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Case Reports

Serum Alpha-Fetoprotein-Positive Gastric Carcinoid with Liver Metastasis

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Summary. The patient was a 60-year-old Japanese male. He complained of epigastralgia and right chest pain of 4 month's duration, and general malaise, nausea and vomiting of 2 month's duration. Physical examination revealed on the right third rib a tender mass with a diameter of 2 cm and hepatomegaly with a multi-nodular surface and red palms. There were no signs of carcinoid syndrome, such as cutaneous flushing. Laboratory examinations disclosed certain biochemical alterations; alkaline phosphatase 810 IU/l, γ-glutamyl transpeptidase (γ-GTP) 2090 IU/l, carcinoembryonic antigen (CEA) 23.5 ng/ml and α-fetoprotein (AFP) 6,800 ng/ml. Both HBs-Ag and HBs-Ab were negative. The patient died in a uremic state, with rapid increases of jaundice and ascites.

Autopsy revealed gastric carcinoid with extensive metastases to the liver and the bone marrow. Tumor cells showed argyrophilia but not argentaffinity. Immunofluorescence specific for AFP was positive in the hepatocytes, particularly those adjacent to the metastatic tumor cells but not in the tumor cells, either primary or secondary.

79 Cases reported in Japan of serum AFP-positive malignant tumor other than hepatocullular carcinoma and certain other malignancies of germ cell origin are reviewed and discussed.

Key words: α-fetoprotein (AFP) – Carcinoembryonic antigen (CEA) – Carcinoid (tumor) – Gastric cancer – Metastatic liver tumor.

Introduction

Alpha-fetoprotein (AFP) found in the serum of mice with primary liver cancer (Abelev et al., 1963) was originally detected in the serum of patients with hepatocellular carcinoma by Tatarinov (1964). Thereafter, the detection of AFP has

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been quite useful for diagnosis of hepatocellular carcinoma with exception of certain types of teratomatous or germ-cell derived malignancies, i. e., malignant teratoma and embryonal carcinoma (Abelev, 1968; Masopust et al., 1968). Since the report of "false-positive" cases by O'Conor et al. (1970), reports of serum AFP-positive malignant tumors other than hepatocellular carcinoma and teratoma group tumors have been accumulating in the literature (Alpert et al., 1971; Bernades, 1971; Kozower et al., 1971; Mehlman et al., 1971; Bierfeld et al., 1973; Montplaisir et al., 1973). This accumulation seems to have accerelated with the development of radioimmunoassay technique for detecting AFP. Most of these malignant tumors are adenocarcinomas of the stomach of endodermal origin with or without liver metastasis. This paper reports a case of serum AFP-positive carcinoid of the stomach, which is considered to be of neuroectodermal origin.

Case Report

A 60-year-old Japanese male was admitted to the Ehime University Hospital on July 25, 1978, with general malaise, nausea and vomiting of 2 month's duration. He had also complained of lumbago for 6 months as well as epigastralgia and right chest pains for 4 months. The blood pressure was 146/60 mmHg. No cutaneous flushing was seen. Physical examination revealed a tender mass of 2 by 2 cm on the right third rib. The liver was palpable seven finger breadths below the right costal margin, and was nodular and firm. Laboratory findings are shown in Table 1. Alpha-fetoprotein (AFP) determined by radioimmunoassay, were 6,800 ng/ml. Both hepatitis B surface antigen (HBs-Ag) and antibody for HBs-Ag (HBs-Ab) were negative. Concentrations of carcinoid-related amines, such as serotonine and 5-HIAA were not recorded in both blood and urine. X-ray examination of the stomach showed no significant alteration.

Just after admission, jaundice and ascites developed, followed by oliguria and hyperkaremia. The patient died in a uremic state on the 16th hospital day.

