

SOME HORMONE STUDIES IN NORMAL AND TOXEMIC PREGNANCY¹

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The steroids of the adrenal cortex assist in, and are necessary for, normal excretion of water and electrolyte (1). In general, these steroids tend to cause water excretion and salt retention, the relative amounts of these responses being dependent upon the structure of the steroid. The hormones of the posterior pituitary gland, on the other hand, cause water retention and, usually, sodium and chloride excretion (1). A reciprocal relationship between these hormones has been proposed (2).

Since the suggestion by Hofbauer (3) on theoretical grounds that posterior pituitary hormones might play a part in the development of eclampsia and the report by Anselmino, Hoffmann, and Kennedy (4) of an increased level of an anti-diuretic material in the plasma of toxemic patients, a large number of studies of the role of antidiuretic materials in toxemia have been carried out. Some of these studies have failed to demonstrate an anti-diuretic substance or have failed to show a correlation between antidiuretic activity and toxemia of late pregnancy. The failures to find increased antidiuretic activity have been mostly when antidiuretic activity has been sought in serum or plasma (5-10). A good many workers have been able to show an increased urinary antidiuretic activity (11-13) or an increased sensitivity to exogenous posterior pituitary hormone in patients with pre-eclampsia or eclampsia (10, 14-17).

Several studies of corticosteroid excretion during pregnancy have found the level of these steroids to be increased in toxemia (18-21). It has recently been demonstrated in normal non-pregnant subjects and in patients with abnormalities of water excretion that during a state of positive water balance, a relatively high level of antidiuretic activity in serum and a relatively low urinary cor-

ticosteroid excretion are found; conversely, in the presence of a negative water balance, relatively low levels of antidiuretic activity and high levels of corticosteroid occur (22). No simultaneous studies have been made during pregnancy of antidiuretic and adrenal cortical substances. Therefore, a study of the possible role of these factors in the water metabolism of normal and toxemic pregnancies was undertaken. Because pregnanediol and chorionic gonadotrophin excretion are also altered during toxemia (23-26), it was felt worthwhile to attempt to correlate the urinary excretion of these materials with the other measurements.

METHODS

Twenty-four hour urine specimens were refrigerated during collection or, when refrigeration was impossible, were preserved with chloroform. All of the determinations were carried out as soon as possible after completion of the collection, usually within two or three hours. Pregnanediol was measured by the method of Guterman and Schroeder (27). Chorionic gonadotrophin was measured by the method of Behnken, Lloyd, and Hughes (28). For this method, 1 rat unit equals from 2½ to 3 international units of chorionic gonadotrophin. Corticosteroid was determined by the method of Lloyd and Lobotsky (29). For all specimens the "total corticosteroid" was measured. For many specimens the "freely-water-soluble corticosteroid" and the "poorly-water-soluble corticosteroid" were also determined. In almost all non-pregnant women, the "freely-water-soluble corticosteroid" is essentially equivalent to the "total corticosteroid" and very little "poorly-water-soluble corticosteroid" is found. The formaldehyde formed is converted to milligrams of corticosteroid by multiplication by a factor of 11.5 (30). The range of normal values for non-pregnant women of the same age as those studied in this series is from 0.200 mg. to 0.750 mg. for 24 hours, averaging 0.420 mg. per 24 hours.

The serum antidiuretic activity (ADS) was estimated by the modification of the method of Birnie and his associates (31) described by Lloyd and Lobotsky (22). Antidiuretic activity is expressed in the following way: The percentages of a water load excreted at the end of a 90-minute period by rats that have been injected with

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the serum being assayed or with physiological saline solution are calculated. The difference between these percentages represents the antidiuretic activity. The antidiuretic activity is expressed as a negative value when the serum of the patients causes the rats used as test animals to retain water; whereas, it is expressed as a positive value when it causes the animals to excrete water. In a series of 39 determinations carried out on 5 normal men and 5 normal women an average antidiuretic activity of -17 for each sex was found with a range of from -41 to $+13$.

Several specimens of urine from patients with toxemia have been subjected to chromatographic separation of the adrenal steroids by a modification of the method of Zaffaroni, Burton, and Keutmann (32) using a benzene-formamide solvent system on paper. By this method the major portion of the steroids excreted by non-pregnant individuals move at a rate characteristic of steroids containing 5 oxygens; a lesser amount of steroid has a rate of flow similar to those containing 4 oxygens.

