STUDIES ON BLOOD SPECTRUM

II. EVALUATION OF BLOOD SPECTRUM AS THE MEANS OF DIAGNOSIS OF HEPATOBILIARY DISTURBANCES

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Owing to the remarkable progress in clinical biochemistry of the recent two decades the tests for hepatic function have assumed a new aspect.[1-3] Many of the early qualitative methods have been displaced by the up-to-date quantitative tests of higher specificity and sensitivity, but most of the difficulty inherent in the diagnosis of the damage to hepatic parenchyma and of the disturbance in the biliary outflow has not as yet been overcome. The best that we can do at present is to make a synthetic interpretation of the combined results of the excellent hepatic tests that are available; an appraisal of hepatobiliary function by a single test is of course impossible at present and will remain so in the future. Such a systematic examination of hepatobiliary function by combined results of the various quantitative tests was already adopted by *Shibata*[4] in 1954. Based on his experiences of studies on hepatic functional tests lasting for several years, he has introduced into his blood spectrum the following ten tests: (1) icteric index (Ict I or Ii) and total bilirubin in serum (Bil), (2) cephalin cholesterol flocculation test (CCF), albumin (Alb), globulin (Glob) and serum cholinesterase (ChE), and (3) one-minute or 15 minute bilirubin (1-m or 15-m Bil), alkaline phosphatase (Alk. P), cholesterol (Chol) and phenol turbidity test (Ph. t) for the detection of jaundice, damage to hepatic parenchyma and biliary obstruction, respectively.[5,6] The results of these tests were divided into three groups, and graphs of jaundice, of hepatic parenchyma and of biliary obstruction were made. By studying these groups the author (*Shibata*) has stated that it is possible to arrive at the diagnosis of hepatobiliary disorders from the purely clinico-biochemical viewpoint (Figure 1).

In the past three years studies have been carried out in our laboratory on 662 cases of the liver and the bile duct diseases to determine whether or not the blood spectrum is useful or appropriate in diagnosing the hepatobiliary disorders. Histological picture of the liver obtained by needle biopsy[7-10] was also compared with the blood spectrum on 159 patients for the same purpose. In the present paper the author aims to present the results accumulated in this study with special

reference to the graphs of hepatic parenchyma and biliary obstruction.

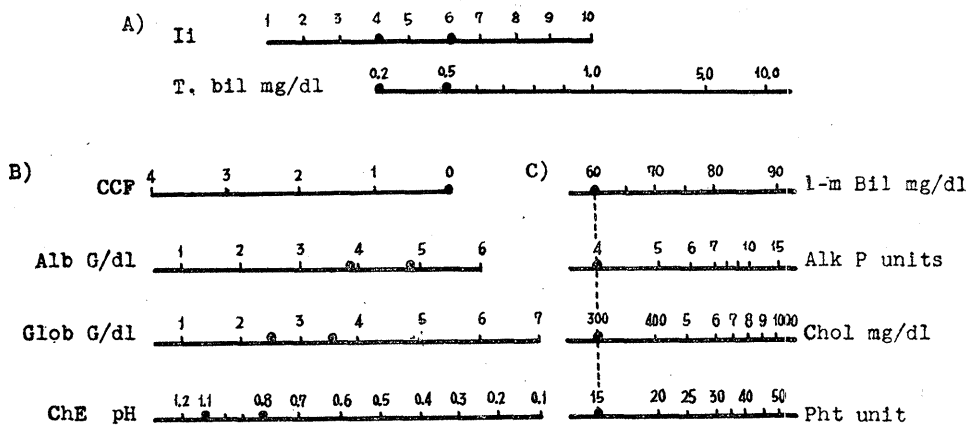


Fig. 1. Graph A for the detection of jaundice, Graph B for the assessment of the damage to hepatic parenchyma and Graph C for the appraisal of the degree of biliary obstruction.

METHOD

Samples of blood taken on empty stomach in the early morning from six hundred and sixty-two patients with hepatobiliary diseases (Table I), were

TABLE I

Cases examined
(Clinical cases)

Gall stone	163
Hepatitis (acute)	81
Hepatitis (chronic)	79
Liver cirrhosis	73
Biliary cirrhosis	31
Hepatic cancer	29
Liver abscess	28
Cancer of the biliary tract.	12
Cholangitis	12
Acute yellow atrophy	11
Others	143
Total	662 cases

subjected to the procedures listed in Table II[11-22] to determine the behavior of the ten chemical constituents. Graphs of (1) jaundice, (2) hepatic parenchyma, and (3) biliary tract were constructed with the results obtained, and their values marked on the relevant parallel, graduated lines were connected with straight

lines as shown in Figure 1. The resultant forms of connecting lines were examined for their shape to study the distribution of specified pattern of connect-

TABLE II

Procedures employed

1.	Icteric index	(Ii)	Comparator colorimetry with potassium-dichromate-cobaltous-sulfate solution as standard series.[11]
2.	Bilirubin	(Bil)	Methanol-diazo method (Malloy-Evelyn).[13]
3.	Cephalin cholesterol flocculation test	(CCF)	Sumitomokagaku's Hanger antigen (Hanger).[14]
4.	Alubumn & Globulin	(Alb & Glob)	Sodium-sulfate-salting-out method adapted to biuret reagent.[15]
5.	Cholinesterase	(ChE)	Phenol-red-comparator methed.[16,17]
6.	Alkaline phosphatatase	(Alk. P)	Modified Shinowara's glycerophosphate method.[19, 20]
7.	Cholesterol	(Chol)	Modified Bloor's method.[21]
8.	Phenol turbidity	(Ph. t)	Kunkel's method.[22]

ing lines in the representative hepatobiliary diseases. In addition, the bits of hepatic tissue obtained by needle biopsy[7] (Vim-Silverman needle) from 159 patients with non-hepatobiliary as well as hepatobiliary diseases (Table III)

TABLE III

Cases examined
(Biopsy)

Hepatitis	20
Liver cirrhosis	13
Gall stone	9
Obstructive jaundice	8
Hepatic cancer	4
Others	105
Total	159 cases

were fixed in *Carnoy*'s fluid, stained with hematoxylin and eosin and their histological pictures studied (Table IV)[8,9,23,] in order to find if there is correlation between them and the graphs of hepatic parenchyma and biliary obstruction.

