paraffin. This procedure seems to be the most suitable for detecting AFP in tissues (60). The superiority of paraffin embedding has also been confirmed by Engelhardt et al (61).

In the present study, the direct immunofluorescence technique has been used to demonstrate the localization of AFP in tumour cells by fluorescence-labeled alphafetoprotein conjugated antiserum in dilution 1:5.

AFP in the tumours were localized in the following 3 areas:

1. Cytoplasm of the tumour cells
2. Cytoplasmic membrane of the tumour cells
3. Fibroblasts and blood vessels

Fluorescence of tumour cells were found tobe localized in the following 3 forms.

1. Diffuse finely granular fluorescence of the cytoplasm.
2. Brightly fluorescent line of the cytoplasmic membranes.
3. Bright fluorescence on the fibroblasts and blood vessels.

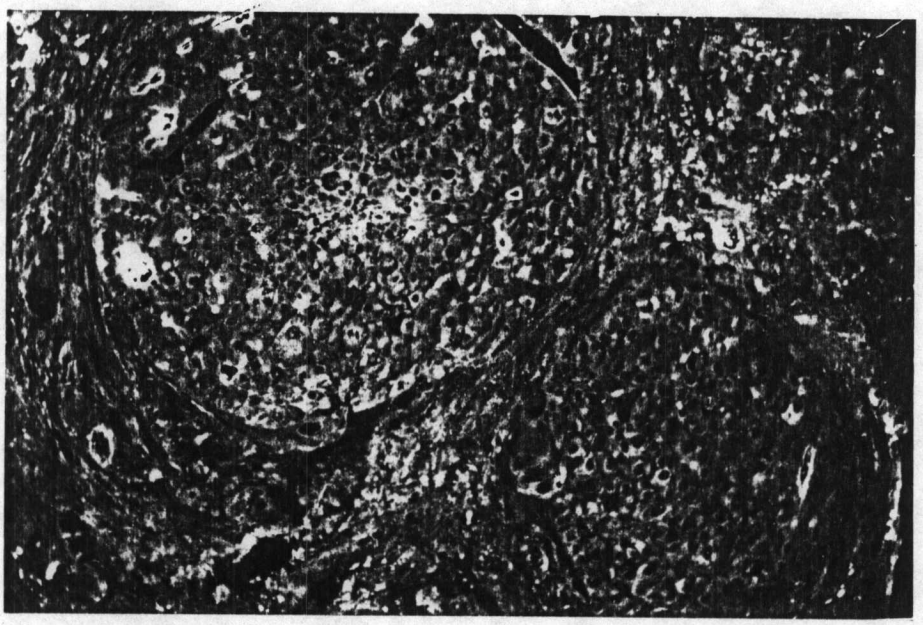

Fig. 12 Faint immunofluorescent staining of AFP in grade II, moderately differentiated carcinoma.(X 100)

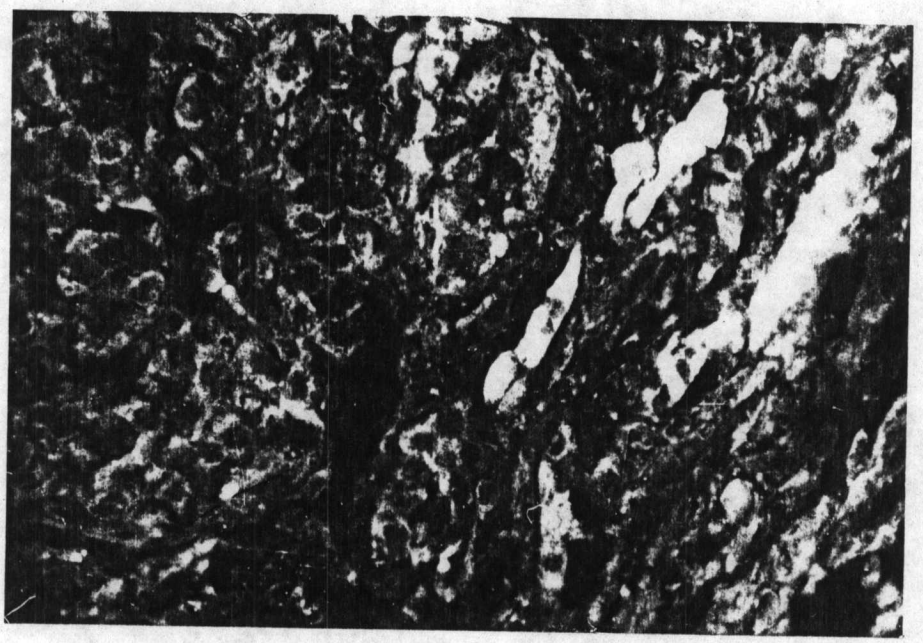

Fig. 13 A moderate type of immunofluorescence for AFP was shown in the cytoplasm and cytoplasmic membrane. There were small clusters of tumour cells showing increased amount of AFP. (X 400)

