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COIL PLANET CENTRIFUGATION: ALTERED PATTERNS OF HEMOLYSIS BAND IN HEPATOBILIARY DISORDERS, AND THEIR DIAGNOSTIC SIGNIFICANCE

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Abstract

Blood samples were collected from 36 patients with various hepatobiliary disorders (acute, subacute, and chronic hepatitis, liver cirrhosis, hepatoma, and biliary tract obstruction), and examined by coil planet centrifugation periodically at intervals of approximately a week for 1 to 3 months. The shape of the hemolysis bands appearing in the coils (with hemolysis peak to the left side: L, at the midpoint: M, forming a table-like plateau: T, and to the right side: R) were recorded by a photoelectric densitograph, and collated with the shift of hemolysis end point toward the hypo-osmolar side to observe the altered properties of erythrocyte membrane in these diseases. The densitography disclosed significant changes in the shape of hemolysis bands according to the diseases of different types and with the vicissitudes of the clinical course.

(1) There was a correlation between the shape of hemolysis band and the severity of diseases. Shapes L, M, T and R were indicative of disturbances of slight, slight to moderate, moderate and severe grades, respectively.

(2) In subacute yellow atrophy of the liver (subacute hepatitis) the shape R hemolysis band was prevalent.

(3) In acute hepatitis which took a favorable course the hemolysis band was of R type during its active stage and assumed L type in recovery stage, passing through T and M types during the intermediate stage.

(4) In chronic hepatitis the hemolysis band was commonly of T type in shape, and with the progress in recovery it took the shapes of M and then L in the order listed.

(5) In liver cirrhosis the hemolysis band was of L type (with narrow width). The hemolysis starting and end points both shifted to the hypo-osmolar side. This pattern remained stationary during the whole course of disease.

(6) In hepatoma both the hemolysis end and starting points shifted to the hypo-osmolar side gradully with the advance in illness, and the hemolysis band was commonly of R type.

(7) In biliary obstruction the hemolysis band was wide with a plateau at its peak (T type), resembling chronic hepatitis in shape. The hemolysis end point shifted toward the hypo-osmolar side. The pattern was of T type so long as the obstruction was persistent.

INTRODUCTION

It was Kitazima and Shibata[1] who first noticed the shift of hemolysis end point (HEP) toward the hypo-osmolar side in hepatobiliary disorders by coil planet centrifugation of the patient's blood samples. According to their observation hypo-osmolar-ward shift of HEP mirrored the severity of the hepatobiliary disturbances. They could predict the prognosis (aggravation or improvement) by the degree of deviation of HEP from its normal range.

The author, who was interested in their investigation, attempted to extend the diagnostic utility of coil planet centrifugation (CPC), and made further observations on hemolysis bands in the coils obtained in these diseases.

As a result of such observations the author found that the hemolysis bands could be classified into four types; namely, L, M, T, and R types by the shape of their photoelectric densitograms, and he discovered that this classification of hemolysis bands was a useful guide index for pursuing the clinical course of the hepatobiliary disorders by CPC in addition to the shift of HEP which had been utilized as a criterion by Kitazima and Shibata[1].

SUBJECTS AND METHODS

The subjects of observations consisted of 36 patients with hepatobiliary disorders (acute hepatitis 11, subacute hepatitis [subacute yellow atrophy of the liver] 2, chronic hepatitis 6, liver cirrhosis 8, hepatoma 4, and biliary tract obstruction 5) whose diagnoses had been established by combined use of routine tests of clinical chemistry (including blood hemoglobin, serum protein, fasting blood sugar, A/G ratio; icteric index, direct bilirubin, total bilirubin; thymol turbidity, albumin, globulin, cholinesterase, cholesterol, alkaline phosphatase, plenol turbidity test, transminases; namely, GPT and GOT, urea N, uric acid and amylase), isoenzymes (lactate dehydrogenase LDH, leucine aminopeptidase LAP, alkaline phosphatase AlK Phos), and electrophoretic fractionation of serum protein[2]. Tests of Au-antigen (HB antigen)[3], α-fetoprotein[4], needle biopsy of the liver[5], percutaneous transhepatic cholangiography[6] and ultrasonic diagnosis[7] were also employed for the diagnosis whenever necessary.

The blood samples (about 1 ml from the antecubital vein, preventing coagulation with a drop of Anticlot Et) were collected periodically (once a week for 1 to 3 months) from the patients, and examined by the standard method of CPC technique which was described by the author in the previous communication[8,9]. The hemolysis bands appearing in the coil were subjected to a photoelectric densitograph to observe the shape of their recorded curves (densitograms).

