THE URINARY EXCRETION OF 17-KETOSTEROIDS AND KETOGENICOSTEROIDS IN NORMAL PREGNANCY AND TOXAEMIA.—I

by

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The function of the adrenal cortex during pregnancy has been extensively studied. The consensus of opinion is that during pregnancy the production of corticosteroids increases and that this rise is associated with alterations in the metabolism of these hormones (Diczfalusy and Troen, 1961). Therefore, the pattern of urinary corticosteroid excretion during pregnancy will vary, depending upon the type of the compounds measured, and possibly more so in toxaemia of pregnancy.

A perusal of literature showed great inconsistency in the level of urinary 17-keto-and ketogenic steroids, which are also known as 17-oxo-and 17-oxogenic steroids respectively, both in normal pregnancy and in toxaemia of pregnancy. For instance, in late pregnancy 20 to 100 per cent higher levels of urinary 17-ketosteroids than those in non-pregnant women were reported by many workers (Venning, 1946; Huis in't Veld, 1954; Schuller, (1957); Martin and Mills, 1958; Steinbeck and Theile, 1962). On the other hand, there are reports showing no increase (Dingemmanse *et al*, 1937; Hain, 1939; Pearlman and Pincus, 1943; Jailer, 1951 and Tampan *et al*, 1956) or a progressive decrease throughout pregnancy (Dobriner, 1943 and Patti *et al*, 1963). Similarly, 17-ketogenicsteroids (17-hydroxysteroids) in late pregnancy have been reported to rise by about 40 per cent over the non-pregnant level (Martin and Mills, 1956; Venning and Dyrenfurth, 1956; Norymberski and Stubbs, 1956; Jones *et al*, 1959; and Cope and Black, 1959), whereas others have found the excretory levels within the range of the normal non-pregnant women (Appleby and Norymberski, 1957; Martin and Mills, 1958; and Steinbeck and Theile, 1962).

Although among the various endocrinological factors implicated the role of adrenocortical hormones in the causation of toxaemia of pregnancy has been given great importance, the existing literature hitherto indicated contradictory reports in the excretion of 17-ketosteroids. Thus, increased excretion (Davis and Davis Venden Eeckhoudt, 1950; Tampan *et al*, 1956), no change (Eiber, 1962; and Skalicky, 1963) and a diminished level (Braga, 1958; Salvadori and Cassano, 1958; and

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Akasu and Nishida, 1965) of the urinary 17-ketosteroids in comparison with that of normal pregnant women were observed by individual workers from different parts of the world. Because of the lack of such a data in Indian women the present investigation was conducted in two parts in the normal healthy non-pregnant, pregnant and toxaemic Punjabi women. The first part consists of the study of the urinary excretion of neutral 17-keto and ketogenicsteroids and the second part deals with the urinary excretion of free 17-hydroxy-corticosteroids in these three groups of women.

Material And Methods

Twenty-five normal non-pregnant healthy females in the reproductive age group, 25 normal pregnant women in the third trimester of pregnancy, and 25 pregnant patients with toxaemia (according to the classification of the American Committee on Maternal Welfare-Eastman, 1956), admitted in the Maternity Ward of the Nehru Hospital were selected for the study.

The general physical and obstetrical examination and routine laboratory investigations such as, haemoglobin level and urine analysis, were done in all cases. For toxaemic patients additional investigations of blood urea, uric acid and fundus examination were also carried out.

Collection of sample

A 24 hour urine sample was collected from the subjects in clean bottles without addition of any preservative (Dixon and Pennington, 1966). The total volume of the specimen was measured and an aliquot (100 ml) was brought to the laboratory and preserved at 4°C. At this temperature the sample could be preserved without any destruction of the hormones under study for a week. One single collection was made in each case and estimations were done in duplicate on each sample.

17-Ketosteroids

The urine was hydrolysed with concentrated HCl (Enrion, 1965) extracted with benzene-petroleum ether (40°-60°C) mixture (1:1 V/V, Patterson and Price, 1960), evaporated to dryness under vacuum at 40°-45°C., and the Zimmerman reaction (Callow *et al*, 1938), was done at 37°C by incubating the final reaction mixture for 25 minutes (Enrion, 1965). The interfering materials were removed by extraction with dichloromethane (Patterson and Price, 1960) and the pink colour produced was read in the Klett Summerson Colorimeter using the green filter. The standard used was epiandrosterone (100 μg/ml.) processed similarly. Recovery experiments performed gave a mean value of 98.73 per cent with S.D. ±4.31.

