STUDY ON PREGNANEDIOL EXCRETION IN THE URINE OF NORMAL PREGNANCY

(A Review of 120 Assays)

By

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In women progesterone is largely eliminated from the body as pregnanediol, an end-result of the metabolism of the hormone. A rise or fall in the pregnanediol content of urine is an index of the progesterone content of the body and its metabolism.

Pregnanediol is a relatively inert saturated steroid which was first isolated from the urine of pregnant women by Marrian (1929). Its chemical constitution was determined by Butenandt (1930-1931) who first called it pregnanediol.

Progesterone was chemically isolated some years later (1934). It is an unsaturated steroid with two ketonic groups and a double bond in its molecule.

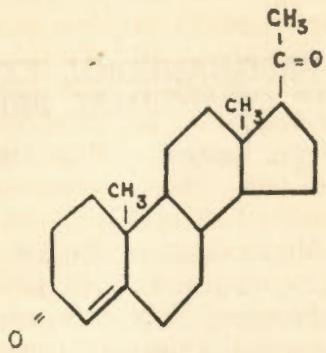
Pregnanediol has the same carbon skeleton as progesterone and could be produced by reduction of the two ketonic groups and the double bond.

Venning and Browne (1936-38) found that the actual elimination product in the urine is a sodium salt of glucoronic acid (sodium pregnanediol glucuronidate). Its presence during the luteal phase and early pregnancy indicated its close relationship with the corpus luteum. Later, Venning, Henry and Browne, finally proved it to be an excretory product of progesterone from the observation that injection of progesterone was followed by excretion of pregnanediol in the urine.

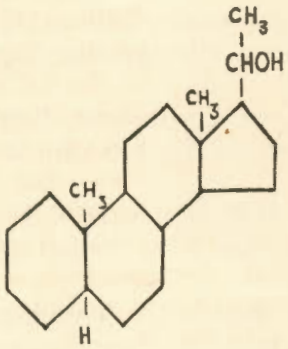
Chemical Structure

Pregnanediol is not the only excretory product of progesterone. Other compounds allied to progesterone have been detected in urine.

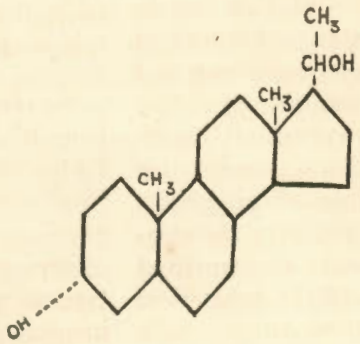
Marker, Kamn and McGrew (1937) isolated an isomer of pregna-



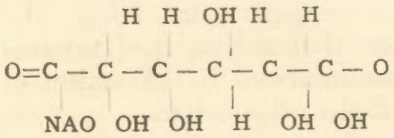
PROGESTERONE



PREGNANEDIOL



PREGNANEDIOL GLUCCURONIDATE SODIUM



nediol—allopregnanediol and another progestin-epiallo-pregnane-ol-3-one-20- from the urine of pregnant women.

Butler and Marrian (1937) identified pregnane-3-17-20-triol in the urine of patients with adrenal hyperplasia.

Westphal and Buxton (1939) were unable to detect pregnanediol in urine of pregnant monkeys, rabbits and cats after injection of progesterone.

Kyle and Marrian (1951) isolated allopregnane-3-20-diol from the urine during progesterone treatment. It

was found that the proportion of pregnane-3-20-diol (pregnanediol) to allopregnane-3-20-diol was about 30 1 in the urine of pregnant women, postmenopausal women and also in men after progesterone treatment.

Inspite of the above facts, there exists a constant relationship between the amount of pregnanediol excreted and the total amount of endogenous progesterone or that fraction of it which produces physiological action on the target organs.

Metabolism and Excretion.

Four factors are mainly concerned in the metabolism and excretion of pregnanediol (Hamblen, Ashley and Baptist, 1939).

1. *Ovary:* Concerned with the formation of progesterone from the corpus luteum. During pregnancy however, the placenta comes into the field.

