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Serum α-Fetoprotein in Patients with Neoplasms of the Gastrointestinal Tract[1]

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SUMMARY

Serum α-fetoprotein levels were measured by a sensitive double-antibody radioimmunoassay in 580 patients with a variety of malignant and nonmalignant gastrointestinal diseases to determine the incidence of levels elevated above 40 ng/ml. Over 200 normal control subjects have all had levels below 40 ng/ml. Fifteen % of 95 patients with gastric carcinoma, 3% of 191 patients with colorectal carcinoma, 24% of 45 patients with pancreatic carcinoma, 25% of 8 patients with biliary tract carcinoma, and 70% of 73 patients with hepatocellular carcinoma had elevated serum α-fetoprotein. None of 14 patients with esophageal or small bowel carcinoma had elevated levels. In contrast, 1% of 154 patients with nonmalignant, nonhepatic gastrointestinal disease had elevations of serum α-fetoprotein. α-Fetoprotein appears to be a potential marker for tumor activity in some patients with certain gastrointestinal cancers.

INTRODUCTION

AFP[3] was first described in the human fetus in 1956 (6) and became a marker protein for tumors with the demonstration of its reappearance coincident with the development of primary liver cancer in mice (1) and in humans (35). Quantitative assays for AFP have demonstrated the usefulness of this protein for monitoring as well as diagnosis in patients with hepatocellular carcinoma (23, 29). The development of radioimmunoassays for AFP (11, 15, 31-33, 36) has increased the sensitivity of the assay 100- to 1000-fold and has increased its usefulness for describing tumor activity correlated with serum AFP level (A. Primack, K. R. McIntire, C. L. Vogel, and T. A. Waldmann, Effect of Treatment on α-Fetoprotein Levels in Patients with Hepatocellular Carcinoma. Presented at the Third Embryonic and Fetal Antigens in Cancer Conference, November 4 to 7, 1973; Refs. 27 and 30). The assay of serum AFP in embryonal cell carcinoma (2, 22) has provided a 2nd valuable marker, in addition to human chorionic gonadotropin (19), for evaluating the effectiveness of therapy of that tumor (10, 12).

AFP was initially thought to be specific for hepatocellular carcinoma and embryonal cell carcinoma (3). It has occasionally been reported in other primary neoplasms, most often associated with the gastrointestinal tract (28), but one of the chief advantages of this oncofetal marker protein was the rarity of these so-called "false" positive reactions (3). Recently, there have been several reports of AFP associated with gastric cancer, with and without metastases to the liver (4, 5, 7, 9, 13, 18, 24, 26).

We have examined the sera of 426 patients with a variety of gastrointestinal neoplasms as well as appropriate benign disease controls with a sensitive radioimmunoassay for AFP to determine the incidence of AFP elevation in these conditions.

MATERIALS AND METHODS

AFP Radioimmunoassay. The double-antibody radioimmunoassay has been described previously (37). The specific antiserum to AFP was the generous gift of Dr. P. P. Sizaret, International Agency for Research on Cancer (34). AFP was purified from both fetal plasma and from plasma of a patient with hepatocellular carcinoma, using the procedure of specific antibody precipitation and dissociation of the immune complex (26). The purity of the antigen was checked by radioimmunoelectrophoresis (37) after labeling with ^{125}I by a modification of the chloramine-T method (16). A standard curve was constructed for every assay, and the lower limit of the sensitivity of the assay was 5 ng/ml. The standard curve demonstrated linearity between 2 ng and 17.5 ng/reaction tube, corresponding to 20 to 175 ng AFP per ml of serum. All of the more than 200 normal individuals, each over 1 year of age, studied had a serum level less than 30 ng/ml, and most of these were below 20 ng/ml. To avoid the ambiguity of slight serum AFP elevations occasionally seen in various acute and chronic (nonhepatic) diseases and to make a meaningful discrimination between benign and malignant disease, levels greater than 40 ng/ml were considered significantly elevated. All samples were run in duplicate and those above 40 ng/ml were run in quadruplicate. The coefficient of variation between assays was 13.4% at the level of 33 ng/ml and 9.7% at 107 ng/ml.

