URINARY EXCRETION OF 17-KETOSTEROIDS IN PREGNANCY AND ITS SIGNIFICANCE IN TOXAEMIA OF PREGNANCY

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Several workers have observed hypertrophic change in the adrenals of experimental animals during the pregnant state. These changes have been attributed to the greater concentrations of sex hormones which are said to stimulate not only the pituitary but also the adrenals. Of late, methods have been developed to assess the adrenal activity by the estimation of substances in urine which are derived from the metabolism of adrenal hormones. Since the urinary 17-ketosteroids are derived from the adrenals and testes in human males and only from the adrenals in females, the measurement of 17-ketosteroids excretion in normal and pregnant states would throw light on the activity of the adrenal gland in both.

Perlman and Pincus (1943) have measured the 17-ketosteroids excretion and found that there is no appreciable change in the 17-ketosteroid excretion in pregnancy. Venning (1946) also came to the same conclusion but however she noted that there is some difference in the values of 17-ketosteroids obtained by the Pincus (1941) reaction and by Holtroff-koch (1940) modifications of Zimmerman reaction. These differences were attributed to the presence of Ketosteroids other than 17-ketosteroids which are said to be excreted, as gestation progressed. But, however, Dobriner et al (1948) found the ketosteroid components in the urine of pregnant women to differ qualitatively and quantitatively from those found in the urine of non-pregnant women.

The factors responsible for these changes are not known in spite of much investigation. Of late, there is a tendency to attribute the cause of toxaemia of pregnancy to the dysfunction of adrenal cortex. Several studies (Venning 1946, Tobian 1949, Davis & Davis 1949, Jailor & Knowlton 1950) of corticoid excretion during normal pregnancy have disclosed that the level of these urinary corticoids are increased and more so in

cases of toxaemia. Barnicott and Wolfson (1952) in Africa studied the output of 17-ketosteroids in 24 hours' specimen of urine and found that the mean value was lower than in European males. Recently Friedman (1954) studied the 17-ketosteroid output in Indian males and observed that it is significantly lower than in the healthy European males. In our enquiry into the etiological factors in toxaemia of pregnancy, we have first concerned ourselves in the study of neutral 17-ketosteroid excretion in normal pregnancy and toxaemia of pregnancy. Since no data in the 17-ketosteroid excretion on normal Indian healthy females are available, we attempted first to establish standards for the same and then study the alterations in normal pregnancy and toxaemia of pregnancy to assess thereby the possible role of the adrenal cortex in the causation of toxaemia of pregnancy. With this view, a systematic study was undertaken. 100 cases of normal nonpregnant healthy women; 59 normal pregnant women and 50 cases of toxaemia of pregnancy were studied.

Materials and Methods

100 healthy South Indian female nurses were selected at random, aged between 18 and 30 years, and 17-ketosteroids output of 24 hours' urine specimen in them was estimated according to the method of Pincus. Since there is likelihood of diurnal variation in steroid excretion (Pincus (1943, Callow et al. (1939)) only 24 hours' urine specimen was used and the samples were collected in a bottle containing 10 ml. con. hydrochloric acid and 1 gm. of copper sulphate which prevents the development of inconvenient amounts of ammonium carbonate. (Medical Research Council 1951). All the samples of urine for the cases of normal and toxaemia of pregnancies were obtained from the patients admitted in the Government Hospital for Women and Children, Egmore, Madras.

100 ml. aliquts of these urine samples were hydrolyzed with hydrochloric acid, boiled to free ketosteroids from their conjucates and ketosteroids were extracted with carbon tetrachloride according to the method of Robinson and Warren 1947 as modified by King 1951. After evaporating the carbon tetrachloride, the neutral 17-ketosteroids were extracted in aldehyde free alcohol and estimated by the method of Pincus (1943). The colour produced by androsterone was used as a standard.

The ketosteroid in some of the urine samples belonging to the nonpregnant healthy females was estimated by both the methods (Zimmerman and Pincus) and the values that were obtained were identical (Borell et al. 1951). The ketosteroids in the urine samples of the normal pregnant and toxaemic cases in the present series were estimated only by the method of Pincus, as the Zimmerman reaction gave higher values according to Venning, because of the associated steroids other than 17-ketosteroids. The same finding was also observed in this present study.