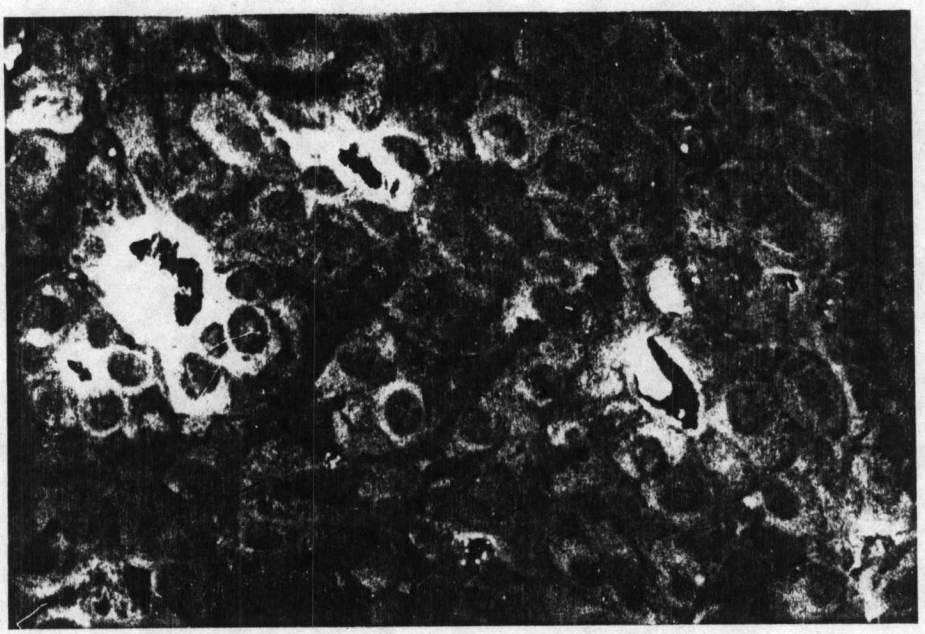

Fig. 14 Diffuse finely granular with marked fluorescence of the cytoplasm was found. The brighter cells were found usually near the blood vessels. (X 400)

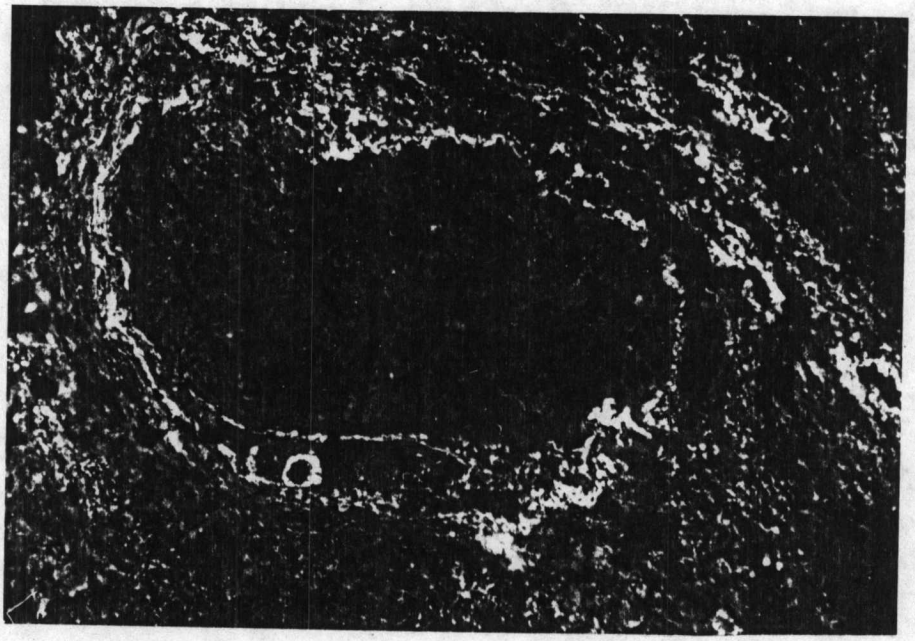

Fig. 15 Linear immunofluorescence for AFP was shown along the fibroblast cells in grade III, poorly differentiated carcinoma with positive serum-AFP. (X 100)