The graphs constructed are as shown in Figure 1. In this figure the parallel lines were adequately graduated so that the connecting line might run within the normal area (circumscribed by dots), which formed an upright short rectangle, an oblique long strip from upper right to lower left, and a space to the left of an imaginary vertical line connecting the upper limits of the normal range for the graphs of jaundice, the hepatic parenchyma and the biliary obstruc-

tion, respectively. In diseased persons the connecting lines naturally protruded frequently from the normal area, either partly or totally, presenting various patterns, since the concentration of chemical constituents was often abnormal. The

TABLE IV

Examination of the hepatic histology [23]

A) Damage to the hepatic cells.
   1. Variation in the size of nucleus.
      Unequality in or loss of stainability of nucleus.
   2. Decreased basophilism of the cytoplasm.
   3. Formation of acidophilic granulations in cytoplasm.
   4. Increased distinctness of the cell border.
   5. Coagulation necrosis.

B) Inflammation.
   1. Round cell infiltration (lymphocyte, polymorphonuclear leukocyte, plasma cell and histiocyte).
   2. Mobilization of Kupffer cells.
   3. Focal necrosis of the liver cell cords.
   4. Perilobular infiltration of round cells.
   5. Round cell infiltration in the portal triads.
   6. Increased proliferation of fibroblasts.

C) Scarring.
   1. Formation of pseudo bile ducts.
   2. Increased formation of interlobular bile ducts.
   3. Angiogenesis (Increased production of capillary vessels).
   4. Altered reconstruction of the hepatic lobule.
   5. Proliferation of fibrocytes.
   6. Increase in the amount of fibrous tissue in the portal triads.

D) Obstruction to biliary outflow.
   1. Bile pigment granules in Kupffer cells.
   2. Bile pigment granules in hepatic cells and pigment thrombi in bile canaliculi.

Histological pictures listed above were graded as 0,1+,2+ and 3+ according to the increased distinctness (Popper and Szanto[23] were followed strictly in this regard). The number of+was summed up with respect to the groups A, B, C and D separately in order to appraise the degree of the lesion in hepatic tissue as classified into damage to hepatic cell, inflammation, scarring and biliary obstruction, respectively.

patterns were classified into N, n, d and D (graph of hepatic parenchyma) or into N, n, ω and 0 (graph of biliary obstruction) according to the blood ingredients which assumed abnormal concentration being 0 (or 1), 2, 3 and 4 in number, respectively (Figure 2). The patterns N and n were further divided into subtypes, marking the dots (.) beside the letter representing the pattern in order to specify the individual ingredients which were outside normal range. The patterns d and ω were similarly subdivided, marking a dash (′) beside the letter to specify

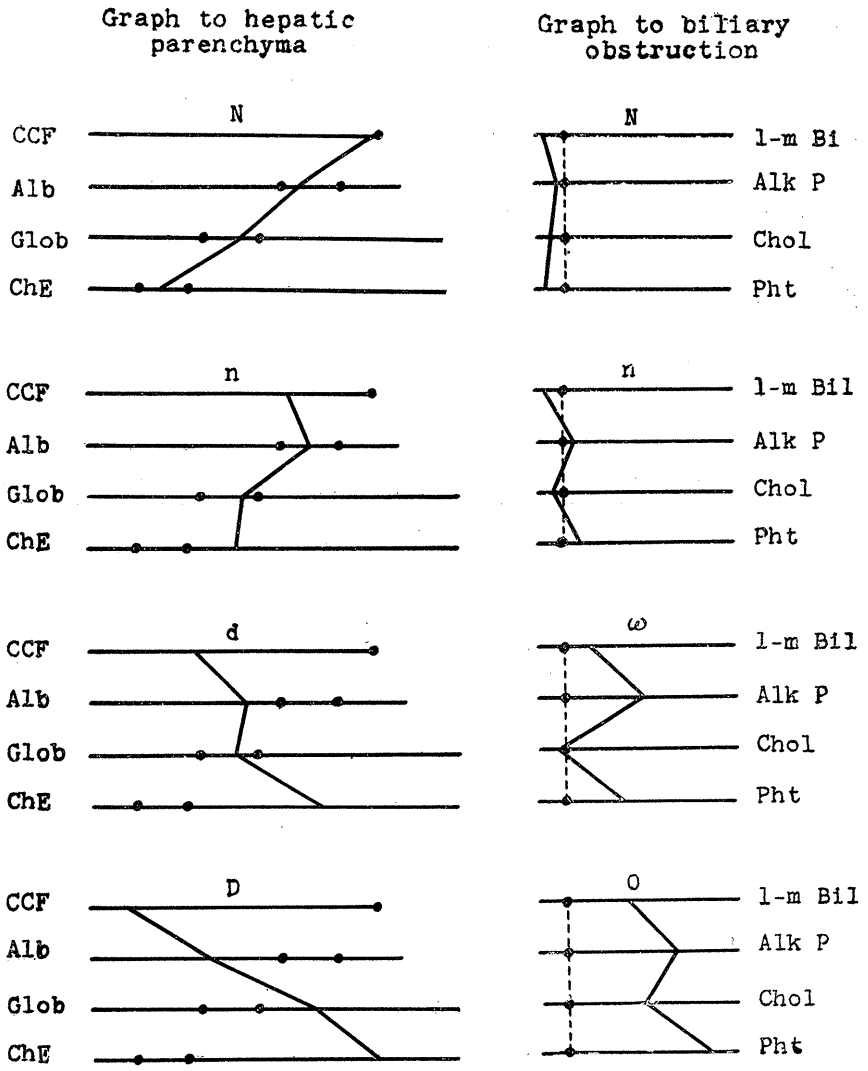


Fig. 2. Normal and abnormal patterns of graphs B and C.