Table 1. Laboratory data on admission

		Biochemical exam.		Total protein	6.2 mg/dl
Urinalysis					
PH	6.0	Bilirubin	1.6 mg/dl	Albumin	50.8%
Protein	+	GOT	144 IU/l	α_1	6.4%
Sugar	−	GPT	58 IU/l	α_2	13.0%
Urobilinogen	±	ALP	890 IU/l	β	10.1%
Blood analysis		LDH	153 IU/l	γ	19.3%
RBC	$394 \times 10^4/mm^3$	γ-GTP	2,090 IU/l	ESR	27 (1°), 51 (2°)
Hb	12.2 g/dl	LAP	202 IU/l	CRP	++
Ht	37.6%	ChE	55 IU/l	RA	±
Thrombocyte	$14.5 \times 10^4/mm^3$	Cholesterol	266 mg/dl	HBsAg	−
Reticulocyte	$16^0/_{00}$	Uric acid	9.8 mg/dl	Anti-HBs	−
WBC	$6{,}000/mm^3$	Urea-N	34 mg/dl	CEA	23.5 ng/ml
Bleeding time	5 min	Creatinine	1.7 mg/dl	AFP	6,800 ng/ml
Coagulation time	7.5 min	Na	140 mEq/l		
		K	4.1 mEq/l		
		Cl	103 mEq/l		
		Ca	12.8 mg/dl		

Materials and Methods

In addition to routine H.E., P.A.S. and alcian blue stains, formalin-fixed materials were stained according to Masson-Fontana's method for argentaffinity and Grimelius' method for argyrophilia.

To demonstrate the localization of AFP-producing cells, paraffine sections from formalin-fixed tissues were examined by direct immunofluorescence technique using FITC-conjugated purified goat anti-human AFP or by an indirect immunofluorescence using goat anti-human AFP antibody followed by staining with FITC-conjugated rabbit anti-goat IgG according to the procedure of Purtilo and Yunis (1971). The specificity of these purified immunoreactive reagents were checked with immunoelectrophoresis and single radial immunodiffusion by the commercial company (Fujizoki Pharmaceut. Co., Tokyo). The specificity of the AFP immunofluorescence was evaluated by the following controls: The use of hepatoma positive for AFP as a positive control; the use of normal adult liver as a negative control, and the use of blocking tests which consisted of layering unconjugated goat anti-human AFP and unconjugated rabbit anti-goat IgG for direct and indirect techniques, respectively, before the respective FITC-conjugated antisera were treated.

Small pieces of formalin-fixed tumors were post-fixed with osmium tetroxide and were examined with a JEOL-100B electron microscope to demonstrate the specific granules of carcinoid tumor.

Results

Autopsy Findings

The stomach had two slightly elevated tumors on the mucosa at the pyloric anterior wall (Fig. 1). One tumor was 28 by 25 mm in diameter, yellowish-white in color and was slightly depressed in the central region with a well-defined margin; the other tumor was adjacent to the former and was 3 mm in diameter. Neither tumor seemed to have invaded the serosa. In addition to these tumors, a scarred ulcer was observed on the posterior wall of the corpus.

The liver was 3,365 g in weight. A surface view showed many metastatic nodules of various sizes up to 9 cm in diameter. The nodules were soft and white to yellowish or reddish in color. The smaller nodules were white, and changed to yellow as the size increased. The cut surface disclosed that a major portion of the liver was replaced by the metastatic nodules, but there was no definite necrosis (Fig. 2). There was no sign of cirrhosis in the remaining liver parenchyma.

There were metastases on the lymphnodes of the pyloric, hepatic hilar, peripancreatic and retroperitoneal regions.

Bone marrow metastasis was extensive. In addition to a tumor mass on the right third rib, the fourth to seventh ribs of both sides, sternum and vertebrae of Th8 and L3 were fragile with pyogenous marrow.

Microscopic Examinations

The larger tumor of the stomach consisting of cell nests of various structures invades into the muscularis propria (Fig. 3) but the smaller one remains in the submucosal tissue. The tumor cells are arranged predominantly in sharply defined islands and trabecular or ribbon-like structures forming an occasional anastomosing pattern (Fig. 4). Some portions of the tumors display distinct

