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Trypsin determination : with particular reference to the laboratory diagnosis of fibrocystic disease of the pancreas

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INTRODUCTION

During the past two decades there were many clinical and autopsy reports which showed an awareness of the disease process, but it was not until 1938 that Dorothy Anderson (1), and Blackfan and May (2), presented to medical literature the new disease entity, fibrocystic disease of the pancreas*.

This is a congenital disease in which the clinical evidence of pancreatic exocrine insufficiency is combined with severe pulmonary disease, such as, chronic bronchitis, bronchiolitis, recurrent bronchopneumonia, and bronchiectasis.

The characteristic lesion is a generalized change in the mucous secreting glands of the body. Those found in the pancreas are but one manifestation of the disease. The atrophy and fibrosis of pancreatic acini and ducts are the result of obstruction by inspissated secretion (1).

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* Congenital pancreatic deficiency, cystic fibrosis of the pancreas, pancreatic fibrosis, congenital pancreatic steatorrhea, congenital pancreatic disease, congenital family steatorrhea, chronic interstitial pancreatitis in infancy, pancreatic insufficiency.

The commonly accepted laboratory aid to diagnosis rests upon the demonstration of markedly decreased or absent pancreatic enzyme trypsin in aspirated duodenal juice or in feces.

A review of the several methods of determining trypsin or trypsin activity in duodenal juice and in the stools is made in this paper. There is, however, a consistent difficulty in evaluating the findings of different investigators because of the difference in methods of estimation and the difference in figures the several methods yield for the same enzyme value. Several investigators have seen fit to run parallel observations using different methods of assay in order to compare and evaluate results. These will be discussed.

METHODS OF ASSAYING TRYPSIN IN DUODENAL JUICE

The importance of the pancreas as a digestive organ has long been recognized. Investigators have also realized that accurate determination of the enzymes in duodenal juice would yield much more valuable information than their determination in feces. However, it was not until Einhorn (3) devised his tube in 1910 that there was a reliable way of collecting duodenal fluid. One year later Hess (4) developed his tube for infants. Since then many different methods for the estimation of pancreatic enzymes have been used.

The following methods have been employed in the assay of trypsin in aspirated duodenal fluid. (It is interesting to note that some of them were devised long before fibrocystic disease of the pancreas was recognized as a clinical entity.)

1. Northrup and Hussey (5) in 1924 devised a very accurate method based upon the proteolytic action of trypsin on casein. They then computed trypsin activity on the basis of a non-protein nitrogen determination.
2. Agren and Lagerlof (6) in 1936 employed a method of estimating trypsin by a titration process. A 6% casein solution was digested by duodenal juice for 20 minutes at 30° C. The determination was made

by titrating the difference in acidity after digestion of the casein-duodenal juice solution against a blank. (No digestion of casein.) The difference between the titration values gave the trypsin activity per cc. of duodenal juice expressed in cc. 0.1N KOH. The trypsin concentration was expressed in arbitrary units.

3. Anderson (7) in 1942 employed a viscosimetric method modified after the method of Waksman and Davidson (8). This consisted of adding differing amounts of duodenal juice (trypsin) to gelatin, thus producing varying degrees of digestion of the gelatin, and, therefore, varying the viscosity. The relative viscosity was determined by measuring the rate of flow from calibrated tubes.

4. Later in the same year (1942) Anderson and Early (9) developed a modification of the Fermi (10) method which they belived to be less reliable and less sensitive but simpler and more suitable for routine diagnostic work than the viscosimetric method they had previously used.

With this method trypsin activity is estimated by adding melted gelatin to tubes of the same quantity of differing dilutions of pancreatic juice in 5% Sodium bicarbonate. Trypsin in the duodenal

juice when incubated at 37% for one hour digests the gelatin so that it does not solidify when refrigerated for 20-24 hours. The reading is taken as the greatest dilution of duodenal juice which liquifies the gelatin. Normal pancreatic juice will liquify gelatin in amounts of 0.02 ml. or less.

5. Vighelgi (11) in 1949 employed a modified Michaelis (12) adsorption method. Duodenal juice was tested in neutral Hcl and NaOH solutions with kaolin, talc, animal charcoal, aluminum oxide and Ferric oxide. The proportion adsorbed was found by determining the strength of the ferment left in the solution.

METHODS OF ASSAYING TRYPSIN IN FECES

1. Shwachman, Farber and Maddock (13) in 1943 devised a simple test for estimating trypsin in the feces by utilizing standard X-ray film. Serial dilutions of feces in distilled water were made. A large drop of each dilution was placed on unexposed, unfixed gelatin film and incubated for one hour at 37° C. Room temperature was satisfacoty if the incubation period wasprolonged to 1½-2 hours. The film was then washed in a stream of cold water with gentle rubbing. A clearing at the site of a drop indicated enzyme activity. Results were read as: 1, 2, 3, or 4 plus.

   In comparing X-ray gelatin film to photofilm in a more recent article (14) the authors discovered that photofilm gave less reliable results.

   In 1950 Schwartz (15) modified the Shwachman technique utilizing the vest pocket as an incubator. The stool suspension was placed in a wasserman tube, then a strip of dental film which extended under the surface of the suspension was placed in the tube. It was capped, placed in the vest pocket for one-half hour, then the film was examined for clearing.

