volume of the isolated segment may vary because of differences in the calibre of the segment and the bore of its lumen. The length of the segment isolated is of course subject to experimental error. The reaction to progesterone may also vary according to differences in the blood supply of the isolated segment. Finally the reaction to progesterone in the individual may be subject to individual variation caused by the interaction of other endocrine secretions.

It is apparent, however, from McGinty's work that the local application of progesterone is superior as a qualitative test to the previous methods of intramuscular injection because of its relative sensitivity.

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Indoluria and Its Relation to Sulfur Deficiency.

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Indole is a product of the action of bacteria on protein. Whether this action takes place in the gastrointestinal tract or in the body proper the organism is faced with the problem of the disposal of the resulting indole. Normally, as far as can be determined by analysis of excretions, the indole is oxidized to indoxyl, this in turn conjugated with potassium hydrogen sulfate and the indican thus formed is excreted in the urine. It is conceivable that if oxidation is deficient, if the liver fails in its function of conjugation, or if the supply of sulfate is deficient, free indole may appear in the urine.

Indoluria has been reported by various investigators, Cantelli,[1] Schour and Rosengarten,[2] Forbes and Neale[3] but Vaughan[4] failed to observe it. It has also been shown by Vaughan[5] and Carnes and Lewis[6] that the urine contains some compound which is readily acted upon by *E. coli* to produce indole.

If the indoluria is due to a lack of sulfate to effect the conjugation, the sulfur deficiency should be manifested elsewhere in the body. It is known that practically all the urinary sulfate enters the organism

1 Cantelli, O., *Riforma Medica*, 1922, **38**, 481, abstract in *J. Am. Med. Assn.*, 1922, **79**, 508.

2 Schour, M., and Rosengarten, C., *Klin. Wochschr.*, 1930, **9**, 1751, 1968.

3 Forbes, J. C., and Neale, R. C., *J. Lab. Clin. Med.*, 1934-35, **20**, 1017.

4 Vaughan, S. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 623.

5 Vaughan, S. L., *J. Lab. Clin. Med.*, 1937, **22**, 399.

6 Carnes, H. E., and Lewis, G. T., *J. Lab. Clin. Med.*, 1938, **23**, 459.

as unoxidized sulfur and hence, it is not necessary to find an actual sulfate deficiency in the excretions to prove the point. Lightbody and Lewis[7] as well as Mitchell and associates[8] have shown that when the sulfur supply is insufficient in the rat, the sulfur and cystine content of the hair is lowered. Presumably if the same condition should occur in the human, it would extend to the fingernail, another epidermal tissue whose cystine content has recently been repeatedly determined. If, therefore, free indole appears in the urine as the result of sulfur deficiency, the fingernails might be expected to reflect the condition in a lowered cystine content.

It seemed to us worth while to determine whether indoluria and low fingernail cystine co-exist in the same individual. Since qualitative tests for free indole can be carried out fairly rapidly in quantity, it was decided to attack the problem from this angle first and then to determine fingernail cystine in those cases which showed a positive test.

A number of difficulties were encountered in the establishment of a sensitive and reliable qualitative test for indole in the urine. False positives might result from (1) bacterial contamination before or after voiding, (2) decomposition of indole precursors during manipulation of the specimen, (3) the presence of compounds which give color reactions similar to those given by indole. In order to rule out any possible bacterial activity after the voiding of the sample, all positive indole tests were checked on freshly voided specimens. In this way the only danger of encountering indole produced subsequent to the excretion of the urine was in cases of G.-U. tract infection. Only one such case (No. 19) is known to have been present in our series.

We have felt that possibly the method used by Forbes and Neale[3] in their studies of indoluria might give rise to false positive tests because of the extensive manipulations involved. For this reason as well as for the saving of time in carrying out an extended series we turned to the method of Vaughan.[4] After rather extensive trial we adopted the following modified procedure.

Two cc portions of redistilled petroleum ether are pipetted into 6 inch test tubes graduated at 7 cc. Urine is poured in up to the mark, the tube tightly stoppered with a clean cork stopper and the specimen extracted by inverting the tube 50 times. Usually a slight

[7] Lightbody, H. D., and Lewis, H. B., *J. Biol. Chem.*, 1929. **82**, 485, 663.

