

# SIMULATED ADRENO-CORTICAL ACTIVITY DURING PREGNANCY IN AN ADDISONIAN PATIENT<sup>1</sup>

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Interest in the role of the adrenals in pregnancy has been stimulated recently by Venning (1). In normal pregnant women, she observed a marked increase in urinary glucocorticoids and a slight rise in 17-ketosteroids which occurred in the latter months of pregnancy. Subsequently, this has been confirmed by Jailer (2). These changes were ascribed by Venning to increased maternal adrenal activity during gestation.

The pregnant patient with adrenal cortical insufficiency affords an unusual opportunity, by contrast with normal patients, to study more exactly adrenal function in pregnancy. Two such studies have been reported, that of Samuels, Evans and McKelvey (3) on one patient and that of the authors on four such patients (4). In both reports an unexpected finding has been a rise in 17-ketosteroids which occurred during the latter months of pregnancy in a manner similar to that observed in normal patients. Samuels, Evans and McKelvey (3) suggested that this increase might be accounted for on the basis of fetal secretion of these steroids; however, assays of newborn infants' urine have yielded amounts of these steroids which are inadequate to account for the observed rise in maternal excretion (5, 6). Consequently, the possibility may be entertained that the placenta rather than the fetus serves as the source of these steroids. This view would also be compatible with the observed survival of adrenalectomized animals without supportive therapy during pregnancy, which again might be due to adrenal-like hormones produced by the placenta.

Within the past year one of the Addisonian patients previously reported (4) has undergone another pregnancy. It seems worthwhile to report

observations made during this pregnancy, since with newer methods for study of adrenal function, additional data have been obtained which serve to strengthen the belief that the placenta plays an important role in the production of "adrenal-like substances."

## METHODS

At approximately monthly intervals during her pregnancy, this patient was admitted to the Metabolism Ward of the Presbyterian Hospital for several days' study. Gonadotropins, estrogens and pregnanediol determinations were performed by methods previously described (7-9). 17-Ketosteroids were determined by the Holtorff-Koch (10) modification of the Zimmerman reaction. The neutral reducing lipids were performed by the phosphomolybdic acid reducing procedure of Heard, Sobel and Venning (11) with a slight modification allowing for more complete hydrolysis. Freshly collected urine was acidified to pH-1 and allowed to stand for 24 hours at room temperature before extraction with chloroform. Serum sodium was determined by the use of an improved internal standard flame photometer (12). Eosinophil tests were performed with 0.3 mg. epinephrine injected subcutaneously and also with 25 mg. ACTH<sup>2</sup> intramuscularly, as outlined by Thorn and his co-workers (13).

## CASE REPORT

*P. H. 814847, S. R.* was a 24-year-old white housewife with known Addison's disease, first admitted for vaginal bleeding in the eighth week of her first pregnancy in 1946. A diagnosis of adrenal insufficiency had been made eight months previously at the Mt. Sinai (New York) Hospital, at which time she gave a history of increasing pigmentation and asthenia which had progressed alarmingly following an appendectomy two months before her admission to Mt. Sinai Hospital. There was no history or X-ray evidence of tuberculosis.

At the time of her admission to Presbyterian Hospital she showed evidence of generalized pigmentation of the skin with many black freckles and several gray-brown spots on the lips, gums, tongue and buccal mucous membranes.

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<sup>2</sup> Kindly supplied by Dr. John R. Mote, Medical Director, Armour Laboratories, Chicago, Ill.

Her blood pressure was 100/70. There was a brownish vaginal discharge but the cervix appeared to be closed. Laboratory studies revealed values for serum sodium of 139 mEq./L. and for fasting blood sugar of 69 mg. per cent. Urinary 17-ketosteroid excretion as determined subsequently in the non-pregnant state was less than 1 mg. in 24 hours. X-ray examination of the chest and adrenal area revealed nothing abnormal.

On her third hospital day the patient had a spontaneous complete abortion, with no exacerbation of her adrenal disease.

The patient was discharged, and two months later 375 mg. of desoxycorticosterone acetate in pellet form were implanted at the Mt. Sinai Hospital. Four months after this she again became pregnant. Her condition during pregnancy remained on the whole very satisfactory and she gained a total of 8 kilograms. During this pregnancy she was admitted to the hospital on four occasions for observation or for intercurrent infection. She remained in the hospital during the ten weeks prior to delivery. Details of the findings in this pregnancy have been reported previously (4). Following delivery, the patient continued in good health, her adrenal insufficiency was controlled with implantation of pellets of desoxycorticosterone acetate at approximately yearly intervals, plus varying amounts of sodium chloride by mouth.