17-Ketogenicsteroids

The 17-ketogenic steroids were estimated as the difference of total ketosteroids obtained after the oxidation with sodium bismuthate and the ketosteroids obtained without sodium bismuthate treatment. Sodium bismuthate in 50% acetic acid (Norymberski *et al*, 1953) was used to oxidise the C_{21} steroids possessing a hydroxyl group at C_{17}. They are mostly cortisols, cortisone, their

tetrahydro derivatives, alpha- and beta-cortols, alpha- and beta-cortolones and pregnanetriol. The progesterone and pregnanediol type of steroids are not oxidised by sodium bismuthate to give 17-ketosteroids. Therefore, an additional step of sodium bismuthate treatment of the urine was included before the above procedure was followed to obtain the total 17-ketosteroids.

Results

The urinary excretory levels of 17-ketosteroids in the healthy normal non-pregnant, the pregnant and the toxaemic women are given in Table 1. In the case of non-pregnant women the range of excretory levels was 3.2-15.6 mg per 24 hours except in one case (1.8 mg per 24 hours). Among the 25 cases of normal pregnant women, three cases had very low levels of 17-ketosteroid excretion, i.e. 1.7, 2.8 and 2.9 mg per 24 hours respectively and the duration of pregnancy was 37, 36 and 30 weeks respectively. The rest of the values varied from 3.7-9.9 mg per 24 hours. The duration of pregnancy for the whole group was 28-32 weeks in 5 cases, 32-36 weeks in 5 cases and 36-40 weeks in 15 cases. Out of the 25 cases with toxaemia of pregnancy, 15 were classified as mild toxaemia and 10 as severe toxaemia. Their duration of pregnancy was 28-32 weeks in 4 cases, 32-36 weeks in 8 cases, and 36-40 weeks in 13 cases. In the mild toxaemic patients the excretory level was in the upper range of the normal levels, i.e. 6.0 to 16.5 mg per 24 hours with three exceptions in which it was 1.9, 2.5 and 3.9 mg. per 24 hours respectively. There was no correlation between the number of weeks of pregnancy and the excre-

TABLE I

The urinary excretion of 17-ketosteroids (mg/24 hrs) of the subjects under study

Status	No. of cases	Range	Mean	S.D. (±)	't'α*	't'b	't'c
Normal non-pregnant women	25	3.2-16.5	7.6	3.3	—	—	—
Normal pregnant women	25	1.7-9.9	5.4	1.9	2.00**	—	—
Toxaemic women	25	1.9-16.5	7.0	3.7	0.64	1.84	—
Mild toxaemic women	15	1.9-16.5	8.2	4.0	0.53	2.94**	—
Severe toxaemic women	10	2.3-9.5	5.4	0.7	2.07**	0.62	2.15**

* 't'α—All samples compared with normal non-pregnant women applying Student's 't' test.
't'b—Comparison of normal pregnancy with toxaemia, mild and severe toxaemic groups.
't'c—Comparison between mild and severe toxaemia.
** P <0.05; change is statistically significant.

tion of the 17-ketosteroids in any of these groups.

Table II represents the urinary excretory levels of the 17-ketogenic steroids in the different groups of subjects studied. No significant differences were observed among the various groups studied.

It was noted that although the excretion of 17-ketosteroids in normal healthy women was similar to that reported by Western workers (Venning and Kazmin 1946; Cauz, 1959; Williams, 1962; Varley, 1962) the excretion during normal pregnancy was much lower, 3.0-9.9. mg/24

TABLE II

The urinary excretion of 17-ketogenic steroids (mg./24 hrs.) of the subjects under study.

Status	No. of cases	Range	Mean	S.D. ±	't'a*	't'b	't'c
Normal non-pregnant women	25	2.6-16.5	6.9	4.0	—	—	—
Normal pregnant women	25	2.9-20.5	10.1	4.2	2.88**	—	—
Toxaemic women	25	2.2-15.3	8.4	3.7	1.35	1.50	—
Mild toxaemic women	15	2.9-14.3	8.1	2.6	1.04	1.60	—
Severe toxaemic women	10	2.2-15.3	8.8	4.2	1.21	0.83	0.44

* and ** — As given under Table 1.