PLAN OF STUDY

Seven normal pregnant women and eleven patients with pre-eclamptic toxemia were studied. The normal women collected 24-hour urine specimens at approximately two-week intervals. At the conclusion of the collection period a specimen of venous blood was drawn and the serum antidiuretic activity was estimated within $\frac{1}{2}$ hour after the blood was taken. The measurements were carried out as frequently as possible on the toxemic patients.

RESULTS

Normal Pregnancy

The findings for normal pregnancy are summarized in Tables I and II. Pregnancy has been divided roughly into three trimesters. The last trimester has been considered as beginning at the

TABLE I

The serum antidiuretic activity, total corticosteroid, pregnanediol, and chorionic gonadotrophin levels in normal pregnancy

Type and number of subjects	Serum antidiuretic activity		Total corticosteroid mg./24 hrs.		Pregnanediol mg./24 hrs.		Chorionic gonadotrophin r.u./24 hrs.	
	Number of samples	Mean \pm S.E.*	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.
Non-pregnant (10 subjects)	39	-17 ± 1.9	64 (31 subjects)	$.420 \pm .016$				
1-13 Wks. pregnancy (3 subjects)	6	-21 ± 5.5	7	$.400 \pm .052$	10	12.2 ± 2.2	13	$10,300 \pm 1410$
14-26 Wks. pregnancy (5 subjects)	18	-6 ± 3.5	21	$.496 \pm .026$	19	30.4 ± 3.7	21	855 ± 200
27th Wk.-delivery pregnancy (6 subjects)	15	-12 ± 3.8	22	$.600 \pm .036$	14	50.1 ± 8.4	27	990 ± 128

* S.E. = Standard Error.

TABLE II

Normal pregnancy—specimens on which benzene to water partitions of corticosteroids were performed

Type and number of subjects	Total corticosteroid mg./24 hrs.		Freely-water-soluble corticosteroid mg./24 hrs.		Poorly-water-soluble corticosteroid mg./24 hrs.	
	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.
Non-pregnant (13 subjects)	22	$.419 \pm .029$	22	$.386 \pm .028$	22	$.054 \pm .016$
1-13 Wks. pregnancy (3 subjects)	6	$.393 \pm .061$	6	$.330 \pm .064$	6	$.070 \pm .034$
14-26 Wks. pregnancy (5 subjects)	20	$.496 \pm .026$	20	$.375 \pm .024$	20	$.107 \pm .015$
27th Wk.-delivery pregnancy (6 subjects)	20	$.633 \pm .037$	20	$.472 \pm .032$	20	$.161 \pm .024$

TABLE III

The serum antidiuretic activity, total corticosteroid, pregnanediol, and chorionic gonadotrophin levels in toxemia of pregnancy

Type and number of subjects	Serum antidiuretic activity		Total corticosteroid mg./24 hrs.		Pregnanediol mg./24 hrs.		Chorionic gonadotrophin r.u./24 hrs.	
	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.
All cases of toxemia (11 subjects)	18	-15 \pm 3.9*	25	1.010 \pm 0.204†	19	34.5 \pm 5.9	39	5,130 \pm 850
Severe toxemia (6 subjects)	12	-17 \pm 4.9	10	1.635 \pm 1.27‡	14	34.8 \pm 7.3	27	6,320 \pm 1,190
Moderate and mild toxemia (5 subjects)	6	-10.5 \pm 5.5	15	.700 \pm 0.067	5	34 \pm 12.5	12	3,683 \pm 960

* The difference between this value and the average serum antidiuretic activity of all normal pregnancies (27th week to term) is not significant.

† The difference between this value and the average total corticosteroid of all normal pregnancies (27th week to term) is not statistically significant.

‡ The difference between this value and the average total corticosteroid of all normal pregnancies (27th week to term) is highly significant.

t = 1.7
p = .1
t = 1.65
p = .1
t = 3.5
p = .01

TABLE IV

Toxemia cases in which benzene to water partitions of corticosteroids were performed

Type and number of subjects	Total corticosteroid mg./24 hrs.		Freely-water-soluble corticosteroid mg./24 hrs.		Poorly-water-soluble corticosteroid mg./24 hrs.	
	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.	Number of samples	Mean \pm S.E.
All cases of toxemia (11 subjects)	19	.795 \pm .105	19	.530 \pm .083	19	.253 \pm .136*
Severe toxemia (6 subjects)	5	1.200 \pm .109	5	.800 \pm .26	5	.390 \pm .170†
Moderate and mild toxemia (5 subjects)	14	.680 \pm .068	14	.435 \pm .05	14	.210 \pm .033

* The difference between this value and the average poorly-water-soluble corticosteroid of all normal pregnancies (27th week to term) is not significant. t = 1.91, p = > .05 < 0.1

† The difference between this value and the average poorly-water-soluble corticosteroid of all normal pregnancies (27th week to term) is probably significant. t = 2.5, p = > .01 < .02

twenty-seventh week to permit a closer comparison of the patients with toxemia, all of whom were in the twenty-seventh week of pregnancy or later.

