STUDIES ON SERUM CHOLINESTERASE*

IV. SERUM CHOLINESTERASE IN RELATION TO THE OTHER CHEMICAL CONSTITUENTS OF BLOOD AND TO SOME TESTS FOR HEPATIC FUNCTION

HIROSHI TAKAHASHI

Department of Clinical Pathology, Yamaguchi Medical School, Ube

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Distinct decrease in the activity of serum cholinesterase was indeed common in the hepatobiliary diseases, particularly in liver cirrhosis,[1-16]** bst equal diminution of this enzyme activity was not rare in some of the non-hepatobiliary diseases.[1,2,8,9,16,17] There was a significant correlationship between the fall of serum cholinesterase activity and the alteration in the histologic picture of the liver so far as hepatobiliary diseases were concerned, though such correlation seemed dubious in non-hepatobiliary diseases,[18] Nevertheless, the activity of liver cholinesterase paralleled roughly the serum cholinesterase activity in every kind of diseases including the non-hepatobiliary as well as the hepatobiliary. The reduction in serum cholinesterase activity was accordingly understandable as a rough indication of diminished activity of the liver cholinesterase, which was thought to be associated with the disturbance in hepatic function, provided maintenance of normal level of liver cholinesterase really required the elaboration of the liver. Decreased activity of serum cholinesterase might for this reason be accepted as an indicator of hepatic dysfunction even in the non-hepatobiliary diseases which did not entail any anatomical lesion in the liver. These were the purport of arguments stated in the second and the third papers of this series of study.[16,19]

Of course, the arguments demand critical examination from another viewpoint, namely verification by the comparison with conventional tests for hepatic function. If the comparison failed to reveal a significant correlation of serum cholinesterase to the conventional tests in non-hepatobiliary diseases, the measurement of serum cholinesterase will not be qualified for a hepatic test in the diseases of the extra-hepatobiliary organs, and if the reverse is the case, it can be

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** More detailed list of the works on the serum cholinesterase in hepatobiliary diseases was presented in the second paper of this study.[16]

regarded as an indicator of hepatic disturbance for both non-hepatobiliary and hepato-biliary diseases.

Bearing this idea in mind 1667 patients with non-hepatobiliary diseases were examined for the serum cholinesterase activity in conjunction with the twelve chemical constituents of blood available for hepatic tests, the urobilinogen tests and the glucuronic acid test, which were listed in Table II. In additon 906 patients with hepato-biliary diseases were similarly studied with the intention of real firming the usefulness of serum cholinesterase as a hepatic test in such conditions, which had been discovered in the preceding papers. Comparison with the indicators of nutritional state or general condition, i.e. blood hemoglobin, and total protein and albumin in serum,[20-24] was also attempted in order to verify whether or not the serum cholinesterase is intemately related to nutritional state.

METHODS

Patients numbering 2573 composed of 906 cases with hepatobiliary diseases and 1667 cases of non-hepatobiliary diseases (Table I) were determined for the

TABLE I.

A list of patient observed in this study

		Cases
A.	Hepatobiliary diseases	906
(1)	Diseases of the liver	483
	Acute hepatitis	178
	Acute and subacute yellow liver atrophy	28
	Chronic hepatitis	39
	Laennec's liver cirrhosis	39
	Biliary liver cirrhosis	25
	Liver abscess	28
	Malignant neoplasms of the liver (primary and secondary)	25
	Liver syphilis (gumma)	6
	Other liver diseases	57
(2)	Diseases of the bile ducts	110
	Cholelithiasis	65
	Cancer of the gall bladder and the common bile duct	12
	Cholangitis and Cholecystitis	33
(3)	Diseases of the liver and the spleen	64
	Banti's syndrome	49
	Swelling of the liver and the spleen	15
B.	Non-hepatobiliary diseases	1667
(4)	Diseases of the stomach	192
	Ulcer of the stomach	107
	Ulcer of the duodenum	25
	Carcinoma of the somach	155
	Stenosis of the pylorus (due to ulcer or carcinoma)	45