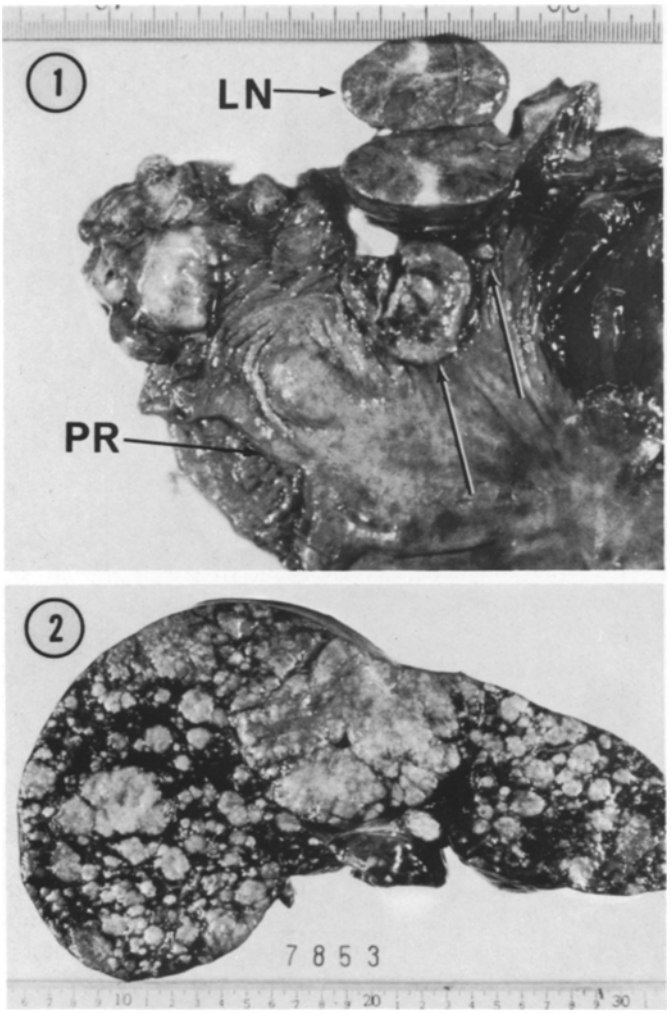

Fig. 1. The stomach has two slightly elevated tumors on the pyloric mucosa (*arrows*). *LN*, cut surface of a regional lymphnode involved, *PR*, pyloric ring

Fig. 2. Cut surface of the liver, a major portion of which is replaced by numerous metastatic nodules

glandular structures and rosette-like formations (Fig. 5). The lumen contains occasionally PAS-positive and alcian blue-weakly positive mucin.

The tumor cells of the liver metastasis show various patterns of arrangement as in the primary tumor of the stomach, although they tend to be arranged in trabecular fashion or as solid islands separated by narrow collagenous stroma by imitating hepatic cords (Fig. 6). Liver parenchymal cells adjacent to the metastatic nodules are compressed and atrophic with a pycnotic, small nucleus and eosinophilic, elongated cytoplasm.