The densitograms were classified into the following four types according to the positions of the peak in the hemolysis bands. (1) Type L: the peak lies biased to the left half of the band, producing a shoulder-like protrusion, (2) Type M: the peak is situated in the middle portion, (3) Type T: the peak makes a table-like plateau in the mid portion of the band, and (4) R: the peak is deviated to the right half of the band, making a shoulder-like prominence.

RESULTS

Fig. 1 to 6 illustrate the typically representative hemolysis bands and clinical courses of acute hepatitis, subacute hepatitis, chronic hepatitis, liver cirrhosis, hepatoma, malignant biliary obstruction, respectively. Other cases were more or less similar in pattern to the relevant representative cases.

Table 1 summarizes the results of our observation, in which the

TABLE 1

Incidence of various pattern (L, M, T and R) in common hepatobiliary disorders.

Diseases \ Patterns (%)	R	T	M	L
Acute hepatitis	2	19	10	69
Chronic hepatitis	0	30	30	40
Liver cirrhosis	2	34	29	36
Hepatoma	31	23	12	35
Obstructive Jaundice	7	63	20	10

incidences of the characteristic shapes of hemolysis bands are arranged according to the difference in the nature of hepatobiliary disorders for the sake of comparison.

DISCUSSION

(1) Acute hepatitis (Fig. 1 a, b):

As had been pointed out by Kitazima and Shibata[1], the HEP of CPC hemolysis band shifted to the hypo-osmolar side when the hepatic disturbance was severe (bilirubinemia, and hypertransaminasemia [GPT] were distinctly present), and returned to the normal range at recovery stage. This will be clearly seen in Fig. 1. The patient illustrated in this figure was a 59-year old male. He had been feeling general lassitude since the 4th of June, 1976. He became pyrexic and anorexic on the next day and came to our hospital on the 9th of June; his skin jaundiced generally, and the liver was palpable 1 finger breadth below the costal arch,

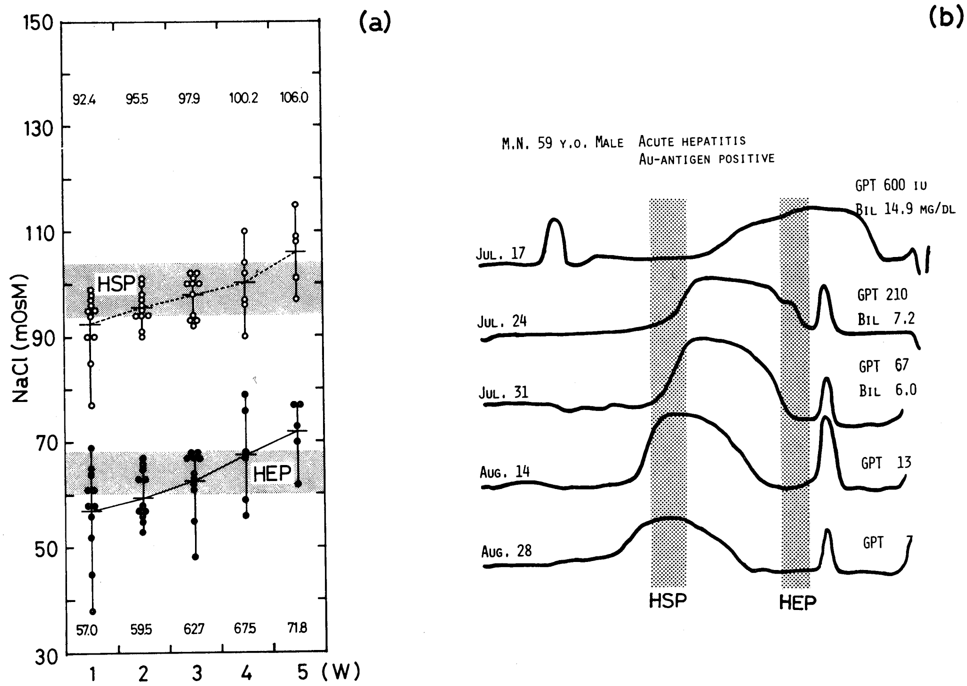

Fig. 1. Acute hepatitis. a: Week-to-week changes of the hemolysis end point (HEP) and the hemolysis starting point (HSP).
b : Illustrative case showing changes in the shape of hemolysis band with improvement in clinical course. Shaded areas refer to the normal ranges of HSP and HEP.

and tender. The diagnosis of acute hepatitis (type B) was made on the basis of laboratory examinations; namely, his GPT and GOT were increased above the level of 1,000 IU/l. The HB antigen was positive. After 3-month's admission, transaminases returned to the normal range and the HB antigen became negative, and he was discharged. There was an interesting change in the shapes of the hemolysis bands simultaneously with the restoration of HEP from the hypo-osmolar side to the hyper-osmolar side in the course of recovery.