(5)	Diseases of the kidney	126
	Acute glomerulonephritis	38
	Chronic glomerulonephritis and nephrosclerosis.	36
	Nephrolithiasis	21
	Tuberculosis of the kidney	13
	Cysts and tumors of the kidneys	18
(6)	Pulmonary tuberculosis	209
	Slight pulomonary tuberculosis	63
	Moderately advanced pulmonary tuberculosis	72
	Advanced pulmonary tuberculosis	53
	Miliary tuberculosis	13
	Complication of intestinal tuberculosis	8
(7)	Gyneco-obstetric diseases	249
	Carcinoma of the uterus	184
	Toxemia due to pregnancy	65
	(total cases)	2573

Table II

Chemical constituents of blood and hepatic test, which were measured in this study and the methods for their measurement

A. Blood constituents as indicators of the hepatic damage

1. Serum albumin
2. Serum globulin
3. Serum albumin to globulin ratio

} Na_2SO_4-Salting-out combined with biuret reaction.[25]

4. Gros' reaction ..... The reaction is expressed in the volume of Hayem's solution consumed.[26]
5. Cephalin cholesterol flocculation test (CCF) ..... Hanger's antigen offered by Sumitomo-kagaku was used.[27]
6. Zinc turbidity test ..... Kunkel's method[26,28]
7. Thymol turbidity test ..... Maclagan's method[26,29]
8. Saline ammonium-sulfate turbidity test (SAST. T.) ..... Method of Popper et al.[26,30]

B. Blood constituents as indicators of the disturbance of the bile ducts.

9. Total bilirubin in serum ..... Malloy-Evelyn's diazo method[31]
10. Alkaline phosphatase in serum ..... Modified Shinowara-Jones-Reinhart's method[32]
11. Serum cholesterol ..... Modified Bloor's method[33]
12. Phenol turbity test ..... Kunkel's method[26,28,34]

C.* Loading test for hepatic function

13. Urobilinogen test ..... Mizuta-Akama's method[35]
14. Glucuronic acid test ..... Modified Snapper's method[36,37]

D. Blood constituents as indicators of nutrition and general condition

15. Blood hemoglobin ..... Cyanmethemoglobin method of Stadie[26,38]
16. Serum protein ..... Refractometry with "Hitachi" Protein meter[26,39]
17. Serum albumin ..... Na_2SO_4-Salting-out combined with biuret reaction[25]

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TABLE III

A list of the tests carried out in comparison with the serum cholinesterase, and the number of patients subjected to these tests

	Diseases of the liiver	Diseases of the bile ducts and the gall bladder; Banti's syndrome	Diseases of the stomach	Diseases of the kidneys	Tuber-culosis of the lungs	Carcinoma of the uterus, and Toxemia of pregnancy
Albumin	365	205	281	113	156	203
Globulin	365	205	281	113	156	203
A/G Ratio	365	205	281	113	156	203
Gros' R.	262	95	161	55	99	179
C C F	200	142	177	72	91	59
Zinc T.T.	138	66	86	28	59	100
Thyml T.T.	174	65	85	30	50	88
SAST.T.	198	76	109	35	69	116
Bilirubin	129	79	24	—	—	—
Alkaline Phosphatase	377	219	234	81	132	67*, 48**
Cholesterol	315	185	229	108	153	107
Phenol T.T.	401	226	315	108	184	201
Hemoglobin	405	236	317	112	181	215
Serum Protein	454	242	124	128	182	226

* Carcinoma of the uterus

** Toxemia of pregnancy

fiften chemical constituents of blood which were listed in Tables II and III by the methods presented in Table II as well as for the serum cholinesterase by the phenol-red comparator method. Correlation tables were constructed with the serum cholinesterase and the other constituents of blood to calculate the coefficients of correlation:* Fifty-seven hepatobiliary and sixty-seven non-hepatobiliary patients were studied for the comparison with the urobilinogen test,[35] while seventeen hepatobiliary and eighteen non-hepatobiliary cases were examined for the correlation to the glucuronic acid test.[36,37] The coefficients of cerrelation were tested individually by their levels of significance ($\alpha=0.05$) and confidence limits ($\alpha=5.05+0.05=0.10$).[40]**

* Coefficients of correlation pertaining to the objects which do not form Gaussian distribution are not very reliable. On calculating the coefficients of bilirubin, alkaline phosphatase in serum and thymol turbidity tests were therefore converted into the approximate Gaussian distribution by substituting their logarithmic values for their determinations as they were, because they gave the skew distributions instead of the Gaussian curve.

