STUDIES ON SERUM CHOLINESTERASE*

III. SERUM CHOLINESTERASE IN RELATION TO HEPATIC HISTOLOGY AND TO CHOLINESTERASE CONTENT OF THE LIVER

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It is the concensus of the studies which have hitherto been made on serum cholinesterase that a significant derease in the activity of this enzyme is encountered in hepatic disorders.[1-10] Determination of serum cholinesterase has therefore been advocated by some authors[4,5] as a useful tool of hepatic tests, but by others[7] it has not yet been approved as a reliable indicator of hepatic function. In the previous paper[10] which refered to the examination in various diseases a suggestion was advanced as to the possibility of a fairly intimate correlationship between the diminution of this enzyme and the hepatic dysfunction, and it was thought that impairment in the hepatic function would be, for a considerable part, responsible for the reduction of serum cholinesterase activity, although hepatic dysfunction was apparently not all of the causative factors. Two pathways of approach will be conceivable to the investigation of this correlationship, namely inquiries into the relation of serum cholinesterase to the pathological histological picture of the liver, and into the connection of the cholinesterase in serum with the cholinesterase in the liver. If serum cholinesterase really bears any relation to the liver, the diminution of this enzyme activity will mirror some definite change in the hepatic histology and its variation will parallel the increase as well as the decrease in the cholinesterase content of the liver, which has been regarded by some workers[11] as the site of production and the reservoir of "pseudo" cholinesterase[10]. A study was accordingly undertaken bearing this idea in mind, with 157 patients as material. These were biopsied or their liver was probationarily excised either for the examination of hepatic histology or for the determination of hepatic cholinesterase.

METHOD

1. Serum cholinesterase and hepatic histology.

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The liver of one hundred and fifty seven patients with hepatobiliary (76 cases; hepatitis and subacute yellow atrophy 23, liver cirrhosis and Banti's syndrome 29, benign as well as malignant biliary obstruction and hepatic neoplasm 24) as well as non-hepatobiliary diseases (81 cases; cancer of the stomach and other organs 23, gastritis and ulcers of the stomach and duodenum 25, and other diseases 23) was either biopsied with the Vim-Silverman needle[12] (110 cases) or laparotomied for the wedge-shaped excision of the liver (2–3 g. in weight, 47 cases). The tissue fragment thus obtained was fixed, dehydrated, embedded in paraffin, cut in microscopic section and stained with hematoxylin and eosin in the conventional way. The blood was withdrawn on the day before biopsy or liver excision for the estimation of serum cholinesterase by means of the phenol-red comparator method[13].

Popper and Szanto[14] were followed in the description of the alteration of the hepatic histology, and its degree was graded into +, + + and + + +. The histological changes were assorted into the liver cell damage, the inflammation, the scarring (increase in the fibrous tissue), the fatty metamorphosis, and the jaundice (obstruction to the biliary outflow), and their grades were summed up individually. They were compared with the level of serum cholinesterase in a correlation table in order to calculate the relevant coefficients of correlation.

2. *Serum cholinesterase and the liver cholinesterase*

The edge of the liver of thirty-one patients bearing the hepatobiliary (10 cases; cholelithiasis 5, liver cirrhosis 3, and hepatoma 2) and non-hepatobiliary diseases (21 cases; gastro-duodenal ulcer 12, gastric cancer 6, pancreatic cancer 2, and uremia 1) was excised to obtain 2 to 3g. of the tissue fragment, which was immediately estimated for cholinesterase content. Serum cholinesterase was determined by the phenol-red comparator method[13] on the day before the liver excision. A correlation graph was constructed and the coefficient of correlation between serum cholinesterase and liver cholinesterase was computed.

The procedure for the estimation of liver cholinesterase is as follows. The fragment of liver was cut into frozen sections of about 30μ thickness, t g. (about 300mg.) of the liver thus sectioned was weighed in a balance, and it was homogenized with $(30t-t/1.1)$ ml.* of physiological saline to prepare a homogenious suspension whicn contained 100mg. of liver in 3.0ml.

Three ml. aliquots of the homogenate were introduced into test tubes A and B, two drops of eserine solution**,*** were added to tube A, 1.5ml. of barbital

* Specific gravity of the liver tissue was estimated 1.098 by measuring the floatation of a fragment of the liver in distilled water. It was accordingly assumed that the liver had the specific gravity of 1.1 in approximation.