Results

Table I gives the frequency distribution among the three classes of cases under study, viz.,

(a) Normal non-pregnant women;

(b) Normal pregnant women and

(c) Cases of toxaemias of pregnancy.

TABLE I.

Frequency Distribution.

Range.	Normal	Non-pregnant. (A)			Normal pregnant. (B)			Toxaemia. (C)		
	f1	f2	f3	f1	f2	f3	f1	f2	f3
1	2	3	4	5	6	7	8	9	10
1 — 2	2	9	38	—	3	9	—	0	3
2 — 3	7			3			—		
3 — 4	12	29		3	6		—	3	
4 — 5	17			3			3		
5 — 6	12	26	45	3	7	16	4	8	24
6 — 7	14			4			4		
7 — 8	13	19		4	9		5	16	
8 — 9	6			5			11		
9 — 10	5	7	12	6	13	23	2	3	11
10 — 11	2			7			1		
11 — 12	3	5		5	10		2	8	
12 — 13	2			5			6		
13 — 14	3	3	4	4	7	8	6	8	9
14 — 15	—			3			2		
15 — 16	—	1		1	1		1	1	
16 — 17	1			—			—		
17 — 18	1	1	1	1	2	3	1	1	2
18 — 19	—			1			—		
19 — 20	—	—		1	1		—	1	
20 — 21	—			—			1		
21 — 22	—			—			—	1	1
22 — 23	—			—			1		
23 — 24	—			—			—		
24 — 25	—			—			—		

The range of variation of the values as well as the mean value for each of the above three types are given in Table II.

TABLE II.

Daily urinary excretion of 17-Ketosteroids.

	No. of cases.	Range (mg.)	Mean (mg.)
Normal Non-pregnant	100	1.7 — 17.4	6.4784
Normal pregnancy	59	2.0 — 19.4	9.5195
Toxaemia of pregnancy	50	4.03 — 22.6	10.0762

A statistical analysis of the results obtained will be found in Table III.

TABLE III.

Statistical constant.

Statistical constant.	Normal Non-pregnant.	Normal pregnant.	Toxaemia.
Size of sample	100	59	50
Arithmetic mean	6.4784	9.5195	10.0762
Median	5.975	9.54	8.79
Mode	4.486	10.056	8.141
Standard Deviation ..	3.1385	3.9592	3.9883
Range	15.7	17.4	18.57
(Range/Median)	2.6276	1.8239	2.1126
Coefficient of variation ..	0.4845	0.4159	0.3958
Standard error of mean. ..	0.31385	0.51545	0.56403

Discussion

The values in 100 normal non-pregnant South India nurses, aged between 18 and 30 years, ranged from 1.7 and 17.4 mgs. per 24 hours, with a mean of 6.48. From a survey of the literature, Mason and Engstrom (1950) concluded that the mean values lie between 8.2 and 11.9 for normal healthy non-pregnant women in England and America. The values reported in the present study are lower as could be seen from the tables compared to the figures obtained by the above authors. (Ref. Table IV).

TABLE IV.

Twenty-four-hour-Urinary Excretion of 17-Ketosteroids in Normal Females.

S. No.	Investigator.		No. of cases.	Range (mg.)	Mean (mg.)
1.	Fraser et al.	(1941)	14	5.1 — 14.2	9.0
2.	Engstrom.	(1943)	18	4.8 — 17.0	10.0
3.	Barnett et al.	(1946)	20	5.3 — 18.1	11.9
4.	Venning.	(1946)	14	6.0 — 18.0	11.0
5.	Forbes et al.	(1947)	65	3.8 — 16.9	8.2
6.	Kenigsberg et al.	(1949)	20	3.0 — 16.0	9.3
	Present investigation		100	1.7 — 17.4	6.48

Barnicott and Wolfson (1952) observed that the mean 24 hours' urinary 17-ketosteroid output in African Negroes was lower than in Europeans living under the same conditions and they were unable to explain this difference on the basis of any of the known environmental factors. Recently, Friedman (1954) after studying 17-ketosteroid output in Indian males, observed that it is significantly lower than the output for the healthy European males and concluded the possible difference may be due to the ethnic relationship rather than the environmental factors like diet or climate. He discussed these findings and the differences observed in relation to climate, diet, body size, ethnic affinities, basal metabolic rate and pigmentation.