** Contingency coefficients were employed to examine the relation between the serum cholinesterase and the urobilinogen or the glucuronic acid test, since the tests could be measured only qualitatively, but not quaatively.

RESTULTS

The results obtained in this study are presented in Tables IV and V as

TABLE IV

Coefficients of correlation between serm cholinesterase and other chemical constituents of blood (including hepatic tests) in various diseases (Figures in brackett refer to the levels of significance ($\alpha=0.05$))

	Diseases of the liver	Diseases of the bile ducts and the gall bladder; Banti's syndrome	Diseases of the stomach	Diseases of the kidneys	Tuberculosis of the lungs	Carcinoma of the uterus, and Toxemia of pregnancy
Albumin	+0.660	+0.602	+0.591	+0.397	+0.478	+0.451
Globulin	−0.416	−0.306	(−0.093)	(−0.033)	(−0.137)	(+0.024)
A/G Ratio	+0.601	+0.499	+0.363	+0.233	+0.288	+0.202
Gros' R.	+0.518	+0.604	+0.428	+0.254	+0.360	+0.284
C C F	−0.367	−0.244	(−0.093)	(−0.085)	−0.209	(+0.096)
Zinc T.T.	−0.189	−0.314	−0.428	(−0.375)	−0.296	−0.195
Thymol T.T.	−0.232	(−0.160)	(−0.171)	(+0.299)	(−0.206)	(−0.044)
SAST.T.	−0.364	−0.289	(−0.116)	(−0.186)	(+0.111)	(+0.001)
Bilirubin	−0.296	−0.512	(−0.140)	—	—	—
Alkaline Phosphatase	−0.131	−0.380	(−0.055)	(+0.120)	(+0.045)	(+0.060)* (−0.008)**
Cholesterol	+0.308	(+0.132)	(+0.037)	(+0.168)	(−0.014)	(−0.082)
Phenol T.T.	+0.167	(+0.107)	+0.129	+0.386	+0.177	+0.206
Hemoglobin	+0.461	+0.509	+0.564	+0.293	+0.369	+0.440
Serum Protein	+0.397	+0.512	+0.422	(+0.134)	+0.312	+0.451

* Carcinoma of the uterus
** Toxemia of pregnancy

TABLE V

Coefficients of correlation between serum choslinesterase and serum tests for hepatic function in hepabiliary and non-hepatobiliary diseases (A-Hepatobiliary diseases, B-Non-hepatobiliary diseases)

		Number of cases	Level of significnce ($\alpha=0.05$)	Coefficient of correlation
Gros' R.	A	361	0.103	+0.590
	B	501	0.086	+0.352
C C F	A	330	0.108	+0.369
	B	400	0.098	−0.050
Zinc T.T.	A	205	0.139	−0.277
	B	259	0.124	−0.262
Thymol T.T.	A	240	0.130	−0.184
	B	225	0.132	−0.026
SAST. T.	A	243	0.127	−0.308
	B	329	0.108	−0.062

well as in Figures 1 to 3. They are briefly summarized as follows.

(1) No coefficient of correlation exceeded 0.80, the level of intimate correlationship, varying from 0 to 0.70. Accordingly any of the chemical constituents of blood and the hepatic tests which were studied could not supplant the serum cholinesterase, and vice versa. The coefficient of correlation was