Serum Samples. Patients with neoplasms were primarily from the Mayo Clinic and the NIH and had histologically

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[3] The abbreviations used are: AFP, α-fetoprotein; 5-FU, 5-fluorouracil; methyl-CCNU, methyl cyclohexylchloroethylnitrosourea.

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proven cancer. Patients with nonneoplastic gastrointestinal disease were all studied at the Mayo Clinic.

Immunological Studies. Antigenic comparison of serum AFP in different disease conditions was made by double diffusion in agarose using a specific horse anti-AFP.

RESULTS

Controls. Serum samples from 180 normal adults and 20 normal children (1 to 10 years of age) were measured for AFP levels and all were less than 40 ng/ml. Patients with nonneoplastic, nonhepatic disease of the gastrointestinal tract served as additional controls and are shown in Table 1. Only 2 of these 154 patients had an elevated AFP concentration (higher than 40 ng/ml). One patient with regional enteritis and complete bowel obstruction had a level of 56 ng/ml. One patient with *in situ* cellular atypia in an adenomatous colonic polyp had a minimal elevation at 44 ng/ml. Thus, AFP is only rarely elevated and only to a minor degree in patients with nonmalignant, nonhepatic diseases of the gastrointestinal tract.

Patients with Gastrointestinal Neoplasms. The serum of 426 patients with primary neoplastic disease of the gastrointestinal tract were assayed for AFP. The incidence of initial AFP levels higher than 40 ng/ml is shown in Table 2, and the relative levels of AFP are depicted in Chart 1. An elevated AFP was demonstrated in 24% of 45 patients with pancreatic carcinoma, 25% of 8 patients with carcinoma of the biliary tract, 15% of 95 patients with gastric carcinoma, and 3% of 191 patients with carcinoma of the colon or rectum. None of the 14 patients with esophageal or small-bowel carcinoma had elevated serum AFP levels. For comparison, 70% of the 73 patients with primary liver cancer had elevated AFP levels.

A summary of patients with gastric carcinoma and initial serum levels greater than 40 ng/ml is given in Table 3. Nine of the 14 patients had either histological or surgical evidence of metastases to the liver or abnormal liver function studies (elevated Bromsulphalein or alkaline phosphatase). The other 5 patients did not appear to have metastases to the liver. Chart 2 shows the association of serum AFP with the clinical course in 1 patient (Patient 7) with an unresectable carcinoma of the cardia with moderately severe dysphagia and laboratory evidence of hepatic metastases. She had no measurable indicator lesion but achieved a very striking clinical response to 5-FU therapy, with complete relief of dysphagia and accompanying weight gain as well as return to normal of alkaline phosphatase and serum glutamic oxalacetic transaminase. The 1st serum AFP level was just before a scheduled dose of 5-FU, and the next level was

Table 1

Occurrence of elevated serum AFP (>40 ng/ml) in nonneoplastic diseases of the gastrointestinal tract

Patient condition	No. of patients	No. with elevated AFP
Normal adult	180	0
Normal children[a]	20	0
Hiatal hernia	3	0
Hypertrophic gastric rugae	2	0
Gastritis	6	0
Gastric ulcer	12	0
Duodenal ulcer	31	0
Regional enteritis	19	1
Granulomatous colitis	11	0
Chronic ulcerative colitis	29	0
Diverticulitis	3	0
Chronic diarrhea	9	0
Polyps of colon	25	1
Cholecystitis	1	0
Pancreatitis	3	0

[a] One to 10 years of age.

Table 2

Occurrence of elevated serum AFP (>40 ng/ml) in patients with gastrointestinal tract neoplasia

Primary tumor site	No. of patients	No. elevated	% elevated
Esophagus	4	0	0
Stomach	95	14	15
Small bowel	10	0	0
Colon-rectum	191	5	3
Pancreas	45	11	24
Biliary tract	8	2	25
Liver	73	51	70