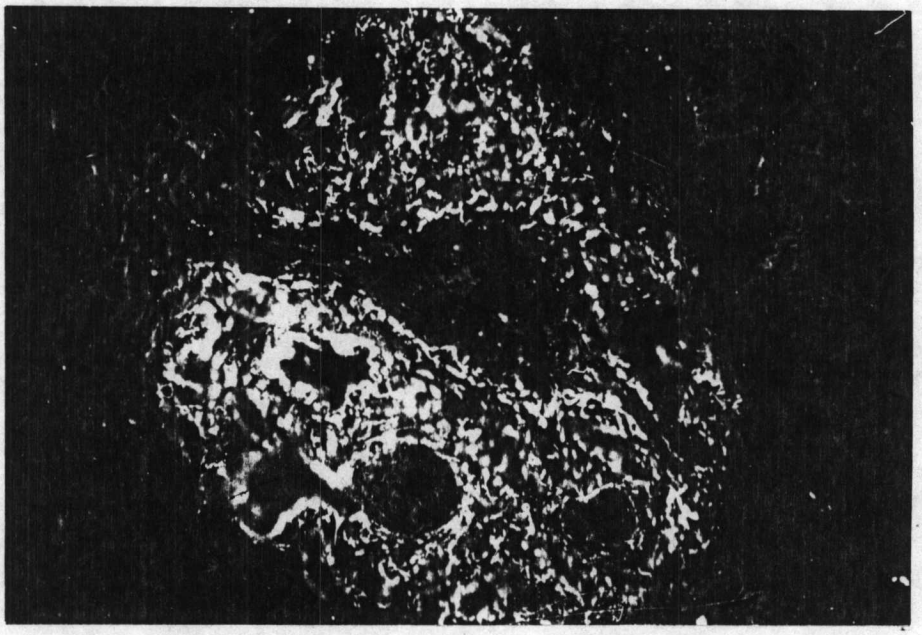

Fig. 16 Moderate intensity of AFP localization in metastatic carcinoma of the liver. (X 100)

AFP is detected in 12 of 14 patients (85.7 %) with histologically confirmed primary hepatocellular carcinoma. In Table 17, page 61,presents the histological gradings, serum AFP and the appearance of fluorescence.

AFP was also detected in 2 of 11 patients (18.2 %) with metastatic carcinoma of the liver, as shown in Table 18, page 62.

There was no fluorescence in the normal adult liver used for control.

As can be seen in Table 19, page 63,12 (85.7 %) of 14 cases with hepatocellular carcinoma were AFP-positive. Among these 14 cases, 6 cases (42.8 %) were grade II, and 7 (50 %) were grade III. The case of grade IV hepatocellular carcinoma was small in number. The highest incidence of AFP-positive was in grade II and grade III hepatocellular carcinomas, 5 of 6 cases (83.3 %) and 6 of 7 cases (85.7 %) respectively.

The results of AFP-positive localization of metastatic carcinoma of the liver are presented in Table 20, page 63, The AFP-positive was detected in only 2 of 11 cases.

In addition to Table 21, page 64,the relationship between the intensity of AFP localization and histological patterns of hepatocellular carcinoma are shown. Intense fluorescence in the cytoplasm of hepatocellular carcinoma, appearing in moderate intensity in high degree, where as 8 out of 14 cases (57.1 %).

Compared with hepatocellular carcinoma, metastatic carcinoma of the liver do not have bright fluorescence, as shown in Table 22, page 64. Particularly, intensity was in the fibroblasts only in 2 cases (18.2 %).