[8] Beadles, J. R., Braman, W. W., and Mitchell, H. H., *J. Biol. Chem.*, 1930, **88**, 623; Smuts, D. B., Mitchell, H. H., and Hamilton, T. S., *J. Biol. Chem.*, 1932, **95**, 283.

emulsification results but this can be quickly dispelled with a drop or 2 of 95% ethyl alcohol. One cc of the petroleum ether layer is transferred to a small agglutination tube and 0.5 cc of Ehrlich's reagent (2 g p-dimethylaminobenzaldehyde in 100 cc 20% HCl) added. If indole is present a red color results on shaking.

If this presumptive test is positive it must be repeated after the removal of as many interfering substances as possible. To this end an excess (usually 3 cc) of saturated lead acetate solution is added to 5 cc of a freshly voided urine specimen. Filter and to 2 cc of the filtrate add 1 cc of Ehrlich's reagent. The precipitate of lead chloride which forms does not interfere with the observation of the pink color. The color can be extracted with amyl alcohol if desired.

Urobilinogen very readily gives a color with Ehrlich's reagent (Wallace and Diamond[9]) but is precipitated by the lead acetate. Skatole and indole acetic acid which might perhaps be expected to arise by the same reaction which gives rise to indole, give a color with the reagent but only when relatively much larger amounts are present and in addition the color is distinctly different in quality and easily distinguishable from the pink given by indole. Indole gives a distinct color in a dilution of 1 :1,000,000. It should be pointed out, however, that the p-dimethylaminobenzaldehyde must be of the highest purity if false positive reactions are to be avoided. Our failure to realize this forced the discarding of several weeks' work at the beginning of our study.

We felt, therefore, that we had a procedure for the qualitative detection of indole in urine which was delicate and quite specific.

Using this procedure we have examined urine samples from 275 hospital patients from the colored medical ward of Grady Hospital. The patients have in practically all cases been males. From this number 22 patients have shown indoluria. Table I contains the clinical diagnoses for these individuals. The total is not that of all the patients whose urines were examined because clinical conditions which never showed an indoluria are not represented.

Fingernail clippings were taken from those patients who showed a positive test for indole. The clippings were brought to the laboratory and soaked for several hours or overnight in 70% alcohol. This served to soften any dirt and cuticle adherent to the fragments of nail and these were then removed by scraping with a dull scalpel. The clean clippings were dried in an electric oven at 100°C overnight.

Fifty to 60 mg portions of the clean, dry nail parings were weighed out and placed in 5 cc of 20% HCl. Hydrolysis was effected by immersion of the flask in an oil bath maintained at 120°-125°C for

[9] Wallace, G. B., and Diamond, J. S., *Arch. Internal Med.*, 1925, **35**, 698.

TABLE I.

Diagnosis	No. of patients	Indolurics	% cystine in fingernails
Pneumonia	58	6	(9.0-10.7)*
Heart disease	54	6	(10.3-11.4)*
Pneumonia and heart disease	3	1	8.4
Pulmonary tuberculosis	13	2	11.2, 9.7
Cerebral hemorrhage	3	3	9.4, 9.7, 10.2
Diabetes mellitus	17	1†	10.5
Pyelo-nephritis	1	1	10.4
Typhoid fever	8	1	11.4
Bronchiectasis	1	1	8.5
Total	158	22	10.0 avg

* Parentheses indicate range of values observed.

† This single positive case from the diabetic series was in diabetic coma when the specimen was obtained.

6 hours. Cystine was determined in the hydrolysate by the Rossouw and Wilken-Jorden modification[10] of the Sullivan method. Duplicate analyses were read against different standards and the determinations were repeated until agreement within 0.2% of cystine was obtained.

As will be seen from Table I, the cystine values varied between 8.4% and 11.4% with an average of 10%. The cystine content of fingernail clippings from presumably normal individuals of the same race and approximately the same age as determined by this method in our laboratory ranged from 7.9% to 11.7% with an average of 9.9%.

It is evident from these data that indoluria as we have encountered it is not accompanied by a lowered content of cystine in the fingernails. And, if the level of cystine in the fingernails be accepted as an index of the available supply of sulfur in the body, the explanation of the presence of free indole in the urine does not appear to be failure of conjugation because of lack of available sulfur.

Summary. Free indole was found in 22 of 275 specimens of urine obtained from hospital patients. Fingernail clippings from the patients having indoluria had a normal cystine content. It seems improbable that indoluria is due to failure of conjugation because of lack of available sulfur.

[10] Rossouw, S. D., and Wilken-Jorden, T. J., *Onderstepoort J. Vt. Sci. and An. Indus.*, 1934, **2**, 361.