

# MUCOLYTIC ENZYME SYSTEMS. X. SERUM HYALURONIDASE INHIBITOR IN LIVER DISEASE<sup>1</sup>

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The hyaluronidase inhibiting property of blood serum has been found to be increased in such diverse clinical states as infectious diseases of bacterial and viral origin (1-4), neoplastic disease (5, 6), and the postpartum state in women (7) as well as in several of the so-called "collagen diseases" of unknown etiology, notably rheumatic fever (8, 9), disseminated lupus erythematosus (2) and dermatomyositis (2). Intensive studies to determine the anatomical site of origin and to understand better the nature of this substance have been undertaken, and are still under way in this laboratory.

Clear evidence of the site of origin of the inhibiting substance is lacking. Wattenberg and Glick (10), in an analysis of a wide variety of tissues from the rabbit, found it in none of the material studied. In the course of their investigations they noted that certain hemoglobin derivatives and steroids possessed inhibitory properties; extending their observations, they noted that many similar chemical compounds also produced inhibition, but that such compounds were uniformly heat stable and not, as is the serum inhibitor, heat labile. The interest in these investigations, as well as in the current one, has been in the nonspecific inhibitor which will act on the enzyme regardless of its source, rather than the specific antibody inhibitors that develop when hyaluronidase is used as an antigen (11). It has also been found that the inhibitor migrates with albumin in an electrophoretic field (12, 13).

This migration with serum albumin, and the finding of an inhibiting property inherent in various products of liver metabolism (10), prompted an evaluation of the serum inhibiting property in disorders of the liver, since it was felt that a de-

nominator common to the protean conditions showing increased serum inhibition might be alteration, in greater or lesser degree, of hepatic function. The previously noted lack of evidence of inhibitor in liver tissue (10) was held to be possibly explainable by the premise that the substance might be manufactured in an organ but not stored therein. The current work concerns itself with an attempted correlation between serum inhibitor and the clinical course and laboratory findings in a selected group of patients with liver disease.

## MATERIALS AND METHODS

The technic of the preparation of materials, the viscosimetric assay method, and the method of calculation of results follow the procedure as described by Glick and Gollan (1) and modified by Wattenberg and Glick (10). The hyaluronic acid employed was obtained from human umbilical cords, and the hyaluronidase from bull testes. The assays were carried out on 0.02 ml. samples of serum, and when repeated analyses were to be run on the same patient, the material was stored at  $-25^{\circ}$  C. until all could be run at the same time, thus eliminating possible variations between different batches of enzyme and substrate. In most instances the patient was used as his own control, via comparisons in serial determinations. The degree of inhibition has been expressed as per cent inhibition, and in a large series of normal humans the mean was found to be  $21.5 \pm 0.7$ . This range of normal values was found to hold for an age group well beyond that covered by the patients in the group studied in this investigation.

Concomitant determinations were available of routine laboratory studies of blood and urine, and in most instances serial values of erythrocyte sedimentation rate, total and fractional serum protein, serum bilirubin (both one minute and total), cephalin-cholesterol flocculation, thymol turbidity, bromsulfalein retention and urine urobilinogen, serum alkaline phosphatase activity, and cholesterol and cholesterol esters. Routine control sera from normal individuals were run at frequent intervals throughout the entire investigation; these were found to fall within 1 per cent of the previously established normal average value. Serial determinations of inhibitor were made during periods believed to represent changes in the clinical course of the disease process, though in some

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\* Subjective symptoms classified according to severity, with 4+ representing maximum subjective discomfort, and 0 representing the asymptomatic state.

TABLE III  
Correlation of hyaluronidase inhibitor  
with liver function tests

Measurement	No. determinations	Coefficient of correlation <sup>(a)</sup>	(P)
Alkaline phosphatase	40	0.88	<0.001
Total cholesterol	46	0.58	<0.001
Bromsulfalein	44	0.54	<0.001
Bilirubin (1')	58	0.78	<0.001
Total protein	48	0.07	0.6
Cephalin flocculation	75	0.11	0.3
Thymol turbidity	59	0.14	0.2
Urinary urobilinogen	63	0.20	0.8
Cholesterol ester	46	0.12	0.4

complete loss of hyaluronidase inhibition in these sera after heating to 60° C., indicating that their inhibitory property differed from the heat stable inhibitor of the chemical compounds found in bile.

A calculation of the coefficient of correlation for the hyaluronidase inhibitor in the serum with several of the laboratory tests of liver function is shown in Table III. As indicated by these data, a positive correlation is found with serum alkaline phosphatase, total cholesterol, bilirubin, and bromsulfalein retention. No statistically significant correlation was found with the remainder of the liver function tests followed in this investigation. Further analysis of the data with respect to these four liver function tests showing good correlation was undertaken; no significant difference was noted between the several types of liver disease, particularly between the major divisions of hepatitis and cirrhosis. This finding suggested that the measurement of hyaluronidase inhibition in the sera of patients with liver disease provided a measure of nonspecific liver damage which was in no way unique to a particular type of liver disease.

Of interest was the finding, in several cases of liver disease where the patient could be followed beyond the early convalescent period, that the elevated inhibitor value persisted to a time at which the orthodox tests of liver function had returned to normal; the possibility that alteration in hyaluronidase inhibitor may be a more sensitive measure of abnormal hepatic function must be considered, although insufficient data are available at present to permit conclusions.

#### SUMMARY AND CONCLUSIONS

1. Studies were carried out on the inhibitory effect on hyaluronidase activity of sera from pa-

tients with liver disease, and an attempt at correlation of this inhibition with clinical course and with laboratory tests of hepatic function was undertaken.

2. A definite correlation was found between serum hyaluronidase inhibitor and both clinical course and several laboratory tests of liver function, *i.e.*, alkaline phosphatase, bilirubin, bromsulfalein, and total cholesterol. Several other tests showed no significant correlation. Elevated values were noted when the disease process was at its height, and these values diminished as the patients recovered.

3. An exception to this correlation was noted in several cases of severe hepatic coma; here previously elevated values became subnormal in the very terminal stages of hepatic failure. This finding has suggested the interesting speculation that the integrity of a certain amount of hepatic function is necessary for the development of both normal and elevated levels of serum inhibitor.

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