The tumor cells of lymphnodes at the pyloric region have a clear and fairly

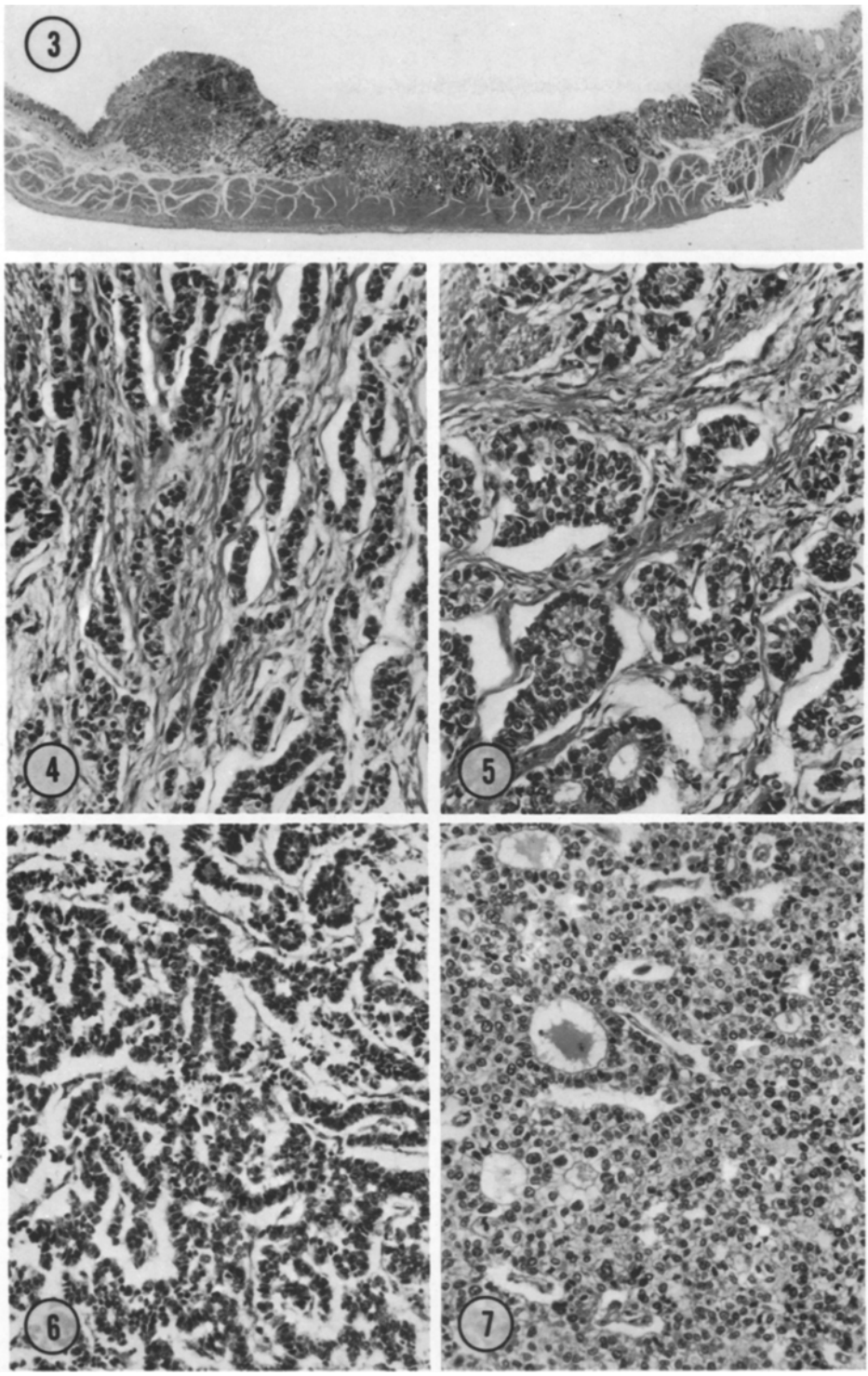

Fig. 3. Survey view of the larger tumor of the gastric carcinoid. ×4.4

Fig. 4. Gastric carcinoid, arranged in trabecular and ribbon-like structures. ×140

Fig. 5. Portion of the gastric carcinoid, showing a glandular appearance. ×180

Fig. 6. Metastatic hepatic tumor in anastomosing ribbon-like arrangement. ×150

Fig. 7. Metastatic carcinoid to the pyloric lymphnode. Clear, fairly abundant cytoplasm and glandular lumina are conspicuous. ×160