Two years after this delivery, and two months after the implantation of 375 mg. of DCA, the patient again became pregnant. During this pregnancy she was ad-

mitted to the Presbyterian Hospital on six occasions for study or for treatment of an intercurrent infection. In general, her condition remained excellent, and she gained approximately 6 kilograms. Satisfactory control of her adrenal disease was achieved initially by DCA absorption from pellets, plus enteric coated sodium chloride given in gradually increasing doses to a maximum of 7 gm. daily. In the middle of the seventh month, daily injections of DCA, 2 mg., were begun and at this time the added sodium chloride was reduced to 3 gm. Her blood pressure, weight, serum sodium, hematocrit and therapy during pregnancy are outlined in Table I. A normal weight gain occurred and blood pressure remained normal. Serum sodium determinations on several occasions were slightly below normal; however, since her clinical condition during pregnancy remained satisfactory, her DCA and sodium chloride dosage was not increased. Serum sugar values were at times significantly low.

Nineteen days prior to the expected date of delivery, her membranes ruptured and 13 hours later the patient went into labor. At the end of eight hours and 46 minutes, she was delivered under cyclopropane anesthesia, by Dr. Anthony D'Esopo, of a male infant weighing 3,240 gm. During the course of the labor, the patient received a liter of glucose in saline and 500 cc. of whole blood intravenously, 10 cc. of "lipo-adrenal cortex" (Upjohn) intramuscularly, and, in addition, her daily dose of 2 mg. DCA. At delivery the patient's placenta was saved for subsequent study. In Table I, the outline of her course

TABLE I

Date	Wgt.	B.P.	Blood sugar	Serum sodium	Gonadotropins	Estrogens	Preg.*	DCA	NaCl	ACE†
	<i>kilo.</i>		<i>mg. %</i>	<i>mEq./L.</i>	<i>M.U.</i>	<i>µg.</i>	<i>mg.</i>	<i>mg./day</i>	<i>gm./day added</i>	<i>cc./day</i>
May	56.1	108/64	74	134.0	180,000	15.2	7.3	375 mg. pellets implanted Jan. '49		
June	54.4	100/55	63	131.4		60				
July	56.3	110/60	76	135.2	14,000					
Aug.										
Sept.	60.5	102/64	67	137.6					7	
Oct.	58.6	104/54	51	133.6		292	115	2	3	
				133.3						
Nov.	62.6		58	132.4		445		2	3	
				138.8						
12/1 Delivery		110/70	67	133.5		814		2	20	10
<i>Post-partum</i>										
12/2		130/90	62	139.3		108		2	12‡	15
12/3	60.1	140/80	79	136.6				2	6‡	10
12/4	61.4	134/86						2	6	5
12/5	61.4	132/80		141.4				2	2	
12/6	61.6	160/100				68		2	6	
12/7	61.4	158/98		133.5				2	3	5
12/8	58.0	146/84						2	3	
12/9	58.0	150/90		138.0				2	6	
12/10	57.4	140/92						2	6	
12/11		142/78						2	6	
12/12		130/82		137.4				2	6	
12/13		130/72						2	6	
12/14	56.1			136.5				2	6	

\* Pregnanediol.

† Upjohn's lipo-adrenal cortical extract.

‡ Includes parenteral NaCl.

and therapy during delivery and post-partum are presented. Prior to, and throughout her pregnancy, her blood pressure had ranged from 90-120/65-76. Following delivery diuresis did not occur for seven days and during this time a significant rise in tension occurred which decreased somewhat at the end of the first week post-partum. This rise in blood pressure was not accompanied by any significant albuminuria. Her blood pressure has persisted at levels higher than those observed prior to delivery. During the four months since delivery, the patient has remained in satisfactory health.

## RESULTS

### *Excretion of gonadotropins, estrogens and pregnanediol*

Gonadotropic hormone, estrogen and pregnanediol excretions in the urine were studied at various times during this pregnancy and were found to be within normal limits, as shown in Table I.

### *Excretion of 17-ketosteroids*

The excretion of total neutral 17-ketosteroids in the urine were determined at various intervals

during the pregnancy (Figure 1). It was found that the value for these steroids rose from 4.5 mg. in the sixth week to a high of 9.9 mg./day immediately prior to delivery. (A value of 1.0 mg. had been obtained about a year prior to the onset of this pregnancy.) Immediately following delivery, the excretion of 17-ketosteroids gradually fell so that on the seventh post-partum day, a value of 1.9 mg. was obtained. Subsequent daily determinations fluctuated somewhat but were all within the range characteristic of Addison's disease.