** The reagents used here are the same as those employed for the estimation of serum cholinesterase.

*** Eserine was used to stop the activity of cholinesterase. The enzymes other than cholinesterase will not be inhibited by eserine. Tube A therefore constituted a blank which compensated for the decomposition of acetylcholine by the non-cholinesterase enzymes which might be contained in the liver.

glycerophosphate buffer solution** and 0.5ml. of 5g./dl. acetylcholine** solution were added to both A and B, and mixed. They were incubated at 37C for an hour, and at the end of the specified time measured for their pH (a for A and b for B, respectively) after two drops of eserine solution was poured into B. The cholinesterase activity in 100mg. of the liver tissue was represented by ΔpH= a-b.

Results

The correlation of serum cholinesterase to the hepatic histology is shown in Table I, from which the coefficient of correlation pertaining to fatty metamorphosis was omitted, because extremely low value was apparent even from a mere glance at the preparatory correlation table. In Table I only the figures which

Table I.

Correlation between the histological picture of liver and the activity of serum cholinesterase

(Figures in brackett refer to questionable correlation.)

	number of cases	level of significance (0.05)	hitological alteration as a whole	liver cell damage	inflammation	scarring	jaundice
Total cases	157	0.158	−0.461	−0.295	−0.361	−0.449	−0.214
Hepatobiliary diseases	76	0.228	−0.523	−0.439	−0.296	−0.486	(−0.095)
Infectious hepatitis	23	0.406	−0.435	−0.548	−0.418	−0.410	(+0.095)
Liver cirrhosis	29	0.356	−0.646	(−0.322)	−0.500	−0.466	(−0.236)
Obstructive jaundice	24	0.389	−0.437	−0.430	(−0.057)	−0.516	(−0.263)
Non-liver diseases	81	0.216	(−0.186)	(+0.006)	(−0.072)	(−0.079)	(−0.186)
Gastric cancer	23	0.398	(+0.020)	(−0.332)	(−0.050)	(+0.050)	(+0.238)
Gastric ulcar	25	0.381	(−0.150)	(+0.054)	(−0.227)	(0)	(−0.394)
Other diseases	33	0.334	(−0.294)	(−0.071)	(−0.241)	(−0.193)	(−0.340)

exceed the corresponding level of significance (α=0.05 in the third row) ought to be understood as suggestive of the presence of correlation. It is consequently obvious that serum cholinesterase bears an appreciable relation to the alteration of hepatic histology so far as the hepatobiliary diseases are concerned, but little in the non-hepatobiliary diseases. In the former conditions the enzyme seems to mirrror the scarring, the liver cell damage, and the inflammation to a certain extent, while it is unrelated to the obstruction of biliary outflow and fatty meta-

morphosis. The correlation varies, of course, individually with the difference in the kinds of diseases.

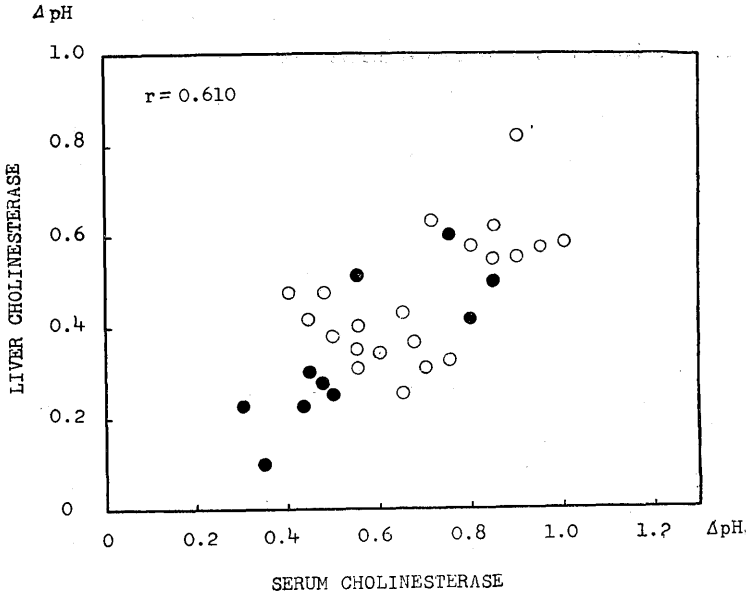

Fig. 1 Serum cholinesterase in relation to liver cholinesterase
Solid circles: hepatobiliary diseases
Open circles: non-hepatobiliary diseases

Figure 1 illustrates the correlation between the serum cholinesterase and the liver cholinesterase. A significiant coefficient of correlation, +0.610, is calculated from the figure. Non-hepatobiliary diseases are the same as hepatobiliary diseases in the attitude of serum cholinesterase, which varies in activity in association with the change in the cholinesterase content of the liver.

