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# Comparison Between Gravimetric and Reduction Methods for Determination of Pregnandiol Glucuronidate.*

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The evaluation of luteal activity in the human has been facilitated by recognition of pregnandiol glucuronidate (P.G.) as a metabolic derivative of progesterone.[1] Quantitative evaluation of luteal activity, however, is not obtainable by P.G. estimation in the absence of quantitative information regarding the efficiency of the extra-ovarian factors necessary for the excretion of P.G.[2] Further, the presence or absence of urinary P.G. may not serve as a qualitative test for luteal activity, since it is possible to obtain zero values for P.G. in the presence of circulating progesterone.[3] Recognizing these difficulties, it is still possible to use P.G. determinations for useful information. The presence of urinary P.G. may be taken as strong suggestive evidence for functional lutein tissue and the quantity of P.G. excreted represents a minimal estimate of the amount of metabolized progesterone.

The practical utility of P.G. determinations for evaluation of luteal function presupposes that the method of P.G. estimation is satisfactory. In dealing with the small amounts of P.G. excreted by non-pregnant women, Venning's modified gravimetric method[4] affords no absolute guarantee that the material obtained by that procedure is actually P.G. The need for a quantitative check of the purity of the P.G. isolated by the method of Venning[4] has led to the development of a simple method of P.G. determination, which depends on the estimation of reducing activity obtained after acid hydrolysis of P.G. The results found with this reduction method are compared to P.G. values obtained gravimetrically.

*Methods and Results.* A sample of sodium pregnandiol glucuronidate (Na P.G.) (Ayerst, McKenna and Harrison) recrystallized from ethyl alcohol (M.P. 271°C uncorrected) was used in the de-

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1 Venning, E. H., and Browne, J. S. L., *Endocrinology*, 1937, **21**, 711.

2 Hamblen, E. C., Ashley, C., and Baptist, M., *Endocrinology*, 1939, **24**, 1.

3 Venning, E. H., and Browne, J. S. L., *Endocrinology*, 1940, **27**, 707.

4 Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 595.

velopment of the method. Preliminary experiments revealed that maximal reducing activity was obtained by hydrolysis of Na P.G. with 0.2 ml of 10 N $H_2SO_4$ per cc of P.G. solution for 60 minutes in a boiling water bath; and that the hydrolysis of Na P.G. by acid under these conditions proceeds as well in 50% acetone as in water.

The calibration curve was obtained in the following manner: Known amounts of recrystallized Na P.G. dissolved in 50% acetone were added to hydrolysis tubes graduated at the 5 ml volume mark. Acetone and water were added so that the final volume was 3 cc and the acetone concentration was 33%. 0.6 cc of 10 N $H_2SO_4$ was added to the P.G. solution and the tubes were placed in a boiling water bath for one hour. A long piece of glass tubing, inserted through a rubber stopper, was used as a reflux condensor to minimize the vaporization of acetone during the hydrolysis. After the hydrolysis period, the tubes are removed, cooled and then neutralized as follows: 1 drop of phenolphthalein is added to each tube, 15 N NaOH is added drop by drop till the solution becomes colored, and then 1 N $H_2SO_4$ is added until 1 drop brings the solution back to the colorless state. Upon completion of the neutralization, the contents are diluted with water to the 5 ml mark, mixed, and then filtered to remove a white precipitate which appears during the course of hydrolysis. 2.0 ml samples of this filtrate are treated for Cu reducing activity according to the method of Folin and Wu,[5] adapted to the Leitz photoelectric colorimeter. The final blue solution is diluted to the 12.5 ml mark when less than 4 mg of Na P.G. is analyzed, and to the 25 ml mark when 4-8 mg are present. When light absorption is plotted against concentration the curve is almost linear from 0.25 to 8 mg.

This method is sufficiently sensitive to allow detection of as little as 0.25 mg of Na P.G. and is accurate to ±0.1 mg. It is recognized that higher sensitivity might be obtained by utilization of a more

TABLE I.
Comparison of Gravimetric and Chemical Determination of Pregnandiol Glucuronidate in the Urine of Normal Women.

| Case | Pregnandiol Glucuronidate in mg per 24 hours | | |
|---|---|---|---|
| | Gravimetric | By reduction | % impurity |
| 1. | 4.0 | 3.8 | 5.0 |
| 2. | 3.0 | 3.0 | 0 |
| 3. | 3.8 | 3.7 | 2.6 |
| 4. | 1.6 | 1.6 | 0 |
| 5. | 5.3 | 5.1 | 3.5 |
| 6. | 4.8 | 4.4 | 8.3 |
| 7. | 2.3 | 2.1 | 8.7 |

[5] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

sensitive reducing procedure and that the accuracy might conceivably be increased. However, the qualitative information regarding the progesterone activity obtained from P.G. does not appear to warrant further quantitative refinements of the reduction method at this time.

