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Alpha-feto-protein during development and in disease

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In fetal life, as the tissues develop and mature, a variety of biochemical modifications occur. There is a switch from the synthesis of a number of specific proteins and cellular antigens to the production of other substances with similar biological properties but a different structure. Some proteins and specific antigens are synthesized predominantly during particular phases of development. These are usually referred to as phase-specific antigens (PSA).

Current interest in phase-specific antigens has been stimulated by observations on the biochemical changes which occur in certain forms of cancer and other diseases. These changes may lead to the synthesis, in adult life, of proteins or other antigens which are otherwise produced mainly during the early stages of development. Studies of the mode of activation of the genes which control the synthesis of phase-specific antigens are therefore of great biological interest in clinical medicine. It is now evident that the detection of PSA may also provide important clinical information regarding the diagnosis of tumours and in monitoring progress and response to therapy.

The concept that cancer and embryonic cells have much in common is an old idea. The morphological resemblance between cancer cells and the cells of fetal tissues has been repeatedly discussed in the pathological literature. In 1932, Hirszfeld and his collaborators published the first immunological data suggesting a relationship between tumour and embryonic antigens. More recently, Prehn (1967a; 1967b) was able to show that the growth of syngeneic tumour transplants could be inhibited by previous immunization with embryonic tissues. This work has now been expanded to other tumours induced by viruses or by chemicals (Anderson and Coggin, 1971; 1972), and interesting similarities between tumour and embryonic cells have also been detected using plant agglutinins (Marx, 1974). The thesis that cancer is a disease of the mechanism of differentiation is strongly supported by the relationship between teratogenesis and oncogenesis.

In recent years, a human fetal plasma protein (alpha-feto-protein, AFP) has been found to be present in high concentration in many patients with primary hepatic tumours or with teratoblastoma. The detection of this protein has become a well-recognized aid to the diagnosis of these diseases (Abelev, 1971; Masseyeff, 1972; Abelev, 1973). Several fetal antigens other than AFP have also been found to be produced by cancer cells. Some are released in the circulation and others are mainly expressed on the cell surface (Alexander, 1972). This paper will analyse some of the biological and physicochemical properties of AFP, one of the first phase-specific antigens to be associated with cancer. It will also discuss how the estimation of AFP in amniotic fluid may help in the diagnosis of specific fetal malformation or fetal distress.

Methods of detection

Several immunological techniques have been used to detect and measure AFP in human sera, each with its own advantages (Table I). By immunoelectrophoresis the AFP appears as a line of precipitation in the α_1-globulin region (Fig. 1). Counter-current, cross-over electrophoresis (Kohn, 1970) provides a rapid and sensitive method for the demonstration of concentrations higher than 250 ng/ml. The 'rocket technique' or electroimmunodiffusion (Laurell, 1966) has been shown to be capable of detecting 0.5–1.0 μg/ml and two recent modifications have increased its sensitivity to 50 ng/ml (Nørgaard-Pedersen, 1973; Alpert *et al*, 1974). The commercially available radial diffusion technique, though less sensitive, can still measure concentrations in excess of 2.5 μg/ml. Recently, the

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TABLE I

SOME IMMUNOLOGICAL METHODS FOR THE DETECTION AND ESTIMATION OF AFP*

Methods	Sensitivity†	Application
Double diffusion in agar gel	2–5 μg/ml	Detection
Counter-current electrophoresis	0.25–0.5 μg/ml	Detection; semi-quantitative
Single radial diffusion	2.5 μg/ml	Quantitative
Electroimmunodiffusion (rocket)	0.5–1.0 μg/ml (50 ng/ml)‡	Quantitative
Radioimmunoassay	2–5 ng/ml	Quantitative
Enzyme-linked immunoassay	5 ng/ml	Quantitative
Reverse agglutination	5 ng/ml	Quantitative

* For references see text.
† Lowest sensitivity using conventional technique.
‡ The sensitivity is increased using an immunoradioassay or an immunoperoxidase test.

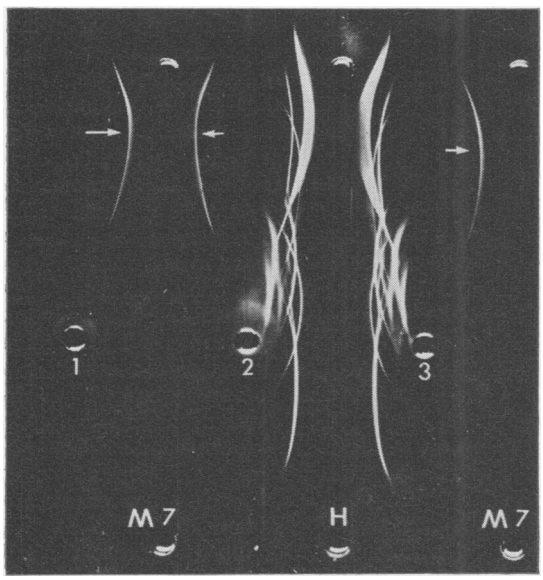

FIG. 1. Immunoelectrophoretic pattern of two cord serum (1 and 2) and a serum from a patient with hepatoma (3) tested against rabbit immune serum against AFP (M7) and horse immune serum against 'adult type' human plasma proteins (H). AFP appears as a line of precipitation in the α-globulin region (arrows).

radioimmunoassay method based on the coprecipitation-inhibition of anti-AFP and ^{125}I-labelled AFP has been extensively used to measure levels of AFP higher than 2–5 ng/ml.

Other methods include a 'reversed' passive haemagglutination technique (Olovnikof and Tsvetkov, 1969) in which formalin-treated red cells are coated with an antibody which reacts with AFP. In the presence of AFP, agglutination of the treated cells occurs.

An enzyme-linked immunoassay has also been proposed, with a sensitivity which is fully comparable to the radioimmunoassay technique (Bélanger *et al*, 1973b).