### *Excretion of neutral reducing lipids*

No studies of the neutral reducing lipids (PMA) were performed until the last trimester and at that time values as high as 6.22 mg./day were attained (Figure 1). These values are within the range of those found during the course of a normal pregnancy (2). During the immediate post-partum stage, these values also fell,

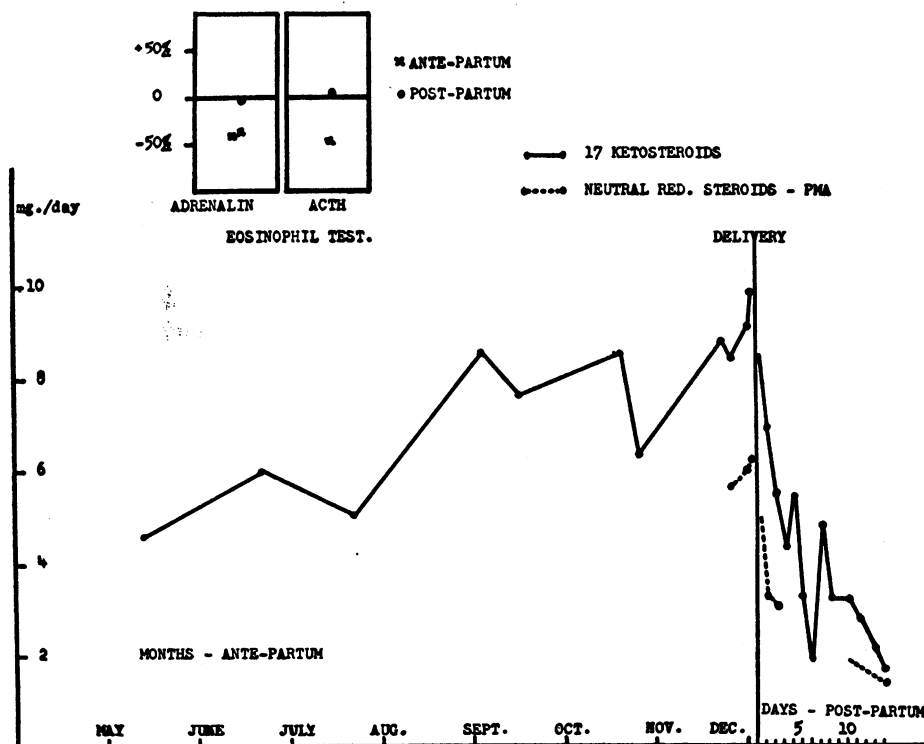


FIG. 1. THE EXCRETION OF 17-KETOSTEROIDS AND NEUTRAL REDUCING LIPIDS DURING PREGNANCY AND IMMEDIATELY POST-PARTUM

Insert shows the percentage eosinophil fall as a result of the injection of epinephrine and ACTH in both the ante and post-partum periods. Neutral red. steroids—PMA = neutral reducing lipids as determined by the phosphomolybdic acid reducing method.

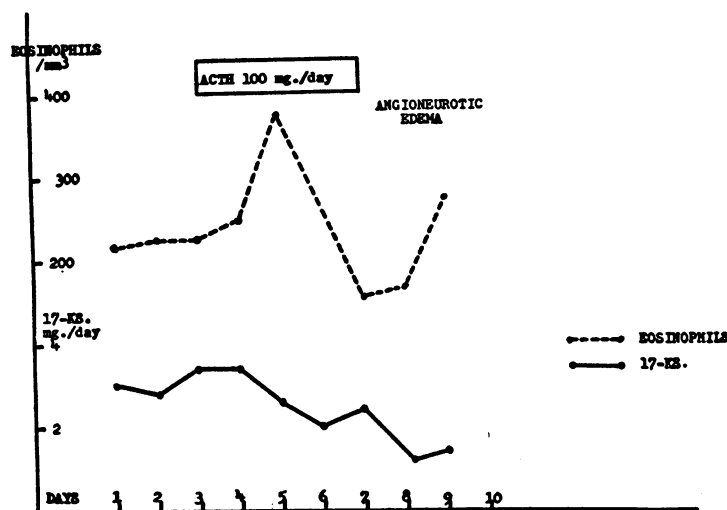


FIG. 2. THE EFFECT OF ACTH ON EOSINOPHILS AND EXCRETION OF 17-KETOSTEROIDS IN THE POST-PREGNANT STATE IN THE ADDISONIAN PATIENT

reaching a low of 1.5 mg. 13 days after delivery. Normal values obtained by the technique employed in this laboratory are 2-4 mg./day.

