CHAPTER I

INTRODUCTION

Primary liver cell carcinoma (hepatoma) is one of the most common malignant tumour of the male population in Thailand. (Nilprabhassorn, Vootprux and Suvonge, 1976). Usually the clinical course of hepatoma is stormy, over 90 per cent of patients die within 3 months after the diagnosis has been established. There is no sensitive test which can verify the early stage of hepatoma. Certain malignant tumours may have some molecular biological changes of protein synthesis especially in the early stage of the disease.

To detect the early stage of malignant disease is the hope of all physicians. Investigators from various institutions have tried to invent a new test for the early diagnosis of cancer. This study indicated that the identification of serum protein patterns of patients with hepatoma and some other liver diseases using rocket immuno-electrophoresis and polyacrylamide gel electrophoresis (PAGE) may be of value.

Plasma proteins

Plasma is a complex mixture in which proteins form the major macromolecular component. The precise number of proteins

in plasma has not been determined finally, although in 1966 Schultze and Heremans listed almost two hundred recognizable immunological distinct entities or enzymatic activities.

The liver has unequivocally been shown to synthesize plasma proteins such as albumin, α_1-acid-glycoprotein, haptoglobin, α_1-antitrypsin, α_2-macroglobulin, α_2-Gc-component, transferrin, high and low-density lipoproteins including chylomicrons, fibrinogen and prothrombin. Studies with radioactive-labeled amino acids also point to the liver as the probable source of ceruloplasmin (Hochwald, Thorbecke and Asofsky, 1961), and possibly of hemopexin (Hochwald et al., 1961). The predominate role of the liver in the synthesis of plasma proteins is best illustrated by the classical experiments of Miller and his associates (Miller et al., 1951; Miller, and Bale, 1954).

The purpose of this work is to study some plasma proteins in liver diseases such as prealbumin, albumin, haptoglobin, α_2-macroglobulin, ceruloplasmin, α_1-acid-glycoprotein, transferrin, hemopexin and Gc-globulin.

ALBUMIN is the major protein produced in the liver, comprising as much as 50 per cent of the productive effort at any one moment. The concentration of this protein in the plasma has long been used as an indicator of health and disease. The basic properties of albumin were recognized as early as 1839 by Ancell, who

noted that "albumin" was needed for transport functions, for maintaining fluidity of the vascular system and for the prevention of edema. He recorded values of protein level in different age groups, species, males and females. These fine early observations have not been disproved during the past 140 years.

Serum albumin is a highly flexible molecule of about 65,000 molecular weight containing 575 amino acids. Albumin is characterized by its extreme solubility in water, by its negative charge of 10 at pH 7.4 and by lack of a carbohydrate moiety. Albumin serves in the plasma to maintain osmotic pressure and as a carrier of metals, ions, fatty acids, amino acids, metabolites, bilirubin, enzymes, drugs and hormones. The binding of one substance may lead to conformational change within the albumin molecule or alter its stability. Albumin can also exist in the plasma as a polymer (Ancell, 1839 - 1940; Rothschild et al., 1969; Peters, 1970; Schultze and Heremans, 1970).

Albumin is synthesized by the hepatocyte. It finds its way directly into the hepatic veins and hence to the systemic circulation. Itshalf-life is 20 days in man, and its site of degradation is yet unknown.

The works of Miller and of Kukral (Miller and Bale, 1954; Kukral et al., 1961) indicated that albumin was produced in the liver, and no other site for its production has been demonstrated.

The reduced rate of albumin degradation which has been observed in cirrhosis, shows greater correlation with the degree of reduction in plasma concentration than with the intravascular pool size (Sterling, 1951, Berson and Yalow, 1954; Wilkinson and Mendenhall, 1963; Dykes, 1968; Rothschild et al., 1970). In patients with cirrhosis and ascites studied in the steady state, a reduced rate of degradation, both fractional and absolute, may coexist with a normal or elevated total exchangeable albumin pool size. In cirrhosis without ascites the hepatic synthesis of albumin may be depressed. In the majority of these patients the plasma albumin pool and total exchangeable albumin pool are decreased. However, in one hypoalbuminemic patient with cirrhosis and hepatoma the synthesis rate was elevated (Tavill et al., 1968). The reduced concentration of plasma albumin that is usually seen in severe liver disease is not always matched by a similar reduction in the plasma concentration of other proteins (Rosenoer, 1976), and the reduced rate of albumin synthesis cannot be correlated with abnormalities of other liver function tests (Hasch et al., 1967).