AFP during fetal life and in neonates

AFP was first detected in the serum of human fetuses less than 5 months old by Bergstrand and Czar (1956; 1957) and, using paper electrophoresis, by Halbrecht and Klibanski (1956). Several investigators confirmed these findings (Galdo *et al*, 1959; Andreoli and Robbins, 1962). Further information about the properties of AFP was obtained only after immunological methods and specific immunosera were introduced (de Muralt and Roulet, 1961; Tatarinov, 1964b; Masopust and Kotal, 1965; Gitlin and Boesman, 1966; Adinolfi and Gardner, 1967; Burtin *et al*, 1967; Gitlin and Boesman, 1967a; 1967b).

The immunodiffusion techniques provided clear evidence that AFP was present in sera of human fetuses more than 4 weeks old. Levels of the protein in the serum increase rapidly during the next 4–6 weeks of life *in utero*, and the highest values have been found at 12 to 16 weeks of gestation (Fig. 2; Gitlin and Boesman, 1966; van Furth and Adinolfi, 1969; Adinolfi, 1971). In the older fetus the level of AFP decreases and in infants at term the concentration ranges between 2 and 17 mg/100ml (Gitlin and Boesman, 1966; Adinolfi and Gardner, 1967; Marklein and Rings, 1972).

After birth, AFP disappears rapidly from the circulation during the first 3 weeks of life. However, levels between 25 and 200 ng/ml can still be detected in a small percentage of normal infants up to 1 year old (Masseyeff *et al*, 1974).

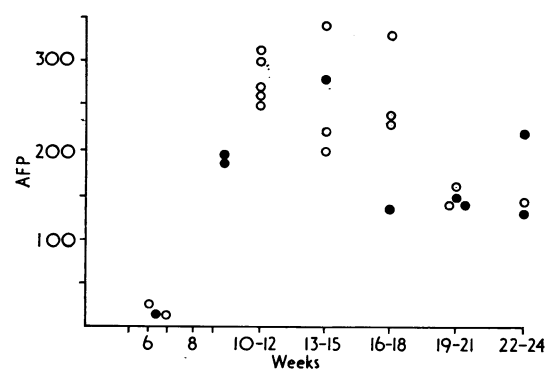

FIG. 2. Individual levels of AFP in sera of human fetuses (● = from Gitlin and Boesman, 1966; ○ = from van Furth and Adinolfi, 1969).

With the introduction of radioimmunoassay methods serum levels between 2 and 20 ng/ml have also been detected throughout childhood and in normal adults. Male and female blood donors have comparable values (Ruoslahti and Seppälä, 1972; Masseyeff *et al*, 1974).

Since the amount of AFP decreases during the last trimester of gestation and reaches low levels at term, it has been suggested that the serum values of AFP in fetal blood may reflect the degree of fetal maturity (Bergstrand *et al*, 1972; Karlsson *et al*, 1972; Hyvarinen *et al*, 1973). Since premature infants have a weight that relates to the degree of maturity—ie, the gestational age—it might be expected that such infants would have higher levels of AFP than more mature infants of the same weight. Bergstrand and his colleagues (1972) have shown that the levels of AFP vary significantly with the degree of maturity. They found no statistically significant differences between full-term infants, whatever their size, but infants at term tended to have lower levels of AFP than premature infants. According to Bergstrand *et al* (1972) AFP levels gave a closer correlation with the gestational age than did birth weight, serum albumin or the total serum protein level. On the other hand Lardinois *et al* (1972) have not observed a correlation between gestational age and levels of AFP.

AFP in amniotic fluid

Using immunodiffusion methods, traces of AFP were first detected in apparently normal human amniotic fluid by Gitlin and Boesman (1966) and by Adinolfi and Gardner (1967) and Seppälä *et al* (1967). This finding has been confirmed (Brock and Sutcliffe, 1972) though the reported levels have varied with the standards used.

In amniotic fluid from normal pregnancies, the mean values of AFP are as follows: between 10 and 14 weeks $\bar{x} = 20\ \mu g/ml$ (range 4 to 36 $\mu g/ml$); between 15 and 18 weeks $\bar{x} = 15\ \mu g/ml$ (range 4 to 32 $\mu g/ml$), and between 19 and 22 weeks $\bar{x} = 6.5\ \mu g/ml$ (range 2 to 16 $\mu g/ml$). These estimations need to be confirmed using a large group of normal pregnancies, and also using an international standard for the estimation of AFP, which is now available from the International Agency for Research on Cancer, Lyon, France.

AFP has also been detected in meconium and in the urine, cerebrospinal fluid, and bile of the human fetus.

AFP in normal pregnant women

The question of whether AFP crosses the placenta has been the subject of controversy. In 1959, Bodman described the presence of a fetal α-globulin in normal pregnant women and in 1964, Tatarinov (1964b) detected AFP in sera of a high percentage of women after spontaneous abortion.

Using an immunodiffusion technique, Foy and his collaborators (1970a; 1970d) were able to detect traces of AFP in about 50% of normal pregnant women after 30 weeks of gestation. These findings were not confirmed by Alpert and Zuckerman (1970). However, using the counter-current technique, with the lowest limit of detection near 0.25 $\mu g/ml$, AFP was detected in normal pregnant women at delivery. The fetal protein was found to disappear from maternal serum within 1 week, suggesting that it was derived from the fetus (Adinolfi and Kohn, 1971). Although, theoretically, there is an alternative possibility that the hormonal changes of pregnancy might lead to a temporary de-repression of AFP synthesis, it is notable that no rise in AFP has been detected following the hormonal changes induced by the contraceptive pill (Seppälä, 1973a).

High levels of AFP have since been demonstrated in all maternal sera (Fig. 3) from the first trimester of gestation (Seppälä and Ruoslahti, 1972; Garoff and Seppälä, 1973). In multiple pregnancies the mean levels of AFP are higher—as they are for placental lactogen—than those present in single pregnancies (Garoff and Seppälä, 1973). Maternal antibodies against AFP have not yet been detected in normal pregnant women either by the counter-current technique (Adinolfi and Kohn, 1971) or by radioimmunoassay (Seppälä and Ruoslahti, 1972).