Serum antidiuretic activity: There was no difference in antidiuretic activity of serum between any of the three trimesters of pregnancy and no difference between the pregnant and the non-pregnant woman.

Excretion of "total corticosteroid": Excretion of "total corticosteroid" increased during pregnancy, with an average during the last trimester which is near the upper limit of the normal range for non-pregnant women. During this trimester, many normal pregnant patients excreted amounts of corticosteroid considerably above the normal non-pregnant range.

Excretion of "freely-water-soluble" and "poorly-water-soluble corticosteroid": During the first trimester almost all the "total corticosteroid" was water soluble. During the second and third trimester, an appreciable amount of corticosteroid that did not pass from benzene into water was present. During the third trimester, approximately 25 per cent of the "total corticosteroid" was poorly-soluble in water.

Excretion of pregnanediol: An increase in pregnanediol excretion occurred throughout pregnancy, reaching a peak just before delivery.

Excretion of chorionic gonadotrophin: The urinary chorionic gonadotrophin level was highest during the first trimester, subsequently falling and

remaining at a low level throughout the rest of pregnancy.

Patients with Toxemia of Late Pregnancy

Eleven patients with pre-eclamptic toxemia were studied. All these patients were in the twenty-seventh week of pregnancy or later. These studies are summarized in Tables III and IV. A comparison of the values of normal pregnancy and of pre-eclamptic toxemia is shown in Figure 1.

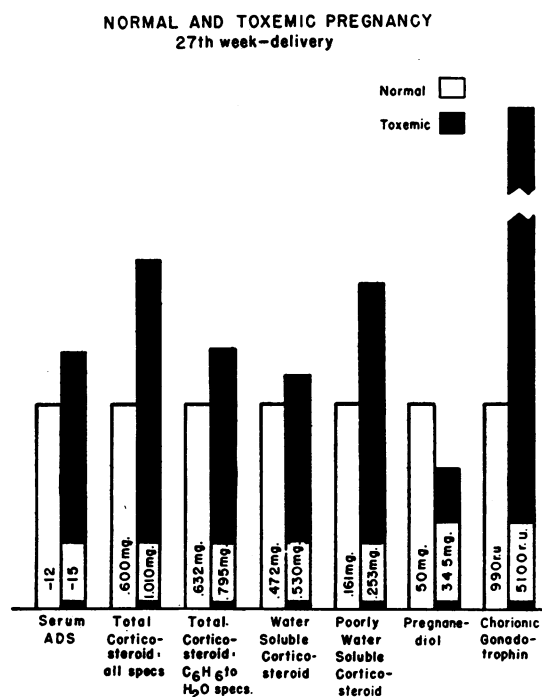


FIG. 1. SERUM ADS, URINARY CORTICOSTEROID, PREGNANEDIOL AND CHORIONIC GONADOTROPHIN IN SEVEN NORMAL AND ELEVEN TOXEMIC PREGNANCIES

Serum antidiuretic activity: The average of the 18 determinations was -15. The range of these values was large and the difference between the activity of the patients with pre-eclamptic toxemia and the normal pregnant women was not statistically significant.

Excretion of "total corticosteroid": The twenty-five determinations on specimens from the 11 patients showed an average of 1.010 mg. of corticosteroid in 24 hours with a range of from 0.265 mg. to 4.750 mg. per 24 hours. This average was 61 per cent higher than the average for the third trimester of normal pregnancy.

Excretion of "freely-water-soluble" and "poorly-water-soluble corticosteroid": In addition to the "total corticosteroid" determination, benzene to water partitions were carried out on 19 specimens collected from patients with pre-eclamptic toxemia. The average "total corticosteroid" for these 19 specimens was 0.795 mg. per 24 hours. Of this corticosteroid 0.530 mg. was freely-water-soluble and 0.253 mg. was poorly-water-soluble.

Excretion of pregnanediol: The average value of pregnanediol for nineteen 24-hour specimens was 34.5 mg., which is considerably lower than the average for the normal subjects.

Excretion of chorionic gonadotrophin: A total of 39 measurements were carried out on specimens from the eleven patients with toxemia. The average excretion was 5,130 r.u. for 24 hours which was over five times higher than the average for normal pregnancy.