Table I illustrates the comparative values of P.G. in the urine of 7 non-pregnant women when obtained by gravimetric analysis of Venning[4] and by the reduction method described above on the isolated precipitates of P.G. In general, it will be seen that there is good agreement between the two methods. However, this situation is not true in all cases. Table II gives results on 2 patients on whom aberrant results were obtained.

With patient 8, no luteal activity (as evidenced by the vaginal smear method) was noted although a mean of 3.1 mg of P.G. was found from the 19 to 25 days of the cycle, after obtaining a zero value on the 17th day. The conclusion that progesterone was being secreted in amounts sufficient to produce P.G., but insufficient to affect the vaginal mucosa seemed unsatisfactory, since crucial evidence was lacking to establish that the isolated material was actually P.G. Two cycles later, simultaneous vaginal smears and daily urine specimens from this patient were studied. As was the case in the previous cycle, the smears showed no evidence of luteal

TABLE II.

Comparison of Gravimetric and Chemical Determination of Pregnandiol Glucuronidate in the Urine of 2 Patients Failing to Show Luteal Activity as Evidenced by Vaginal Smears.

| | | Pregnandiol Glucuronidate in mg per 24 hrs | | |
|---|---|---|---|---|
| Patient | Day of cycle* | Gravimetric | by reduction | Impurity, % |
| 8 | 17 | 1.6 | 0 | 100 |
| | 18 | 3.0 | 0.2 | 93 |
| | 19 | 3.6 | 0 | 100 |
| | 20 | — | — | — |
| | 21 | 1.6 | 0 | 100 |
| | 22 | 2.0 | 0 | 100 |
| | 23 | — | — | — |
| | 24 | 4.0 | 0 | 100 |
| 9 | 5-12† | 13.9 | 0.5 | 96 |
| | 13 | 0.4 | — | — |
| | 14 | 0.3 | — | — |
| | 15 | 1.3 | — | — |
| | 16 | 6.7 | 0 | 100 |
| | 17 | 1.5 | 0 | 100 |
| | 18 | 1.5 | 0 | 100 |
| | 19 | 1.5 | 0 | 100 |
| | 20 | 0.2 | — | — |
| | 21 | 6.0 | 0.9 | 85 |

*Onset of menstruation—1st day.

†Gravimetric values on 8 successive days were 2.4, 2.7, 2.2, 2.0, 2.5, 0.5, 0.8, and 0.8 mg respectively.

activity. The material isolated and gravimetrically determined as P.G. is clearly not that substance, since it is apparent that 93-100% of this material consists of impurities. This P.G.-like material appears indistinguishable from amorphous Na P.G. by color and solubility properties. The second acetone precipitation used by Venning[4] for final purification precipitates this impurity as well as Na P.G. Similar results were obtained on patient 9, who also failed to exhibit luteal activity as determined by vaginal smears.

*Summary.* The results presented indicate the necessity for determination of the purity of the P.G. isolated from non-pregnant urine. It has been clearly shown that the modifications reported by Venning[4] do not necessarily remove all impurities from the final product. The implications of these findings, for much of the data in the literature, are clear. In the light of our results, the finding (first reported by Hamblen, *et al.*,[2]) that P.G. may be present in the absence of a secretory endometrium, particularly warrants critical reëxamination.

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## Intrapleural Tuberculosis Following Talc Pleuritis.

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The artificial induction of aseptic pleuritis by the injection of talc into the pleural space has been reported by a number of different workers.[1] This procedure has been recommended for the purpose of producing adhesions which will prevent recurrent spontaneous pneumothorax from collapsing the lung, or may allow a selective collapse, with only an affected portion of the lung collapsed. It has also been advised as a preliminary to operations on the lung, such as drainage of abscesses, cysts or cavities, or before partial or complete amputation of a lung, to diminish the extent and severity of subsequent infection.[2] In view of the frequency of tuberculous involvement of the pleura, it seemed desirable to study the effect of such talc pleuritis on intrapleural tuberculosis before endorsing its use in such conditions.

[1] Bethune, Norman, *J. Thoracic Surgery*, 1935, **4**, 251.

[2] Singer, J. J., Jones, J. C., and Tragerman, L. J., *J. Thoracic Surgery*, 1941, **10**, 251.