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Spectrophotometric Method for Assay of Serum Antiprotease: Clinical Applications.*

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(Introduced by S. C. Harvey.)
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Variation in the level of antiproteolytic activity in the serum has been studied in some detail since the presence of this factor was first reported in 1893.[1] The methods for determination of this activity in the past have largely been antifibrinolytic in nature,[2-5] and are not entirely satisfactory because of the subjectivity of the determinations and the variations in result due to differences in substrate and slight variations in handling of the tubes containing clots. A simpler and more objective procedure was described recently from this laboratory.[6] This was still time-consuming, and a more rapid, objective method of assay, to be described, was developed. The results obtained on examination of 250 sera by this method and by the previously reported clot formation method were collected and a statistical analysis was made of the correlation between the two tests.

Materials. 1. Trypsin‖ solution 70 mg% dissolved in veronal buffer, shaken well and filtered, kept cold until used and prepared daily.

2. Fibrinogen¶ solution, 0.5% in veronal buffer.

3. Trichloracetic acid (USP), 16% solution.

4. Sodium hydroxide (reagent grade), 1 N.

5. Saline solution, 0.9%.

6. Veronal buffer,[6] pH 7.4.

7. Folin-Ciocalteau phenol reagent,†† diluted 1:3 with distilled water.

Method. Blood is drawn into dry centrifuge tubes using a dry syringe and needle. Serum is obtained by centrifugation. The serum is diluted to 1:40 with saline, and 0.5 ml of the serum dilution is transferred to a 13 x 100 mm pyrex tube. Two similar tubes for uninhibited trypsin are set up with 0.5 ml of saline. A blank is prepared with 1.5 ml veronal buffer and is carried through the routine, except that trypsin is not added.

All tubes, with the blank tube first, are placed in an ice water bath. When temperature equilibrium has been reached (10 minutes), 1 cc of trypsin solution is added to each tube, the tubes are shaken, and returned to the bath at 30 second intervals (in order). After 30 minutes, in the same order and with the same time interval, 2 ml of fibrinogen are added to each tube. After the addition, each tube is shaken slightly and placed in a water bath at 37°C for 30 minutes.

At the end of this time 5.0 ml of trichlor-

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‖ Armour trypsin powder.

¶ Armour bovine fibrinogen; Fraction I from bovine plasma.

†† Hartman-Leddon Company.

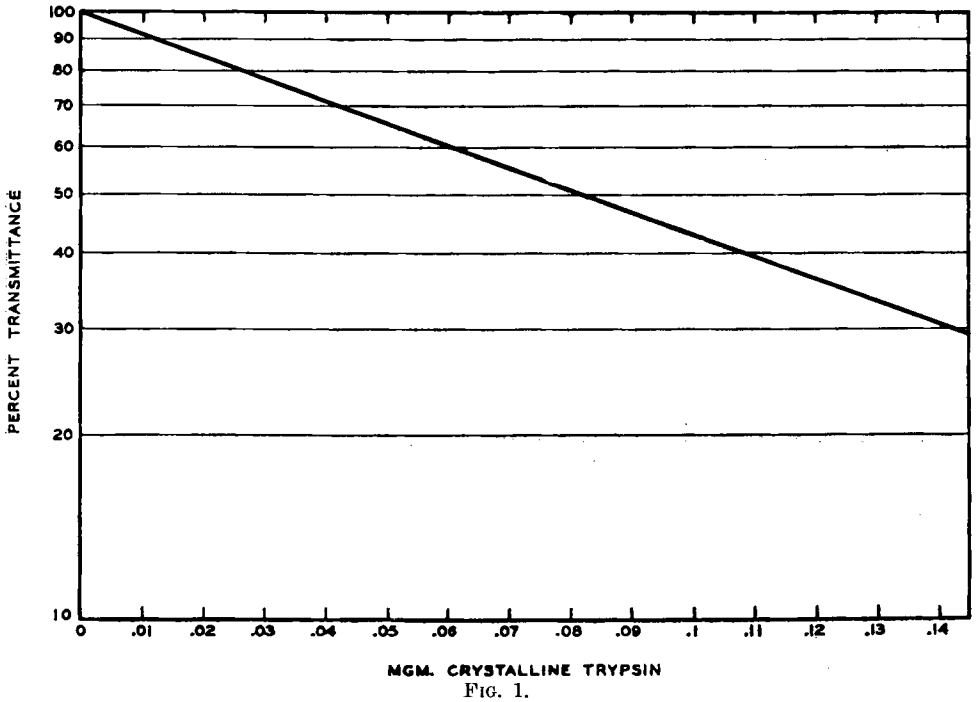

FIG. 1.

acetic acid are added to each tube without shaking and they are left at room temperature for 15 minutes.