Placenta. Progesterone has not as yet been isolated from extracts of placenta, but it is believed that this organ is responsible for the elaboration of progestationally active material. Since histochemical studies indicate that the chorionic syncytium elaborates steroids, possibly progesterone may also be secreted by these cells. The corpus luteum had been removed during pregnancy with hardly any alteration in the preganediol level in urine. Mazor and Goldstein detected progestin in placenta of a six months' pregnancy. In some cases, Smith and Smith detected progestin in full term placentae.

2. *Endometrium:* Concerned with the conversion of progesterone into pregnanediol. Whether endometrium is or is not the main site of conversion is still debatable. Buxton and Westphal were able to recover pregnanediol from the urine of men after progesterone injection. Strover and Pratt found continued excretion of pregnanediol for 5 days after hysterectomy. On the other hand, Venning and Browne could not detect any pregnanediol from urine of hysterectomised women after injection of progesterone, but in the presence of active corpus luteum (when the endometrium is in the secretory phase) injection of progesterone led to the excretion of sodium pregnanediol glucuronidate in the urine. The foregoing observations support the view that the endometrium in the progestational phase may be responsible, though not essential, for the conversion of progesterone into pregnanediol.

3. *Liver:* Brings about the conversion of progesterone into pregnanediol and the conjugation of the latter with glucuronic acid.

4. *Kidney:* Involved in the excretion of sodium pregnanediol glucuronidate. This organ, it is said, probably takes part in the metabolism also.

The role of testes in the elaboration of pregnanediol is still undetermined. Unlike males of most species, bull's testes have been found to excrete large quantity of pregnanediol. It has also been found that hog's testes excrete pregnanolones a probable precursor of progesterone in the biogenesis of steroid hormones. So it has been suggested that the testes probably elaborate pregnanolones, which are converted into progeste-

rone in the adrenal cortex and excreted as pregnanediol in the urine.

Review of Literature.

From the literature during the last 10 years, we find extensive work on urinary pregnanediol estimation both in pregnant and nonpregnant women.

The subjects for investigation in nonpregnant women are:—

(a) Cases of sterility.
(b) Menstrual disorders.
(c) Certain pathological conditions of the breast.
(d) Adrenal tumours.

In the pregnant women investigations were carried on for:—

(a) Early diagnosis of pregnancy.
(b) Threatened and habitual abortions.
(c) Normal pregnancy, labour and puerperium.
(d) Complications of pregnancy.
    (i) Missed abortion.
    (ii) Hydatidiform mole.
    (iii) Antepartum haemorrhage.
    (iv) Dystocia.
    (v) Toxaemia of pregnancy.

Hain and Robertson (1939) have estimated the pregnanediol content in urine of nonpregnant women and found it absent in the first half of the menstrual cycle, though relatively large amount is present between the 17th day and the last day of the menstrual cycle. The urinary assays were correlated with endometrial biopsies and the result was that no pregnanediol could be detected in the proliferative stage. Stover and Pratt investigating on nonpregnant women found that maximum excretion (8 mgm./24 hours) takes place between the 21st to the 26th day of menstrual cycle—pregnanediol appears in the urine 48 hours after ovulation and ceases to be excreted in recognizable amount about 1-4 days before menstrual flow.

Cope in 1940 said that pregnanediol was excreted during luteal phase of menstrual cycle in women, disappeared from the urine before uterine bleeding began and reappeared on about the 14th day of the next cycle. He suggested that continued excretion of pregnanediol may be used as a very early indication of pregnancy.

Venning and Browne in 1940 found that in a normal menstruating woman, with cycle between 25-27 days, excretion of pregnanediol in the urine occurred from the 14th to the 25th day of the cycle, the maximum being on the 18th, the 19th or the 20th day.

During pregnancy, pregnanediol excretion is maintained at or slightly above the maximum attained during the menstrual cycle for about 12 weeks. Then it rises continuously and sharply upto or till near about term. The result obtained during the few days before labour is contradictory. According to Marrian the level reaches a maximum of 60-110 mgm./24 hours at about the 266th day. From there on until parturition, the quantity gradually fell till no pregnanediol could be recovered after 48 hours of the puerperium.