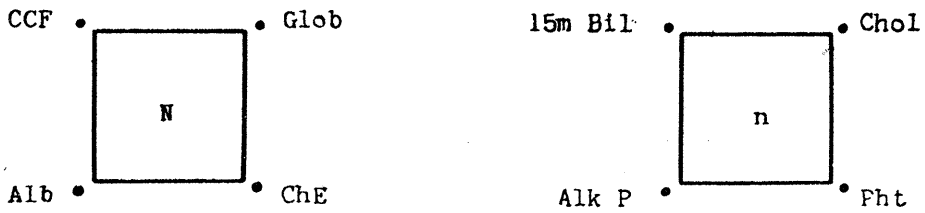


Fig. 3. Classification of subpatterns of graph B and C. Dots are marked to the notation (N,n, etc.) which represents the subpattern in order to indicate the blood chemical constituents of abnormal concentration. Position of dots in relation to the individual chemical constituents is illustrated in this figure.

the ingredient whose connecting line remained within the normal limits. The detailed description of the notation will be apparent from Figure 3.*

RESULTS

The graphs of hepatic parenchyma and of biliary obstruction encountered in hepatobiliary disorders were greatly variegated in their pattern, as shown in Table V. The frequency with which patterns N, n, d and D (hepatic parenchyma)

TABLE V

Graphs of hepatic parenchyma & biliary obstruction

A) GRAPH OF HEPATIC PARENCHYMA		B) GRAPH OF BILIARY OBSTRUCTION	
N pattern	148	N pattern	431
N	49	N	194
˙N	33	.N	103
N.	33	N.	74
N˙	27	˙N	34
.N	6	N˙	26
n pattern	137	n pattern	125
˙n˙	39	n:	34
˙n.	37	.n.	33
.n.	26	:n	27
.n˙	19	.n˙	17
n:	13	˙n.	9
:n	3	˙n˙	5
d pattern	155	ω pattern	63
′d	50	′ω	31
,d	46	ω,	15
d′	38	ω′	14
d,	21	,ω	3
D pattern	203	0-pattern	31

and patterns N, n, ω and 0 (biliary obstruction) appear in the representative diseases of the liver and biliary tract was as follows:-

(1) Infectious hepatitis and liver cirrhosis: Great advance in the knowledge of infectious hepatitis was made possible by the recent study on its pathology.[24-28]

* Figure 3. Notation of the patterns of graphs.
Left shoulder, left foot, right shoulder and right foot of the letter indicating the pattern are assigned to cephalin cholesterol flocculation test, albumin, globulin, and cholinesterase, respectively, in the graph of hepatic parenchyma, while to one minute (or 15 minute) bilirubin, alkaline phosphatase, cholesterol and phenol turbidity tests respectively, in the graph of biliary obstruction. In patterns N and n dots are marked to the assigned position to indicate the ingredients of abnormal value, and in patterns d and ω a dash is put to indicate the constituent residing within normal range. Example:- ˙N refers to either the graph of hepatic parenchyma in which all the tests (or ingredients) except CCF are normal or the graph of biliary obstruction where all the tests except one-minute (or 15 minute) bilirubin are normal. Notation ˙n. indicates the pattern (CCF positive; Alb and Glob normal; ChE decreased) or the pattern (1-m. Bil increased; alkaline phosphatase and cholesterol normal; Pht positive). Notation ,d refers to the pattern (CCF positive; albumin normal; Glob increased; ChE decreased) and notation ω′ represents the pattern (1-m. Bil increased; Alk P increased; Chol normal; Pht positive).

In non-fatal cases of this disease[27] there appear destruction of liver cells, mobilization of Kupffer's cells and round cell infiltration in the lobule and the portal triads (signs of inflammation) early before jaundice manifests itself, constituting the icteric stage and occupying first two weeks from the onset of clinical symptoms. Toward the time of the appearance of jaundice (icteric stage; the third and fourth morbid weeks) the histological picture of the liver is further complicated by the entry of bile thrombi, bile pigment in liver cells, and the dilatation of bile canaliculi and bile ducts (signs of biliary stasis) as well as by the mitotic division of liver cells and tortuous bile ducts (signs of reparation). Biliary stasis disappears in the fifth morbid week, but inflammation persists until the eighth morbid week (*Mallory*).[27] When inflammation does not subside completely even in eight weeks, either acute inflammation recurs or the hepatitis passes into the chronic stage. According to *Amano*[28),29] the liver cirrhosis is the end-result of hepatitis, either overt or latent, because hepatic inflammation gives rise to fibrous tissue increase in the portal triads and the distorted reconstruction of the hepatic parenchyma (pseudo lobule) during the long course of reparation in hepatitis. On the contrary, in the fatal hepatitis (acute or subacute yellow atrophy of the liver)[24,26] there is an extensive destruction of hepatic tissue in the icteric stage: dissolution of hepatic cells with the loss of stainability of nucleus, hemorrhage and intensive infiltration of round cells. Accordingly, viewed from the standpoint of pathological anatomy, the hepatic damage is thought to be the severest in the acute or subacute yellow atrophy, next in order of severity in the preicteric and icteric stage (within four weeks after the onset of disease), and relatively slight in the stage of convalescence (the fifth to eighth morbid weeks). In the ordinary acute cases, reparation of hepatic lesion will be almost complete after the eighth morbid week, and the anatomical lesion of the liver is consequently supposed to be less serious in chronic hepatitis[29] than in the liver cirrhosis.[20,31]

The relative frequency of the patterns of the graph of hepatic parenchyma in hepatitis is illustrated in Figure 4, which reveals the overwhelmingly large number of pattern D with a few cases of pattern d in acute or subacute yellow atrophy, which makes a sharp contrast with the distinct majority of patterns N and n with minority of patterns d and D in the ordinary hepatitis of the eighth or later morbid week. The correlation of this figure with the pathological anatomy of hepatitis described above will naturally lead to the conception that patterns D and d refer to the severe damage to the liver, while patterns N and n indicate the slighter damage. Comparison of chronic hepatitis with liver cirrhosis (Figure 5) was also corroborative of this view.