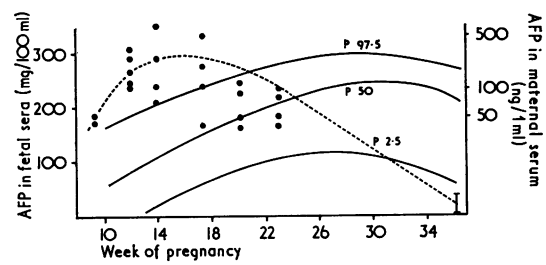

FIG. 3. Levels of AFP (mg/100 ml) in sera from human fetuses at various stages of gestation (●) compared with the 97.5, 50, and 2.5 centiles in maternal blood (ng/ml). The highest values of AFP in fetal and maternal samples are observed at different weeks of pregnancy.

Sites of synthesis and catabolism

The sites of synthesis of AFP have been investigated using *in-vitro* cultures of fetal tissues in the presence of labelled amino acids. When the culture fluids are harvested and dialysed the presence of newly synthesized proteins can be demonstrated by

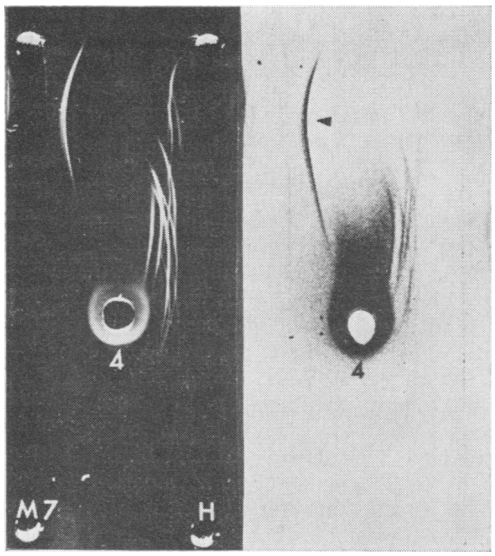

FIG. 4. Immunoelectrophoretic pattern and autoradiography of the liver culture fluid from an 18-week-old fetus. The autoradiography shows the labelled AFP newly synthesized *in vitro*.

immunoelectrophoresis and autoradiography of the immunoelectrophoretic plates (van Furth and Adinolfi, 1969; Gitlin and Perricelli, 1970). These studies have shown that AFP is synthesized in fetal liver (Fig. 4). The highest rate of synthesis was observed in liver cultures from fetuses of between 10 and 20 weeks of age. *In-vitro* synthesis of AFP was also observed in the culture fluid of human yolk sac from fetuses between 5.5 and 11.5 weeks old (Gitlin and Perricelli, 1970) and in the human placenta in two out of seven cases which were tested (van Furth and Adinolfi, 1969). Occasionally traces of newly synthesized AFP have also been detected in cultures of fetal gastrointestinal tissues (Gitlin *et al*, 1972). Newly synthesized AFP has not been detected in the culture fluids of fetal spleen, thymus, or lung tissues.

By studying the incorporation of labelled amino acids into organ cultures of rabbits, newly synthesized AFP has also been detected in cultured placental cells from two 19-day-old fetuses (Branch and Wild, 1972).

Kekomäki and his collaborators (1971) have shown that AFP is produced by the perfused human fetal liver at a rate ranging between 19 and 26 μg of AFP/min/total liver. The rate of synthesis of albumin was 50 μg/min at 14 weeks and 196 μg/min at 20 weeks, but during this phase of embryonic life the rate of synthesis of AFP did not seem to vary.

When these data are compared with plasma levels, it appears that AFP has a higher turnover rate than albumin (Gitlin and Boesman, 1966), and that its half life is near 3–5 days at birth. Similar values are obtained when ^{125}I-labelled AFP is injected intravenously into the adult patient with a hepatoma (Hirai *et al*, 1973).

Site of synthesis of AFP at cellular level

In order to localize the site of production of AFP single fetal cells have been studied using immunofluorescence (Engelhardt *et al*, 1969; Abelev, 1971; Engelhardt *et al*, 1971). With this technique AFP was detected almost solely in the liver parenchyma. It is not yet clear if the synthesis occurred in all hepatocytes, nor if other 'adult types' of serum protein were produced in the same cells.

One drawback of the immunofluorescence technique is that it does not discriminate between proteins which are produced by the cell and those which are passively absorbed onto the cell surface, a process which is especially likely to occur with cells such as hepatocytes, which have fragile membranes.

Studies have also been made using antisera against IgG by way of controls. Since it is known that IgG is not produced by liver cells the demonstration of these molecules in the liver tissues would indicate their absorption onto the cell membrane, presumably as a result of membrane damage. When this non-specific effect was excluded it was found that only a small proportion of cancer liver cells shows evidence of synthesizing AFP. The AFP 'positive' cells were detected around capillaries of the tumour sinuses and did not exceed 15% of the total (Goussev *et al*, 1970; Engelhardt *et al*, 1971; Abelev, 1971; Masseyeff, 1972).

Using another approach to the localization of AFP, Uriel and his colleagues (1973) exploited the oestrophilic property of AFP in order to develop an autoradiographic method. Fixed histological sections were taken from rat fetal liver and hepatoma tissues and incubated in solutions of radio-labelled oestrogens. The sections were then washed in order to remove free hormone molecules. Autoradiography showed the localization of the radioactive label in small groups of single, hepatocyte-like cells, with some preferential localization in the vicinity of large blood vessels. Haemopoietic, duct, and Küpffer cells were free of radioactivity and were therefore assumed to contain no AFP. In sections of rat hepatoma there were only a few labelled cells disseminated among neoplastic hepatocytes. These cells were frequently at the border of sinusoid capillaries, suggesting the interesting possibility that in the rat hepatoma the cell type producing AFP is distinct from other neoplastic hepatocytes.