Browne, Henry and Venning in 1937 maintained that large amount of pregnanediol was excreted when actual labour began and the level did not fall appreciably until delivery. Later in 1938 Venning observed that levels as high as 60-100 mgm./24

hours could be obtained on the 280th day. So their normal curve showed a more or less continued rise till term.

Smith and Smith pointed out the wide range of normal values and concluded that the curve of pregnanediol excretion throughout the pregnancy was more important than the actual level at any given time. They found in individual curves the highest peak at about 2 weeks before term; this was followed by decreasing values before and during labour. Highest individual value of Smiths was 120 mgm./24 hours.

Strover, Pratt and Bachman failed to reveal any decline of pregnanediol before labour. Lyon found peak of pregnanediol about 2 weeks before delivery, followed by decreasing values and then a precipitous drop during 5-7 days prepartum, regardless whether labour was premature, at term or postmature. Hain (1940) stated that during the last 3 weeks of pregnancy, the urinary pregnanediol rose, the largest outflow occurring 8-10 days before parturition.

In 1950, Mouzey made a systematic study of the excretion level during the last 4 weeks of pregnancy, labour and puerperium in 46 assays done on 6 patients. He showed that there was a distinct drop, sometimes of a dramatic nature, during the last week of pregnancy (a levelling of phase) followed by a rise in excretion prior to labour, a continued excretion during labour and its absence from the urine within a relatively short time following delivery.

Table below shows the results of some of the previous workers as compared with the authors' result at the peak period.

TABLE I

Period of pregnancy.	Author	Maximum pregnanediol excretion/24 hrs.	Minimum pregnanediol excretion/24 hrs.	Mean
280th day	Venning	105 mgm.	60 mgm.	75 mgm.
Last month of pregnancy	Cope	90 „	30 „	55 „
„	Witson & Randel	—	—	53.8 mgm.
10 days before labour	Marrian	100	60	—
266th day	Jayle	105	60	—
280th day	Present Authors	115	25	48.0650
40th week	*(Corrected data)	86	26	46.5292

* Corrected data were arrived at by excluding a few of the abnormally high and abnormally low figures in the peak period (40-week).

Technical Feature.

Clinical Material. Our present study was started in 1951 to find out the etiological and prognostic correlationship between pregnanediol excretion and toxaemia of late pregnancy as against normal pregnancy.

The clinical materials for this publication were drawn from normal cases alone throughout the pregnancy from 8 weeks till term and also upto the 42nd week when pregnancy was prolonged.

The cases, declared as normal, were uncomplicated, healthy pregnant women. Special attention was given to clinical manifestations such as oedema, hypertension and albuminuria, etc., as well as some biochemical and clinical laboratory tests of blood and urine. The cases having other general diseases, like anaemia, etc., were excluded from our series.

Altogether 120 assays were made upto the end of June 1952 in normal cases as against 152 in toxaemia. Of these, in 10 cases excretion was studied periodically from 28 weeks till term to observe the excretion pattern with the progress of pregnancy.

A special antenatal ward is maintained for the selection of cases and collection of 24 hours urine accurately. In all cases, whenever possible, renal clearance and hepatic function tests (Hippuric acid test) are made.

Method. After reviewing all the methods given in Table II we have found Astood and Seegar Jones gravimetric technique most suitable for our purpose. It is simple, accurate and gives free and stable pregnanediol instead of the sodium salt which is hygroscopic and is prone to error of calculation.

The principles involved in Astood and Jones gravimetric estimation* of pregnanediol are:—

1. Collection of 24 hours' urine.
2. Liberation of free pregnanediol by acid hydrolysis of urine in presence of toluene.
3. The separation of most of the impurities from toluene extract by precipitation with alcoholic alkali.
4. The final quantitative crystallization of the pure pregnanediol from aqueous alcohol.