(2) Various hepatobiliary disorders. The patterns of the graph of hepatic parenchyma were studied in comparison with the histological picture of the liver tissue obtained by needle biopsy in a variety of hepatobiliary diseases. It was

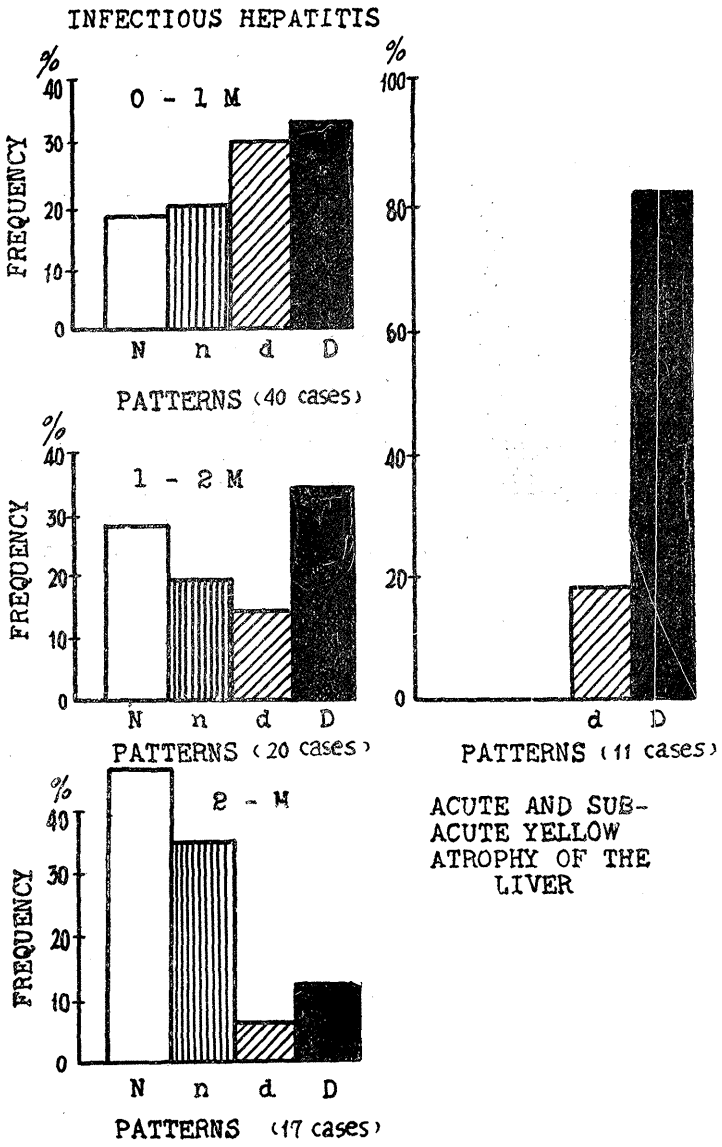


Fig. 4. Frequency distribution of the various patterns of graph B in infectious hepatitis and acute (and subacute) yellow atrophy of the liver. (0-1M, 1-2M and 2-M indicate the stages of 0-1, 1-2 and more than 2 months after the onset of illness).

discovered that the patterns varied in the order N→n→d→D with increase in the severity of the hepatic damage (Figure 6). Furthermore, there was a close parallelism between the average pattern of the graph and the mean hepatic damage, when such a comparison was made in each individual disease (Figure 7).

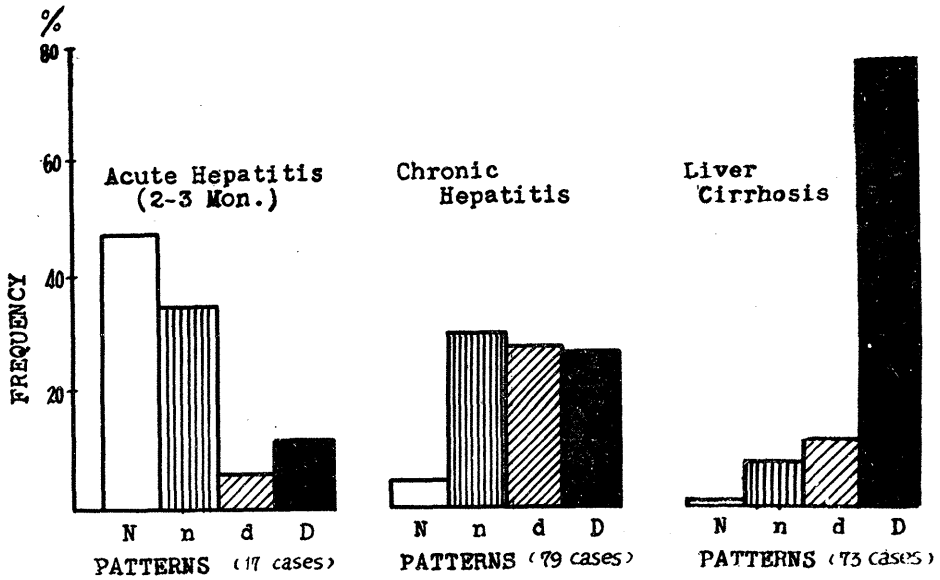
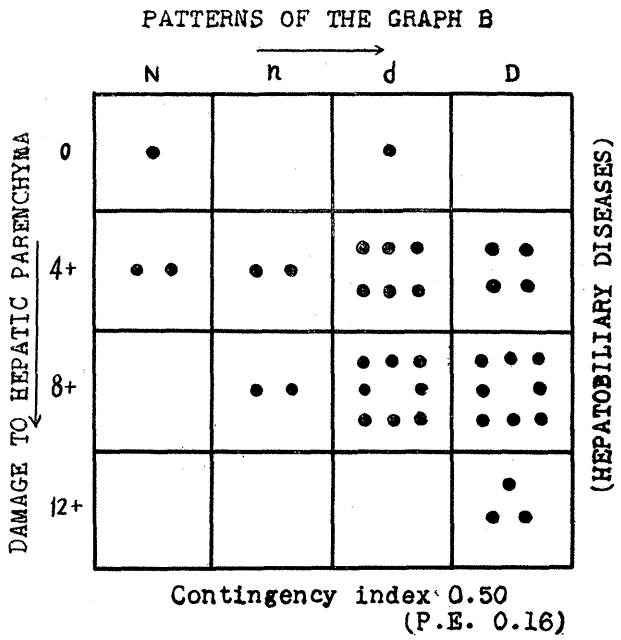


