CHAPTER I

INTRODUCTION

Haptoglobin is an alpha-2-globulin found in human serum and plasma. It is specifically capable of binding haemoglobin both in vivo and in vitro (Jayle and Boussier, 1955; Polonovski and Jayle, 1940). Haptoglobin contains about 20 per cent carbohydrate, namely, sialic acid, fucose, hexoses and glucosamine; and 80 per cent protein (Javid, 1967; cheftel et al., 1960). The protein portion of haptoglobin consists of alpha and beta-polypeptide chains which are designated as Hpα and Hpβ. The beta chain which is similar in all individuals, acts by binding to free haemoglobin when it is available (Gordon and Bearn, 1966; Shim and Bearn, 1964). In contrast, the alpha chain is not identical but is subjected to genetic variation (Smithies et al 1962).

Haptoglobin was originally identified and named by Polonovski and Jayle in 1938 from the Greek root "Hapto" meaning "to hold fast", because it binds free and circulating haemoglobin to form a very stable complex.

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; Kashiwagi et al., 1968; Krauss and Sarcione, 1966; Merrill et al., 1964; O'Hara et al., 1968).

Haptoglobin could be obtained by purification. The original pure haptoglobins were obtained from the urine of nephrotic patients. Pure haptoglobin was also extracted from the ascitic fluid by Laurell (1959).

Subsequently, a method of preparing pure haptoglobin from normal serum using column chromatography has been worked out in detail by Connell and his colleagues in 1961 and this has formed the basis for most recent studies of haptoglobin structure.

Haptoglobin has the characteristic property of binding with free haemoglobin to form a stable haemoglobin-haptoglobin complex. This complex remains soluble and does not precipitate. Haptoglobin does not appear to bind myoglobin, deoxyhaemoglobin, haemoglobin H or Bart's although it will bind with the alpha-chains and weakly with the beta-chains of haemoglobin (Wheby et al., 1960; Chiancone et al., 1968; Giblett, 1968).

The nature of the bond between the haemoglobin and haptoglobin is not clear. Several haptoglobins are now known by their different electrophoretic migration patterns, and it is now established that the types of haptoglobins possessed by any given individual is genetically determined by a two allelic system without

dominance (Bearn, 1961; Giblett, 1968; Louderback and Shanbrom , 1968; Smithies, 1955; Smithies and Walker, 1956).

The precise significance of the function of the haptoglobin is not known. The quantity of haemoglobin usually required to saturate the binding capacity of the haptoglobin present in normal serum is only about 50-150 mg per 100 ml (Nyman 1959), and any excess haemoglobin remains in the free state.

Haptoglobin-haemoglobin complexes are formed as a result of red cell break-down. They are rapidly removed from the serum and are degraded in the tissues while only free haemoglobin is mainly excreted in the urine . So it is possible that one function of haptoglobin is to minimise the loss of iron of the body. Haptoglobin may also be significant in bile pigment formation because it has been shown that the haeme in native haemoglobin is resistant to the attack of a liver enzyme, known as α-methenyl oxygenase This enzyme converts haeme in the haptoglobin-haemoglobin complex to a precursor of biliverdin (Nakajima et al., 1963).

Smithies (1955) discovered that when serum from different individuals is examined by starch gel electrophoresis,

several distinct and quite characteristic haptoglobin types can be recognised (Fig.1). The patterns may be visualised more easily by using the peroxidase reaction of haemoglobin to bring about colour reaction with benzidine, o-tolidine, leucomalachite green, guaiacol and o-dianisidine.

The types are genetically determined and the initial family studies led to the suggestion that a pair of alleles at an autosomal locus were involved (Smithies and Walker 1955, 1956). These were called Hpl and Hp2, and it was supposed that Hp 1-1 individuals were homozygous for Hpl, Hp 2-2 individuals were homozygous for Hp2 and Hp 2-1 individuals were heterozygous Hpl Hp2. It appeared therefore that the homozygotes differed from one another both in the mobility and the number of protein components they formed, and also that the heterozygotes formed a series of protein components qualitatively different from those present in either type of homozygotes.

Sera from Hp 1-1 persons give only a single haptoglobin component which is an intensely staining band somewhat slower than free haemoglobin at pH 8.6 in borate buffer.