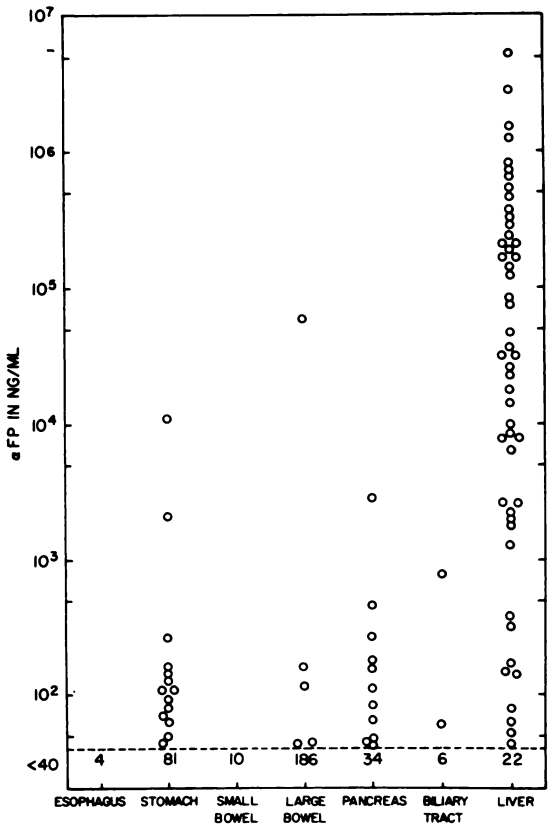

Chart 1. Distribution of initial serum AFP (αFP) levels in 426 patients with gastrointestinal cancer plotted in log scale. ----, upper limit of normal.

Table 3
Carcinoma of the stomach

Patient	Age	Sex	Liver metastases	Abnormal liver function test	AFP (ng/ml)
1	70	M	+	–	110
2	68	M	+	–	260
3	56	F	–	–	45
4	76	F	+	+	78
5	42	M	+	+	78
6	51	M	–	–	94
7	67	F	–	–	10,300
8	37	M	+	+	70
9	68	F	+	+	68
10	57	M	–	+	44
11	62	M	+	–	2,100
12	80	F	+	+	70
13	64	M	–	–	74
14	72	M	–	–	43

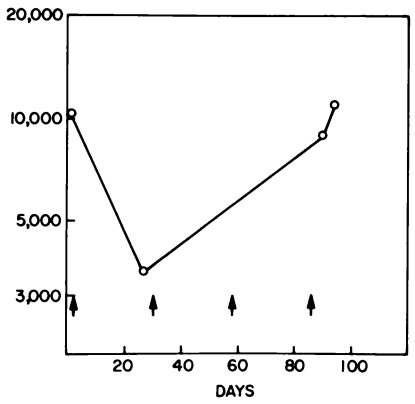

Chart 2. Change in serum AFP level of a patient with gastric carcinoma during therapy with 5-FU. This is Patient 7 in Table 3; *arrows*, times of therapy.

Table 4
Carcinoma of the colon

Patient	Age	Sex	Liver metastases	Liver function test	AFP (ng/ml)
1	47	F	+	+	160
2	70	M	–	+	44
3	65	M	+	–	60,000
4	69	F	–	–	44
5	60	M	+	–	115

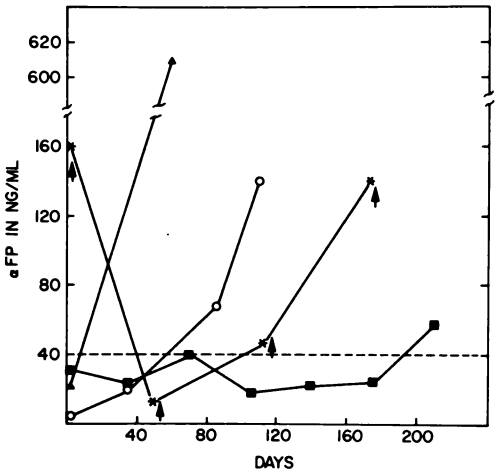

Chart 3. Change in serum AFP (*αFP*) levels in patients with colon carcinoma. *, serum levels of Patient 1 in Table 4; *arrows*, therapy with methyl-CCNU (225 mg/sq m). The other 3 patients are not included in Table 4 because the initial serum AFP level was below the upper limit of normal, 40 ng/ml (- - - -).

obtained at the time of greatest antineoplastic effect and just before another scheduled treatment. Two months later her serum AFP level had risen to the previous levels and she had evidence of progressive disease, which was no longer responsive to 5-FU therapy.