Fig. 5. Frequency distribution of the various patterns of graph B in acute and chronic stages of infectious hepatitis and liver cirrhosis. (Acute hepatitis in this figure is related to the stage of 2–3 months after the onset of clinical symptoms, excluding the earlier stages. Later stages are dealt with as chronic hepatitis.)



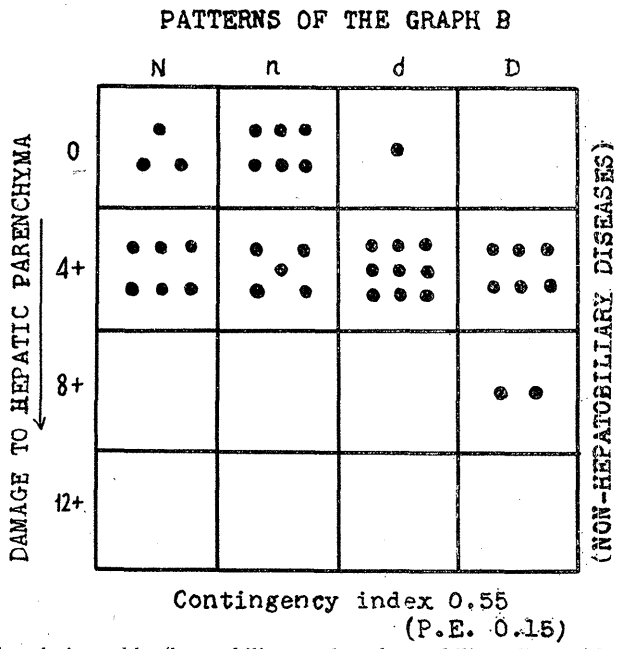


Fig. 6. Correlation tables (hepatobiliary and nonhepatobiliary diseases) between the patterns of graph B and the damage to hepatic parenchyma as observed by the microscopic examination of the liver fragment obtained by biopsy.

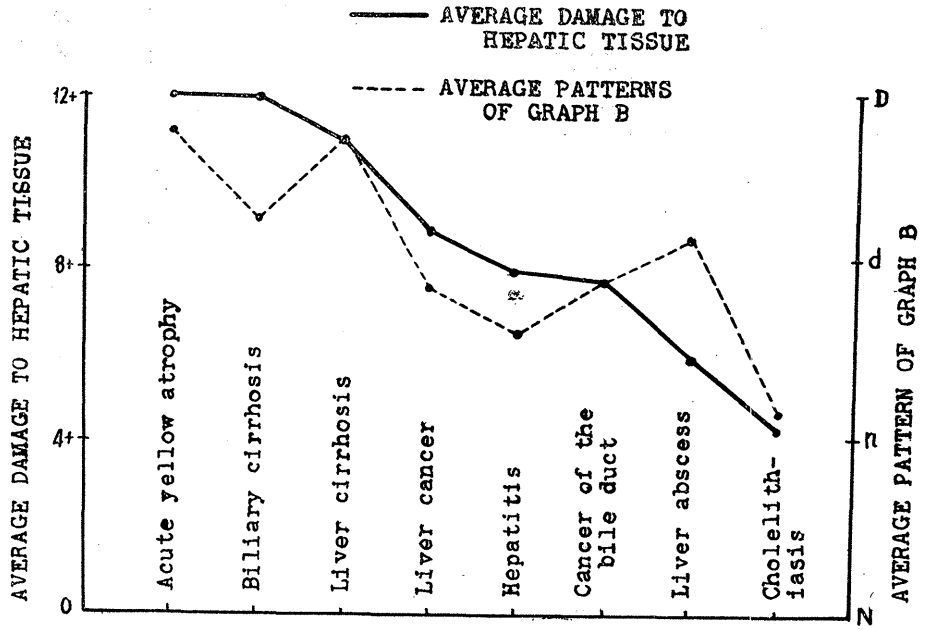


Fig. 7. Average patterns of graph B in comparison with the average damage to hepatic tissue as corroborated by the microscopic examination of the liver fragment obtained from the patients with various hepatobiliary diseases by needle biosy.