In the previous report it was stated by the author[9] that the CPC hemolysis band assumed the shape of type L in normal subjects, occasionally it was of type M, and rarely of type T or R. Therefore, the types T and R were thought to indicate an abnormality, possibly a latent pathological process.

In the cases of acute hepatitis, the type R shape was frequent at its active stage, and the hemolysis band underwent gradual and sequential transition of shapes, R → T → M → L with improvement in clinical manifestations and laboratory examinations. Occasionally the shape of hemolysis band passed from R directly to L, skipping the intermediate types (T and M). The patient shown in Fig. 1 was an example of such a case.

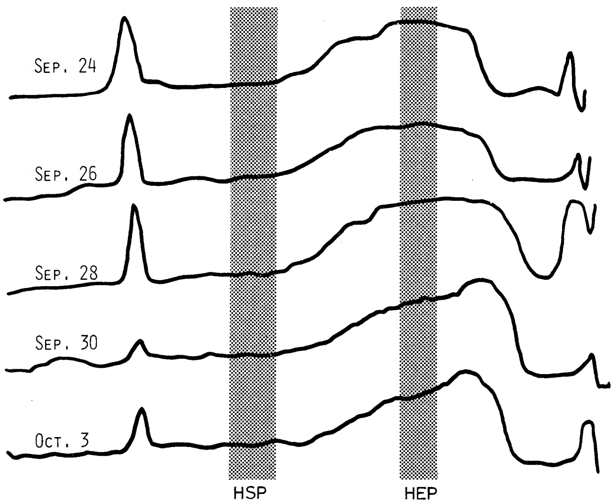

Fig. 2. Altered shapes of hemolysis band encountered in subacute hepatitis. The band is of R type from the onset of the illness. Its summit becomes increasingly prominent with simultaneous shift of the hemolysis end point (HEP) toward the hypo-osmolar side in parallel to the aggravation in hepatic disturbances.
Shaded areas refer to the normal ranges of HSP and HEP.

(2) Subacute hepatitis (Fig. 2):

With subacute hepatitis, the HEP shifted to the lower osmolar side remarkedly, and the hemolysis band was R type in shape. The right shoulder soared more saliently in proportion to the advance in aggravation. The patient of Fig. 2 was a female aged 30 who complained of general lassitude late in June, 1976 and became pyrexic (38°C) early in August. She was diagnosed as hepatitis on the basis of routine tests of clinical chemistry on the 10th of August. She was treated in the out-patient clinic without significantly favorable improvement and was admitted to the hospital on August 26. However, jaundice appeared abruptly on September 19, and serum bilirubin rose to 14.5 mg/dl, transaminase (GOT and GPT) increased gradually and she became dysarthritic. Diagnosis of subacute hepatitis was thus made. The hemolysis band in the CPC coil assumed a shape of type R which was characteristic of severe hepatic disturbance.

(3) Chronic hepatitis (Fig. 3 a, b):