Structure of AFP

The data on the physicochemical properties of AFP are still scanty (Table II). Although partial purification of AFP has been achieved by different techniques, contamination with albumin has presented a major problem. It can, however, be separated from other serum proteins by precipitation with ammonium sulphate at between 40 and 65% saturation. This procedure is normally followed by gel filtration on Sephadex G 150 and DEAE chromatography. Several modifications of this method have been published (Masopust *et al*, 1971b), but none avoids contamination with traces of albumin and α_1-globulin. Isolation by immunochemical methods has, however, been achieved by Nishi (1970), Adinolfi *et al* (1971), Nishi and Hirai (1971), Ruoslahti and Seppälä (1971), and Hirai *et al* (1973).

TABLE II

SOME PHYSICOCHEMICAL PROPERTIES OF HUMAN AFP*

	From Fetal Sera	From Hepatoma Sera
Molecular weight	64.000	64.000
Sedimentation constant S20w	4.50	4.50
Isoelectric point pI	4.70	4.70
$E^{1\%}$ 1 cm (278 nm)†	5.30	5.26
Nitrogen	14.70	14.90
Carbohydrate	—	3.40
$T\frac{1}{2}$ (days)	3.50	3.50

* For references see text.
† Optical density of a 1% solution in 1-ml cuvette at 278 nm.

There is good agreement that the molecular weight of AFP is near 64 000 (Nishi, 1970; Adinolfi *et al*, 1971; Hirai *et al*, 1973) and that the protein is formed by a single polypeptide chain (Adinolfi *et al*, 1971; Ruoslahti and Seppälä, 1971). The carbohydrate content of the protein is near 3.4% (Hirai *et al*, 1973). Treatment with neuraminidase slightly reduces the electrophoretic mobility of AFP derived from fetal or hepatoma serum.

AFPS which have been isolated from fetal and hepatoma sera appears to be indistinguishable from one another as judged by immunological tests, molecular weight, amino-acid composition, and the analysis of tryptic digests of the isolated protein (Hirai *et al*, 1973). A microheterogeneity has, however, been observed when serum samples containing the fetal protein have been analysed by isoelectric agarose electrophoresis (Alpert *et al*, 1973).

As with other glycoproteins which contain variable amounts of sialic acid residues, it has been suggested that the AFP variants may differ in the amount of sialic acid present in the molecules, possibly as a result of the low activity of sialyl transferase which is found in fetal tissues (Zimmerman and Madappally, 1973).

Smith and Kelleher (1973) have shown that the two variants of human and rat AFP have a different affinity for Concanavalin A and that therefore they can be separated by affinity chromatography.

AFP in other mammals

The fetal serum of several mammalian species contains an alpha-feto-protein with properties similar to AFP (Gitlin and Boesman, 1967b; Masopust *et al*, 1971c; Zizkovsky and Masopust, 1974). It has been detected in dog, cat, sheep, horse, cow, monkey, armadillo, rat, mouse, rabbit, opossum, and harbor seal. There is evidence that AFP is present in birds (Gitlin and Kitzes, 1967), and other species have been investigated, including sharks, which have been shown to have an alpha-feto-protein in 'fetal' serum but not in the adult (Gitlin, 1974). In the shark this protein was found to have a molecular weight of about 75 000 Daltons and to be synthesized in liver, stomach and, to a lesser extent, in the intestine and yolk sac. Since the earliest mammals seem to have emerged during the period in which the modern order of sharks first appeared —about 180–200 million years ago—it could be argued that an archaic homologue of AFP may well have been present in a common ancestor.

Human AFP was at first considered to be similar to bovine fetuin, a protein which was discovered by Pedersen in 1944. However, since 1956, Bergstrand and Czar have reported a difference in the percentage of the carbohydrate moiety of the two fetal proteins.

Recent studies by Kithier and his colleagues (1968; 1972) have shown that bovine fetuses possess a second serum protein that resembles human AFP. Not only is the structure similar but the level of this bovine protein increases in animals that have cancer of the liver. Immune sera which were raised against this bovine AFP were found to react against goat, sheep, and pig AFP. In addition, and in contrast to antisera against bovine fetuin, rabbit antiserum against bovine AFP also reacted against human AFP. This immunological cross-reactivity emphasizes the similarity between the AFP of one species and another.

Nishi, Hirai, and their colleagues (Nishi *et al*, 1972; Hirai *et al*, 1973) have shown that rabbits and horses, when injected with human AFP, produce specific antibodies which cross-react with their own homologous AFP. Common antigenic determinants must therefore be present. Attempts to produce antibodies with homologous AFP in rabbits, rats, and dogs were not successful.

Table III summarizes the cross-reaction of anti-human and anti-rat AFP immunosera tested against fetal sera from several mammalian species (Hirai *et al*, 1973).

TABLE III

EVIDENCE OF COMMON ANTIGENIC DETERMINANTS IN HUMAN AND OTHER MAMMALIAN AFP*

Fetal sera	Anti-human AFP Produced in:			Anti-rat AFP Produced in:	
	Horse	Rabbit	Rat	Horse	Rabbit
Human	+ + +	+ + +	+ +	—	—
Horse	+ +	+ +	+	—	—
Calf	(+)	(+)	+	—	—
Sheep	(+)	(+)	(+)	—	—
Pig	+	+	+	—	—
Rabbit	+ +	+	+	—	—
Dog	+ +	+ +	+ +	—	—
Cat	+ +	+ +	+ +	—	—
Rat	+	+	—	+ + +	+ + +
Mouse	+	+	—	+ +	+ +

* Based on the cross-reaction of antisera raised in various species against human or rat AFP. Data from Hirai *et al* (1973).

