THE URINARY EXCRETION OF FREE 17-HYDROXY CORTICOSTEROIDS IN NORMAL PREGNANCY AND TOXAEMIA OF PREGNANCY.—II

by

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The present communication is a continuation of a previous paper in which the authors had reported a marked distinction between mild and severe toxaemic patients in their urinary excretion of 17-ketosteroids, whereas there was no difference in the ketogenic steroid excretion.

An increased excretion of free 17-hydroxycorticosteroids in normal pregnancy has been observed by all workers (Tobian, 1949; Parviainen *et al.*, 1950; Lloyd *et al,* 1952; Venning *et al,* 1954; Daessler and Kyank, 1963; Nadel, *et al,* 1964; Pal, 1966. Comparatively very little data is available on the excretory level of the free 17-hydroxycorticoids in toxaemia of pregnancy. Increased levels were observed by Tobian (1949), Venning *et al.*, (1954), Hughes *et al,* (1954) and Tampan *et al,* (1956), while Daessler (1964) and Parviainen *et al* (1950) did not find any difference from those of normal pregnancy. The great interest and emphasis attributed to the role of adrenal cortex in the causation of toxaemia of pregnancy warranted a study of the excretion of free 17-hydroxycorticosteroids in non-pregnant, pregnant and toxaemic women.

Material and Methods

The twenty-five subjects selected in each group, namely, non-pregnant, pregnant and toxaemic women, and the method of collection of urine samples were the same as in the previous communication referred to. All chemicals were of the highest grade analytical reagent and ethyl alcohol was specially purified (Sunderman and Sunderman, 1960). The reference standard hydroxycortisone acetate was supplied by Ciba. The extraction procedure was modified from the existing methods in the respect that the urine samples were saturated to 20% with sodium sulphate prior to the extraction with chloroform and washings were also conducted with 20% aqueous sodium sulphate instead of pure distilled water as in the case of 6-B-hydroxycortosoles (Frantz *et al.*, 1960). The free 17-hydroxycorticosteroids were estimated in this chloroform extract by the phenyl hydrazine reaction (Sunderman and Sunderman, *a,* 1960). A standard and water blank were also treated similarly. The opti-

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cal densities of the final reaction products were measured at three wave lengths. 370, 410 and 250 mμ respectively in a Beckman DU spectrophotometer. Allen correction was applied as suggested by Reddy (1962) before the application of the Lambert-Beer's law which was found to obey up to 50 μg of hydrocortisone acetate. A recovery of 90 to 104 per cent was obtained by this procedure.

Results and Discussion

The mean free 17-hydroxycorticosteroids excretion in 25 cases each of normal non-pregnant, pregnant, and toxaemic women are given in Table 1. Among the normal non-pregnant women, 18 cases showed an excretory level ranging from 0 to 2.0 mg; 4 cases 2.0 to 4.0 mg and 2 cases 4.0-6.0 mg. per 24 hours. In the case of normal pregnant women, out of 25 cases an equal distribution of 8 in each of the above range with one single exception having 8.3 mg. per 24 hours was observed. The lower limit was 1.2 mg per 24 hours in these cases. The toxaemic women were classified as mild (15 cases) and severe (10 cases) cases. Their distribution pattern of the urinary free-corticosteroids was quite uneven, ranging from 1.2 mg. to 14.3 mg. per 24 hours.

A significant increase (40%) in the free 17-hydroxycorticosteroids was observed in both normal and toxaemic pregnancy (Table 1) as compared to the non-pregnant level. There was an elevation of 22% in the toxaemic groups as a whole, 26.5% in the mild toxaemia and 12% in severe toxaemia, from that of the normal pregnancy. In severe toxaemia the mean value was almost equal to that of the normal pregnancy but 19% less than that of mild toxaemia. None of these changes were statistically significant. The ratios (Table 1) of free-17-hydroxycorticosteroids to ketogenic steroids showed 2.4 and 3 times increase with statistical significance in normal pregnancy and toxaemia from those of the non-pregnant excretory levels. The

TABLE I

Free 17-hydroxycorticosteroids excretion and the ratio of Free 17-hydroxycorticosteroids (FHCS) to 17-ketogenic steroids (KGS) in normal non-pregnant, pregnant and toxaemic women

Status of Subjects.	No. of cases	FHCS Mean mg./24 hrs.	FHCS S. E. (±)	FHCS/KGS Mean	FHCS/KGS S. E. † (±)
Normal non-pregnant	25	1.35	+0.22	0.22	0.04
Normal pregnant	25	4.19*	+0.51	0.52*	0.06
Texaemia	25	5.12*	+0.73	0.62*	0.09
Mild toxaemia of pregnancy	15	5.70*	+0.76	0.75*	0.13
Severe toxaemia of pregnancy	10	4.82*	+0.91	0.62*	0.11

†Data for 17-ketogenic steroids were obtained from the same patients reported in an earlier communication referred to in the text.

* Astericks indicate that ' t ' values are greater than 1.99 when $P<0.05$ and the change is statistically significant according to Students ' t ' test.

changes observed in the ratios were similar to those described above and supported those findings.

From Table 1 it is evident that not only the free 17-hydroxycorticosteroids increased significantly but the ratio, i.e., in relation to ketogenic steroids also did show significant increase. As the increase in ketogenic steroids is not significant, (indicated in the second paper submitted), the increase in the ratio is only due to the free corticosteroids. This is an additional proof for the increase observed in free 17-hydroxycorticosteroids. Further, the increase paralleled the increase in different groups in the same order. Therefore to avoid repetition it was expressed in this way.

A similar observation was recorded by Venning *et al* (1954). A significant increase was found in the free and conjugated formaldehydogenic corticosteroids in the patients with mild and moderate symptoms. In the severe cases the mean value for the free fraction was lower than that found in the more moderate cases, but higher than that found in normal pregnancy, while the conjugated fraction was lower than that in normal pregnancy. This lower output of the conjugated or the higher excretion of the free corticosteroids was postulated to be due to liver damage. Tobian's study (1949) had also showed that most patients with essential hypertension showed a normal corticosteroid excretion. However, others (Lloyd *et al*, 1952; Hughes *et al*, 1954; Tampan *et al*, 1956) had reported very high levels (61% higher or 4 times that of the normal pregnancy).

The present study showed that in pregnancy, and more so in mild toxaemia, the peripheral metabolism of the corticoid hormones is increased resembling that of the administration of a massive dose of cortisol (Katz *et al*, 1962, and Burstein *et al*, 1954). This appeared to be a defensive mechanism rather than a low level of liver glucuronyl transferase (Ulstrom *et al*, 1960) or an impairment of the liver (Venning *et al*, 1954).

Summary

The urinary excretory levels of free 17-hydroxycorticosteroids were determined in 25 cases each of normal non-pregnant, pregnant and toxaemic Panjabi women. The mean values with S.E. was found to be 1.35 ± 0.22, $4.19 + 0.51$ and $5.3 + 0.73$ mg per 24 hours in the three respective groups. The increases in the latter two groups were significant as compared to the non-pregnant level. In the case of toxaemia of pregnancy there was an increase of about 20 to 30% as such, as well as in relation to ketogenic steroids from those of the normal pregnant women. Although these changes were not statistically significant, a distinctive difference between mild and severe toxaemia was observed.

Conclusion

The present observation suggested that the peripheral metabolism of the adrenal hormones in pregnancy and toxaemia of pregnancy was increased to a marked level from those of the the non-pregnant level. This was most pronounced in mild rather than in severe toxaemia. This distinctive feature as in the case of ketosteroids requires further confirmation so that it could be of some

use for differentiating these two conditions.

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