Technique. Usually 1000 c.c. of urine is taken when available and boiled with 50 c.c. toluene for 15 minutes. 100 c.c. concentrated hydrochloric acid is added, boiled for 10-15 minutes and cooled. Toluene and toluene emulsion layers are separated by the separating funnel and urine layer is re-extracted twice with toluene. The toluene and toluene emulsion layers thus collected are filtered through the Buckner funnel and the filtrate is treated with sodium hydroxide after dispelling all water by boiling. The solution is now reduced to half its volume on water bath. The solution is cooled and filtered through Buckner funnel —the clear greenish yellow filtrate is now dried and treated with 10 c.c. of 95 per cent ethyl alcohol and 40 c.c. of hot aqueous 0.1 N sodium hydroxide is added—cooled over-night in

* Jour. of Biological Chemistry—January 1941.

the refrigerator. The clear precipitate is collected, dissolved in 10 c.c. of alcohol and precipitation repeated in the same manner with 40 c.c. distilled water instead of sodium hydroxide solution. The precipitate is collected in a weighing tube with ethyl alcohol, dried and weighed.

The criteria of purity of the substance is the melting point. Usually, the melting point of pregnanediol ranges between 218° to 228° (Astood and Jones). In our series the melting point ranged between 218°—220°. We have checked the genuineness of pregnanediol extracted by us in our laboratory, comparing it with a specimen of pure pregnanediol supplied to us by the London University Hospital. This specimen had a melting point of 218°.

Data

TABLE II (WATTEVILLE)

Year	Authors	Principle	Minimum quantity estimable
1937	Venning	Extraction, precipitation and weighing of glucuronidate of sodium	3 mgm.
1941	Astood Jones	Acid hydrolysis, extraction, precipitation and weighing of pure pregnanediol	3 mgm.
1941	Allen, Viergiver	Titration—compound precipitates as lead salt and finally determined by measuring amount of glucuronic acid liberated by acid hydrolysis	3 mgm.
1941	Talbot & Coll	Colorometric estimation after extraction and precipitation (Astood, Jones) by sulphuric acid	
1943	Talbot, Coll	Extraction of glucuronidate, hydrolysis by glucuronidase, precipitation according to Astood and Jones and colorometric estimation by sulphuric acid	3 mgm.
1943	Jayle, Coll	Exraction of glucuronidate and colorometric estimation by naphthoresorcin.	
1944	Westphal	Extraction according to Venning, precipitation and weighing of glucuronidate of barium	1 mgm.
1947	Huber & Borth	Hydrolysis and extraction according to Astood and Jones, chromatographic isolation and weighing of pure pregnanediol	0·5 mgm.

TABLE III

Maximum, minimum and average output of pregnanediol in mgm/24 hours during different periods of normal pregnancy.

(Total number of Assays—120)

Weeks	No. of assays	Pragnanediol in mgm. 24 hours Maximum	Minimum	Average
Below 12	5	7.5631	3.4500	5.7563
12-15	5	7.8674	5.1160	6.5068
16-19	3	11.2544	6.5562	9.5313
20-23	3	13.0095	9.1620	11.5419
24-27	12	29.1104	10.0605	15.7055
28-31	14	30.9259	10.7695	20.1473
32-33	14	40.2310	14.3840	25.2789
34-35	9	41.7490	20.4330	29.5014
36-37	18	66.6194	22.3789	33.0938
38-39	8	66.2150	21.6450	38.2227
40-	19	115.2097	30.8662	45.5729
41-42	10	60.7200	19.7781	33.2496

TABLE IV

Pattern of rise of pregnanediol excretion rate in 10 normal cases with the progress of pregnancy (period of assay 28th week to 40th week).

Cases	Weeks of pregnancy	Pregnanediol in mgm./24 hrs.
I	34	26.0650
	37	40.3210
	40	65.1004
II	36	20.1460
	38	30.0879
	40	60.7200
III	32	18.3438
	36	25.1370
	38	40.2320
IV	28	10.3070
	32	14.3842
	36	15.4350
	38	21.9005
	40	30.6460
V	28	11.8790
	32	30.6674
	36	54.3179
	40	35.1056
VI	28	21.3003
	32	21.7854
	36	33.0839
	40	53.8192
VII	35	25.3410
	36	27.9628
	38	37.2228
VIII	32	14.3840
	37	22.3789
	40	40.0500
IX	28	19.8920
	38	33.4560
	40	51.1266
X	28	10.7695
	30	12.4245
	40	25.9240

Comment

From our data based on 120 assays it was evident that in normal pregnancy there was a steady and orderly rise from the 12th week till the 40th week. The rise was rather sharp from the 36th week onwards. After the 40th week in cases where preg-

nancy continued till the 42nd week, we saw a rapid fall. We have studied 10 such cases of postmaturity (Table III).