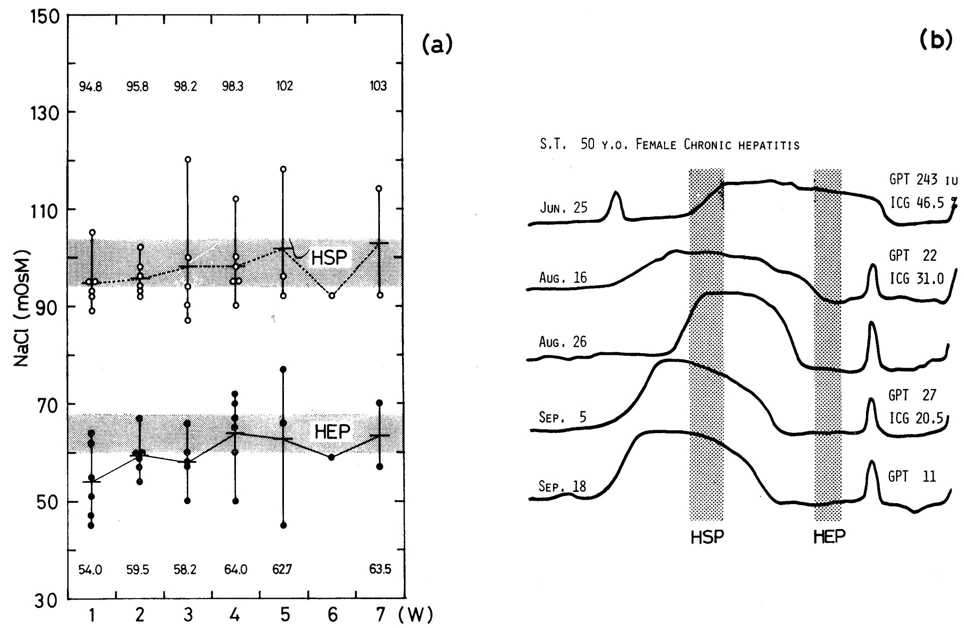

Fig. 3. Chronic hepatitis. a: Weekly observed change of hemolysis end point (HEP) and hemolysis starting (HSP).
b: Representative typical case of the change in the shape of hemolysis band (T → TL → ML → L).
Shaded areas refer to the normal ranges of HSP and HEP.

It is worthy of emphasis that the T type hemolysis band pattern was commonly seen in chronic hepatitis. Of course, there is simultaneous shifting of the HEP to the hypo-osmolar side with broadening in the width of the band (HSP → HEP). With improvement in clinical manifestation the left portion of the T-shaped plateau rises slowly, approaching the shape of type L to a certain extent, although the band itself still remains to be broad (type TL). Then the band becomes narrow and, at length, resumes the shape of type L of a normal subject.

The patient whose hemolysis band is depicted in Fig. 3 was a 46-year old female who had general lassitude and vomiting on March 23, 1976. She was admitted to a hospital with the diagnosis of acute hepatitis. After admission hepatic dysfunction became increasingly apparent with intensification of jaundice. She was therefore brought to our hospital on the 22nd of June. At first the patient seemed to have subacute hepatitis, but needle biopsy of the liver established the diagnosis of chronic hepatitis. When the patient was in aggravation, her hemolysis bands took the transition of shapes from type T to type R.

(4) Liver cirrhosis (Fig. 4 a, b):

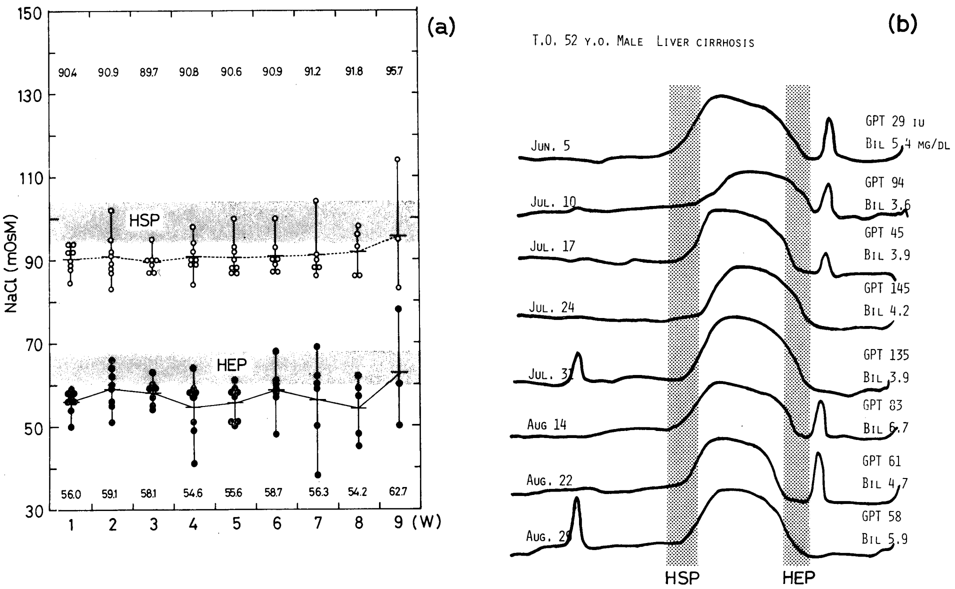

Fig. 4. Liver cirrhosis. a: Weekly observation of the change in hemolysis end point (HEP) and hemolysis starting point (HSP). b: Typical changed pattern of hemolysis band. Note that the band is of M or ML type.
Shaded areas refer to the normal ranges of HSP and HEP.