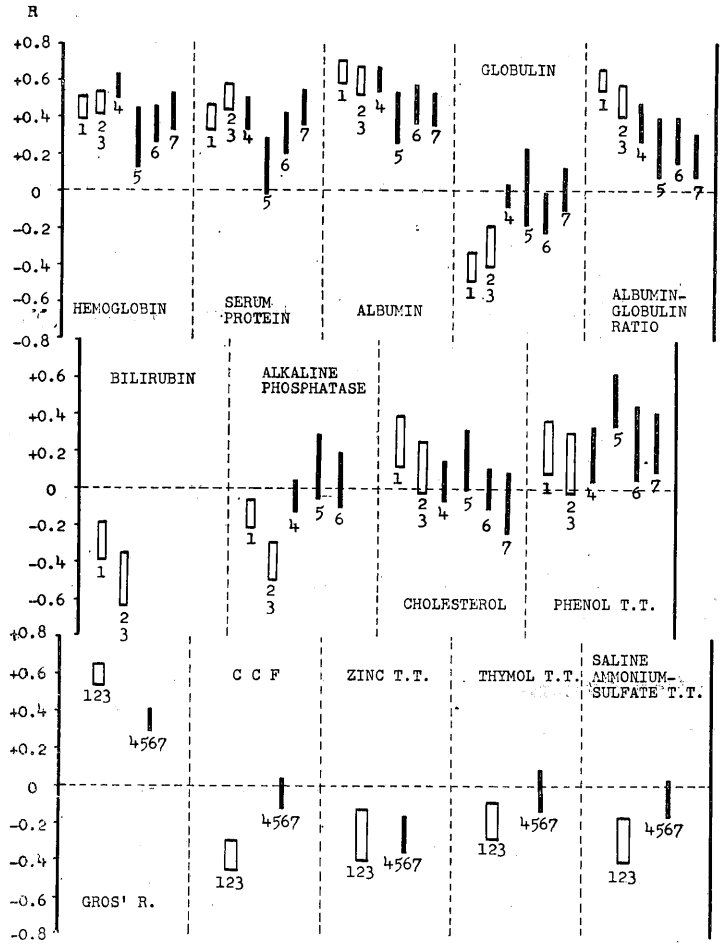

Fig. 1 Confidence limits of the coefficients of correlation (R) between serum cholinesterase and other chemical constituents of blood (including the liver function tests by means of the determination of serum constituents)

Notation: 1–Diseases of the liver
2–Diseasis of the bile ducts and the gall bladder
3–Bati's syndrome
4–Diseses of the stomach
5–Diseases of the kidney
6–Tuberculosis of the lungs
7–Carcinoma of the uterus, and toxemia due to pegnancy

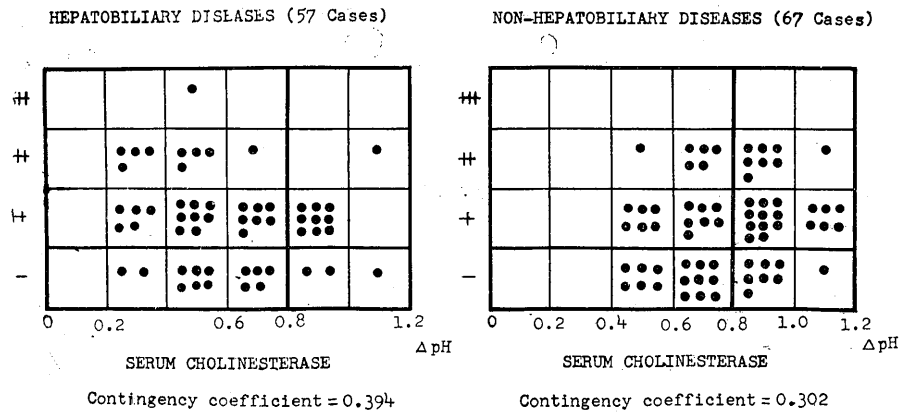

Fig. 2 Serum cholinesterase activity as compared with urobilinogn test in hepatobiliary and nonhepatobiliary diseases.

