

EXCRETION OF URINARY PREGNANEDIOL IN NORMAL PREGNANCY

BY

R. G. CHITRE and K. R. JUVALE,

*Biochemistry Section, Department of Physiology,
Seth G. S. Medical College, Parel, Bombay.*

In 1936 Venning and Browne described the isolation of water-soluble pregnanediol complex from pregnancy urine. It was observed that larger quantity of pregnanediol is excreted in urine during normal pregnancy than during the luteal phase of the menstrual cycle. The function of the corpus luteum is necessary for the differentiation of endometrium in order to form nidus for the fertilized ovum and that the continued function of the corpus luteum is necessary to maintain a decidual reaction of the endometrium. As the corpus luteum of pregnancy is much larger and persists for a longer time than does the corpus luteum of the menstrual cycle, the natural inference is that greater amount of the progesterone is produced by former than by latter. It has been regarded that increase in the pregnanediol (a progesterone degenerated product) level early in pregnancy would serve as a basis for the diagnostic test of the pregnancy.

In 1937, Venning reported a gravimetric method for pregnanediol estimation, in which 24 to 48 hrs. urine collection was required for the estimation.

In 1944 Guterman published a qualitative method for the determin-

ation of pregnanediol, based on the method of Astwood and Jones and Talbot et al. In this method 100 c.c. sample of the first morning void was used. The method consists of extraction of urine with toluene of the acid hydrolysed pregnanediol complex urine, followed by purification and precipitation of the pregnanediol by alkali. The characteristic colour was developed by H_2SO_4 . This method was extended and modified by Guterman et al and developed on a quantitative basis, applicable as a diagnostic aid for pregnancy and threatened abortion. McCormack modified a slightly different technique. Davis and Fugo gave another method based on Guterman's method but used 24 hrs. urine collection. Morrow and Beuna following Guterman's procedure used artificial colour standards of $K_2O_2O_7$.

Sommerville, Marian and Kellar suggested that Guterman's method was liable to give erratic results at low levels of the pregnanediol excretion and fictitiously high results in the presence of abnormally large amounts of cholesterol or 17-Ketosteroids. He gave an alternative method.

It was shown by Brown and Venning and Backman Leekly and

Hirschman that from 3 to 10 mgm. of pregnanediol were excreted daily during the last half of the menstrual cycle. After conception the amount excreted begins to increase, reaching a maximum of 60 to 105 mg. per day on or about 266 days. After this time until parturition the pregnanediol excretion falls down.

Even though pregnanediol levels in normal pregnancy were determined in foreign countries, no such values are available for Indian women. In view of the normal variations in the excretion of pregnanediol in women living in cold countries and in India, the problem was considered worthwhile investigating.

Experimental

Investigation was carried on normal pregnant women from 6th to

9th months duration. Twelve cases of 6 months duration, 9 cases of seven months duration, and 11 cases of 8th and 9th months duration were studied. In five cases the excretion of pregnanediol was studied 72 hrs. after delivery. The women studied were from Nowrosjee Wadia Maternity Hospital. They were given six ounces sample bottle containing 1 to 2 c.c. of toluene as a preservative and were asked to collect first morning sample directly into the bottles. They were requested not to urinate throughout the night.

The estimation of pregnanediol was carried out according to Somerville et al. The results were expressed per 1000 c.c. of urine. These are shown in Table I and are represented graphically in Graph I.