Though there was a wide range of variation, as seen in our data, the output for a particular individual showed a general pattern of excretion with the progress of pregnancy. In our graphical record No. II and Table IV we have shown the quantity of pregnanediol excretion in 10 cases, starting from the 28th—40th week. The rise in the later weeks, precisely from the 36th week was rather sharp (except in Case No. V).

The variation in normal range have also been observed by previous workers (Marrian, Mouzey, Venning, Smiths, etc.).

Our normal curve compares favourably with the pioneer work of Venning and Browne, as well as Strover, Pratt and Bachman; although others (Marrian, Lyon, etc.) noticed prepartum drop.

Another significant fact is also noticeable from our detailed data. The subjects who show low excretion in the beginning, have persistently a low excretion rate all throughout the pregnancy, although the general pattern of rise is there; and the subjects who show higher level in the first assay maintain a persistently high level and yet follow the general pattern of rise.

This may be due either to an inherent property of the progesterone metabolism itself or the pregnanediol excretion, or to some other unknown factor which plays some role in the metabolism and the excretion in respect to different individuals.

Though confusing, the general trend of excretion being uniform, it was the general and considered opinion that urinary pregnanediol although very insignificant in the early months of pregnancy, increased gradually and steadily upto or near term.

No work has yet been done in cases of postmaturity. The steady fall in our 10 cases of postmaturity till labour set in, seemed significant so far as the diagnosis of postmaturity was concerned.

Summary and Conclusion

1. 120 assays for pregnanediol were made in normal pregnancy.
2. 10 cases were followed and pregnanediol estimated periodically from the 28th to the 40th week.
3. 10 proved cases of postmaturity were investigated till labour set in.
4. In normal pregnancy, the urinary pregnanediol followed a steady and orderly rise from the 12th to the 40th week.
5. In postmature cases, the urinary pregnanediol rapidly declined from the 40th week till delivery.
6. 10 cases which were followed periodically showed steady rise of pregnanediol with rather a sharp rise from the 36th week.
7. No assays were made during labour or puerperium.
8. Our data were subjected to statistical scrutiny and found to be significant.
9. Inspite of the wide range of variation in pregnanediol values during normal pregnancy, the assay shows that the actual production of

progesterone from the placenta or at least the progestational activity of the placenta increases gradually and steadily from the 12th week onwards till term, without any drop before labour sets in and declines appreciably if or when the pregnancy is continued after the 40th week. This decline might possibly be due to ageing of the placenta, the source of the steroid hormones, and chorionic gonadotrophin during pregnancy.