PREALBUMIN (tryptophan-rich prealbumin, thyroxin binding prealbumin) has been studied metabolically in four normal subjects (Oppenheimer, 1965) and has proven to be extremely short-lived, having a metabolic half-life of only 1.9 days and a turnover rate of 0.361 day^{-1}. Prealbumin has molecular weight of about 61,000

(Schultze and Heremans, 1966). Quantitative determinations of serum prealbumin levels in normal adults yield values between 20 and 50 mg per 100 ml, a mean value of around 30 mg. (Schultze and Heremans, 1966; Stabilini et al., 1968; Rossi et al., 1970). Prealbumin level varies in physiological conditions for example, according to sex (Stabilini et al., 1968) and in pregnancy (Stabilini et al., 1968, Rossi et al., 1970). It also varies in various pathological conditions, for example, viral hepatitis and Laennec's cirrhosis (Inada and Sterling, 1967; Van den Schrieck, 1969) and other non-thyroidal illness (Bellabarba et al., 1968). Low levels of prealbumin are shown in stress, inflammation and surgical trauma (Surks and Oppenheimer, 1964; Oppenheimer, 1968). Metabolic studies with ^{131}I-labeled prealbumin indicate that decreased synthesis is responsible for the depression of prealbumin levels in acutely and chronically ill patients. (Socolow et al., 1965).

TRANSFERRIN was named by Holmberg and Laurell (1947) to designate the iron-binding glycoprotein present in plasma and other biological fluids. The normal concentration of transferrin in human sera is 200 - 400 mg/100 ml. (Schultze and Heremans, 1966). The protein has a molecular weight of about 80,000. It is formed by a polypeptide chain and two carbohydrate groups linked to the protein moiety through an asparaginyl-glycosamine linkage. The carbohydrate content is approximately 5 per cent (Bowman, 1968; Giblett, 1969).

Transferrin plays a central part in iron metabolism. While the extracellular pool of iron, which is bound to transferrin, amounts to approximately 4 mg in man, some 20 - 25 mg of iron are utilized each day for the production of hemoglobin in the erythroid marrow; thus, the extracellular iron pool turns over several times each day. In normal circumstances, most of the iron used for heme synthesis is derived from the hemoglobin of red cells that are broken down in the reticuloendothelial system. Reticuloendothelial cells donate this iron to transferrin and transferrin exchanges iron with the erythroid marrow. The movement of iron is one way; there is little evidence that a significant amount of iron is transferred from transferrin to the reticuloendothelial system. Apart from this cycle of iron, transferrin reversibly exchanges iron with the storage pool (ferritin and hemosiderin) in the hepatic parenchymal cell. Mechanisms involved in transferrin-ferritin exchange of iron are not known, although various hypotheses will be considered later.

Apart from childhood, raised transferrin levels are found in pregnancy with or without clinical iron deficiency. Transferrin concentrations are increased in iron deficiency and in females taking estrogen-containing oral contraceptives. Higher than normal plasma transferrin levels may occur in acute hepatitis. (Laurell et al., 1970).

Deficiency of iron stimulates transferrin synthesis. Other factors that generally increase protein synthesis rates also increase transferrin producting, e.g. acute blood loss and proteinuria, and the effects of some hormones, notably estrogens (Weinfeld, 1965; Lane, 1966; Lane, 1968). Conversely, synthesis rates are generally depressed by protein-calorie malnutrition and in severe systemic and liver disease.

HEMOPEXIN, a heme-binding serum protein, was first described by Neale, Aber and Northam in 1958. The high carbohydrate content (20 - 22 per cent) and immuno-electrophoretic identity of this macromolecule led to its designation by Schultze, Heide, and Haupt, 1961, as β_{1B}-glycoprotein. The name "hemopexin" reflects the ability of the protein to bind heme in an equimolar ratio. (Hrkal and Muller 1971).