Patients with carcinoma of the colon or rectum with initial serum AFP levels greater than 40 are shown in Table 4. Three of these 5 patients had surgical evidence of hepatic metastases or abnormal liver function tests. Changes in serum AFP during the course of the disease for 4 patients are shown in Chart 3. Three patients, not included in Table 4, showed a rising serum AFP associated with evidence of progressive disease. Two of these who later developed a rise in AFP after initial normal values had evidence of hepatic metastases; 1 patient, however, had no evidence of liver involvement at later necropsy examination. The 4th patient was a 47-year-old woman with a previously resected carcinoma of the colon followed by biopsy-proven hepatic metastases. She had gross nodular hepatomegaly and an AFP value of 160 ng/ml. Following treatment with a single p.o. dose of methyl-CCNU, she had a striking remission of her malignant disease. The liver was no longer palpable. The patient became asymptomatic, resumed normal activity, and her serum alkaline phosphatase level fell from 171 units to 71 units (normal, <60 units). Her AFP level fell to 13 ng/ml. Methyl-CCNU was continued at 8-week intervals. Six months later she remained asymptomatic and the liver was not palpable. Her alkaline phosphatase level, however, had risen to 264 ng/ml and her AFP level had risen to 140 ng/ml. Over the next 8 weeks, she progressively deteriorated, in spite of continued methyl-CCNU therapy. She again developed gross hepatomegaly, and she died shortly thereafter.

Eleven patients with carcinoma of the pancreas and serum AFP levels higher than 40 ng/ml are shown in Table 5. All except 1 of these patients had evidence of hepatic metastases. Both islet cell carcinoma and carcinoma of the exocrine pancreas were represented in those found to have elevated AFP.

With an antiserum specific for normal human fetal AFP, it was demonstrated that the protein responsible for elevated serum levels in carcinoma of the stomach, pancreas, and colon was immunologically identical to AFP in serum of patients with hepatocellular carcinoma, embryonal cell carcinoma, and fetal serum (Chart 4).

Table 5
Carcinoma of the pancreas

Patient	Age	Sex	Liver metastases	Liver function tests	AFP	Type
1	57	F	+	+	2800	Islet cell
2	50	F	+	+	44	Islet cell
3	60	F	+	+	470	Adenocarcinoma
4	59	M	–	–	180	Adenocarcinoma
5	78	M	+	+	110	Adenocarcinoma
6	51	M	–	–	42	Adenocarcinoma
7	62	M	+	+	86	Adenocarcinoma
8	46	M	–	+	64	Adenocarcinoma
9	68	F	+	+	270	Adenocarcinoma
10	61	M	+	+	154	Adenocarcinoma
11	69	M	+	+	43	Adenocarcinoma

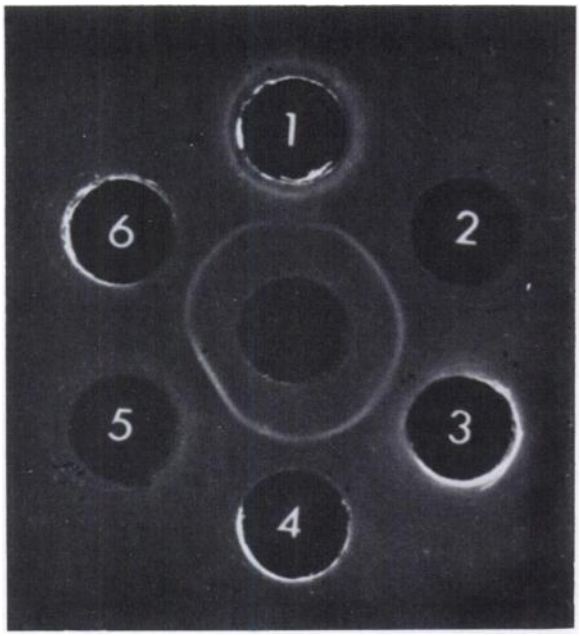

Chart 4. Double diffusion in agar identification of AFP in different conditions. *Center well* contains horse anti-AFP. Other wells contain serum as follows: *Well 1*, hepatocellular carcinoma; *Well 2*, gastric carcinoma; *Well 3*, embryonal cell carcinoma of testis; *Well 4*, pancreatic carcinoma; *Well 5*, fetal serum; *Well 6*, colon carcinoma. AFP in each instance gives a reaction of antigenic identity with the 2 adjacent serums.