Biological function of AFP

Uriel and his colleagues (1972) have found that some hormones are firmly bound by rat AFP. This seemed to suggest a possible role for this fetal protein in the transport of hormones and in the feto-maternal relationship across the placental barrier. Oestrone, oestradiol, oestriol, and diethylstilboestrol all bind to rat AFP (Uriel *et al*, 1972; Anssel *et al*, 1974) regardless of which AFP variant is tested (Belanger and Dufour, 1974).

Swartz *et al* (1974) were unable to confirm the binding of oestrogens to human AFP. This, conceivably, may have been due to a previous saturation of binding sites with fetal hormones, but it also suggests the possibility that alpha-feto-proteins may have a different role in different species. Indeed in man, oestrogen binding is mainly associated with albumin and a β-globulin.

The effect of mouse AFP on primary and secondary antibody response has been investigated by Murgita and Tomasi (1974a; 1974b). Using an *in-vitro* plaque technique, AFP from amniotic fluid was found to have a non-cytotoxic inhibitory effect on the primary and secondary synthesis of antibodies to sheep red cells. AFP was also found to suppress the mitogenic effect of phytohaemagglutinin, concancavalin A, and lipopolysaccharide on mouse spleen cells and to inhibit allogeneic lymphocyte stimulation in the one-way mixed lymphocyte reaction. These results suggest that AFP may play an important immunoregulatory function in the normal fetal development, the protection of the fetus against the maternal immunological attack and in certain disease states associated with high levels of AFP.

AFP in patients with primary cancer of liver

Tatarinov (1964a) was the first to make the observation that AFP may be present in the blood of patients with primary cancer of the liver. This, and similar observations in mice (Abelev *et al*, 1963), prompted a whole series of systematic studies on the incidence of AFP in patients with hepatocellular carcinoma and other diseases of the liver (Abelev, 1965; Kithier *et al*, 1966; Uriel *et al*, 1967; Abelev, 1968; Alpert *et al*, 1968; Masopust *et al*, 1968; Masseyeff *et al*, 1968; Purves *et al*, 1968; Uriel *et al*, 1968; Alpert, 1969; Alpert *et al*, 1969; Endo *et al*, 1969; Foli *et al*, 1969; Hull *et al*, 1969b; de Nécheaud *et al*, 1969; Economopoulas *et al*, 1970; Foy *et al*, 1970b; Hull *et al*, 1970; O'Conor *et al*, 1970; Purves *et al*, 1970a; Abelev, 1971; Kresno *et al*, 1971; Masseyeff, 1972; Purves *et al*, 1973b).

In various ethnic groups, it has been found that carcinoma of the liver is not always associated with AFP levels in the same proportion of cases. The data reported in Fig. 5 have been obtained using immunodiffusion techniques. When radioimmunoassay methods are used a higher incidence of positive cases is observed but some differences persist between the various ethnic groups. It has been suggested that the fetal AFP gene may be 'activated'

FIG. 5. Percentage of patients with primary carcinoma of liver with high levels of AFP detected by immunodiffusion techniques. Data in USA refer to estimations in Caucasians and Negroes (•) by immunodiffusion (31 and 71) and radioimmunoassay (51 and 76). (For references see text.)

more readily by certain genetic or aetiological factors and at younger ages. Raised levels of AFP are found in the large majority of patients with primary cancer of the liver who are less than 30 years old, but in only about half of patients who are more than 60

years old Bagshawe and Parker, 1970; Mawas *et al*, 1970; Masseyeff, 1973). Since the age of onset of the disease varies in different populations, it is possible that the activation of AFP in some patients reflects an earlier onset of the disease in Africans as compared to Caucasians. Independent studies by Purves *et al* (1973b) and Abelev *et al* (1974) have also shown that apparently normal individuals in Senegal and Congo may have levels of AFP between 30 and 500 μg/ml. Whether these represent 'high-risk' subjects for primary carcinoma of the liver remains to be established. There is good agreement that the presence of AFP in patients with hepatocellular carcinoma is not related to histological differences, the volume of the tumour, or the duration of the disease (Masseyeff *et al*, 1968; Foli *et al*, 1969; Purves *et al*, 1970b).

In several studies raised levels of AFP have been found in a higher percentage of male than female patients (Masseyeff *et al*, 1968; O'Conor *et al*, 1970; Alpert *et al*, 1971; Hull and McIntire, 1972). However, in a group of adult Japanese patients, it was difficult to discern either age or sex differences in the prevalence of AFP or its concentration in serum (Nishi and Hirai, 1973).

In-vitro cultures of liver tissues obtained from cancer patients with high levels of AFP have suggested that this protein is produced exclusively by the liver cancer cells. Normal liver tissue, obtained by biopsy from the same patient, does not synthesise AFP *in vitro* (Adinolfi and Adinolfi, 1971). There is also evidence to suggest that only between 10 and 20% of the total liver cancer cells produce and release AFP *in vitro* (Abelev, 1971).

The serum level of AFP often falls temporarily after the surgical removal of a liver tumour (Masseyeff *et al*, 1968; Lin, 1970; Mawas *et al*, 1971). Occasionally, it then reaches a steady level (Alpert *et al*, 1971), and it may eventually disappear from the circulation. It has been suggested that in these cases incomplete removal of the tumour may leave viable cells which produce AFP, and this appears to be a very good explanation of the persisting steady level. The final disappearance of AFP cannot be explained with such conviction, but it has been suggested that the remaining tumour cells may be rejected by the host cellular immune response. When a disappearance or decrease in the level of AFP is followed by an increase this would indicate a recurrence of the disease.

It is generally agreed that AFP levels are little affected by the agents which are used to treat patients with hepatocellular carcinoma, including methotrexate and cyclophosphamide, radiotherapy, and corticosteroids (Purves *et al*, 1970b; Lin, 1970).

