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ONE-DROP TEST FOR URINE PROTEIN DETERMINATION

Edwin M. Knights, Jr. M.D. and Joseph C. Erwin, M.D.*

Although tests for proteinuria have been a fundamental part of laboratory urinalysis for over one hundred years, most of the technics currently employed are still based on the original precipitation phenomeon principle which was originally employed. Recently a new colorimetric tablet test has been devised for the detection of protein in urine. This test is called Albutest⁺ and is supplied as soft tablets along with color chart and directions.

To perform a test, a drop of urine is placed on the tablet followed by two drops of water. An immediate color development takes place if protein is present, and the surface of the tablet is then compared with a color chart to establish the extent of the reaction. If there is no protein in the urine, the tablet will remain yellow, whereas in the presence of protein a blue-green spot will persist on the tablet. Increased amounts of protein influence the intensity of the blue-green reaction.

The Albutest tablet contains cellulose which absorbs on the surface of the tablet any protein in the urine. The color reaction is obtained by means of the indicator bromphenol blue, and a salicylate buffer is also present. The function of the buffer is to provide in the moistened tablet a reaction which will maintain the yellow color of the indicator.

In the presence of protein, the indicator exhibits the "protein error of indicators" first described by Sorenson in 1909.1 This effect, which has been an obstacle for many years to biochemists attempting to use indicators to measure pH of body fluid, has been studied quite extensively in urine and body fluids. Exton² devised a colorimetric test for albuminuria in 1925 using sulfosalicylic acid and the indicator bromphenol blue. Qualitative tests for proteins have been devised by Feigl and Anger³, by Ishidate and Sakaguchi⁴, Joukovsky and Vandervelden⁵, and Ketomaa and Ruosteenoja⁶. Many ph indicators have been found to be affected in this manner and the ethyl ester of tetrobromphenolphthalein has been found particularly desirable for serum protein methods. Klotz found that albumin was quite unique in its affinity for small anions^{7,8}, and in 1953 Bracken and Klotz reported a simple method for rapid determination of serum albumin using methyl orange (sodium p-die-methylaminoazobenzene-p'-sulfonate)9. With this dye equilibrium between zwitterionic acid form and anionic basic form is at its midpoint near pH 3.5 and upon addition of serum albumin, color shifts markedly towards yellow because the protein combines with anions.

The urine tablet test has been studied extensively in the laboratories of the Department of Pathology of Harper Hospital and compared with sulfosalicylic and Kingsbury-Clark technics10. It was found to be a rapid, easy method requiring a minimum amount of urine and no heating. Use of a common drinking straw for transfer of the urine allows the complete elimination of glassware from the procedure, making it an attractive procedure for routine scanning of large numbers of urine

^{*}Department of Pathology, Harper Hospital. Presented by title at the Henry Ford Hospital Medical Association, June 1st, 1957, Detroit, Michigan. †Albutest is a registered trademark of Ames Co., Inc. Elkhart, Indiana.

Urine Protein Determination

specimens either in the office or in the hospital laboratory. Ability of technologists and physicians to interpret the roughly quantitative yellow to blue color change was found to improve considerably with experience. When used by an experienced person, the tablet proved very reliable in picking up human albumin concentrations of 20 mg./100 ml. or greater in more than 2000 urine specimens studied. False positive tests for albumin were infrequent; differences of reaction between the sulfosalicylic acid test and the colorimetric tablet test occurred less than 1% of the time. A decomposed, strongly ammoniacal urine will give a false positive reaction because bromphenol blue is also an indicator on the alkaline side. Tablets appear to be stable at room temperature for at least 5 months. Table I shows the results of examinations of 180 coded urine specimens by 18 persons participating in a recent workshop in ultramicro methods. Human albumin was added in varying concentrations to urine specimens testing negatively by the sulfosalicylic acid technic. No false positive tests were recorded in this group and interpretations were remarkably accurate. An additional set of 18 urines, which have been allowed to decompose and become ammoniacal, gave the following results: test called questionable by five examiners, recorded as negative by eight, 1 plus by two, 2 plus by one, and 3 plus by two examiners.

Table I								
Urine P	rotein	Test	Results					
180 Coded Specimens 18 Examiners								

Human Albumin Co	ontent (mg. %)		Interpretations			
		0	1+	2+	3+	Totals
500			1	1	16	18
400			1	3	14	18
250			1	4	13	18
120			6	7	5	18
100			2	10	6	18
80			8	9	1	18
50		2	12	3	1	18
20		7	10	1		18
0		36				36

SUMMARY

Evaluation of a new tablet test for albuminuria (Albutest) reveals the test to be a rapid, efficient method of qualitative screening for albuminuria requiring neither heat nor glassware. Previous studies showed roughly quantitative results to agree well with sulfosalicylic acid Kingsbury-Clark methods when albumin was present in concentrations above 20 mg./100 ml. Tablets appear to be stable at room temperature for at least 5 months.

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