DISCUSSION

The occurrence of elevated levels of serum α-fetoprotein has been reported with several tumors other than hepatocellular carcinoma and embryonal cell carcinoma. These have largely been associated with neoplasms of the gastrointestinal tract (3–5, 7, 9, 12, 13, 18, 28). We have shown that there is a significant elevation of AFP in patients with gastrointestinal neoplasia and that the incidence of elevation is dependent upon the site of origin. Patients with carcinoma of the pancreas, biliary tract, and stomach were found to have elevations with a greater incidence than did those with carcinoma of colon, esophagus, and small bowel. Many patients, but not all, were followed with serial serum AFP determinations, and in this group an additional 2 patients with gastric carcinoma and 3 with carcinoma of the colon were found to have elevations of AFP, although they were not elevated on initial examination (see Chart 3). Almost all of the patients with elevated serum AFP who did not have hepatocellular carcinoma (35 of 37) had levels below 10,000 ng/ml, which is below the level detected by conventional double diffusion in agar (32). In other words, if these 426 patients with gastrointestinal neoplasia had been tested by conventional qualitative methods, only 2 would have been detected as having serum AFP present.

All 23 patients with nonneoplastic gastric disease studied had serum AFP levels under 26 ng/ml, whereas 14 of the 95 patients with gastric carcinoma had initial levels above 40 ng/ml. Only 3 patients with pancreatitis and 1 with cholecystitis were assayed, but none had AFP levels above 11 ng/ml, while one-fourth of the patients with carcinoma of the pancreas and biliary tract had levels higher than 40 ng/ml.

Five of 191 patients with carcinoma of the colon or rectum had serum AFP higher than 40 ng/ml, but only 1 of 53 patients with chronic nonneoplastic disease of the large bowel had AFP greater than 38 ng/ml. One of 25 patients with adenomatous polyps had minimal AFP elevation. Studies of serum AFP in nonmalignant, hepatic disease have demonstrated levels higher than 40 ng/ml in 23% of 373 patients (8). The highest incidence and the highest values of AFP were found in viral subacute hepatic necrosis, and no elevations were seen among 42 patients with inactive Laennec's cirrhosis or fatty liver.

Most of the patients with malignant disease reported in this study had evidence of advanced disease, *i.e.*, regional extension or distant metastatic involvement (not necessarily hepatic). The AFP serum levels were those obtained before therapy, either surgery or chemotherapy. Five patients with colorectal carcinoma, 14 patients with gastric carcinoma, and 2 patients with pancreatic carcinoma had only local disease. The remaining patients all had disease spread beyond the organ of origin. All those with elevated AFP were in this category of nonlocalized disease, but at least 8 such patients had no evidence of liver metastases, and it is highly unlikely that occult disease of the liver could produce

serum elevations as high as 10,300 ng/ml (Table 3, Patient 7). Additionally, we have examined serums from over 300 patients with nonentodermally derived neoplasms (breast, kidney, and bladder), many of these having metastatic involvement of the liver but none having elevated levels of AFP. Other studies reporting the incidence of elevated AFP in a variety of cancers other than hepatocellular or testicular cancer have either had small numbers of patients with no breakdown as to organ-site involvement (11, 27) or had suggestive evidence that some neoplasms of the gastrointestinal tract can be associated with elevated levels of AFP (4, 17, 33).

AFP may or may not be a useful marker for the early diagnosis of gastrointestinal cancer, but for certain patients with advanced cancer, especially gastric or pancreatic, it can serve as a serum marker to indicate the extent of disease. Further work is needed to develop the degree of correlation between rising serum AFP levels and increasing tumor cell number.

The demonstration of elevated serum AFP associated with different malignant conditions of the gastrointestinal tract in addition to hepatocellular carcinoma is similar to the identification of elevated carcinoembryonic antigen associated with neoplastic conditions other than carcinoma of the colon (20, 21, 25), for which it was thought to be specific (14). In contrast to the observations with carcinoembryonic antigen, there are very few patients with benign nonhepatic disease and an elevated AFP level.

In the case of AFP, serum levels in some patients have been sufficiently high to allow the demonstration of antigenic identity among the proteins called AFP in the different types of cancer and other conditions where it occurs.