TABLE 17

Relationship between localization of AFP, serum AFP, and histological grading of primary hepatocellular carcinoma

Case No.	Histological grade	Serum AFP	Fluorescent intensity of tumour cells*
1	II	-	+++
2	III	-	++
3	II	-	-
4	III	+	-
5	III	+	++
6	III	+	++
7	II	-	+
8	II	+	++
9	III	-	+++
10	III	+	++
11	IV	+	+++
12	II	+	++
13	II	+	++
14	III	+	++

* +++ marked

++ moderate

\+ faint

\- negative

TABLE 18

Relationship between localization of AFP, serum AFP, and histological grading of metastatic carcinoma of the liver

Case No.	Histological grade	Serum AFP	Fluorescent intensity of tumour cells
1	Well differentiated	-	-
2	Moderately differentiated	-	+
3	Well differentiated	-	-
4	Moderately differentiated	-	-
5	Moderately differentiated	-	-
6	Moderately differentiated	-	+
7	Moderately differentiated	-	-
8	Moderately differentiated	-	-
9	Moderately differentiated	-	-
10	Moderately differentiated	-	-
11	Moderately differentiated	-	-

TABLE 19

Number of cases in each histological grade of hepatocellular carcinoma of AFP localization of tumour cells

Grade	I	II	III	IV	Total
AFP +	-	5	6	1	12 (86 %)
AFP -	-	1	1	0	2 (14 %)
Total	0	6 (43 %)	7 (50 %)	1 (7 %)	14 (100 %)

TABLE 20

Number of cases in each histological grade of metastatic carcinoma of the liver and AFP localization in tumour cells

Localization	Differentiation			Total
	Well	Moderate	Poor	
AFP +	-	2	-	2 (18.2 %)
AFP -	2	7	-	9 (81.8 %)
Total	2	9	0	11 (100 %)

TABLE 21

Intensity of AFP localization and histological grading of hepatocellular carcinoma

Grade	No. of cases	Intensity of fluorescence			
		+++	++	+	-
I	0	0	0	0	0
II	6	1	3	1	1
III	7	1	5	0	1
IV	1	1	0	0	0
Total	14	3	8	1	2

TABLE 22

Intensity of AFP localization and histological grading of metastatic carcinoma of the liver

Differentiation	No. of cases	Intensity of flourescence			
		+++	++	+	-
Well	2	0	0	0	2
Moderate	9	0	2	0	7
Poor	0	0	0	0	0
Total	11	0	2	0	9

3.3 Serum AFP

Serum AFP were determined in the Department of Medicine, Siriraj Hospital.

In 25 cases qualitative determination of serum AFP were done by counterimmunoelectrophoresis. The reaction was positive in 9 out of 25 patients (36.0 %). It was positive in 64.3 % for hepatocellular carcinoma. The results of sera AFP test for hepatocellular carcinoma are presented in Table 23, page 66.

It had been negative in sera of 11 patients with metastatic carcinoma of the liver.

Histopathological findings have no definite relation of the presence of AFP in the sera.

The present experiment reports the analysis of the intense fluorescent staining and the serum-AFP. The results are summarized in Table 24. There was no correlation between the serum-AFP and the occurrence of fluorescence in the tumour cells. Grade II and grade III hepatocellular carcinoma showed variable degrees of AFP production, with the high degree belongs to moderate intensity.

The comparative study of metastatic carcinoma of the liver are shown in Table 25, page 65. Intense fluorescence tended to occur in only 2 cases of moderately differentiated in association with moderate fluorescence, and the rest were negative both in sera and tumour cells.

TABLE 23

Histological grade of hepatocellular carcinoma correlated to serum AFP

Grade	I	II	III	IV	Total
Serum AFP +	0	3 (50 %)	5 (71.4 %)	1 (100 %)	9 (64.3 %)
Serum AFP -	0	3 (50 %)	2 (28.6 %)	0	5 (35.7 %)
Total	0	6 (100 %)	7 (100 %)	1 (100 %)	14 (100 %)

TABLE 24

Interrelation between serum AFP and the intensity of AFP in hepatocellular carcinoma

Grade	Intensity of fluorescence				Serum AFP
	+++	++	+	-	
II	+++				-
				-	-
			+		-
		++			+
		++			+
		++			+
III		++			-
				-	+
		++			+
		++			+
	+++				-
		++			+
		++			+
IV	+++				+

TABLE 25

Interrelation between serum AFP and the intensity of AFP in metastatic carcinoma of the liver.

Differentiation	Intensity of fluorescence				Serum AFP
	+++	++	+	-	
Well				-	-
				-	-
Moderate		++			-
				-	-
				-	-
		++			-
				-	-
				-	-
				-	-
				-	-
				-	-
				-	-