Human hemopexin was first purified by the research team of Schultze et al. 1961. It has molecular weight of about 57,000 (Seery et al., 1972) and consists of a single polypeptide chain. It has no free sulfhydryl groups (Hrkal and Muller, 1971). The assayable half-cysteins can, therefore, be assumed to form intramolecular disulfide bridges rather than to take part in porphyrin binding.

Like many hemeproteins, hemopexin also binds porphyrins rather than heme. The binding of denteroporphyrin and some of its

derivatives by hemopexin has been studied extensively by spectrophotometry, equilibrium dialysis and fluorimetry. (Morgan and Muller, 1972; Seery and Muller, 1973).

The liver was found to be the primary site of hemopexin formation (Gitlin and Biasucci, 1969). Heme catabolism occurs in the hepatocytes rather than in the cell of the reticuloendothelial system of the liver. The actual site of hemopexin catabolism is also yet unknown.

To confirm that the hemopexin concentration is a dependable indicator of hemolysis, other factors that may influence hemopexin levels have been evaluated. Hemopexin does not appear to be an "acute-phase reactant". In severe infections, hemopexin levels usually remain stable (Braun and Aly, 1971; Clarke et al., 1971; Kushner et al., 1972), indicating a rigid homeostatic control of this protein.

Elevated hemopexin levels, although rarely more than twice normal, occur in diabetes mellitus, possibly in muscular dystrophies, and definitely in various forms of cancer (Braun and Aly, 1971; Manuel et al., 1971). Whether this elevation is an early or late manifestation of cancer has not been ascertained. Very high hemopexin levels were encountered in patients with fast-growing melanomas. In the course of severe liver disease, hemopexin levels decline presumably because of decreased synthesis rather than hemolysis.

Gc-GLOBULIN was discovered by Hirschfeld in 1959. He noted that one of the α_2-globulins of human serum displayed inheritable differences in immuno-electrophoretic mobility. This protein, which he called the "group specific component" (Gc) was later isolated by Schultze et al. in 1962 and characterized as a glycoprotein.

Immuno-electrophoresis has been the method of choice for the study of the Gc-polymorphism. In the electrophoresis pattern Gc-globulin appears among the so-called post-albumins. Indeed, the post albumin polymorphism described in starch gel patterned by Smithies in 1959 presumably corresponded to variation in the Gc-globulin.

Three common phenotypes are observed among populations. Some sera contain a fast-migrating (inter-α) component, Gc 1-1; others a slow-migrating (α_2) component, Gc 2-2; whereas the third phenotype, Gc 2-1; contain both forms in approximately equal amounts. Extensive studies on families, twins, mother-and child pairs have made it abundantly clear that this polymorphism is created by two autosomal co-dominant alleles, Gc^1 and Gc^2.

HAPTOGLOBIN is an α_2-globulin with several unusual properties. It is the chief component of the "acute-phase", its concentration increasing up to several-fold during inflammatory reactions. Haptoglobin (Hp.) exhibits the fundamental property of combinding with hemoglobin (Hb) to give a complex (Hp.Hb.) which is characterized by peroxidase activity. Isolation and purification of free haptoglobin

from human, rabbit or rat plasma was described by Connell and Shaw, 1961, Lombart et al., 1965 and Monray, 1966. The liver is the site of haptoglobin synthesis (Monray et al., 1964; Krauss and Sarcione, 1964; Alper et al., 1965; Peter and Alper, 1965).

Haptoglobin contains about 20 per cent carbohydrate, namely sialic acid, glucose, hexoses and glocosamine, and 80 per cent protein. (Cherftel et al., 1960; Javid, 1967). The protein portion of haptoglobin consists of alpha and beta-polypeptide chains which are disignated as Hp α and Hp β. The beta chain which is similar in all individuals, acts by binding to free hemoglobin when it is available (Shim and Bearn, 1964; Gordon and Bearn, 1966). In contrast, the alpha chain is not identical but is subject to genetic variation (Smithies et al., 1962).