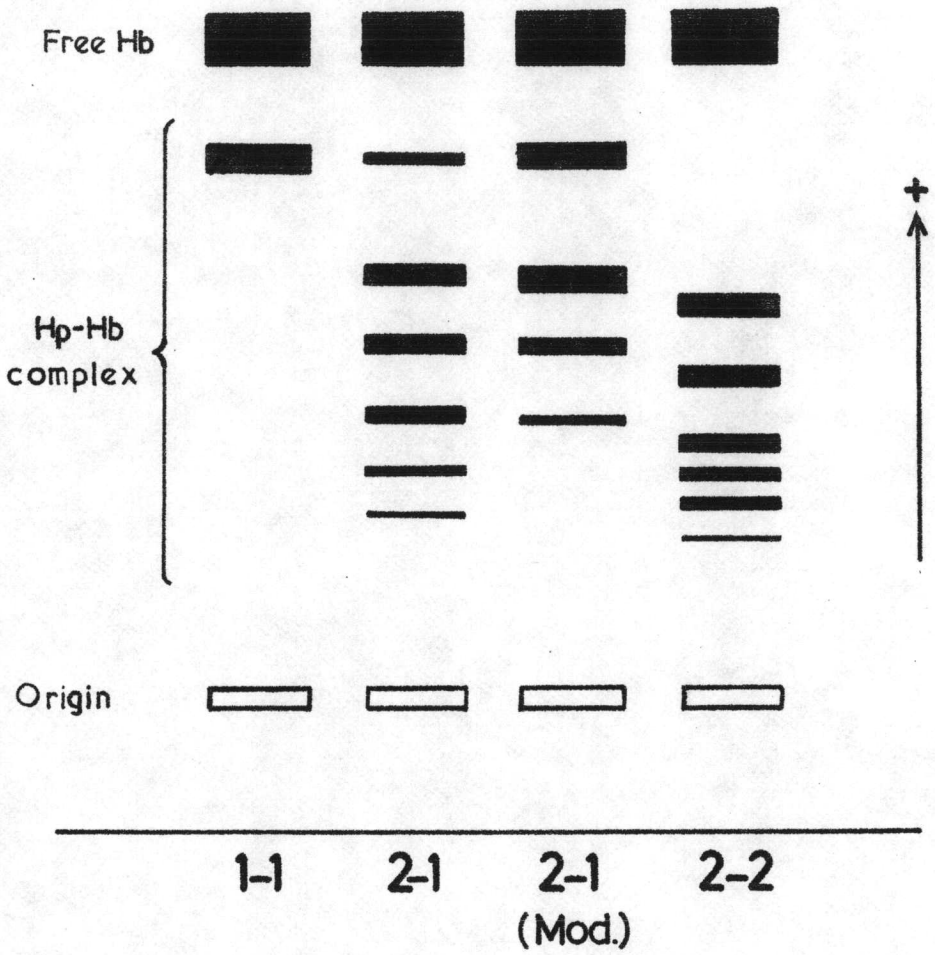

Figure 1. Electrophoretic pattern of haptoglobin-haemoglobin complex.

Hp 2-2 persons give a pattern in which there is no band in the position of the Hp 1-1 component, but a series of slower bands of diminishing intensity towards the origin.

Sera from Hp 2-1 persons, who are genetically heterozygous, give a pattern similar to that for Hp 2-2 , but in which the bands have a slightly greater mobility which displaces them toward the anode, together with a band corresponding to the Hp 1-1 position.

These types of haptoglobins correspond to the existance of a series of polymer association of the haptoglobin molecules, the larger-sized particles moving more slowly through the pores of the starch-gel under the influence of the potential gradient along the gel.

The three common haptoglobin phenotypes above have been detected so far in all population studied, though their frequency varies considerably in different parts of the world. In addition, black African and American Negro populations show an appreciable frequency of a modified Hp 2-1 pattern. This phenotype gives a pattern in which the slower bands of the Hp 2-1 pattern are absent, and in which there is an increased

concentration of the fastest band. Inexperienced observers sometimes classify Hp 2-1 (mod.) as Hp 2-1.