Liver cirrhosis is characterized by the shift of both HEP and HSP toward the hypo-osmolar side. Consequently, the width of the hemolysis band becomes narrower. It is curious that the shape of hemolysis band is almost always of type L notwithstanding the apparent hepatic dysfunction. Another aspect worthy of special mentioning is the sationary status with respect to the shape and position (HSP and HEP) of the band for a long period of observation.

The patient illustrated in Fig. 4 was a male aged 45 who had general lassitude, abdominal distension, slight jaundice. He was found to have esophageal varices by X ray examination in February, 1973. The diagnosis of liver cirrhosis was therefore entertained and he was hospitalized in April, 1975. He was once improved and discharged in January, 1976. After 2 months, he had abdominal distension again and was admitted on March 11, 1976.

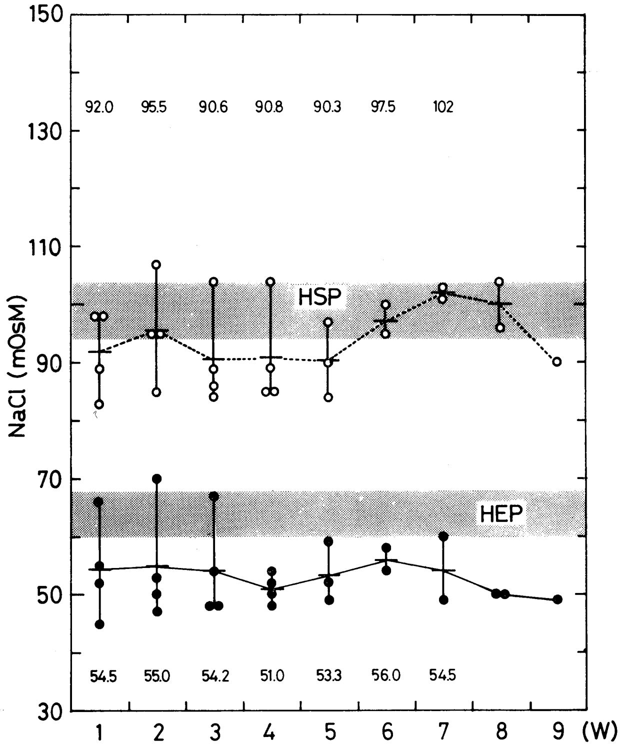

Fig. 5. Hepatoma. a: The changes of hemolysis end point (HEP) and hemolysis starting point (HSP) as observed at weekly intervals. They are gradually shifting to the hypo-osmolar side with lapse of time.
Shaded areas refer to the normal ranges of HSP and HEP.

(5) Hepatoma (Fig. 5):

Unfortunately, representative case of hepatoma was not available. However, it will be seen from Figure 5 that the hemolysis end and starting points shift gradually to the hypo-osmolar side with the advance in hepatic disturbance. The hemolysis band was of R type in 31 percent of cases, suggesting the frequent occurrence of severe hepatic disturbance.

(6) Biliary obstruction (Fig. 6 a, b):

The hemolysis band assumes the shape of type T in biliary obstruction. The peak is wide, and the width of the band is large, resembling those of chronic hepatitis. The HEP shifts to the hypo-osmolar side.