Since there is very little reason for the conclusion that environmental factors such as diet or climate are responsible for the difference in the mean values, we have to look for the reasons probably ethnic or other factors of which nothing is definite.

The values in 59 normal pregnancies ranged between 2.0 and 19.4 per 24 hours, with a mean value of 9.52. These values show that there is a significant increase in the values obtained in normal pregnancy cases. But however it was found that the range of variation is not affected when compared to normal non-pregnant values, but only the main value was found to be increased.

In 1946, Venning studied 9 cases to evaluate adrenal function in human pregnancies by measuring the excretion of ketosteroids throughout gestation, besides corticoids, estrogens, pregnandiol and gonadotrophin. The author concluded that there is no difference in the values obtained in normal pregnancy as compared to normal non-pregnant level.

By biological assays Dingemanse et al (1937) found no increase in the androgenic activity in the urine of the pregnant women, between the 6th and 8th month. In 1939, Hain reported that less androgen was excreted in the urine of pregnant women as compared to normal non-preg-

nant. The same finding was also observed by Dobriner and his co-workers (1943) by using chromatographic methods.

In 1943 Perlman and Pincus in their study did not find any significant difference in the values of ketosteroids obtained in the urine of normal pregnant women. In the same year Samuel et al. (1943) found a rise in the 6th month of pregnancy which dropped to its original level following parturition, at this time the foetal adrenal is known to show some development and they suggested that the ketosteroids were derived from the foetal adrenal. Regneioro Castro (1950) who studied the neutral 17-ketosteroids excretion in normal pregnancy and normal non-pregnant cases found that the excretion of neutral 17-ketosteroid did not exceed the limit regarded as physiological for the non-pregnant women. Recently Maestro (1952) found that the mean value found in normal pregnancy cases was twice the normal value in the absence of pregnancy. Though the mean value was increased in the present series, the values obtained in most of the cases were within the upper normal limit of the normal non-pregnant. But still all the above authors have studied only a very small number of cases and so their values could not be compared with those obtained in the present series. The apparently high values observed in normal pregnancy cases might be an effect of increased adrenal cortical activity.

In cases of toxaemia of pregnancy studied, the mean values for 17-ketosteroid excretion were still more increased when compared to normal pregnancy cases. The values ranged between 4.03 mgs. and 22.6 mgs. with a mean of 10.08. The increased adrenal cortical activity is believed to be the result of a reaction to various non-specific stimuli in some instances (Selye. 1946). In other words it might be an effect of stress. Even though our ideas about stress and stress diseases have not become sufficiently precise, the correct trend is to recognize stress, as an important contributory cause of disease. There are many kinds of stress and the ways how they cause disease are not well understood.

Davis & Davis (1950) studied the 17-ketosteroid excretion in a very small number of cases of toxaemia of pregnancy to find out whether toxaemia of late pregnancy may be considered as the disease of adaptation. They found the ketosteroid excretion to be normal. Mason (1952) concluded, that pregnancy may be interpreted as a resistance phase of the general adaptation syndrome, and toxaemia the disease of adaptation, after studying the blood constituents of normal and toxaemia of pregnancy cases. Other investigators (Pareiainens 1949. Garrett 1950, Garrett 1950-a) also concluded toxaemia of late pregnancy to be a disease of adaptation. Garrett in 1950, concluded that the type of response to stress is due to the hyperactivity of the anterior pituitary with the excessive production of ACTH.

All the above findings suggest that normal pregnancy may be a resistance phase of the adaptation syndrome, and toxaemia a disease of adaptation. The increased values observed in normal pregnancy may be due to hyper-stimulation of the adre-

nal cortical function which does not reach the pathological levels.