AFP in patients with terablastoma

In addition to its presence in patients with primary carcinoma of the liver, high levels of AFP have been detected in many patients with teratoblastoma or embryonal carcinoma of the testis or the ovary. Abelev and his colleagues (1967) found high levels of AFP in 10 out of their 27 patients. These findings have been repeatedly confirmed (Masopust *et al*, 1968; Mawas *et al*, 1969; Abelev, 1971; Mawas *et al*, 1971; Buffe, 1973). Those tumours which are associated with high levels of AFP were found to contain undifferentiated tissues with no indication of an organized structure.

The detection and estimation of AFP in patients with teratoblastoma has a considerable clinical value. After surgical treatment, the levels of AFP decrease to normal values in cases of complete remission, but high levels of AFP reappear in blood in patients who develop metastases. As with primary tumours of the liver, there is a higher incidence of 'positive' AFP findings in the young. Mawas and his colleagues (1970; 1971), reported high levels of AFP in 56% of patients who were less than 15 years old as compared to an incidence of 15% in an older group of subjects.

The reason why only some of these highly undifferentiated tumours of the testis and the ovary produce AFP is not yet clear; it has been suggested that the synthesis is associated with tumours containing hepatic tissue (Masseyeff, 1972) or vitelline cells (Ballas, 1972). High levels of AFP have not been detected in children with tumours such as neuroblastoma, nephroblastoma, lymphoreticulosarcoma, osteosarcoma, embryonal carcinoma, brain tumours, and liver angioma (Masopust *et al*, 1968; Mawas *et al*, 1971; Buffe, 1973).

AFP in experimental cancer of liver

AFP may be detected not only in human patients but also in adult animals with primary carcinoma of the liver. The first report showing an association between a fetal serum protein and tumour of the liver was that of Abelev and his collaborators (1963), who based their studies on the mouse. Hepatomas which produce AFP can be induced readily in mice and rats and are capable of transplantation. The fetal protein may also be excreted in the urine of hepatoma-bearing rats, as it may in pregnant animals (Okon *et al*, 1973). Studies in the rat after the administration of carcinogens have shown that on basal diet containing 3'-methyl-4 dimethyl aminobenzene (3'DAB) or dimethyl nitrosamine, AFP may be detected in the serum after only 2 weeks (Watabe, 1971; Kroes *et al*, 1972; de Néchaud and Uriel,

1973). Once detected, AFP was found to persist in the blood for some time, regardless of whether the administration of carcinogen continued. Those rats which maintained a stable level of AFP in the serum had a high probability of developing a hepatocellular carcinoma. Of those with cancer, 70% had detectable AFP.

A fetal plasma protein with some of the characteristics and common antigenic determinants of human AFP has also been observed in cows with primary carcinoma of liver (Kithier *et al*, 1972). Similar findings have been reported in monkeys (Hull *et al*, 1969a) after the induction of liver tumours by treatment with N-nitroso-diethylamine (DENA). Adamson *et al* (1972) have shown that oral DENA induces a liver tumour in 82% of cases, and 60% of those which developed a hepatoma also produced AFP. On the other hand, AFP was not found in monkeys who received other types of carcinogens. When the DENA was administered intraperitoneally an even more striking effect was obtained. A hepatoma developed in 95% of the monkeys after a relatively short latent period of 20.6 months. In this series 97% had a high serum level of AFP which was usually detectable about 6 months before any histological evidence of tumour could be demonstrated.

Foy and his colleagues (1970c; 1970d) have also observed the synthesis of AFP in baboons maintained on a pyridoxine-free diet for periods varying from one to three years.

AFP in patients with gastrointestinal tumours and liver metastases

High levels of AFP have occasionally been detected in other groups of patients. Using the most conventional immunological assays, abnormal levels of AFP have not been detected in patients with benign adenocarcinoma, haemangioma, or carcinoma of bile ducts.

Masseyeff *et al* (1968) were the first to observe abnormal levels of AFP using an immunodiffusion technique in patients with metastatic carcinoma of liver. In two of them, one with an anaplastic form of cancer of the stomach and the second with a pancreatic adenocarcinoma, post-mortem examination demonstrated the presence of metastases of the liver. Abnormal levels of AFP have also been described in patients with secondary cancer of liver (Bourreille *et al*, 1970; Andrieu *et al*, 1971; Geffroy *et al*, 1971).

The occasional presence of high levels of AFP has been recorded in the literature in other isolated cases with or without secondary cancer of the liver, and from these findings it may be concluded that the synthesis of AFP may increase in a few patients with tumours of the stomach or the pancreas in the absence of a primary liver cancer (Bernades *et al*, 1971).

AFP and fetal alkaline phosphatase

The association of AFP with other fetal antigens has been the subject of a number of studies. A fetal alkaline phosphatase, characterized by a high heat stability (Regan isoenzyme) has been recognized in adults with cancer. It was first isolated from a patient (Regan) with a bronchial carcinoma. The enzyme is usually present in the placenta and serum of pregnant women, but not in normal adult tissues. The Regan fetal alkaline phosphatase has been detected in the sera of 4% of patients with a wide variety of different tumours and its detection has been shown to be a reliable guide for the progression or the regression of the carcinoma following treatment.

Another variant of alkaline phosphatase, which differs from the Regan isoenzyme, has been found in human hepatocellular cancer (Warnock and Reisman, 1969). In a study of primary carcinoma of the liver in Africa, 65% of patients were found to have high levels of AFP and 29% (all with high levels of AFP) had detectable levels of the alkaline phosphatase variant (Portugal *et al*, 1970).

AFP and hepatitis

The first case of a patient with hepatitis and high levels of AFP was described by Alpert *et al* (1968). Another case was reported by Kresno *et al* (1970) and Geffroy and his colleagues (1970) demonstrated the transitory presence of AFP in the serum of a third adult with viral hepatitis.

When AFP is estimated by radioimmunoassay, levels between 100 and 500 ng/ml have been observed in several patients with chronic hepatitis and cirrhosis.