Discussion

It was evident from Table I that the excretion of 17-ketosteroids was significantly decreased in normal pregnancy (5.4 ± 1.93 mg/24 hours), in toxaemia as a single group (7.0 ± 3.66 mg/24 hours) and in severe toxaemia (5.37 ± 0.66 mg/24 hours) as compared to the levels in normal women. A significant rise in the excretion of the steroid metabolites (34.7%) in mild toxaemia with no change in severe cases in relation to normal pregnancy was observed. Thus a significant difference between mild and severe cases was evident and was inconsistent with the observations of Wilson *et al*, 1954.

hours as compared to 10-20 mg/24 hours (Wilson *et al*, 1954; Lodi *et al*, 1959; Cauz, 1959; Eiber, 1962) and only comparable to the low values, 2.64-4.40 mg/24 hours, reported by Japanese workers (Akasu and Nishida, 1965). The fall in excretion during pregnancy was not in keeping with the findings of Venning, 1946; Martin and Mills, 1958; and Steinbeck and Theile, 1962; but in agreement with those of Dobriner, 1943; Patti *et al*, 1963; and Akasu and Nishida, 1965. This discrepancy may be explained by the low level of the production of progesterone and excretion of its metabolites, pregnanolones, in the Indian wo-

men. It may be related to the low weight of the babies and the placenta as compared to those of Westerners. As explained by various workers (Mills, 1961; Davis, 1961; Huis in't Veld, 1954; Birke *et al,* 1958) the rise in 17-ketosteroids in late pregnancy may be due mainly or wholly to a rise in 20-ketosteroids such as pregnanolones, the metabolites of progesterone (Bayliss *et al,* 1955).

The urinary levels of 17-ketosteroids in toxaemia and normal pregnancy were consistent with the reports of Tramontano, 1959; Eiber, 1962 and Skalicky, 1963. The significantly raised excretion in mild toxaemia as compared with the severe group could not be explained in this short study. Whether this finding can be utilized in grading the severity of toxaemia or differentiating the two conditions is questionable.

Urinary excretion of 17-ketosteroids in the normal non-pregnant women was in agreement with the range of 6.5-18.0 mg/24 hours reported by Enrion (1965). The average value of 10.10 ± 4.12 mg/24 hours in normal pregnancy was similar to the observations of Cope and Black (1959), Mills (1961) and Steinbeck and Theile (1962). There was an increased excretion of 45% in pregnancy as compared to the non-pregnant state which was of high statistical significance, and in agreement with the 40% rise reported in literature (Martin and Mills, 1956; Venning and Dyrenfurth, 1956; Norymberski and Stubbs, 1956; Jones *et al,* 1959; Cope and Black, 1959).

In toxaemia as a whole group and classified as mild and severe groups separately, there was a diminished excretion as compared to that in normal pregnancy by 17%, 20% and 13% respectively, but of no statistical significance. Other workers (Stark, 1960; Skalicky, 1963; and Daessler, 1964) have also reported no change in excretion of this metabolite in the two conditions.

Our results with respect to 17-ketogenic steroids are therefore in conformity with the general view that the mineralocorticoids rather than the glucocorticoids have a major role in the pathogenesis of toxaemia and one has to divert attention to their study or better still to the individual metabolites in both groups. Nevertheless, the excretion of 17-ketosteroids showed marked distinction between severe and mild toxaemia which requires further confirmation by taking up the study of the individual metabolites.

Summary

Twenty-five cases each, of normal healthy non-pregnant women of child-bearing age, pregnant women in the third trimester and women with toxaemia of pregnancy formed the basis of the present study. The urinary excretory levels of 17-ketosteroids in the three respective groups were found to be 7.6 ± 3.3, 5.4 ± 1.9, and 7.0 ± 3.7mg/24 hours, and those of 17-ketogenic steroids were 6.9 ± 4.0, 10.1 ± 4.2 and 8.4 ± 3.7 mg per 24 hours respectively. The significant changes observed from those of the non-pregnant women were a decrease in the excretion of 17-ketosteroids in normal pregnancy and in severe toxaemia (5.4 ± 0.7 mg/24 hours) and an in-

crease in the excretion of 17-ketogenic steroids in normal pregnancy. The 17-ketosteroid excretion in mild toxaemia (8.2 ± 4.0 mg/24 hours) has been found to exceed the level of all the other groups and showed a high statistical significance as compared to normal pregnancy and severe toxamia. 17-ketogenic steroid excretion was not affected in this disease.

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