10. Further investigation is necessary to come to a definite conclusion.

Acknowledgment

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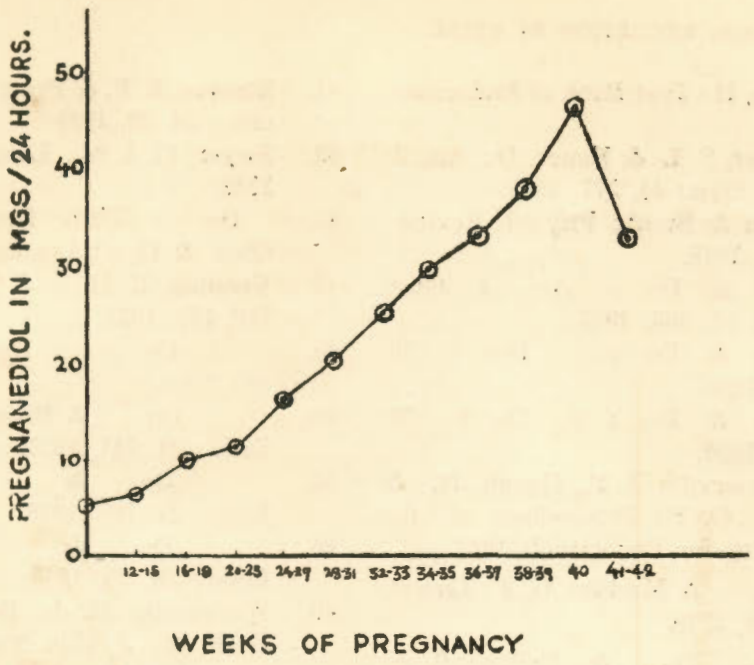
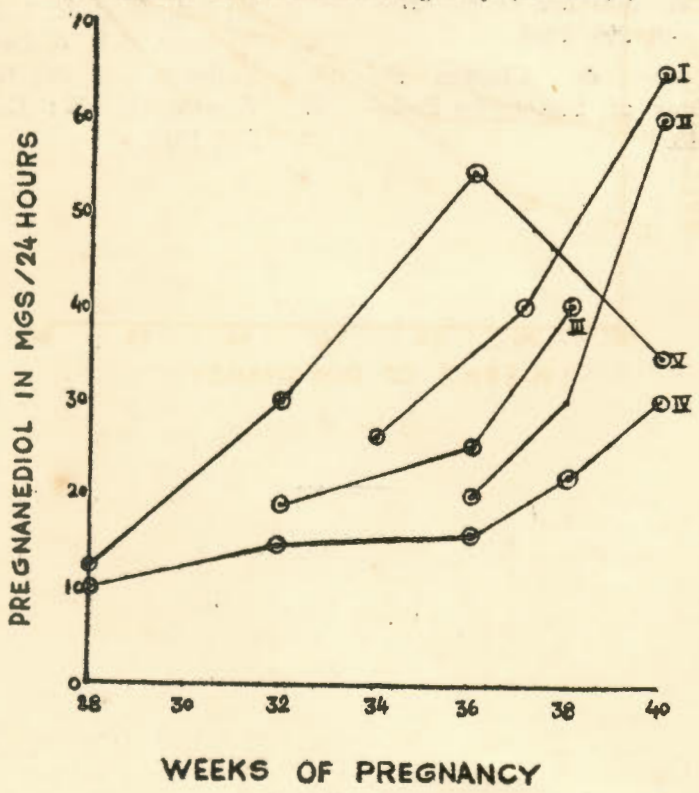


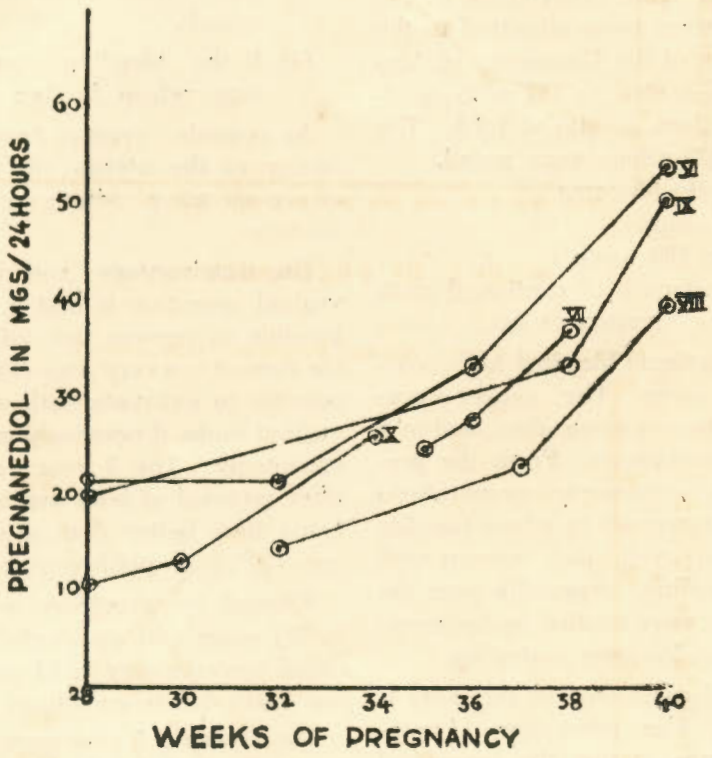
Graph No. I.

Predianediol Curve—Normal Pregnancy (120 assays)



Graph No. II.

Pregnanediol excretion pattern with progress of pregnancy in 10 normal cases.



Graph No. II (contd.)