#### Eosinophil tests

During the seventh month and again during the ninth month, eosinophil tests were performed using 0.3 mg. epinephrine subcutaneously. On both occasions, there occurred a 40 per cent fall in circulating eosinophils (Figure 1), at the end of four hours. The administration of a single dose of 25 mg. of ACTH in the ninth month also was followed in a 40 per cent decline in eosinophils four hours later. These tests were repeated in similar fashion approximately 10 days post-partum and were completely negative (Figure 1).

#### Administration of ACTH

To rule out the possibility that the patient harbored residual adrenal tissue which, during pregnancy, had been stimulated to physiological activity, the patient was given large doses of ACTH beginning two weeks after delivery for a period of five days. Injections of 25 mg. ACTH, freshly dissolved in saline, were administered every six hours. No fall in eosinophils ensued and no rise in 17-ketosteroid excretion was observed (Figure 2). The administration of the hormone was discontinued after the fifth day, due to the development of angioneurotic edema.

#### Excretion of 17-ketosteroids and neutral reducing lipids in patient's infant son

Urine was collected from the newborn male infant for the first three days of its life. The daily urinary excretion of 17-ketosteroids averaged 0.6 mg. and the neutral reducing lipids, 0.3 mg. These values fall within the normal limits of newborn infants (Venning, Randall and Gyorgy [14], Day [6]) and argue against the possibility that the infant's adrenal contributed significantly to the increased excretion in the mother. It is of great interest that more than a week post-partum supervened before the maternal excretion of steroids fell to low levels and yet the infant excreted but traces of these substances at birth. If these were present in high concentration in the fetus, one would expect to find them elevated for several days after birth.

#### Determination of ACTH in patient's placenta

Acetone dried placental tissue from the patient was assayed for ACTH in the newborn rat by the method of Jailer (15). The administration of 102 mg. of the dried powder resulted in a 35 per cent decline in adrenal ascorbic acid (Figure 3) which is the equivalent of approximately 100 gamma of ACTH. Boiled placental produced similar results. This procedure destroys most of the toxicity, gonadotropic hormones and pituitrin but has no effect on ACTH activity. In collabora-

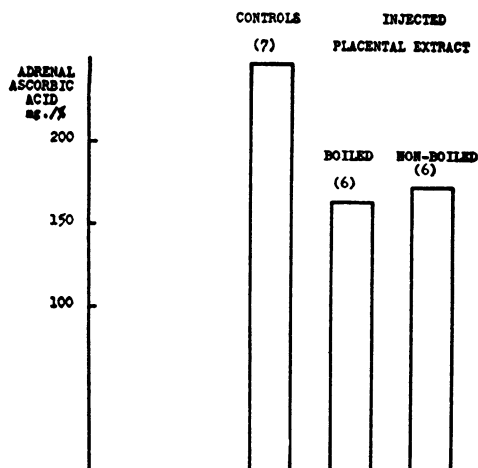


FIG. 3. THE EFFECT OF CRUDE PLACENTAL EXTRACTS ON THE ADRENAL ASCORBIC ACID CONTENT OF THE NEWBORN RAT

The number in parenthesis indicates the number of rats injected. Boiling destroys toxicity but has no effect on ACTH activity.

tion with Drs. Philip E. Smith and F. Agate (16), this finding was checked by assaying the placental powder according to the method of Sayers, Sayers and Woodbury (17), and significant declines in adrenal ascorbic acid in hypophysectomized rats were obtained. (This will be presented in greater detail by Drs. Smith and Agate in a separate publication.)