Haptoglobin is absent, or present in very small amounts, in the serum of new borns, but its concentration increases to normal levels within a few months after birth. In 1958, Allison et al. reported that 30 per cent of sera collected from Nigerian Africans who were not newborns had insufficient haptoglobin to permit typing. Later, using quantitative methods, it was found that some of these people had no detectable haptoglobin. Further studies in Africa revealed other populations with high frequency of ahaptoglobinemia (Barnicot et al., 1960; Blumberg and Gentile, 1961). The populations with the highest frequency were in the middle belt of the continent, south of the Sahara, north of South Africa, and extending from coast to coast. Ahaptoglobinemia or haptoglobin type 0 exists in 4 to 10 per cent of U.S.Negroes, 2 per cent of Micronesians, 1 per cent or less of Europeans, and in other populations.

There appear to be several explanations for this phenomenon. As noted previously, a variety of diseases can cause intermittent ahaptoglobinemia, and this probably accounts for the presence of the trait in many hospitalized patients or

in countries where hemolytic diseases are common. The area of high frequency of ahaptoglobinemia in Africa corresponds to the belt of malaria endemicity, and this disease probably contributes to the high frequency, although other causes are undoubtedly also important. In addition, normal individuals can have intermittent ahaptoglobinemia, for no apparent cause.

The Sub-types of haptoglobin

Reductive cleavage followed by electrophoresis in 8 M urea starch gel requires care to achieve reproducible results. Its application, however, increases the number of recognizable common haptoglonin phenotypes (and genotypes) in most populations to six, and to eight phenotypes for black Africa and American Negro populations.

Since sub-typing is a relatively expensive and difficult procedure, most studies of the inheritance and distribution of the haptoglobin groups have provided data for the types detectable in normal starch gels at pH 8.6. For those populations where the three common phenotypes are Hp 1-1, 2-1 and 2-2, the autosomal two-allele model Hpl and Hp2 appears to be adequate to explain the inheritance, as suggested originally by Smithies and Walker (1955).

Serum haptoglobins are decreased in the following conditions :

CONDITIONS	REFERENCES
Hemolytic conditon : malaria, abnormal haemoglobin, G-6-PD deficiency.	Allison and Rees(1957), Laurell and Nyman, (1957), Brus and Lewis (1959), Shinton et al(1965) Giblett , et al (1966)
Liver diseases : infectious hepatitis, hepatoma or carcinoma of the liver, hepatitis and cirrhosis.	Jayle and Boussier, (1955) Owen et al , 1950), William et al (1961).
Megaloblastic anemia : pernicious anemia, folic acid and vitamin B12 deficiency.	Nyman (1957), Owen et al (1960) Shinton, (1965)
Pregnancy	Neel et al (1961) Giblett et al (1966)

Serum haptoglobin levels are increased in the following conditions :

CONDITIONS.	REFERENCES
Acute and chronic infections	Nyman (1959), Bovornkitti (1962) Jayle and Morretti (1962), Owen et al (1964), Murray et al (1966), Bogdanovich (1968).
Burns and trauma	Neuhaus (1964).
Lipoid nephrosis	Jayle and Morretti (1962).
Nephritis	Owen et al (1964), Rajendran et al (1975)
Myocardial infarction Arterial disease Acute rheumatic fever	Owen et al (1964), Jayle and Morretti (1962).
Ovarian malignancy	Mueller, et al (1971).
Carcinoma of the liver, biliary system and stomach	Jayle and Morretti (1962), O'Hara et al (1968).
Obstructive jaundice	Owen et al (1959), Jayle and Morretti (1962), William et al (1962).
Hodgkin's disease, leukaemia, lymphoma.	Owen et al (1964), Ivanyi et al (1961), Jayle and Morretti (1962), Shinton et al (1965) Krauss et al (1966),

CONDITIONS	REFERENCES
Acute scurvy	Robert et al (1961).
Collagen disease Rheumatoid arthritis	Owen et al (1964), Kobiela et al (1966), Turowska(1969), Anastassea-Vlachou et al (1973).
After injection of pyrogenic substance.	Nyman (1959).
Surgery in man	Owen et al (1961), Crockson et al (1966).
Experimental aseptic inflammation.	Murray and Connell (1960), Robert et al (1961).
Administration of androgen, glucocorticoids, parathormone and purified enzyme including papain, elastase and hyaluronidase.	Borglin and Nyman (1961), Robert et al (1961), Studnitz and Nyman (1957).

The polymorphism of haptoglobin makes this protein a useful genetic maker. Haptoglobin typing may be used as an adjunct to blood grouping in exclusion of paternity, forensically in the identification of blood stains, as well as in anthropological studies of relation among various populations.