Discussion

As apparent from the results described in the preceding section, the reduc tion of serum cholinesterase reflects the scale of the histological lesion in the liver in rough approximation. But this is limited to the hepatobiliary disorders, and in the non-hepatobiliary diseases which commonly entail no appreciable change in hepatic histology the serum chclinesterase is independent of the histological picture of the liver. The correlation between the serum cholinesterase and the liver function may seem dubious in these conditions. However, the activity of serum chlinesterase varies in parallel with the cholinesterase content of the liver not only in the hepatobiliary disorders, but also in the non-hepatobiliary dis-

eases. In view of the prevailing conception put forward by Wescoe and his associtates[11] who regard the liver as the main site of production of "pseudo" cholinesterase, the rise and fall in the hepatic function will in all likelihood be the determinative factor of the variation of serum cholinesterase. The intimate relationship between the liver cholinesterase and the serum cholinesterase, which was confirmed in the present study is compatible with the conception.

Hepatic disturbance is classified into two categories: (1) the morphogical disorder which is demonstrated by the examination of hepatic histology, and (2) the metabolic disorder which is examined by the tests of hepatic function. Recent studies[15,16] on the comparison of hepatic tests with the histological picture of biopsied liver revealed that metabolic disorder did not always run in proportion to the severeness in morphological disorder, and in not a few cases a considerably advanced disturbance in metabolism was found without any indication of the abnormal hepatic histology. Thus the morphological disorder often fails to represent the functional disturbance in the liver. Apparent absence of correlation between the level of serum cholinesterase and the hepatic histology in the non-hepatobiliary diseases will be one of the examples of this dissociation.

Hepatic function is variegated. Metabolism of "pseudo" cholinesterase, namely its production, supplying and reserving, constitute merely a small facet of the multifarious aspects of the functions operating in the liver. But if the liver is really the main producer of "pseudo" cholinesterase and if it is actually the supplyer of this enzyme to the blood, as maintained by some workers[11], it will be most probable that the decreased content of cholinesterase in the liver refers to the disturbance in hepatic function at least with respect to the metabolism of cholinesterase. Reduction of liver cholinesterase is therefore justified as an indication of partial or general dysfunction of the liver in the diseases of extrahepatobiliary organs as well as in the maladies of hepatobiliary system. Inasmuch as the fall of cholinesterase activity in serum parallels the decrase in liver cholinesterase, as stated above, it will naturally be expected to suggest a hepatic dysfunction, either partial or general, depending on the conditions, in both hepatobiliary and non-hepatociliary diseases. This interpretation is not inconsistent with the afore-mentioned absence of correlation between the level of serum cholinesterase and the hepatic histology in the latter conditions, because hepatic dysfunction without morphological lesion in the liver is supposed to be common in the extra-hepatobiliary maladies. Needless to say unlimited application of the interpretation will be hazardous, since consumption of this enzyme in the tissues which has not so far been taken into consideration will similarly participate in the maintenance of the level of serum cholinesterase. In any way it is thought to be most probable that the diminished activity of serum cholinesterase reflects the disordered morphology and function in the liver in the hepatobiliary diseases, and in the diseases of extra-hepatobiliary organs it fre-

quently indicates the possibility of hepatic dysfunction which is not or is rarely accompanied by the abnormal hepatic histology.

Summary and Conclusion

The activity of serum cholinesterase was compared with the histological picture of the biopsied liver and with the cholinesterase content of the surgically excised liver on 157 patients which included 76 cases of hepatobiliary diseases and 81 cases of extra-hepatobiliary diseases. Its results were described, and the following conclusion was derived.

(1) In hepatobiliary diseases the diminution of serum cholinesterase indicated the damages to the hepatic parenchyma such as scarring, damaged liver cells and inflammation.

(2) In non-hepatobiliary diseases it seemed to refer to the functional disturbance of liver which was not or was rarely associated with the definite morphological lesion, although exceptions may be present.

(3) Serum cholinesterase was likely to rank among the reliable indicators of hepatic function, particularly in the hepatobiliary maladies.

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