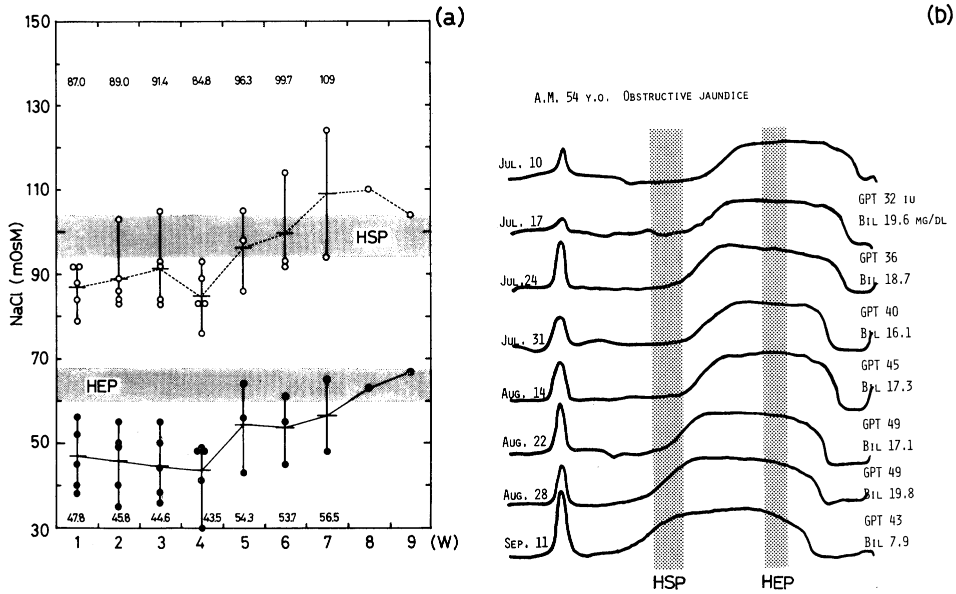

Fig. 6. Biliary obstruction. a: The changes of hemolysis end point (HEP) and hemolysis starting point (HSP) which were observed at weekly intervals. Shift of the HEP is the most remarkable of the various hepatobiliary disorders. This becomes less evident, returning to the normal range with the alleviation of obstruction.
b: Illustrative case showing typical changes of hemolysis band. The shape of band remains to be of T pattern so long as the obstruction is persistent.
Shaded areas refer to the normal ranges of HSP and HEP.

The case shown in Fig. 6 was a 54-year old male who had been hospitalized several times because of gastric ulcer since 1967 and was still attending the clinic in 1973. A dark urine was noted in April, 1973 with appearance of jaundice. A diagnosis of obstructive jaundice was entertained on the basis of laboratory examinations. He was admitted on April 20, and died on October 3, 1973. At autopsy he was found to have intrahepatic cholangiocarcinoma.

Takano[10] was the first investigator who classified the CPC hemolysis band into several groups. During the course of the study on the membrane properties of erythrocytes of normal subjects by means of CPC he noticed the various hemolysis bands corresponding to the L, M, T and R types which were mentioned by the author in this paper. Kitazima and Shibata[1] found in their works on coil planet centrifugation that the clinical course of a patient with hepatobiliary disorder could be nicely pursued by repeated examinations of the HEP of the hemolysis band in the coil, because the HEP shifts toward the hypo- or nomor-osmolar side sensitively to aggravation or improvement, respectively. They propounded a hypothesis that LCAT (lecithin cholesterol acyltransferase) activity in plasma would be in close correlation to the shift of the HEP. In their opinion, LCAT, the enzyme supplied from the liver into the blood plasma, promotes an increase in plasma esterified cholesterol, diminishing the free cholesterol in plasma. The damage to hepatic parenchyma in liver diseases will cause decreased production of LCAT and consequently diminished supply of this enzyme to plasma. This results in stagnation of free cholesterol in plasma. Such free cholesterol passes into the membrane of erythrocytes and fortifies its membrane, so that it becomes resistant to hypo-osmolar stress. The HEP in CPC test shifts, therefore, toward the hypo-osmolar side. This will be one of the cause of the HEP shift in the parenchymatous diseases of the liver. Furthermore, in biliary obstruction bile acids regurgitate from the bile canaliculi to the blood and they rise to a considerably high level. Since bile acids are the inhibitor of LCAT, another cause of decrease in LCAT activity will be conceivable for obstructive jaundice. Thus, the HEP shifts toward the hypo-osmolar side will be understood as an end-result of stagnation of free cholesterol in plasma, because this passes into erythrocyte to strengthen its membrane.

The alteration of the membrane properties of erythrocytes (increased resistance to hypotonic salt solution) in hepatobiliary disorders was discovered as early as in 1907 by Chauffard[11] when he established hereditary spherocytosis as a disease entity on the basis of the increased osmotic fragility of patient's erythrocytes.

However, the technique employed by him for the test of osmotic fragility (or resistance) was so primitive that it did not enable him to make diagnostic use for hepatobiliary diseases. The Parpart method[12] which was developed later is a milestone of improvement in the technique for the measurement of osmotic fragility, but it is a little complicated in manipulation as a routine diagnostic work of hepatobiliary disorders, although it is appropriate for hematological use. In this respect the coil planet centrifugation is recommended as the most convenient and efficient tool. It is particularly useful for pursuing the clinical course of hepatobiliary disorders. The author wants to emphasize that inspection of the densitogram of the hemolysis band together with the observation of the HEP shift to the hypo-osmolar side will give valuable information more abundantly than the simple examination of the HEP shift that was introduced by Kitazima and Shibata[1].

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