#### DISCUSSION

In previous studies in pregnant patients with adrenal cortical insufficiency (4), the unexpected finding of a progressive rise in 17-ketosteroid excretion has been made. The explanation suggested by Samuels, Evans and McKelvey (3), *i.e.*, that the rise was due to a contribution from the fetal adrenal glands, appears no longer tenable in the light of Venning's (14) and also Day's (6) observations that the excretion of these substances in the newborn is extremely low. Nor does Venning's suggestion seem wholly satisfactory, that the rise in 17-ketosteroids, as measured by the Zimmerman reaction, is due largely to pregnanolone or other 20-ketosteroids rather than adrenal steroids. In the patient reported here the pregnanediol complex (which contains pregnanolone) was isolated and the color reaction with *m*-dinitrobenzene determined; 640  $\mu$ g. were found to

give the color intensity of less than 10  $\mu$ g. of dehydroisoandrosterone. From this, it may be calculated that pregnanolone or other 20-ketosteroids could not account for more than 1 mg. of the determined 17-ketosteroid value. In addition, a similar rise in 17-ketosteroids was observed in this patient's first pregnancy (4) at which time determinations were made by the antimony trichloride method in which there is no possibility of inclusion of the 20-ketosteroids.

In the present study, in addition to the suggestion of increased "adrenal-like" activity in the rise in 17-ketosteroids, two other findings offer similar evidence. One of these is the pronounced increase in the excretion of neutral reducing lipids. Although this is known to occur in normal pregnancy (2), along with a rise in glucocorticoids as determined by bio-assay (1), its occurrence in a patient with adrenal insufficiency is surprising.<sup>8</sup> The third indication of "adrenal activity" is the normal response during pregnancy to epinephrine and ACTH as measured by a fall in circulating eosinophils.

These three indications of increased "adrenal activity" were specifically related to the pregnant state, since they could no longer be demonstrated after delivery. The 17-ketosteroids and neutral reducing lipids in the urine returned to values in keeping with hypoadrenalism, and, as might be expected in an Addisonian, no eosinophil response to epinephrine or a single injection of ACTH could be demonstrated in the post-partum period. The presence of only minimal amounts of 17-ketosteroids and neutral reducing lipids in the infant's urine would seem to argue against the possibility that the fetus contributed significantly to the increased amounts observed in the patient's urine. Furthermore, the patient's subsequent lack of response to five days' administration of large doses of ACTH argues strongly against the possibility that the patient harbored residual

<sup>8</sup> During this patient's first pregnancy extractions of urine were made in our laboratory and sent to Dr. Venning who found no glucocorticoid activity present on two occasions. In the light of further knowledge of technique, however, it is likely that biological activity may have been destroyed during extraction by excessive heating in evaporating off the organic solvent.

adrenal tissue which became stimulated during pregnancy by endogenous ACTH.

From these data, it would appear that some extra-adrenal source of adrenal-like hormones was present in this Addisonian patient during pregnancy. The pregnant state differs from the normal in the presence of a fetus and placenta. In view of the aforementioned findings in the infant's urine, the placenta rather than the fetus would seem to be the most likely source of these substances.<sup>4</sup> Since the placenta is known to excrete estrogens, progesterone and chorionic gonadotropin, it is certainly within the realm of possibility that it could elaborate other steroids and pituitary-like hormones.

These results would suggest the elaboration of adrenal cortical-like hormones and adrenotropic hormones by the placenta. However, Venning (14) has not been successful in demonstrating glucocorticoid activity in chloroform extracts of three human placenta. We have also failed thus far to demonstrate adrenal-like material by bioassay from the placenta due to the great toxicity of extracts from this source. Preliminary experiments, however, indicate that there are lipid soluble substances present in placental tissue which react similarly to 17-ketosteroids with *m*-dinitrobenzene and to corticosteroids with the phosphomolybdic acid reagent.

If the placenta is a source of both ACTH or a close analogue and of adrenal cortical-like substances, as is suggested by the present study, it is still perplexing that ACTH (exogenous and endogenous) should stimulate the liberation of adrenal cortical-like substances from the placenta during pregnancy.

#### SUMMARY

1. A patient with Addison's disease was studied during the course of pregnancy.

<sup>4</sup>Of course it is impossible to ascertain the status of the fetal adrenals. However, it should be emphasized that the decline in steroid excretion to Addisonian levels in the mother took more than a week, while the urinary content of steroids in the infant was already low at birth. If the fetal adrenals were responsible for this increased secretion of steroids found in the mother, the concentration in its urine would be expected to decline gradually as it did in the mother.

2. Evidence of increased adrenal cortical-like activity was present since the 17-ketosteroids and neutral reducing lipids of the urine rose to normal values late in pregnancy and a fall in eosinophils resulted from the administration of either epinephrine or ACTH.

3. During the early post-partum period the steroid excretion fell to levels characteristic of Addison's disease and there was no fall in eosinophils as a result of ACTH or epinephrine injection. The newborn male infant excreted but traces of steroids during his first three days of life.

4. ACTH-like activity was found in a crude extract of the patient's placenta.

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