Statistical Notes

Of the 100 observations under normal non-pregnant condition only 12 showed a value greater than 10 while the remaining 88 observations are distributed in the range of 1.7 to 9.8. For the normal pregnant observations, but for four readings that are above 15, the remaining 55 are distributed in the range from 2.0 to 14.6. For the cases of toxaemia also all the 50 observations, with the exception of four, are in the values ranging from 4.05 to 14.62 These observations have been represented first in the form of frequency distributions with a class interval of 1. These frequencies given in F1 of Table I indicate a unimodel skew distribution for the normal non-pregnant cases represented by (A) while the other two distributions have not given any clear indication of the shape of the distribution. So in order to get a definite indication of the type of the distributions, the class interval has been enlarged to 2 and the frequencies thus obtained are shown in Table I. For (A) the skew distribution is shown out prominently while the distribution for the normal pregnant observations (B) has taken almost the shape of a symmetric unimodel distribution. The frequencies for the toxaemia cases (C) indicate possibly a distribution with two modes.

The different statistical constants calculated from the original series of observation are given in Table II. It is seen that the mean value is as high as 10.08 for (C) while it is only 6.48 in the normal condition (A) and during normal pregnancy (B) the average value comes upto 9.52. This shows the general tendency for the value to increase during pregnancy and this is further enhanced in toxaemic condition. But it is necessary to ascertain how far these values reveal statistical significance.

In order to study the significance of the mean values and prescribe limits for variation, the standard errors are calculated and given in Table III. Excluding the chance of about 5% excess value occurring, the mean for (A) (B) and (C) should be within the limits, taking the range of twice the standard error, since the samples studied are large.

$$6.4784 \pm 0.62770$$
$$9.5195 \pm 1.03090$$
$$10.0762 \pm 1.12806$$

To test the significance of difference between observed means the statistic 't' is given by

$$t = \frac{\text{difference between means}}{\text{standard error of difference}}$$

is computed. Under the hypothesis that the samples are drawn from normal universes having same mean and variance, the observed difference will be significant approximately at 5% level.

If F1 and F2 are the standard errors of the means of two samples it is known that F, the standard error of the difference is given by $F=F_1^2 + F_2^2$. This result is utilized to compare the difference between the three mean values.

For (A) and (B) it is found that the observed t=5.039 while for (A) and (C) it is 5.574. The value of t for (B) and (C) is only .729. These results indicate that at 5% level of significance the observed mean va-

lues are significant compared with the normal condition both under (B) and (C) while the condition (C) produces no significant influence on the measuremets compared with (B). Thus these tests of significance indicate that it is not possible to distinguish between (B) and (C) conditions, although it is easy to differenciate either of these from (A).

But from a careful study of the frequency distributions given in Table I and the statistical constants associated with measures of central tendency and dispersion given in Table III it can be observed, that under normal conditions, low values are more frequent giving a skew distribution, whereas in pregnancy under normal conditions, although the general tendency is to increase the values, it is much more for the smaller original values than for the high ones, thus making the distribution almost symmetric. The influence of toxaemia appears to be twofold. On individuals having low or moderately high values in normal condition, the influence is to decrease, while for those having high values, the tendency appears to increase ketosteroids excretion. Thus it appears that although toxaemia has an effect on the measurements, the mean values are not found statistically significant. Hence these observations indicate that it is possible to conduct further work to study under the influence of (C). For this it is necessary to ascertain the normal figure above which condition (C) along with (B) increases the value, while below which condition (C) along with (B) reduces the value. This work can be conducted only on small animals under controlled conditions and can be later used for distinguishing between (B) and (C).

Summary

100 normal non-pregnant cases, 59 normal pregnancy cases and 50 toxaemia pregnancy cases were studied.

1. The mean daily urinary neutral 17-ketosteroid output in Indian healthy females is lower than in healthy European females.

2. The values obtained in normal cases of pregnancy are within the limit regarded as physiological for the non-pregnant women. The mean values are found to be increased. In toxaemia of pregnancy cases the values are still more increased and the possible factor for this increase is fully discussed.