The common application of such simple genetic systems is in excluding paternity in cases of disputed fatherhood. If testing is done for the three common haptoglobin phenotypes; Hp 1-1, 2-1 and 2-2, the possible exclusion of paternity using haptoglobin type is indicated:

MOTHER	CHILD	FATHER CANNOT BE
1-1	1-1	2-2
	2-1	1-1
2-1	1-1	2-2
	2-1	-
	2-2	1-1
2-2	2-1	2-2
	2-2	1-1

Obviously if sub-typing is carried out the power of the method would be considerably increased.

Another potential forensic application is to aid in the identification of dried blood stains. Culliford (1963) has claimed that blood stains dried on the cloth can be treated by moistening

with tris-citrate buffer and then inserting directly into the slot of a starch-gel. After electrophoresis and staining with benzidine reagent identification of the common haptoglobin patterns was possible.

Determination of the haptoglobin level is also helpful in the investigation of anemia caused by hemolysis, because serum haptoglobin determination is of great diagnostic value in differentiating between hemolytic and non-hemolytic anemia.

Estimation of serum haptoglobin

There are several different methods for the quantitative estimation of haptoglobin in serum:

1. Iodometric (Jayle, 1940)
2. Electrophoretic (Nyman, 1959; Hommes, 1959; Brus & Lewis, 1959; Owen, et al, 1959; Lathem & Worley, 1959; Rowe, 1961; Hall, 1964; Valeri et al., 1965; Ferris et al., 1966; Miale, 1964).
3. Spectrophotometric (Connell & Smithies, 1959; Owen, et al, 1960)
4. Immunological (Kluthe, et al , 1965)
5. Methods using gel-filtration on Sephadex (Lionetti,

et al., 1964; Killander, 1964).

These methods require either separation of free haemoglobin from the haptoglobin-bound haemoglobin or the use of special apparatuses. There is still an evident need for a simplified method adaptable to the facilities and requirements of routine laboratory work.

The formation of the haemoglobin-haptoglobin complex is the basis for almost all the methods for the determination of haptoglobin level. Basically, these methods depend upon the addition of an excess of haemoglobin to plasma or other solution containing haptoglobin. This mixture can then be treated in two different ways:

1. The bound and free haemoglobin can be separated from one another by simple techniques . The fraction of the total haemoglobin associated haptoglobin indicated the haemoglobin-binding capacity of the latter, which for practical purposes is taken as the haptoglobin level. The separation of haemoglobin from haemoglobin-haptoglobin complex can be achieved either by electrophoresis on paper (Javid and Horowitz , 1960 ; Nyman, 1959 ; Miale, 1964), Cellulose acetate (Erus and Lewis, 1959 ; Valeri et al, 1965) starch gel

(Smithies, 1959) and acrylamide gel (Ferris, et al 1966) or by gel filtration on Sephadex (Valeri et al., 1963).

If increasing amounts of haemoglobin are added, it is possible to measure how much haemoglobin can be bound by a particular serum by noting when a second band of free haemoglobin appears.

A better quantitative meaurement can be achieved by adding a measured amount of haemoglobin to serum and estimating the amount of bound and free haemoglobin by scanning electrophoretic strips after staining. The concentration of haptoglobin can be expressed as mg of haemoglobin-binding capacity per 100 ml of serum.

2. The peroxidase activity of the haemoglobin-haptoglobin complex can be measured under conditions in which free haemoglobin has negligible activity. Under these conditions the peroxidase activity is directly proportional to the amount of the complex which, in turn, is determined by the amount of haptoglobin present.

Several methods based on this principle are available which are suitable both for clinical and investigative applications.

The correlation among the haptoglobin levels determined by these various methods is good.

As mentioned above, the haptoglobin levels were elevated in a wide variety of inflammatory diseases of tissue destruction and were decreased in intravascular haemolysis, pernicious anaemia and liver cell failure. Determination of the haptoglobin levels is, therefore, helpful in the investigation of anaemia caused by haemolysis. Since the parenchymal cells of the liver are responsible for synthesis of serum proteins including haptoglobin, there might be some alterations in the serum haptoglobin level of patients with hepatic diseases.

The object of the present study is to estimate the level and type of haptoglobin in serum of the pregnant women, patients with malaria, amoebic liver abscess, hookworm, infectious hepatitis and other liver diseases as well as in the normal Thai subjects.