(3) Cholelithiasis. Cholelithiasis represents the disorders of the biliary outflow. Recurrent acute seizures of the biliary obstruction in cholelithiasis have been said to result in biliary cirrhosis, since they give rise to chronic biliary obstruction which is closely related to the destruction of hepatic parenchyma and increased production of fibrous tissue.[31] As shown in Figure 8, the frequency

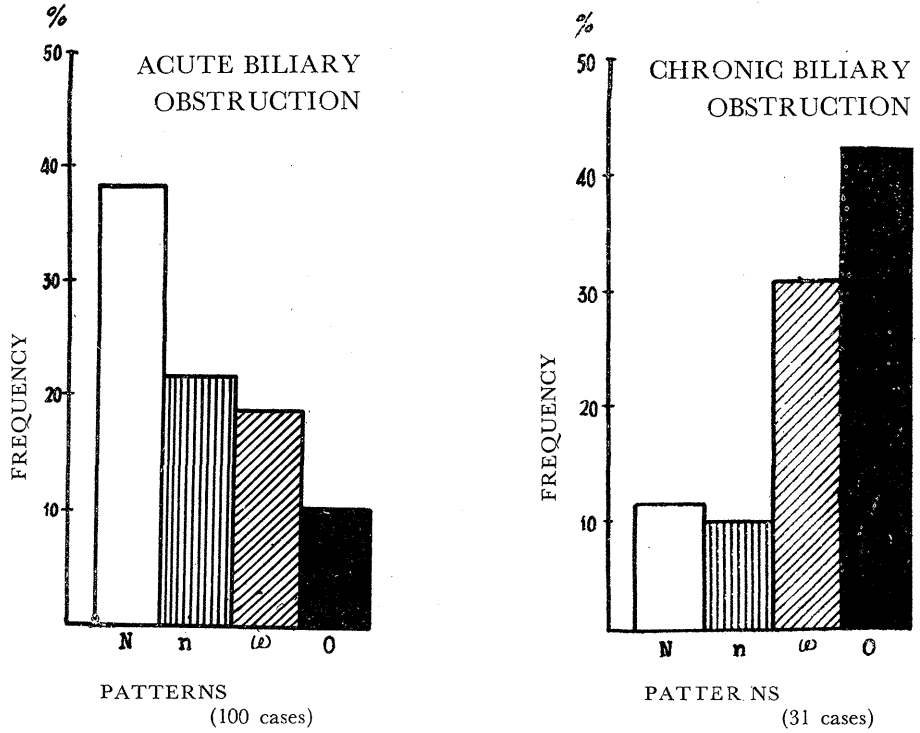


Fig. 8. Frequency distribution of the various patterns of graph C in acute and chronic biliary obstruction. ((Acute biliary obstruction:- Absence of increased activity of serum alkaline phosphatase was found in 28%. Patterns commonly encountered were N (21 cases), :n (8), .n. (7), ′ω (7), ω (5), ω′ (5) and 0 (11). Chronic biliary obstruction:- Absence of increased activity of serum alkaline phosphatase was found in 3.2%. Patterns commonly encountered were .N (4 cases), :n (2) ,′ω (5),ω, (3) and 0 (14).))

of patterns of the graph of biliary obstruction in acute biliary obstruction was quite different from that of chronic biliary obstruction. Patterns N and n constituted the majority in the former condition, whereas the patterns ω and 0 outnumbered the others in the latter. It will therefore be considered that the patterns ω and 0 refer to a long-standing or a large-scaled disturbace in biliary outflow, while the patterns N and n indicate the minor hindrance to the outflow of bile.

It is commonly seen that there is an alleviation or disappearance of jaundice

following the surgical extraction of gall stone from the common bile duct, if the operation has successfully eliminated the major cause of the obstruction. The development resulting from the stone extraction in cholelithiasis is summarized diagrammatically in Figure 9, which clearly demonstrates the successive transition

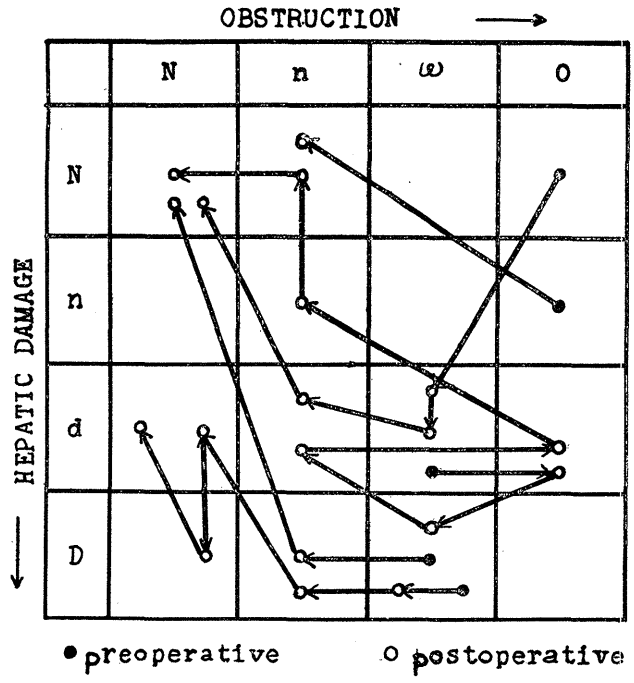


Fig. 9. Change in the patterns of graph B (hepatic parenchyma) and C (biliary obstruction) after the extraction of gall stones from the common bile duct in cholelithiasis. (Arrows indicate the course.)

of the patterns in the order of $0 \rightarrow \omega \rightarrow n \rightarrow N$. The comparison of the average pattern with the mean sign of biliary stasis observed in the histological picture of the biopsied liver in every individual disease of hepatobiliary system substantiated the parallel correlation between them, revealing that the patterns $N \rightarrow n \rightarrow \omega \rightarrow 0$ roughly correspond with the biliary obstructions, increasing in degree in the order listed (Figure 10). However, the parallelism between the histological indication of biliary stasis and the patterns was not so evident, when the hepatobiliary diseases were studied as a whole (Figure 11).