There are now several marker antigens that can be measured in the serum by very sensitive and accurate radioimmunoassays that appear to be elevated in a significant number of patients with neoplastic disease, especially those of the gastrointestinal tract. The combined use of a battery of such assays may provide new criteria for tumor evaluation and allow a more intelligent approach to chemotherapy and immunotherapy, both for earlier instigation of therapy and for monitoring the effectiveness of therapy.

REFERENCES

1. Abelev, G. I. Study of the Antigenic Structure of Tumors. Acta Unio Intern. Contra Cancrum, *19:* 80–92, 1963.
2. Abelev, G. I. Production of Embryonal Serum α-Globulin by Hepatomas. Review of Experimental and Clinical Data. Cancer Res., *28:* 1344–1350, 1968.
3. Abelev, G. I. Alpha-Fetoprotein in Ontogenesis and its Association with Malignant Tumors. Advan. Cancer Res., *14:* 295–358, 1971.
4. Akai, S., and Koto, K. Serum α-Fetoprotein-Positive Cancers of the Stomach Other Than Hepatoma. Gann Monograph, *14:* 149–154, 1973.
5. Alpert, E., Pinn, V. W., and Isselbacher, K. J. Alpha-Fetoprotein in a Patient with Gastric Carcinoma Metastatic to the Liver. N. Engl. J. Med., *285:* 1058–1059, 1971.
6. Bergstrand, C. G., and Czar, D. Demonstration of a New Protein Fraction in Serum from the Human Fetus. Scand. J. Clin. Lab. Invest., *8:* 174, 1956.
7. Bernades, P., Smadja, M., Rueff, B., Bonnefond, R., Tursz, T., Martin, E., Bognel, C., Barge, J., and Uriel, J. Presence de l'Alpha-Foetoproteine Serique dans Quatre Cas de Cancers Digestifs autres que l'Hepatome. Presse Med., *79:* 1585–1587, 1971.
8. Bloomer, J. R., Waldmann, T. A., McIntire, K. R., and Klatskin, G. Serum α-Fetoprotein Levels in Patients with Non-Neoplastic Liver Diseases. Gastroenterology, *65:* 530, 1973.
9. Boureile, J., Metayer, P., and Suager, F. Existance d'Alpha Fetoproteine au Cours d'un Cancer Secondaire du Foie d'Origine Gastrique. Presse Med., *78:* 1277–1278, 1970.
10. Braunstein, G. C., McIntire, K. R., and Waldmann, T. A. Discordance of Human Chorionic Gonadotropin and Alpha-Fetoprotein in Testicular Teratocarcinoma. Cancer, *31:* 1065–1068, 1973.
11. Chayvialle, J. A. P., and Ganguli, P. C. Radioimmunoassay of Alpha-Fetoprotein in Human Plasma. Lancet, *1:* 1355–1357, 1973.
12. Esterhay, R. J., Shapiro, H. M., Sutherland, J. C., McIntire, K. R., and Wiernik, P. H. Serum Alpha-Fetoprotein Concentration and Tumor Growth Dissociation in a Patient with Ovarian Teratocarcinoma. Cancer, *31:* 835–839, 1973.
13. Geffroy, T., Metayer, P., Denis, P., Philippe, J., Matray, F., and Sauger, F. Alpha Foeto-proteine et Cancer Secondaire du Foie. Presse Med., *78:* 1896, 1970.
14. Gold, P., and Freedman, S. O. Demonstration of Tumor-Specific Antigens in Human Colonic Carcinomata by Immunological Tolerance and Absorption Technique. J. Exptl. Med., *121:* 439–462, 1965.
15. Hirai, H., Nishi, S., and Watabe, H. Radioimmunoassay of α-Fetoprotein. Protides of the Biological Fluids, Pergamon Press, *20:* 579–587, 1972.
16. Hunter, W. M., and Greenwood, F. C. Preparation of Iodine^{131} Labeled Growth Hormone of High Specific Activity. Nature, *194:* 495–496, 1962.
17. Ishi, M. Radioimmunoassay of α-Fetoprotein. Gann Monograph, *14:* 89–98, 1973.
18. Kozower, M., Fawaz, K. A., Miller, H. M., and Kaplan, M. M. Positive Alpha-Fetoglobulin in a Case of Gastric Carcinoma. N. Engl. J. Med., *285:* 1059–1060, 1971.
19. Li, M. C. Trophoblastic Disease. Natural History, Diagnosis and Treatment. Ann. Internal Med., *74:* 102–112, 1971.
20. Lo Gerfo, P., Krupey, J., and Hansen, H. J. Demonstration of an Antigen Common to Several Varieties of Neoplasia. N. Engl. J. Med., *285:* 138–141, 1971.
21. Martin, F., Martin, M. S., Bordes, M., and Bourgeaux, C. The Specificity of Carcino-Foetal Antigens of the Human Digestive Tract Tumors. European J. Cancer, *8:* 315–321, 1972.
22. Masopust, J., Kithier, K., Rádl, J., Koutecky, J., and Kotal, L. Occurrence of Fetoprotein in Patients with Neoplasms and Nonneoplastic Diseases. Intern. J. Cancer, *3:* 364–373, 1968.
23. McIntire, K. R., Vogel, C. L., Princler, G. P., and Patel, I. R. Serum α-Fetoprotein as a Biochemical Marker for Hepatocellular Carcinoma. Cancer Res., *32:* 1941–1946, 1972.
24. Mehlman, D. J., Bulkley, B. H., and Wiernik, P. H. Serum Alpha$_1$-Fetoglobulin with Gastric and Prostatic Carcinomas. N. Engl. J. Med., *285:* 1060–1061, 1971.
25. Moore, T. H., Kupchik, H. Z., Marcon, N., and Zamcheck, N. Carcinoembryonic Antigen Assay in Cancer of the Colon and Pancreas and Other Digestive Tract Disorders. Am. J. Digest. Diseases, *16:* 1–7, 1971.
26. Nishi, S. Isolation and Characterization of a Human Fetal α-Globulin from the Sera of Fetuses and a Hepatoma Patient. Cancer Res., *30:* 2507–2513, 1970.
27. Nishi, S., and Hirai, H. Radioimmunoassay of α-Fetoprotein in Hepatoma, Other Liver Diseases and Pregnancy. Gann Monograph, *14:* 79–85, 1973.