Haptoglobin is synthesized in the liver and probably degraded in the same organ. The half-life of haptoglobin is about two days. (Alper et al., 1965; Krauss et al., 1966; Kashiwagi et al., 1968; O'Hara et al., 1968).

The haptoglobin concentration is usually elevated in the blood of patients with infectious diseases, cancer and leukemia, as well as after surgery and burns. Haptoglobin is regarded as a very sensitive indicator of disease persistence. (Jayle and Moretti, 1962; Owen et al., 1964; Werner, 1969).

α_2-MACROGIOBULIN, which forms about one-third the total α_2-proteins, was first purified by Brown et al. in 1954. The amino acid composition of α_2-macroglobulin has been determined by Heimburger et al., 1963 as well as its carbohydrate composition and N-terminal amino acid (Schultze, 1966). α_2-macroglobulin has a molecular weight of 726,004 (Roberts et al., 1974). It was identified as an proteinase inhibitor by Brown et al., 1954. Human α_2-macroglobulin can bind endogenous and exogenous endoprotein in vitro (Barnet and Starky, 1973; Ohlsson, 1974). One of the functions of α_2-macroglobulin may be the binding and clearance of proteolytic enzymes (Ohlsson, 1974), because the complex between α_2-macroglobulin and the proteases are rapidly eliminated by the reticuloendothelial system (Ohlsson, 1971).

The normal α_2-macroglobulin concentration in human serum is between 200 and 300 mg/100 ml (James et al., 1966; Shortridge et al., 1973). A much higher concentration of plasma α_2-macroglobulin is found in infants than in adults, whereas women have about 20 per cent higher α_2-macroglobulin concentration than men (Ganrot and Schersten, 1967). Pregnant women have significantly higher α_2-macroglobulin concentrations (Talamo et al., 1975).

CERULOPLASMIN accounts for the majority of the transportation of copper in plasma and shows oxidase activity against a number of substrates including p-phenylenediamine. Several methods for the

quantitative assay of ceruloplasmin are based on this oxidase activity (Houchin, 1958; Ravin, 1961; Seal, 1964; Sunderman and Nomota, 1970).

The mean serum ceruloplasmin level of 32 ± 4.9 mg per cent was found in human adults by Ravin (1961) by the enzymatic method. A very similar value (30.4 mg per cent) was reported by Cox (1966), but she observed age-dependent variations with the maximum in early childhood. Ceruloplasmin levels appear to be influenced by genetic factors both in man and animals (Cox, 1966; Meier and Macpike, 1968).

In 1955 Wintrobe and co-workers demonstrated that oxidase activity of ceruloplasmin is elevated in sera of pregnant women, patients with infectious diseases and nephrotic syndrome. Rice (1960, 1961) found a good correlation between serum ceruloplasmin and other acute-phase reactants, especially C-reactive protein, in various pathological states. Sass-Kortsak (1965) reviewed the effects of infectious diseases, pregnancy and hormone administration on the ceruloplasmin level and copper metabolism.

α_1-ACID GLYCOPROTEIN (orosomucoid) is a well characterized constituent of normal human plasma. Physicochemical properties of this protein are described in detail by Jeanloz (Jeanloz, 1966).

Several authors have reported plasma concentrations of α_1-acid glycoprotein in healthy individuals of about 75 - 100 mg/100 ml (Schultze and Heremans, 1966) but Schmid et al., (1967, 1968) gave

lower values of about 46 mg per cent. Surgery may double plasma levels of orosomucoid and a sustained elevation of this protein in burned patients was described by Zeineh and Kukral (1970).

The turn-over rate of α_1-acid glycoprotein has been studied in normal man by Weisman et al. (1961) and in burn patients by Zeineh and Kukral (1970). The picture is far from being complete but it appears that the 5 - 6 days half-life of iodine-labeled orosomucoid is not altered significantly by parenchymal liver disease (low level of α_1-acid glycoprotein) or inflammation (higher levels of this protein).

A considerable body of information on the conditions leading to increased concentrations of acute-phase reactants has already been obtained both in clinical studies and with experimental animals, mainly rats and rabbits.

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