It is now known that the levels of the fetal protein increase above the normal values in a large percentage of patients with viral hepatitis. Using immunoradioautography, Abelev (1971) found abnormal levels in 23 out of 176 patients (12%). The level rose between 15 and 30 days after the onset of the disease and returned to normal between 15 and 26 weeks. Raised levels have been found only in Au-SH positive patients (Smith, 1971) or independently of the presence of the Australia antigen and antibodies (Akeyama *et al*, 1972; Ruoslahti and Seppälä, 1972. By studying cryoprecipitates from patients with acute or chronic hepatitis Florin-Christensen *et al* (1974) have shown that autoantibodies with anti-AFP specificity may also be present.

Masopust *et al* (1971a) have shown that the percentage of AFP 'positive' sera is higher in young patients with hepatitis than in adult patients. This finding has been confirmed by other investigators (Buffe and Rimbaut, 1973; Chandra, 1973). The highest levels are found in the most severe cases, and in patients who are in coma it has been noted that when AFP appears in the serum this is followed by recovery (Karvountzis and Redeker, 1974). The suggestion has therefore been made that increased synthesis of AFP may be associated with hepatic regeneration and there is also some evidence for this in experimental animals.

Levels of AFP ranging between 4 and 29 mg/100 ml were detected in 10 out of 11 infants with neonatal hepatitis; in contrast eight infants with biliary atresia had levels lower than 4 mg/100 ml (Zelter *et al*, 1974). It appears that the compensatory response to neonatal hepatitis by the liver parenchyma is proliferation, retrodifferentiation, and increased synthesis of AFP, while the response to biliary atresia is proliferation of tissue incapable of AFP synthesis. A temporary reappearance of AFP in the serum following treatment with carbon tetrachloride has been noted in mice (Bakirov, 1968), rat (de Néchaud and Uriel, 1971; Perova *et al*, 1971), and rabbit (Branch, 1972). In mice the increased synthesis of AFP can be induced in nearly 100% of animals treated with carbon tetrachloride, but the reappearance of AFP in rat and rabbits seems to be age dependent and occurs only in young animals. It has recently become evident that this age-dependent reactivation of the AFP gene is often mirrored in man and that in cancer of liver, as in teratoblastoma or hepatitis, high levels of AFP are more frequently found in young subjects than in old.

Geffroy *et al* (1971) have described a temporary increase of the value of AFP in one out of seven patients with hemochromatosis. No evidence of a hepatoma was found in any of these cases.

AFP in congenital diseases

Elevated levels of AFP have been detected in eight patients with hereditary tyrosinaemia (Belanger *et al*, 1973b; Bélanger, 1973). This inborn error of metabolism, inherited under the control of an autosomal recessive gene, is characterized by a progressive degeneration and cirrhosis of the liver. In two cases reported by Buffe and Rimbaut (1973) the elevated levels of AFP were found only in patients with parallel increased values of blood methionine.

High levels of AFP (from 44 to 2800 μg/ml) have been detected in all 20 patients with ataxia-telangiectasia tested by Waldmann and McIntire (1972). None of the sibs or parents of these patients had levels above normal values. Abnormal concentrations of AFP were not detected in another group of patients with various types of immunodeficiency. These findings have been confirmed by Simons and Hosking (1974). The persistence of AFP in the circulation of patients with ataxia-telangiectasia is connected with the hypothesis that the primary defect in these patients is an abnormality of tissue differentiation.

Using the method of double diffusion in agar gel, a young patient with Down's syndrome was found to have high levels of AFP over a period of 3 years (Adinolfi *et al*, 1967). However, the possibility that the increased values of AFP were due to a mild form of hepatitis could not be excluded. Subsequent studies using radioimmunoassay (A. Leek and M. Adinolfi, unpublished observations) have not revealed abnormal values of the protein in a group of Down's syndrome patients whose ages ranged from 2 to 16 years of age.

Patients with cystic fibrosis (CF) have been found to have levels of AFP ranging from 56 to 8800 ng/ml (mean 690); a moderate but significant increase in serum AFP was observed in their parents and sibs. Samples from patients with coeliac disease and bronchiectasis had normal values of AFP (Chandra *et al*, 1975). Persistent synthesis of AFP may be an associated marker of CF genes and estimation of serum AFP might help to detect heterozygote carriers in families at risk and for prenatal diagnosis of the disease.

Amniotic levels of AFP in neural-tube defects, fetal and placental distress

Anencephaly, with or without spina bifida, is a relatively common congenital anomaly in many countries. Once an affected child has been produced, the chance that a second child will be affected by the disease is 1 in 20. This hazard increases to 1 in 10 if two previous children have been affected (Roberts, 1970). Several attempts have been made to detect a 'marker' in the amniotic fluid which would indicate the presence of an affected fetus. The presence in the amniotic fluid of bilirubin or bilirubin-like molecules (with an extinction coefficient near 450 nm) has suggested a possible leakage or transudation of fetal blood components.

In 1972, Brock and Sutcliffe published a retrospective study and showed that the level of AFP was increased in the amniotic fluid of the anencephalic fetus between 26 and 36 weeks of gestation. Later, again retrospectively, Brock and Scrimgeour (1972)

observed a high level of AFP in the amniotic fluid of an anencephalic fetus that was only 18 weeks old. This suggested that the detection of an abnormal level of this protein could be of diagnostic value at an early stage of gestation. Since then anencephaly and spina bifida have been successfully diagnosed *in utero* on the evidence of a raised level of AFP in the amniotic fluid (Allan *et al*, 1973; Nevin *et al*, 1973; Seller *et al*, 1973; Harris *et al*, 1974).

Allan *et al* (1973) have confirmed the value of estimating the levels of AFP in the detection of open neural-tube defects early enough to allow termination of the pregnancy. Spina bifida was detected by amniocentesis in two out of 20 pregnancies at risk because of at least one previous affected child. Low levels of AFP in the amniotic fluid in the presence of fetuses with neutral-tube defects have been observed during the last weeks of gestation when only a small amount of this fetal protein is synthesized by the fetus.