28. O'Connor, G. T., Tatarinov, Y. S., Abelev, G. I., and Uriel, J. A Collaborative Study for the Evaluation of a Serological Test for Primary Liver Cancer. Cancer, *25:* 1091–1998, 1970.
29. Purves, L. R., Bersohn, I., and Geddes, E. W. Serum Alpha-Feto-Protein and Primary Cancer of the Liver in Man. Cancer, *25:* 1261–1270, 1970.
30. Purves, L. R., Branch, W. R., Geddes, E. W., Manso, C., and Portugal, M. Serum Values in Normal People, Pregnant Women and Primary Liver Cancer High Risk Populations. Cancer, *31:* 578–587, 1973.
31. Purves, L. R., and Purves, M. Serum Alpha-Fetoprotein. IV. The Radioimmunoassay. Levels in Normal People and in Pregnancy. S. Afr. Med. J., *46:* 1290–1297, 1972.
32. Ruoslahti, E., and Seppälä, M. Studies of Carcino-Fetal Proteins. III. Development of a Radioimmunoassay for α-Fetoprotein in Serum of Healthy Human Adults. Intern. J. Cancer, *8 :* 374–383, 1971.
33. Silver, H. K. B., Gold, P., Feder, S., Freedman, S. O., and Shuster, J. Radioimmunoassay for Human $Alpha_1$-Fetoprotein. Proc. Natl. Acad. Sci. U.S., *70:* 526–530, 1973.
34. Sizaret, P. P., McIntire, K. R., and Princler, G. L. Quantitation of Human α-Fetoprotein by Electroimmunodiffusion. Cancer Res., *31:* 1899–1902, 1971.
35. Tatarinov, Y. S. Detection of Embryospecific α-Globulin in Sera of Patient with Primary Liver Tumor. Vopr. Med. Khim., *10:* 90–91, 1964.
36. Waldmann, T. A., and McIntire, K. R. Serum-Alpha-Fetoprotein Levels in Patients with Ataxia-Telangiectasia. Lancet, *2:* 1112–1115, 1972.
37. Yagi, T., Maier, P., and Pressman, D. Multiplicity of Insulin Binding Antibodies in Human Sera. J. Immunol., *90:* 760–769, 1963.