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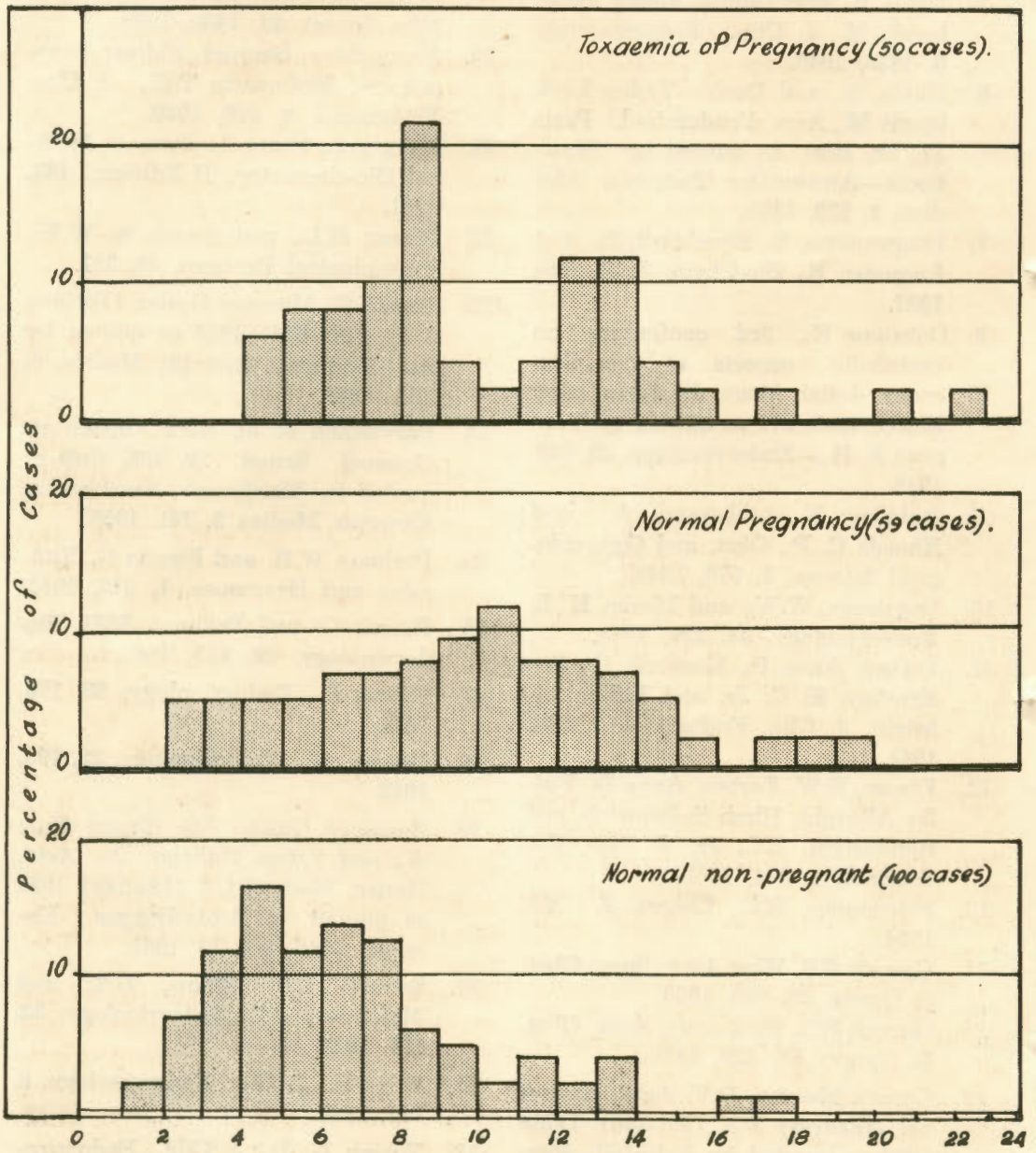

17 Ketosteroids Excretion (in Mgm.) in 24 Hours Urine.