The contents of each tube are then filtered through Whatman No. 3 filter paper. When filtration has been completed, a 2.5 ml aliquot of each filtrate is added to 5 ml of NaOH in a 50 ml Erlenmeyer flask. 1.5 ml of diluted phenol reagent is then added at definite time intervals and the contents of the flask are transferred to a standard cuvette. Ten minutes are allowed for color development, when the samples are read at the same time interval against the fibrinogen blank set at 100% transmittance as a reference. A Coleman Spectrophotometer Model 14, set at a wave length of 675 mμ, was employed.

Calculations. The % T (transmittance) readings of uninhibited trypsin and each serum dilution are converted to crystalline trypsin equivalents (CTE) by use of a conversion graph (Fig. 1). The equivalent number of milligrams of crystalline trypsin inhibited by each serum sample is then determined by subtraction of its CTE from the average CTE of the 2 uninhibited trypsins. Tests performed over a period of four weeks have shown that serum of the average patient with no apparent disease inhibits 0.0167 mg of crystalline trypsin. This figure may be used for statistical purposes; however, it is not dependable for individual daily tests because of variations in the reagents, which are prepared daily. Any daily variations are proportional for normal individuals and for those with malignant neoplasia or other diseases. For this reason at least one normal control serum is analyzed with each daily group and the unknowns are reported as % of the normal arbitrarily fixed at 100%.

Results. Tests were performed on 250 samples of blood from patients with various diseases and control patients without evident disease. The results are essentially the same with the two methods (clot formation and spectrophotometric). Although individual cases do not coincide exactly, definite differences occur but rarely. A statistical analysis**

** The statistical analysis was made by Mrs. Sylvia Johnson of the Department of Public Health and the Department of Surgery (Oncology), Yale University School of Medicine, assisted by Mr. David Votaw of the Department of Mathematics, Yale University.

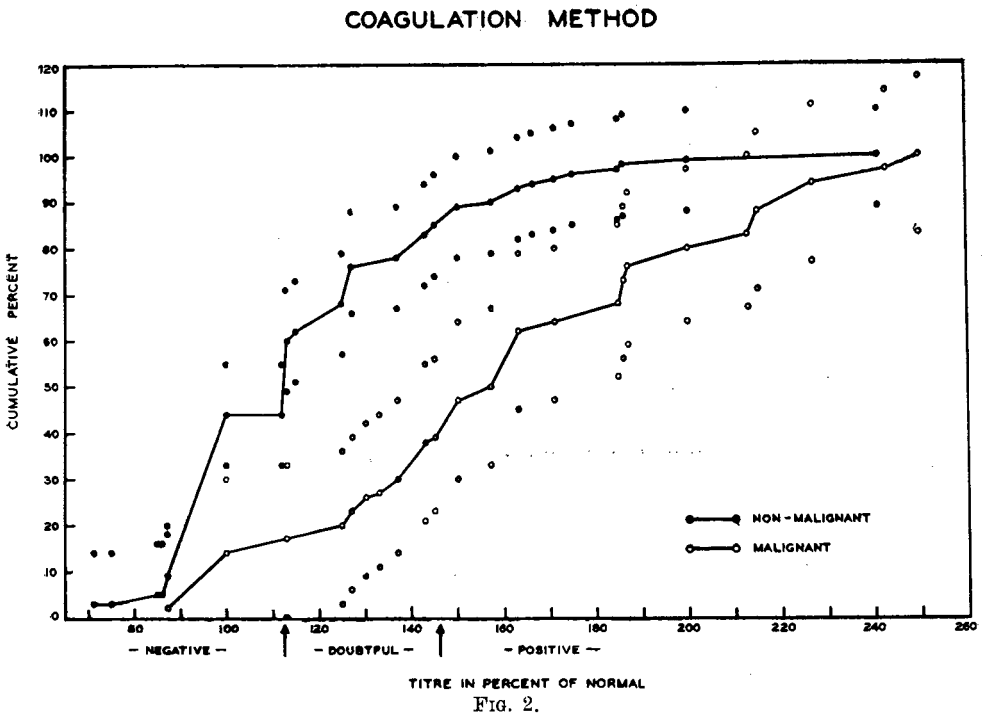

FIG. 2.