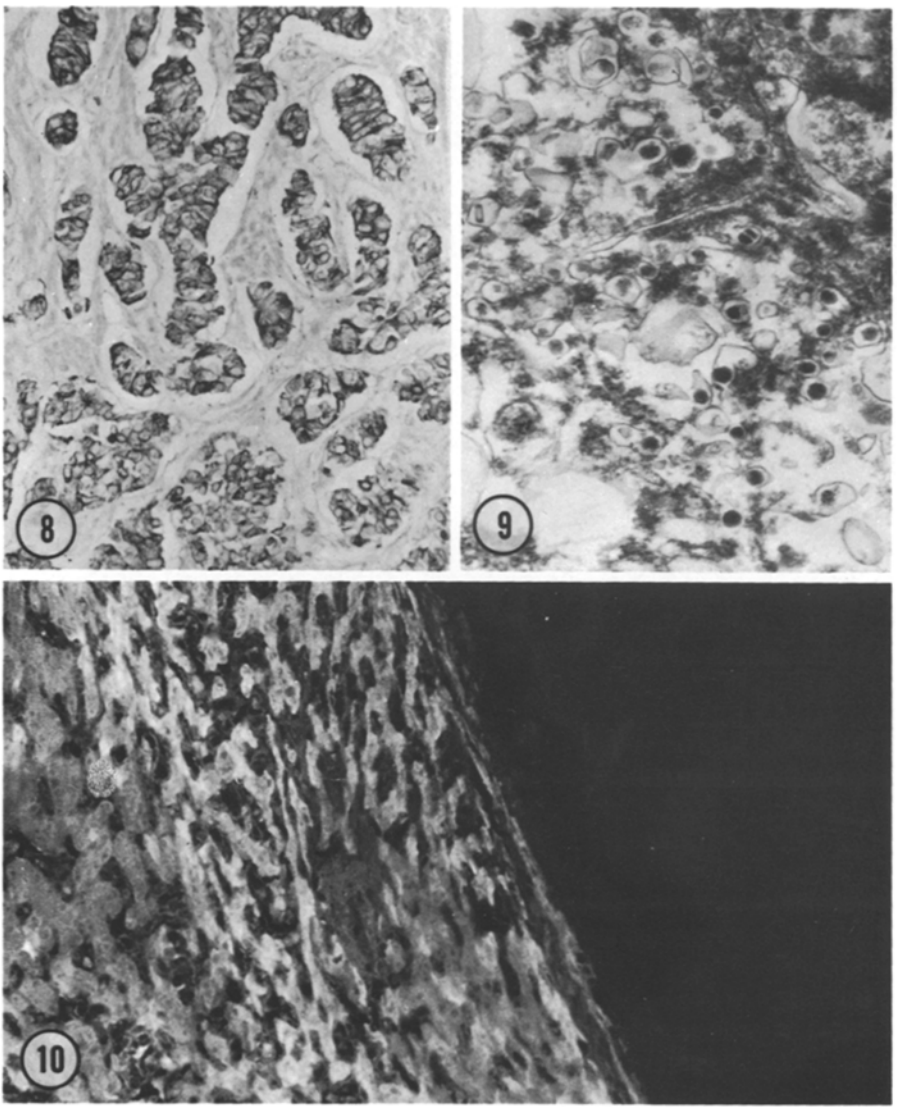

Fig. 8. Carcinoid cells of the stomach, arranged in trabecular pattern are stained intensely with Grimelius' silver impregnation. ×180

Fig. 9. Electron micrograph showing granules of the carcinoid. ×23,000

Fig. 10. Immunofluorescence for AFP is positive in the liver cells, particulary those adjacent to and compressed by the metastatic tumor cells. ×140

abundant cytoplasm and are arranged in medullary fashion with numerous glandular lumina of various sizes (Fig. 7). But those of the lymphnodes of the other regions and bone marrow of the various sites involved show under microscopy trabecular or ribbon-like structure with frequent anastomosis, similar to the prominent pattern of the gastric tumor.

None of the tumor cells is positive for Masson-Fontana's argentaffin stain. The cells of the gastric tumor arranged in trabecular pattern show a strongly positive reaction for Grimelius' stain (Fig. 8) but the cells of the glandular structure were occasionally positive. A few scattered cells of the lymphnodes and the hepatic metastatic tumors are also positive for argyrophilia.

Although the fine structure of the formalin-fixed materials is miserably deteriorated, dense granules can be seen in the tumor cells. The granules of secretory type are from 100 to 300 nm in diameter und are surrounded by a limiting membrane with a broad clear halo between the membrane and the dense core (Fig. 9). They are generally round or oval in shape but vary considerably in electron-density, probably because of poor fixation. The frequency of the occurrence of the granules seems to parallel the intensity of argyrophilia.

The immunofluorescence specific for AFP is strongly positive in the atrophic liver cells adjacent to the metastatic tumor cells, and is from moderately to weakly positive in the normal liver cells (Fig. 10). Although a very few cells in the tumor cell cords of the hepatic metastasis show positive immunofluorescence, none of the tumor cells in the primary gastric carcinoid and in the metastatic lesions other than the liver is positive for AFP.

Microscopic examination of both kidneys reveals the deposition of calcium in the urinary tubules, and desquamation and degeneration of the epithelial cells of the tubules. This deposited calcium seems to have derived from the bones, as shown by the extensive bone metastasis and hypercalcemia. These renal changes may explain a possible cause of oliguria and hyperkaremia just before the patient's death.

Discussion

After the original report of Tatarinov (1964), AFP has been a well-recognized oncofetal marker of hepatocellular carcinoma and malignant teratoma or embryonal carcinoma of germ cell origin (Abelev, 1968; Masopust et al., 1968). However, O'Conor et al. (1970), Bourreille et al. (1970) and Geffroy et al. (1970) detected AFP in the serum of patients with carcinomas of the stomach and the pancreas. After this, reports of serum AFP-positive carcinomas of certain organs have been accumulating in the literature.