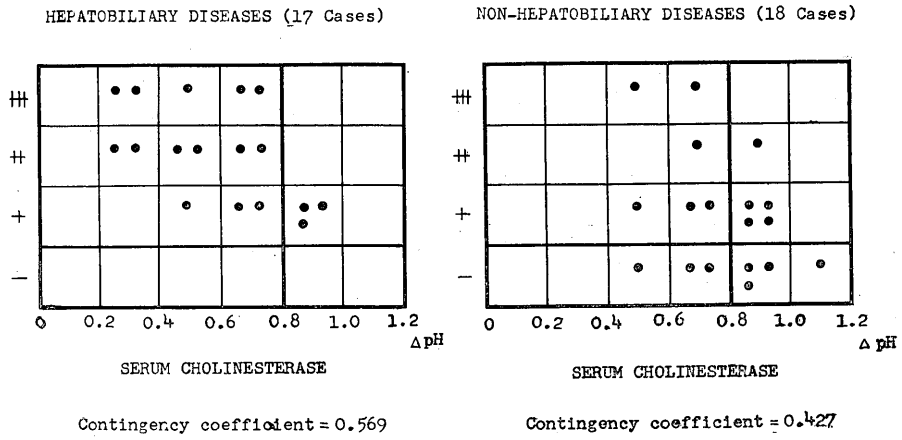

Fig. 3 Serum cholinesterase activity as compared with glucuronic acid test in hepatobiliary and nonhepatobiliary.

maximum for serum albumin, attaining to 0.66.

(2) The coefficients were either positive (for albumin, albumin to globulin ratio, Gros' reaction, cholesterol, phenol turbidity test, hemoglobin and serum protein) or negative (for globulin, cephalin cholesterol flocculation test, turbidity tests of zinc sulfate, thymol, and saline-ammonium-sulfate, bilirubin and alkaline phosphatase).

(3) Hepatobiliary diseases offered a fairly sharp contrast to non-hepatobiliary diseases. The coefficients of correlation were considerably smaller in the non-hepatobiliary diseases than in the hepato-biliary, being frequently below

the level of significance. The tendency was particularly evident in globulin, albumin, to globulin ratio, cephalin cholesterol flocculation test, Gros' reaction, turbidity tests of thymol and saline-ammonium-sulfate, and alkaline phosphatase.

(4) On the contrary the correlation of serum cholinesterase to blood hemoglobin, serum protein or albumin did not varyw ith the difference in the kind of diseases. The individual coefficients of correlation in hepatobiliary diseases were nearly equal to those in non-hepatobiliary diseases.

(5) The coefficients pertaining to bilirubin or to alkaline phosphatase were larger in biliary diseases than in the diseases of hepatic parenchyma.

(6) Significant correlation was demonstrated with respect to the urobilinogen test or the glucuronic acid test, with somewhat larger coefficients of contingency in hepatobiliary diseases than in the non-hepatobiliary.

Discussion*

It will be apparent from the data described in the preceding section that the serum cholinesterase offers reliable information about the scale of the damage to hepatic parenchyma, so far as the hepatobiliary diseases are concerned, because it yelds for these maladies results which compare fairly with those given by the conventional hepatic tests whose evaluation as indiactors of hepatic damage was already established by the comparison with liver biopsy.[18,19] In other words, the present study reaffirms the conception delivered in the previous paper[19] which dealt with the comparison of serum cholinesterase with the histological picture of the liver. Furthermore serum cholinesterase appears to be related to disturbance in the biliary outflow,[1] since it gives significant coefficients of correlation to bilirubin and alkaline phosphatase, the indicators of biliary obstruction. Cholinesterase may therefore contribute little to the differential diagnosis of parenchymatous and obstructive jaundices. In spite of this kind of demerit, the serum cholinesterase still remains to be among the excellent tests for hepatic function in the hepatobiliary diseases, especially when their whole course must be followed up, because it is insusceptible to irregular fluctuation, as clearly shown by Figure 4.

However, serum cholinesterase is unfortunately unreliable for the diagnosis of hepatic disturbance of non-hepattbiliary diseases, in which the coefficients of correlation to other hepatic tests are, as stated above, small and often below the level of significance. Either the low specificity of serum cholinesterase to the hepatic disturbance of non-hepatobiliary origin or the interference of malnutrition resulting from these diseases is suspected to be responsible for the unrelia-

* Poisoning by organophosphorous insecticides which inhibit cholinesterase activity is not dealt with in this article.