Comparison of mild and severe pre-eclampsia: The eleven patients with toxemia were divided into six "severe" cases, one "moderate" case, and four "mild" cases of pre-eclampsia. This purely arbitrary division of cases was made by the obstetrician (E. C. H.) without knowledge of the antidiuretic activity or corticosteroid values of the patients. Those patients with "severe" eclampsia had most of the following features: severe hypertension, severe edema, convulsions, albuminuria of 4 plus or greater degree, premature labor, and fetal death. The "moderate" or "mild" pre-eclamptics had moderate weight gain with slight edema, slight hypertension, and little albuminuria. The patients with more severe toxemia tended to have higher corticosteroid excretion, larger amounts of "poorly-water-soluble corticosteroid," and higher chorionic gonadotrophin excretion than are found in the patients with "moderate" or "mild" toxemia, although the number of determinations is insufficient to be statistically significant. The "moderate" or "mild" toxemia patients showed little, if any, deviation of the values from the normal range.

Fractionation by paper chromatography of urinary corticosteroid: Urines of two patients with pre-eclampsia have been studied by chromatography. In one subject, all the corticosteroid moved at a rate characteristic of steroids containing 4 or 5 atoms of oxygen. The major portion of the steroid moved at a rate characteristic of the 5-

oxygen-containing steroids. The other urine also contained a good deal of material that moved in a manner characteristic of 5- and 4-oxygen-containing steroids. There was, in addition, a considerable quantity of alpha-ketol which moved much more rapidly and had a rate of flow similar to that of desoxycorticosterone in a benzene-formamide system. When it was rechromatographed in a cyclohexane-formamide system it moved slightly more rapidly than desoxycorticosterone.

DISCUSSION

Is there an explanation for the discordance in results reported by those who have studied antidiuretic activity in pregnancy and toxemia? Several investigators have found increases in amounts of antidiuretic activity in normal pregnancy or in toxemia in the urine (11-13), but most of the workers who have looked for antidiuretic activity in serum have failed to find increased amounts of it (5-9). A possible explanation might be as follows: the antidiuretic activity of a material represents the algebraic sum of those factors which cause diuresis and of those which produce antidiuresis. Since adrenal cortical steroids are definitely diuretic in the rat, which is the usual test animal for antidiuretic activity, and since these steroids are considerably increased in toxemia and in normal pregnancy, it seems entirely possible that even if blood or plasma were to contain an increased amount of antidiuretic substance, the activity of this material might be masked by the increased amount of corticosteroid which would be injected into the rats when whole serum or plasma was given. On the other hand, if an antidiuretic material in urine were being sought, there would probably be no corticosteroid in the preparation given to the test animals because the technic of concentration of the urinary antidiuretic material is usually one which removes corticosteroids. For this reason, it is entirely possible that an increased amount of antidiuretic activity might be present in blood, but this activity might be balanced by an equally increased amount of diuretic substances, perhaps as a result of the body's compensatory mechanisms. And it is also possible that if there were increased amounts of antidiuretic activity, it could be detected in urine because the diuretic activity would not be measured.

The major objection to measurements carried

out for urinary antidiuretic activity is that the test animals respond by profound antidiuresis to any noxious substance, and since the urine of women with pre-eclampsia is often extremely foul, containing albumin, bacteria, as well as possible unknown toxic products, it is well within the realm of probability that some of the antidiuretic activity of the urinary residues might be the result of response to a toxic substance.

The significance of the increased poorly-water-soluble material, which is found in the last trimester of pregnancy and which is further increased during toxemia, is at present unknown. Our evidence concerning the identity of this poorly-water-soluble fraction is not complete, but it can be said that if desoxycorticosterone were present in the urine, it would probably fall in this fraction. For example, when desoxycorticosterone was given in a large dose to a patient with adrenal insufficiency, the corticosteroid increase was entirely in this fraction (33). The formation by the body of the poorly-water-soluble material appears to be influenced by the administration of desoxycorticosterone. When a patient who was normally excreting an appreciable amount of poorly-water-soluble corticosteroid was given desoxycorticosterone, essentially no change in excretion occurred until the exogenous steroid was stopped, at which time the poorly-water-soluble material disappeared completely from the urine for several days (29). This suggests that the secretion of the poorly-water-soluble material was inhibited by the dose of desoxycorticosterone. It is not known whether the poorly-water-soluble material corresponds to the substance which moves on paper chromatography at a rate characteristic of 3-oxygen-containing steroids. However, a patient who had a significant amount of the material which moved on chromatography at a rate characteristic of a 3-oxygen-containing steroid also had an appreciable amount of poorly-water-soluble corticosteroid.