2. In 1949 Shwachman, Patterson and Laguna (14) described a diagnostic test tube method for stool

trypsin in which dilutions of stool trypsin were mixed with commercial gelatin and incubated for one hour after which the degree ofliquifaction signified trypsin activity.

The author claimed that discrepancies between the X-ray film method and the test tube method occurred in less than one per cent of determinations. They found the tube method to be more sensitive, but the X-ray film method was simpler and easier to perform, yet reliable enough for clinical use.

COLORIMETRIC DETERMINATION OF TRYPSIN

No clinical application has been made of the following two procedures.

1. In 1908 Roaf (16) found that when fibrin stained with congo red was digested by trypsin or pepsin the congo red was freed. From the depth of the color the amount of fibrin which had been digested could be estimated. His experiments consisted of comparing the ratio of peptic to tryptic activity by placing trypsin plus congo red fibrin in one tube and pepsin plus congo red fibrin in another. The colors of the two solutions were then compared and the deeper color was diluted to a point where it matched the other tube. From this a ratio of peptic to tryptic activity was derived. The author made no mention of what method he used to match his colors. Results when compared with non-protein nitrogen determinations on similar solutions did not correspond exactly, but a parallel between the two methods was shown to exist.

2. Snell and Snell (17) in 1937 developed a similar test but used fibrin stained with alcohol-blue instead of congo red. Furthermore they prepared a series of standard solutions with known quantities of trypsin

therefore, the method was quantitative. Their results were more satisfactory than those of Roaf.

COMPARISON OF THE DIFFERENT METHODS OF TRYPSIN DETERMINATION

The multiplicity of methods, the lack of standardization for evaluating the enzyme and the use of either pancreatic fluid or feces make evaluation of the various methods difficult.

Vighelgi (11) made parallel observations using three different methods of assay on a large group of healthy children. The three methods were reported as showing a good correlation.

He found Agren and Lagerlofs (6), titration method to be themost accurate, but also the most complicated, time consuming and expensive. The Michaelis (12) adsorption method and the Anderson-Early (9) gelatin digestion method he reported as being simple and reliable enough for clinical purposes. However, if all his determinations were run upon healthy subjects, this conclusion is not valid.

Gordon, Levin and Whitehead (18) compared the X-ray film digestion method of Shwachman with the gelatin digestion method of Anderson and Early and the complicated casein digestion-non-protein nitrogen determination method of Northrup. They reported the X-ray film digestion technique as being entirely adequate for differentration of fibrocystic disease from other

conditions. It is simpler, less time consuming and requires less duodenal juice.

The difficulties encountered in duodenal drainage procedures on young children who are often in respiratory distress makes a fecal trypsin estimation desirable.

In general, the concensus of opinion among investigators is that trypsin determinations upon aspirated duodenal juice are more accurate than those made upon stools, however, most investigators agree that the simple fecal X-ray film test is adequate for clinical use, or at least it is an excellent screening procedure.

Shwachman in evaluating his gelatin film test (14) stated that of 50 proved cases of fibrocystic disease seven gave false positive results. He considered the possibility of these resulting from the action of certain bacteria in the feces, but concluded that this was not an important objection to the method.

Johnstone (19, 20, 21), in three reports in which he investigated the role of bacterial flora in feces, found up to 50% false positive gelatin film tests in one series of 78 proved cases of fibrocystic disease. The false positive results, however, were reported as occurring at "one time or another". These were apparently not consistently positive for a given

patient, therefore, his objection to the method is overemphasized. Furthermore, Schwartz (15) stated that a test will rarely be repeatedly positive in a patient with fibrocystic disease.

The colorimetric methods of Roaf and Snell have had no clinical application, however, a quantitative procedure suitable for clinical use might be devised by their modification.

SUMMARY

The various methods for determining trypsin in both duodenal fluid and in feces have been reviewed in this paper and an effort has been made to compare and evaluate these methods with respect to their value as clinical procedures in the diagnosis of fibrocystic disease.

The value of the X-ray digestion spot test as a screening method has been pointed out as has the need for the development of a simple accurate method for quantitatively determining trypsin. Such a method might possibly be developed using fibrin stained with an indicating dye that is released when the fibrin is digested by trypsin. With this in view a fellow student, Roy E. Fredericksen has been elaborating on the colorimetric methods of Roaf and Snell.

I am indebted to Byron Oberst, M. D., Herbert Jacobi, Ph.D. and senior medical student, Roy E. Fredericksen, for their aid in the compilation of this material.

CONCLUSION

1. The determination of low or absent pancreatic trypsin is an important laboratory aid in the diagnosis of fibrocystic disease of the pancreas.
2. In general, trypsin activity is determined by:
   A) Trypsin estimation in duodenal juice.
   B) Trypsin estimation in feces.
3. Comparison of methods of trypsin determination.
   A) Determination of trypsin in duodenal fluid is more reliable but the methods are more complicated.
   B) Estimation of trypsin in feces is not as accurate but the methods are simple and are usually sufficient for clinical diagnosis when correlated with the clinical picture.
4. There is a need for a simple, quantitative method of determining trypsin, either in duodenal fluid or in feces. A fellow student, Roy E. Fredericksen, is working on this problem.

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