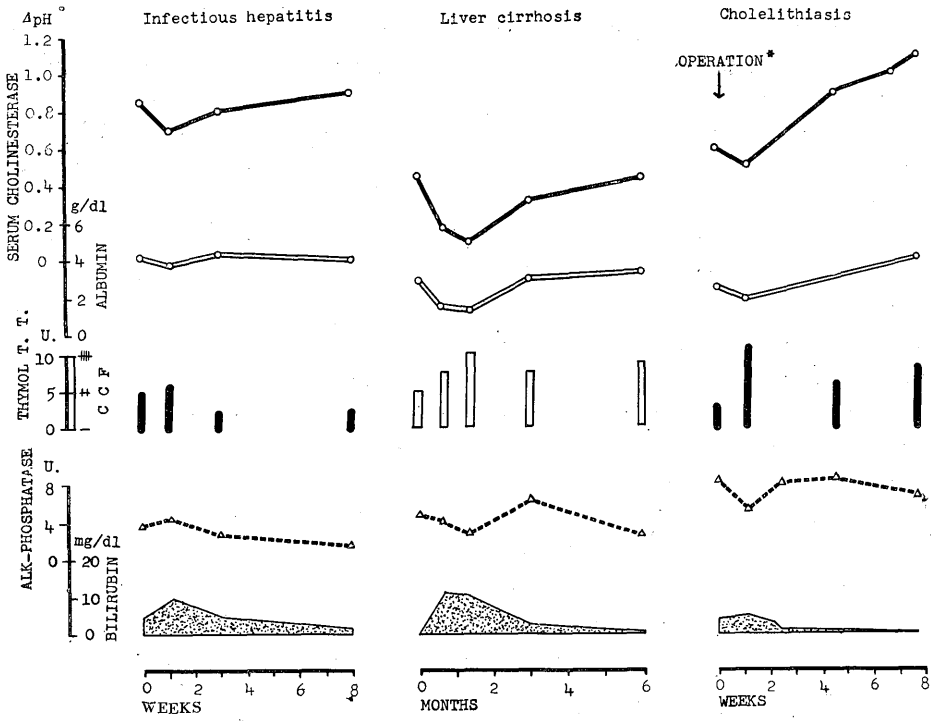

Fig. 4 Three examples of the clinical course of hepatobiliary diseases as observed by the serial determination of rerum cholinesterase activity, which was compared with some of the other hepatic tests.
* OPERATION: Isolation of the gall stone with cholecystectomy

Table VI

The distributions of cholinesterase in various organs of normal dogs and rats.

	Dog* (mean of 4 cases)	Rat** (mean of 2 cases)
Cerebrum	124μM/g	1.21ΔpH/100mg
Thyroid	—	0.26
Lung	95	0.32
Heart	124	1.01
Liver	1421	0.23
Kidney	99	0.14
Spleen	132	0.47
Pancreas	1779	—
Stomach	123	1.62
Duodenum	—	2.02
Small intestine	192	2.07
Appendix	—	1.47
Large intestine	42	1.01
Omentum	—	0.58
Testicle	—	0.15
Bone-marrow	55	—
Muscle	—	0.20

* measured by De La Huerga's method[76] ** measurred by modified Michel's method[19]

bility. In fact pseudo and true cholinesterase are rich not only in the liver, but also in the brain, the pancreas and the stomach[41,42] (Table VI). Obviously the pancreas and the stomach are expected to participate in the control of serum cholinesterase level, though the liver seems to play the greatest rôle.* For instance diminished activity of serum cholinesterase in the gastric diseases[1,2,8,16] (ulcer and cancer of the stomach) may be related to the diminished production of this enzyme in the stomach rather than to the hepatic dysfunction.**

It has been believed by some authors[46,47] who manitain that there is influence of autonomic nervous system upon the serum cholinesterase, that decrease or increase in serum cholinesterase is to a certain extent attributable to the augmented tonus of parasympathetic or sympathetic nervous systems, respectively. Rise in serum cholinesterase in hyperthyroidism,[13,16] diabetes mellitus[13,16] and pulmonary tuberculosis***[8,16,48-50] may be accounted for from such a viewpoint (sympathicotonic state). Its seasonal variation[47,51-56] may also be explained analoguously (alternation of parasympathicotonic and sympathicotonic states). Nevertheless, the portal area which houses various cholinesterase-rich organs, stomach and pancreas for example, is thought to be more important for the fluctuation of serum cholinesterase, since the cholinesterase of the nervous system is different in quality from the serum cholinesterase.