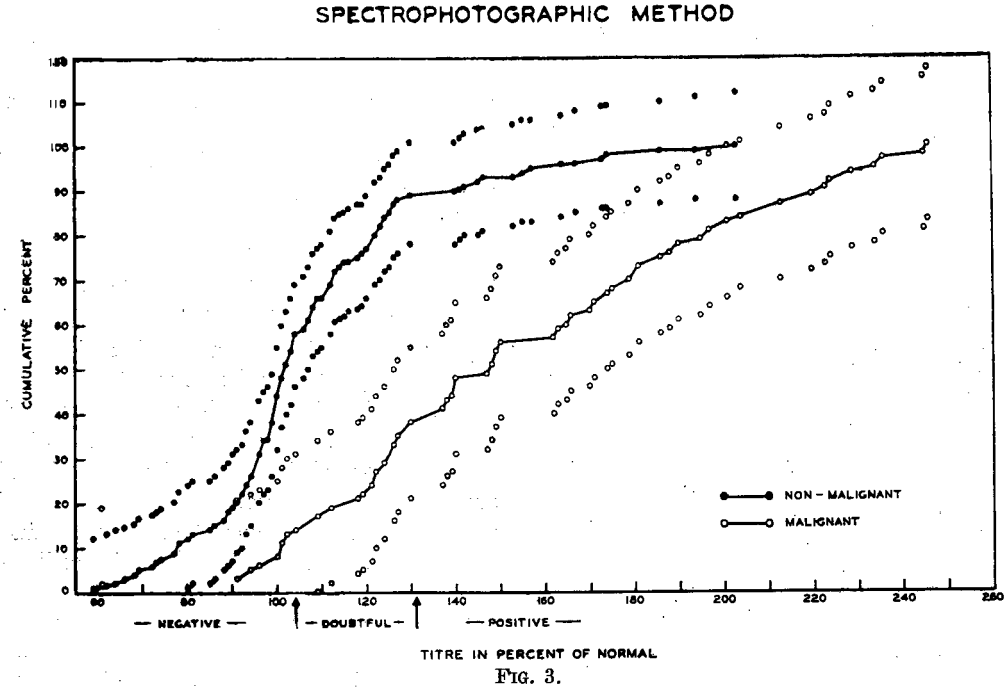

FIG. 3.

of the results in relation to the presence or absence of malignant tumor in the patients studied revealed that the results are comparable (Fig. 2 and 3). This analysis of these cases revealed that using a normal control at 100%, a titre below 112.5% for the clot formation method and below 103% for the spectrophotometric method could be consid-

ered negative with a possible error of 5%, and that a titre above 146% for the clot formation method and above 130% for the spectrophotometric method could be considered as indicative of malignant neoplasia with a possible error of 5%. With the clot formation method, titres between 112.5% and 146% and with the spectrophotometric method titres between 103% and 130% must be considered of doubtful significance.

These results indicate that this spectrophotometric method may be as satisfactory for the detection of patients with malignant neoplasia as the previously reported method.

False positive results with the spectrophotometric method fall in the same groups as with the clot formation method, *i.e.* those patients with acute infectious diseases, especially streptococcal infections, those with advanced tuberculosis of the lungs and those recently subjected to major operations.

Summary. A spectrophotometric method of assay of antiproteolytic material in the serum, using inhibition of trypsin digestion of fibrinogen, has been described. Results obtained in 250 sera by this method have been analyzed statistically and have been found to show a close correlation with the clot formation method.

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Microphonic Manometer for Indirect Determination of Systolic Blood Pressure in the Rat.*

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Although the plethysmographic method for the indirect measurement of systolic pressure in the rat[1] has been improved by modifications devised later by other workers,[2-5] it has not been adopted universally. This has been due primarily to technical difficulties and errors encountered in any method employing plethysmographic changes for determination of blood pressure.

We have devised a method, however, of obtaining the systolic blood pressure of the rat which is similar in principle to that employed in the indirect measurement of systolic blood pressure in man,—namely, the detection of sound at the exact time that the arterial pressure of the caudal artery exceeds the pressure of the occluding cuff. This method was found to be accurate, simple, and rapid.

The apparatus consists essentially of a carbon type of microphone (3 cm in diameter) to the diaphragm of which is attached a thin copper trough (1.5 cm in length and 0.6 cm in diameter). The microphone is connected to a specially designed, low frequency (100 c.p.s.) sound amplifier† operated by direct current supplied by three dry cells. This amplifier affords a voltage gain of approximately 3000. The energy changes obtained by the amplifier may be detected by ordinary earphones or oscilloscope.

In order to take blood pressure readings, a

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† Further details of the construction of this apparatus and the electrical circuit will be furnished by the authors upon request. The microphonic manometer itself may be obtained from Charles Calvert of the Pacific Industrial Electronics Company, San Francisco, Calif.