DISCUSSION

The results presented in the preceding section justifies the interpretation

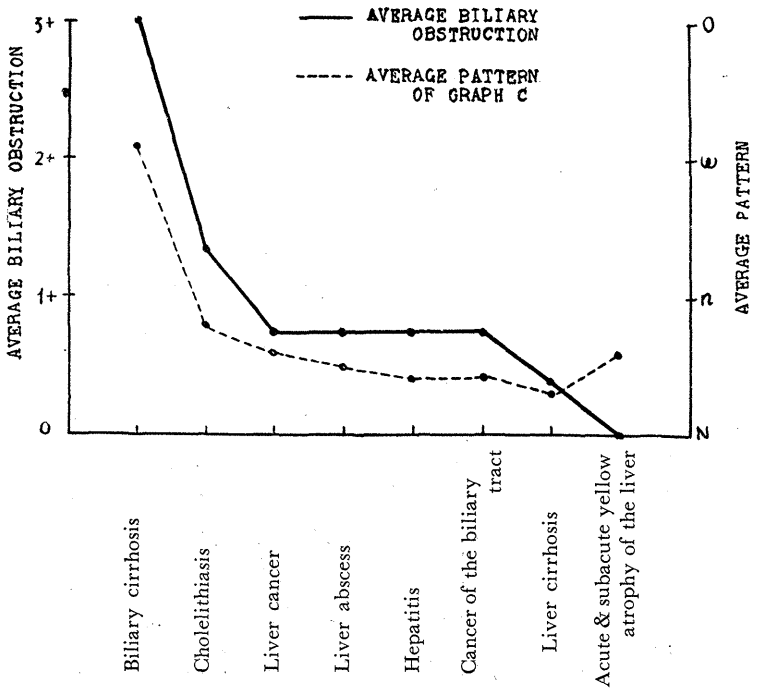


Fig. 10. Average pattern of the graph C in relation to the average biliary obstruction histologically demonstrated by the examination of liver fragment obtained from the patients with various kinds of hepatobiliary disorders by needle biopsy.

that patterns N→n→d→D (hepatic parenchyma) and patterns N→n→ω→0 (biliary obstruction) refer to the hepatic damage and biliary obstructions increasing in degree in the order given respectively, although the relation between the graph of biliary obstruction and the disturbed biliary outflow is not quite so intimate as that between the graph of hepatic parenchyma and the hepatic damage.

The high rate of the incidence of increased serum alkaline phosphatase in biliary obstruction, namely its occurrence in 96.8% of chronic biliary obstruction and in 72.0% of acute biliary obstruction, will deserve special attention. Even among the five patterns belonging to graph N which connotes only the questionable biliary obstruction, the pattern .N is characterized by its relatively close association with the unequivocal disturbance in biliary outflow (Figure 8). Latent biliary obstruction should first be suspected when pattern .N of the graph of biliary obstruction is encountered in the absence of the complication by other conditions causing the elevation of serum alkaline phosphatase. In this respect the pattern .N may be regarded as being equivalent to the group of the pattern n.

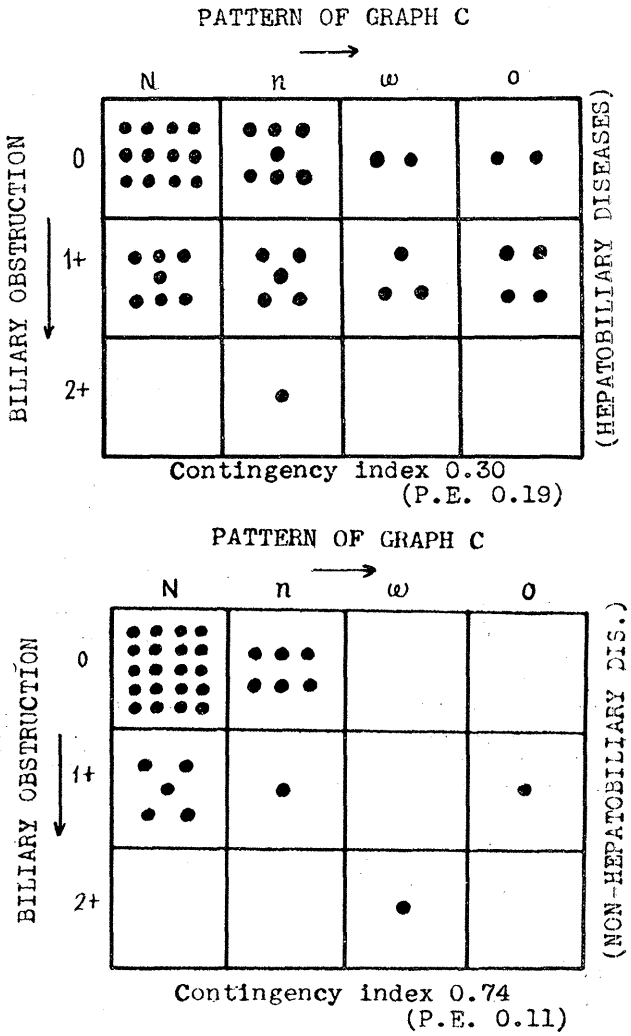


Fig. 11. Patterns of graph C with reference to the biliary obstruction histologically demonstrated by the examination of liver fragment obtained from the patients with hepatobiliary and non-hepatobiliary diseases by needle biopsy.

Pattern N',N. and n: (of biliary obstruction) appearing in anemia, hypertension, nephrosis and other conditions which frequently entail lipemia are commonly unrelated to the obstacle to biliary outflow.