In this connection the nearly equal magnitude of coefficients referring to blood hemoglobin, serum protein and albumin between the non-hepatobiliary and hepatobiliary diseases will deserve special consideration, because this will be understood as an evidence for the correlationship between the serum cholinesterase and nutritional state. These chemical constituents of blood have recently been evaluated as indices of nutritional state or general condition[20-24] and impaired nutrition is equally common both in hepatobiliary and non-hepatobiliary diseases. Therefore our results support concretely the view of early

* Cholinesterase in the brain will not be considered, because it belongs to true cholinesterase which is not found in the blood serum.

** The relation of serum cholinesterase to the pancreas has not yet been established by experimental observation. Esterase are grouped into aliesterase (esterase proper and lipase), cholinesterase and the enzymes which split the esters of procaine, tropin and alkaloids.[43] The so-called serum esterase which is measured by the tributyrin method increases in pancreatic necrosis, whereas serum cholinesterase decreases.[44] Accordingly they should not be put in the same category. Esterase in the human blood serum has been said to be eventually cholinesterase.[43] This conception is correct, if there is really no enzyme except cholinesterase, whose activity is inhibited by eserine.[45] Similary no experimental study has hitherto been made on the effect of gastric function upon the serum cholinesterase. Nevertheless, the clinicobiochemical observation described in the second paper of this series of studies[16] is easily accounted for by the assumption that the diseases of the portal area (cancer and ulcer of the stomach, dysentery and so forsh) directly reduce the serum cholinesterase, but not indirectly by way of the hepatic dysfunction or malnutrition. The assumption will be maintained until contrary evidence is obtained.

*** The activity of serum cholinestesterase falls in severe cases of tuberculosis.[8.15.16.50]

workers[2,55 57-68] who vaguely suspected the effect of malnutrition upon the serum cholinesterase. It has been said that determination of serum albumin will supplant the measurement of serum cholinesterase activity, for the latter is contained in the former as one of its fraction, and both share the same fluctuation in a variety of diseases.[59,72] This conception is incorrect, because the major fraction of serum cholinesterase is included in α-globulin but not in albumin (Table VII), and the coefficient of correlation which represents the similarity in fluctuation of cholinesterase and albumin is 0.30 to 0.70, being too small to corroborate their identical variation.

TABLE VII

Distribution of cholinesterase activity in the fractions of serum protein which were salted out by the addition of various amount of sodium sulfate

Sodium sulfate added to serum up to the final concentration of	26.0g/dl	23.4g/dl	21.6g/dl	18.8g/dl	0.0g/dl
Protein fractions in the filtered fluid*	Albumin	Albumin and α-Globulin			Albumin, α, β and γ Globulins
Cholinesterase activity of the filtered fluid (ΔpH)	0.1	0.8	0.9	0.9	0.9

* Various amount of sodium sulfate was added to the serum. The resultant turbid solution was incubated at 37°C for three hours, and filtered to obtain limpid filtrate. The activity of cholinesterase in the filtrate was measured by the phenol-red comparator method with slight modification.

For the clinical interpretation of serum cholinesterase activity, careful consideration should accordingly be paid to the following three determinative factors···(1) hepatic function, (2) functional state of the organs included in the portal area (stomach, pancreas, etc.) and (3) nutrition or general condition. Undoubtedly, in the hepatobiliary diseases the damage to hepatic parenchyma partly accompanied by the disturbance in biliary passage is chiefly responsible for the decrease in serum cholinesterase, but in the diseases of the extra-hepatobiliary organs impaired nutrition is thought to be the primary factor. For the diminution of serum cholinesterase in the diseases of portal area the disturbance in the function of stomach, pancreas and so forth is supposed to be playing the greatest rôle, being assisted by hepatic damage and impaired nutrition.

Circumspection is thus essentially important to the clinical interpretation of serum cholinesterase activity. Proper appraisal of nutritional state by the simultaneous determination of blood hemoglobin, serum protein and albumin is inevitably required, and collation to other reliable tests is desirable when they

are available.