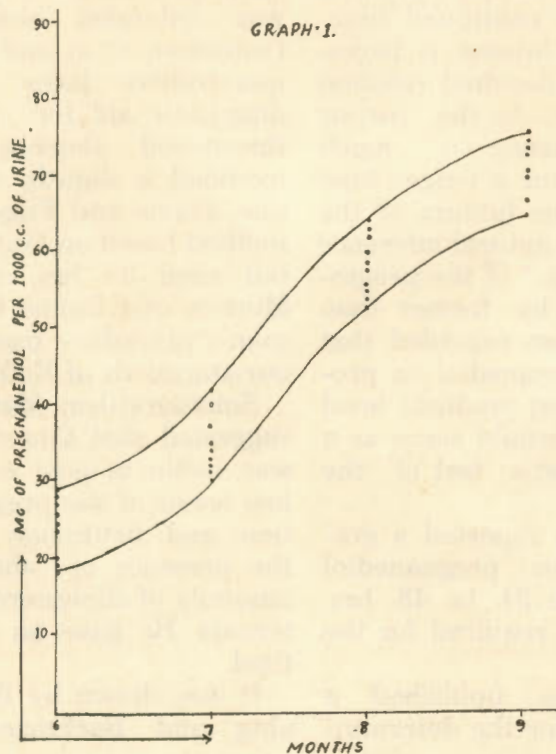


TABLE I

Excretion of the Urinary Pregnanediol in Normal Pregnancy in Different Months.

No.	6th Month Mg. per 1000 c.c.	7th Month Mg. per 1000 c.c.	8th Month Mg. per 1000 c.c.	9th Month Mg. per 1000 c.c.
1.	19.0	30.0	53.0	67.0
2.	24.0	31.0	58.0	73.0
3.	21.0	34.0	54.0	65.0
4.	27.0	33.0	63.0	67.0
5.	29.0	42.0	53.0	70.0
6.	26.0	37.0	56.0	74.0
7.	18.0	32.0	58.0	76.0
8.	28.0	40.0	64.0	67.0
9.	26.0	33.0	60.0	75.0
10.	24.0	—	62.0	76.0
11.	20.0	—	55.0	70.0
12.	21.0	—	—	—
Mean	23.5	34.7	57.8	70.9

Excretion of Urinary Pregnanediol 72 hrs. After Normal Delivery.

No.	Pregnanediol Mg. per 1000 c.c.
1	Nil
2	Nil
3	Nil
4	Nil
5	Nil

Discussion

Estimation of pregnanediol was carried out in normal pregnancy in Indian women from sixth month onwards up to the 9th month and in few cases seventy-two hours after the normal delivery.

The samples were collected from Nowrosjee Wadia Maternity Hospital, Bombay. Patients belonging to the lower class usually attend the hospital. 100 c.c. of the first morning

void was examined for urinary pregnanediol estimation and the calculations were made on the basis of 1000 c.c. urine excretion. The results are shown in Table I and represented in Graph I.

It could be seen from the graph that the excretion of pregnanediol after 6 months gradually increased during normal pregnancy. Brown and Venning reported that up to the 60th day of normal pregnancy the va-

lues found were 4 to 10 mgms. per day i.e. approximately the same as found in normal menstruation. The rate of excretion began to rise from this level between 80th and 100th day. By about 150th day it reached 40 mgms. per day. The rate of excretion continued to rise and peak was reached at about the 8th month. Within 48 hrs. after delivery the pregnanediol excretion disappeared.

Bachman, Leekly et al have shown that, prior to 20th week, mean daily excretion of pregnanediol did not exceed 30 mgms. per 24 hrs. Thereafter the amount of such excretion was found to rise by increments of about 20 mgms. per month until the 36th week when a peak level of about 100 mgms. per 24 hrs. was attained. By the 39th week a mean level was 80 mgms./24 hrs. Comparison of the values of urinary pregnanediol as reported by Smith and Smith and present investigation is as follows:

Month	Average preg- n a n e d i o l (Smith a n d Smith)	Average preg- n a n e d i o l in In- dian women (Present In- vestigation)
	mgms.	mgms.
6	48.0	23.5
7	62.0	34.7
8	72.0	57.8
9	86.0	70.9
After delivery	Nil	Nil

The table shows that the excretion of pregnanediol in Indian women increased from 23.5 mgm. per 1000 c.c. during the sixth month of pregnancy, reaching the maximum of 70.9 mgm/1000 c.c. during the ninth month. No pregnanediol was found in the urine of normal women 72 hours

after delivery, while in the case of European women the excretion of the pregnanediol increased from 48.0 mgm./day in the sixth month, going up to 90 mgm./day in the 9th month. In this case also no pregnanediol was detected 72 hours after delivery. The difference between pregnanediol excretion in Indian and European women might be due to number of factors such as climate, nutrition and heredity. The fact that the women coming to the public maternity hospitals in India were undernourished as compared with those in the foreign countries appeared to be a possible factor which might explain the low figures in Indians. It may be pointed out that the figures of pregnanediol excretion were low also in normal menstruating women. However, nothing definite could be said unless more information is available from different parts of India to evaluate the effects of nutrition, climate and heredity on the excretion of this sex hormone.