In Japan, including the present case, 79 carcinomas of other than hepatocellular carcinoma and malignant teratoma or the embryonal carcinoma have been reported to be serum AFP-positive with a concentration of more than 1,000 ng/ml[1] (Kato et al., 1974; Nishimura et al., 1976; Noda et al., 1977; Okita et al., 1978; Miyasaka et al., 1978). The reason why so many cases were found in Japan may be explained by the fact that the detection of AFP is becoming a routine examination in clinics for digestive diseases, since hepatocellular carcinomas as well as gastric carcinomas are quite common in Japan.

[1] Detection of AFP was carried out by either micro-Ouchterlony, single radial immunodiffusion, immunoelectrosyneresis or radioimmunoassay. The concentration of 1,000 ng/ml or 0.1 mg/dl is the lowest limit in detecting AFP by the above methods, except radioimmunoassay, and is the highest limit for non-cancerous hepatic diseases

Table 2. Serum AFP-positive carcinomas other than hepatocellular carcinoma and malignant teratoma

Primary tumor	No. of cases[a]	No. of autopsy	Liver metastasis		
			+	−	Undetermined
Stomach	67 (84.8)	35	50	14	3
Pancreas	5 (6.3)	4	4	0	1
Gall bladder	2 (2.5)	1	2	0	0
Duodenum	1 (1.3)	1	1	0	0
Liver	1 (1.3)	1	1	0	0
Ovary	2 (2.5)	2	1	1	0
Undetermined	1 (1.3)	0	1	0	0
Total	79	44	60	15	4
(%)	(100.0)	(55.7)	(75.9)	(19.0)	(5.1)

[a] Percentage is given in parentheses

Table 3. Classification of gastric carcinomas with positive AFP in serum[a]

Macroscopy			Microscopy	
Classification		No. of cases[b]	Classification	No. of cases[b]
Borrmann	I	2 (5.7)	Adenocarcinoma	
	II	8 (22.9)	well, moderately differentiated	26 (74.2)
	III	22 (62.8)	poorly differentiated	2 (5.7)
	IV	0 (0.0)	undetermined	4 (11.4)
Undetermined		3 (8.6)	Carcinoid[c]	1 (2.9)
			Undifferentiated	1 (2.9)
			Undetermined	1 (2.9)
Total		35 (100.0)	Total	35 (100.0)

[a] The concentration is more than 1,000 ng/ml
[b] Percentage is given in parentheses
[c] Present case

Except for 2 ovarian carcinomas (granulosa cell carcinoma and clear cell carcinoma) and 1 carcinoma of uncertain origin, the great majority of those cases (76/79=96%, Table 2) consisted of carcinomas of endodermal tissues (stomach 67, duodenum 1, pancreas 5, gall bladder 2 and liver 1). In particular, gastric carcinoma occupied the largest population (84.8%). On a basis of autopsy of gastric carcinoma, 31 cases out of 35 (89%) had liver metastasis. Histological examination showed that the majority of those gastric carcinomas are adenocarcinoma (32/35=91%, Table 3). But there is no report of AFP-positive carcinoid either of the stomach or of other sites, in Japan, as well as in other countries.

The carcinoid is considered to arise from Kulchitsky cells (enterochromaffine cells), which means it is of neuroectodermal origin.

It is postulated that AFP can be produced by either primary tumor cells, or tumor cells which have metastasized to the liver and are transformed in the liver, or liver parenchymal cells (probably regenerating hepatocytes). In the present case, immunofluorescence specific for AFP was demonstrated in the liver cells, particularly those adjacent to the metastatic tumors. But a possibility remains that tumor cells, either primary or metastatic, can produce AFP, even though too small in quantity to be detected by immunofluorescence technique, since the tumor cells make up a large volume and occupy the major part of the enlarged liver.

No matter what cells produce AFP, it has been detected exclusively in patients with carcinomas of endodermal origin and germ cell origin. The appearance of AFP in the serum of this patient with carcinoid of neuroectodermal origin may be explained by the idea that the carcinoid consists of two cell types, neuroectodermal and endodermal in origin, the latter being the cell line for the usual adenocarcinoma. Another explanation comes from the possibility that the ectodermal cells may also produce AFP, since the amniotic fluid of the fetuses with developmental anomalies of the neuronal canal, such as anencephaly or spina bifida, contains AFP (Brock and Sutcliffe, 1972). Recently, Soltani et al. (1978) have demonstrated the localization of AFP in the epidermal cells of Bowen's disease by immunofluorescence.

Examination of additional carcinoids of this type may serve to establish whether or not the (neuro-)ectodermal tissues could produce AFP.

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