Harris *et al* (1974) have carried out a prospective study of mothers at risk; they concluded that when amniocentesis and ultrasound are employed to detect fetuses with anencephaly and spina bifida, most cases are detectable before 20 weeks of gestation, thus allowing selective abortion. Closed neural-tube defects, as well as one case of 'open' spina bifida at 33 weeks of gestation, were found to be associated with normal levels of AFP in amniotic fluid.

However, a word of warning is necessary, since raised levels of AFP have been found in the amniotic fluid in the absence of neural-tube defects; raised levels may be associated with the spontaneous abortions, erythroblastosis, and fetal distress (Seppälä and Ruoslahti, 1972; 1973a; 1973b).

In the case of open neural-tube defects it has been suggested that the high levels of AFP in amniotic fluid are due to the transfer of the protein from cerebrospinal fluid (CSF). Levels of AFP ranging from 1220 to 52 μg/ml) have been detected in the CSF of normal fetuses from 16 to 25 weeks of gestation (Seller and Adinolfi, 1975).

Work is now in progress in several laboratories to evaluate the significance of the levels of AFP in amniotic fluid and in maternal blood. One of the problems concerning amniotic fluid is that it may occasionally be contaminated with fetal blood and so give falsely high readings. It has been calculated (Ward and Stewart, 1974) that a fetal bleed of only 1 ml will produce an abnormally high AFP reading in amniotic fluid at 14 weeks—or less if there is incomplete mixing in the amniotic fluid. Although Harris *et al* (1974) have not found serum-AFP measurements to be reliable, however, others have obtained useful results, especially in the 13th to 18th week (Brock *et al*, 1973; Brock and Scimgeour, 1974; Seller *et al*, 1974; Wald *et al*, 1974). As with amniotic fluid measurements it is clear that the method may not detect 'closed' neural-tube defects. It also seems likely that anencephaly or open spina bifida will sometimes be missed if reliance is placed on maternal blood measurements alone.

In fact, lack of correlation between the levels of AFP in amniotic fluid and those in the corresponding maternal serum has been repeatedly observed (Adinolfi, 1975; Seppälä, 1975). In several instances, high levels of AFP in amniotic fluid were associated with normal values in maternal serum.

High levels of AFP in maternal sera have also been detected in twin and triplet pregnancies; in twin pregnancies the average AFP levels were double those found in singleton pregnancies matched for maternal age, parity and the time of gestation (Garoff and Seppälä, 1973; Wald *et al*, 1975). Intrauterine death is frequently associated with abnormal high levels of AFP in the amniotic fluid and the maternal serum. AFP values above the normal 97.5 centile in maternal circulation were observed in 30 out of 51 cases (59%) in which the fetus died *in utero*. Unequivocally raised levels of AFP in maternal serum were seen in four out of 10 cases when the fetus was still alive (Seppälä, 1975).

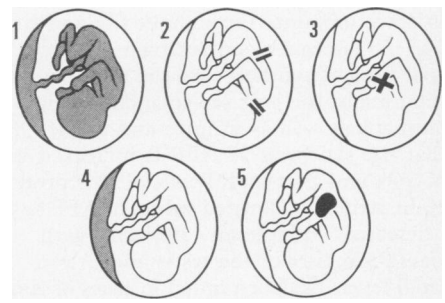

FIG. 6. Possible mechanisms responsible for the increased levels of AFP in amniotic fluid and maternal serum. Fetal death (1) is associated with transendation of AFP in the amniotic fluid and transfer of the protein across the placenta, occasionally even when the fetus is still alive. In 'open' neural-tube defects (2) AFP in amniotic fluid derives from the CSF. Reduced swallowing (3) increases the levels of AFP in amniotic fluid. Placental distress (4), as in diabetic mothers, may be responsible for transfer of AFP in the maternal circulation. Increased synthesis of AFP (5) has been suggested in cases of severe Rh incompatability.

The raised levels of AFP in amniotic fluid and maternal serum in cases of intra-uterine death are likely to result respectively from the transendation of the protein from the fetus and the placental distress (Fig. 6).

Elevated levels of AFP in amniotic fluid have been observed in fetuses with oesophageal atresia

(Seppälä, 1973b). Reduced clearance of amniotic fluid by impaired swallowing (Fig. 6) may be responsible for elevated levels of AFP in other fetal abnormalities (Adinolfi, 1975). On the other hand, placental distress may be the major factor responsible for the elevated levels of AFP in maternal blood of diabetic mothers (Fig. 6).

The results of these studies are being compared with other methods for the prenatal detection of congenital malformations, fetal distress, and placental insufficiency. This may help to justify a more widespread screening of pregnant women who are known to have a high risk of carrying a fetus with a neural tube defect.

Summary

An alpha-feto-protein (AFP) is present in many mammals, in birds, and in sharks during development. The AFP present in different species have similar physicochemical properties and often have common antigenic determinants. Their study, both in health and disease, has provided a useful model for the understanding of other phase-specific antigens and the activation of the genes which control their synthesis.

In the human fetus, the level of AFP falls with increasing maturity. The more sensitive methods of detection have disclosed that this fetal protein persists in trace amounts throughout life and its level increases in maternal blood during pregnancy. The principal sites of synthesis are the fetal liver and in some mammals, the yolk sac splanchnopleur.

In humans as well as in mice and cows, it is notable that the synthesis of AFP is increased in liver cancer cells and that high levels of this protein are present in serum. Elevated values of AFP have also been detected in human subjects with undifferentiated tumours of the testis and ovary. A fall to normal levels has been noted in cases of complete remission after surgery and a return to high levels in patients who develop metastases.

In some patients with hepatitis a temporary rise in the level of AFP has also been observed.

In recent years, the detection of high levels of AFP in amniotic fluid has proved to be of great value for the prenatal diagnosis of neural-tube defects. Abnormal levels have also been found in the amniotic fluid or in maternal serum in cases of spontaneous abortion. Such measurements are now being assessed as a method of monitoring abnormal pregnancy.

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