Summary

Twelve cases of six months' pregnancy, nine cases of seven months' pregnancy and eleven cases each of eight and nine months pregnancy were studied for their urinary pregnanediol excretion. In addition to this, five cases were studied 72 hours after the delivery. The level of the pregnanediol increase rose from 23.5 mgm./1000 c.c. in the sixth month to 70.9 mgm./1000 c.c. in 9th month. It dropped down to zero 72 hrs. after the delivery.

Acknowledgement

The authors have great pleasure in thanking Dr. E. H. Venning, Royal

Victoria Hospital, Montreal, Canada for the supply of sodium pregnanediol glycuromide. They are also thankful to Dr. B. N. Purandare, M.D., F.R.C.S. (E), Hon. Gynaecologist, K. E. M. Hospital, Bombay, for his keen interest.

References

1. Astwood E. B. and Jones G. E. S.: A simple method for qualitative determination of pregnanediol in human urine; *J. Biol. Chem.*, 137, 397, 1947.
2. Backman C., Leekly D. and Hirschmann H.: Excretion of sodium pregnanediol glucuronidate in urine of normal pregnancy; *J. Clin. Invt.*, 19, 801, 1940.
3. Brown J. S. L. and Venning E. H.: Isolation of water soluble pregnanediol complex from human urine in pregnancy; *Proc. Soc. Exper. Biol. & Med.*, 34, 792, 1936.
4. Brown J. S. L., Henry J. S. and Venning E. H.: The corpus luteum hormone in pregnancy; *J. Clin. Invt.*, 16, 678, 1937.
5. Chitre R. G. and Juvalé K. R.: Urinary excretion of pregnanediol glycoronidate in normal menstruating Indian women.
6. Davis M. E. and Fugo N. W.: A simplified method for quantitative determination of pregnanediol excretion in pregnancy; *Proc. Soc. Exper. Biol. & Med.*, 66, 39, 1947.
7. Guterman H. S.: A human pregnancy test based on colour reaction of pregnanediol in urine; *J. Clin. Endocrinol.*, 4, 462, 1944.
8. Gutermann H. S. and Schroeder M. S.: A simplified Technique for Quantitative colorimetric estimation of pregnanediol in urine; *J. Lab. & Clin. Med.*, 33, 356, 1948.
9. McCormack G.: A comparison of colour chemical test with Friedman modification of A. Z. test; *Am. J. Obst. & Gynec.*, 51, 722, 1946.
10. Morrow A. G. and Beuna R. S.: An exaluation of Guterman's pregnancy test; *Ibid* 51, 685, 1946.
11. Smith G. V. S. and Smith O. W.: Urinary pregnanediol values during the last trimester of pregnancy; *J. Clin. Endocrinol.* (quoted from *Endocrinology of Women—Hamblen*).
12. Sommerville I. F., Marian G. F. and Kellar R. J.: Rapid determination of urinary pregnanediol; *Lancet*, 2, 89, 1948.
13. Talbot N. B., Berman R. A., McLachlan E. and Wolfe J. K.: The Colorimetric determination of neutral steroids (hormones) in 24 hour sample of human urine (pregnanediol Total. alpha & beta) 7-ketosteroids; *J. Clin. Endocrinol.*, 1, 668, 1941.
14. Venning E. H.: Gravimetric method for the determination of sodium pregnanediol glucuronidate an excretion product of progesterone); *J. Biol. Chem.*, 119, 473, 1937.