With full realization that identification of the poorly-water-soluble fraction and of the material which chromatographs in a manner characteristic of the steroids with 3 oxygen atoms is essential, and that any hypothesis must remain purely tentative until such identification has been made, we feel that the following question may justifiably be raised at this time. Is it possible that an increased

amount of desoxycorticosterone-like material may be of importance in the pathogenesis of eclampsia? If a material similar to desoxycorticosterone in its salt-retaining effects were present, it could easily explain the salt and water retention which is so characteristic of eclampsia. If its other properties were also similar to those of desoxycorticosterone, an excess of this steroid could produce many of the other aspects of eclampsia, especially the vascular changes which are so pathognomonic of this disease.

The increased corticosteroid excretion in eclampsia is due not only to the poorly-water-soluble material, but also to a considerable fraction of freely-water-soluble steroid. This freely-water-soluble fraction includes steroids with 5 oxygens such as cortisone and 17-hydroxycorticosterone. The increased levels of steroids with 5 oxygens might represent a compensatory mechanism, since in many respects these steroids can antagonize the effects of desoxycorticosterone (1). The antidiuretic material found in the urine of patients with eclampsia might be analogous to the increased antidiuretic activity which Skahen and Green (34) have found in the urine of rats chronically treated with DCA pellets and which is probably the result of stimulation of posterior pituitary secretion by the retained salt, or it might be the result of the deficient antidiuretic hormone inactivation by the damaged liver which is found in this disease (35). The increased level of 5-oxygen-containing steroids in the blood might prevent detection of any increased antidiuretic substance when serum is assayed. The vascular changes of the placenta could, of course, account for the altered excretion of chorionic gonadotrophin and pregnanediol which are seen in this disease (23, 26).

The normal pregnant woman also excretes an amount of poorly-water-soluble material which is greater than is found in the nonpregnant individual. However, this fraction of the corticosteroid is relatively smaller in the normal woman than it is in the toxemic. It seems possible that the relatively greater amount of freely-water-soluble corticosteroid proportionate to poorly-water-soluble corticosteroid found in the woman who does not develop toxemia, might reflect a normal compensation for the development of the poorly-water-soluble fraction. Thus, the inability

to produce the relatively larger amount of freely-water-soluble material might be in some way connected with the development of eclampsia.

There is a small amount of other work which offers at least suggestive confirmation of this concept. Shipley (36) has found an increased amount of corticosteroid which has salt retaining activity when measured by bio-assay in the urine of patients with toxemia. Venning (37) has measured formaldehyde-forming corticosteroid and glycogen-depositing corticosteroid in women with toxemia, and has found that there is present a considerable amount of formaldehyde-forming material which does not cause glycogen deposition and that this material is less in amount in the normally pregnant individual. It is possible that in the human there may be a material analogous to the steroid recently isolated by Grundy, Simpson, and Tait (38) from beef adrenal which is highly active as a salt-retaining steroid.

Further proof of the validity of this concept would of course be afforded by the demonstration that administration of desoxycorticosterone can produce eclampsia and that administration of 5-oxygen-containing steroids would benefit patients with pre-eclampsia. Relatively little evidence is available on these points, but what is known is somewhat conflicting. Sobel (39) has given moderate amounts of DCA to normal pregnant women without effect. The dose was not large nor was it continued for long. Her work does not eliminate the possibility that a desoxycorticosterone-like substance is active in producing eclampsia, but it certainly does not add weight to this concept. Long-continued administration of relatively large doses of DCA would be necessary to furnish a definite answer to this question. It has been reported (40) that cortisone is definitely of value in severe eclampsia when other more usual methods of therapy have failed. On the other hand, Jailer (41) has found no benefit from the administration of ACTH to pre-eclamptic patients.

SUMMARY

1. No increase in antidiuretic activity of serum is detectable during normal or toxemic pregnancy.
2. Total corticosteroid increases throughout pregnancy, reaching a peak before delivery. Freely-water-soluble corticosteroid increases throughout pregnancy. A poorly-water-soluble

material appears during the first trimester and increases until the end of pregnancy, constituting 25 per cent of the corticosteroid.

3. In toxemia corticosteroid is considerably increased above the level found in normal pregnant women at the same stage of pregnancy. The poorly-water-soluble component is also considerably increased above that seen in the normal, representing approximately 35 per cent of the total corticosteroid. In one patient with eclampsia, paper chromatography demonstrated a material which has a rate of flow characteristic of 3-oxygen-containing steroids.

4. The possibility that this material might play a part in the etiology of eclampsia is discussed.

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