In our experience the chemical constituents of blood which decreased in pathological condition, like serum cholinesterase, were convenient and reliable for following up the clinical course of hepatobiliary disorders because they varied within a limited range below the normal limits with little irregular fluctuation, although they seemed to be inferior in the spesificity to hepatic disturbance to the blood ingredients which increased in diseases. Serum cholinesterase has hitherto been said to have a wide range of normal activity with indistinct border line to the pathological activity, thus frequently resulting in difficulty in its clinical interpretation (Table VIII). However the normal range obtained by

TABLE VIII

Normal ranges of human serum cholinesteras activity hitherto reported by various investigators.

Investigator	Normal range	Units	Method
Mann et al.[73]	0.7—1.6	ΔpH	Michel
Vorhaus et al.[68]	0.58—1.37	ΔpH	Michel
Alcalde[74]	0.4—0.8	ΔpH	Michel, modified
Peinhold et al.[75]	0.926—0.145(S.D.)	ΔpH	Michel, modified
De La Huerga et al.[76]	130—310	μMol/ml.	Hestrin, modified
Okinaka et al[8]	50—90	μLCO_2/10min./0.1ml.	Ammon
Kitayama[77]	151—257	μLCO_2/30min./0.1ml.	Ammon
Schaefer[78]	4—5.5	ccm CO_2/hr./ml.	Ammon, modified
Shibata et al.[79]	0.8—1.1	ΔpH	Shibata & Takahashi

phenol-red-romparator method which was employed in our research was fortunately narrow (ΔpH 0.8–1.1). forming a relatively sharp demarcation to the pathological range, presumably because of the urtailement of supernormal activity. Unlike the other methods, the phenol-red comparator method is simple in procedure and demands no expensive apparatus, as was mentioned in the first paper of this series of studies. In view of the obviously limited usefulness of serum cholinesterase as an aid to the diagnosis of hepatic dysfunction, which has been discussed above, the labor for its determination[80-84] will not always be rewarded with good fruits when tedious and expensive methods are used. Phenol-red comparetor method is recommended in this regard. After all, the measurement of serum cholinesterase can ranck among the useful tests for hepatic function, provided that simple procedure is used for its estimation and it is interpreted with careful consideration to the extrahepatic factors (malnutrition and disorders in the portal area). Cholinesterase provides us with an excellent measure for following up the clinical course of hepatobiliary diseases.

Summary and Conclusion

Patients, 2573 cases in number, (pulmonary tuberculosis, gynecological diseases and so forth as well as the diseases of hepatobiliary system, hepatosplenic system, stomach and kidneys) were measured for serum cholinesterase (by phenol-red comparator method), serum protein, albumin, globulin, albumin to globulin ratio, cephalin cholesterol flocculation test, Gros' reaction, turbidity tests of zinc sulfate, of thymol, and of saline-ammonium sulfate, bilirubin, alkaline phosphaphatase, cholesterol, phenol turbidity test and blood hemoglobin in order to study the correlation between the serum cholinesterase and other chemical ingredients of blood. A small number of these patient were also subjected to the urobilinogen test (Mizuta and Akama; 124 cases) and glucuronic acid test (Snapper et al; 35 cases) so that they might be compared with the serum cholinesterase. The conclusions drawn from this study are as follows.

(1) In hepatobiliary disorders the reduction of serum cholinesterase activity is a reliable and excellent indicator of the damage to hepatic parenchyma, although it may not be very helpful for the differential diagnosis of parenchymatous and obstructive jaundices when it is used singly.

(2) In non-hepatobiliary diseses the dimnution of serum cholinesterase activity does not directly imply the hepatic dysfunction, since in such conditions malnutrition and disturbance in the organs of the portal area may often be the primary cause which entail the fall of this enzyme activity. Determination of blood hemogobin, serum protein and albumin (indicators of nutrition) as well as the detailed examination of the portal area, especially of cases of ulcers and cancer of the stomach and duodenum, is indispensable before the diagnosis of hepatic dysfunction can be established.

(3) In view of its limited usefulness for the appraisal of hepatic disturbance in the non-hepatobiliary diseases, which has been mentioned in (2), it is recommended that serum cholinesterase should be measured in combination with some other hepatic tests by a simple technique which dispenses with expensive appratus as well as with skill in manipulation.

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