In figure 12 the average patterns of the graph (biliary obstruction) with careful consideration of the degree of the shift of the individual tests from their normal range (Table VI) are presented in relation to the indication of biliary

TABLE VI

Criteria for the rough classification (0, 1+ and 2+)
of the fluctuation in test results

A)		0	1+	2+
	CCF	0 – 1+	1 – 2+	2 – 4+
	Alb (g/dl)	4.8 – 3.8	3.8 – 2.5	2.5 >
	Glob (g/dl)	2.5 – 3.5	3.5 – 5.0	5.0 <
	Ch.E (⊿pH)	1.1 – 0.8	0.8 – 0.4	0.4 >
	15mB (%)	0 – 60	60 – 80	80 <
	Alk.p (Unit)	0 – 4	4 – 8	8 <
	Chol (mg/dl)	0 – 300	300 – 600	600 <
	Ph.t (Unit)	0 – 15	15 – 30	30 <

B) Added numbers of +	Classification
0–1	(N), (N)
2–3	(n), (n)
4–5	(d), (ω)
6 <	(D), (0)

stasis histologically demonstrable in the biopsied liver for the purpose of comparison with Figure 6 and 11 which refers to the patterns dealt with as they were. The correlation seems to be somewhat closer in Fgure 12 than in Figure 6 and 11 with respect to the hepatobiliary diseases, although there is no difference of correlation with regard to the non-hepatobiliary diseases. Consideration of the

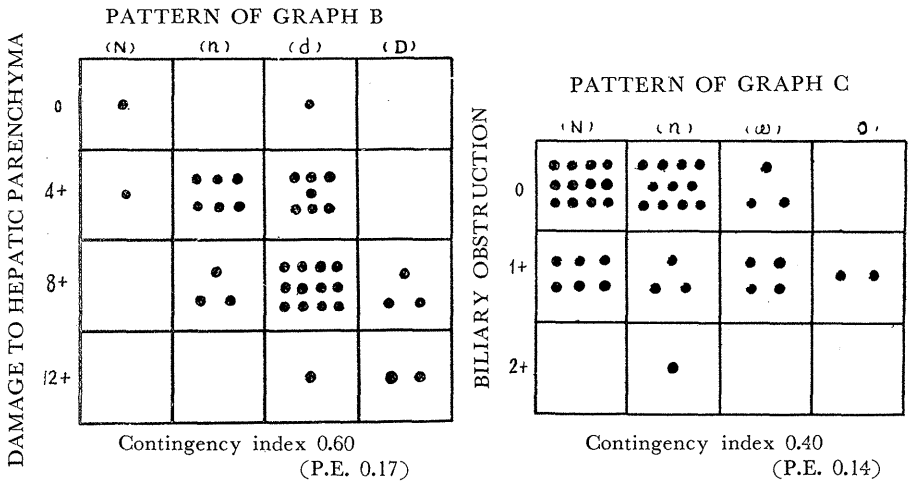


Fig. 12. Corrected patterns of graph B (hepatic parenchyma) and C (biliary obstruction) in relation to the hepatic histology (damage to hepatic parenchyma and biliary obstruction).

degree of deviation of the individual tests composing the graph may therefore be helpful to a certain extent for more accurate appraisal of biliary obstruction in hepatobiliary disorders.

The usefulness of the graphs (hepatic parenchyma and biliary obstruction) for assessing the disturbed hepatic function in the non-hepatobiliary diseases was corroborated by the comparative study of the graphs with the liver biopsy (Figures 6, 11 and 12).*

SUMMARY AND CONCLUSION

The graphs of hepatic parenchyma (CCF, Alb, Glob and Ch. E) and biliary obstruction (1 or 15-m Bil, Alk. P, Chol and Ph. t) which compose the blood spectrum (a system of graphical representation of chemical constituents of blood for the purpose of clinical diagnosis) were studied on 662 patients with hepatobiliary diseases and on 159 patients on whom biopsy of the liver was done. They were classified into the groups of patterns according to the shape of the lines connecting the points of estimation marked on the graduated lines relevant to the individual tests or chemical ingredients of blood: patterns N, n, d and D (graph of hepatic parenchyma) and patterns N, n, ω and 0 (graph of biliary obstruction) depending on the number of abnormal results of the tests or ingredients being 0-1, 2, 3 and 4, respectively. The groups were further divided into subpatterns, taking into consideration the individual tests which shifted in the abnormal range. The graphs thus classified and divided were evaluated as a useful tool for the detection of the damage to hepatic tissue and the biliary obstruction after they were carefully compared with the clinical diagnosis, clinical course and hepatic histology (biopsy). The results are summarized as follows:

1) The graph of hepatic parenchyma: Groups N and n of the patterns referred to the slight hepatic damage, while groups d and D indicated the severer damages. Groups N→n→d→D were related to the hepatic damage which were increasingly severe in the order given. The graph of biliary obstruction: Groups N→n→ω→0 corresponded with the increasingly distinct disturbance in biliary outflow in the order listed.

2) The patterns belonging to the same group were equivalent in clinical significance. However, pattern .N of the group N (biliary obstruction) was the exception, because it was more often associated with the unequivocal disturbance in the biliary outflow, whereas other patterns included in this group generally indicated the unhindered passage of bile. Patterns N', N. and n: (biliary obstruc-

* It has hitherto been said that there is no correlation between the hepatic tests and the liver biopsy.[32] Separate examination of every individual test one by one without consideration of synthetic judgement of the several tests run simultaneously is thought to have been responsible for the failure of establishing the correlation.

tion) were the contrary, being frequently of little significance regarding the biliary obstruction,since they were also common in the diseases entailing the lipemia which was independent of the disturbed biliary outflow.

3) The feature of the hepatic damage and biliary obstruction suggested by the graphs agreed fairly well with the histological picture of the liver obtained by needle biopsy.

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