PREGNANCY AND ADRENOCORTICAL FUNCTION: ENDOCRINE STUDIES OF PREGNANCY OCCURRING IN TWO ADRENAL-DEFICIENT WOMEN.[1]

By A. GORMAN HILLS, ELEANOR H. VENNING, F. CURTIS DOHAN, GEORGE D. WEBSTER, JR.,[2] AND EDWIN M. RICHARDSON

(From the Edward B. Robinette Foundation and the Endocrine Section, William Pepper Laboratory of Clinical Medicine, Medical Clinic, Hospital of the University of Pennsylvania, the Department of Obstetrics and Gynecology, and the Department of Surgery, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; and the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada)

(Submitted for publication February 23, 1954; accepted July 6, 1954)

It has been suggested by Jailer and Knowlton (1) that when adrenal-deficient women become pregnant, they exhibit "simulated adrenal cortical activity" attributable to an extra-adrenal source of "adrenal cortical-like" hormone. Observations which can be interpreted as lending support to this view may be summarized as follows:

1. Pregnancy has been reported to prolong the survival of adrenalectomized animals (2–4).
2. Urinary 17-ketosteroid elimination (as estimated by the Zimmermann reaction) rises during the latter months of pregnancy in patients with Addison's disease (1, 5, 6) as well as in normal women (7).
3. Urinary excretion of neutral reducing lipids rose to the normal range late in pregnancy in one patient with Addison's disease (1).
4. A decrease of circulating eosinophils four hours following the injection either of epinephrin or of corticotrophin was observed on three occasions in one Addisonian patient during pregancy and not thereafter (1).

The occurrence of pregnancy in two women rendered adrenal-deficient by surgery because of severe hypertensive disease has provided an opportunity to conduct certain studies, reported in this communication, which bear upon the question.

[1] This investigation was supported in part by research grants H-1419 and H-922 from the National Heart Institute, U. S. Public Health Service; by a grant from the National Research Council of Canada; and by grants from the Squibb Institute for Medical Research, Eli Lilly and Company, Ciba Pharmaceutical Products, Inc., Merck and Company, Inc. and the Publicker Industries Nutritional Fund.

[2] Trainee of the National Heart Institute.

METHODS

Sodium and potassium were determined on a flame photometer with internal lithium standard (8); creatinine by the procedure of Bonsnes and Taussky (9); urea by Karr's method (10). Urinary 17-ketosteroids were estimated by the Holtorff-Koch modification (11) of the Zimmermann reaction. The measurements were of total neutral 17-ketosteroids, which would be expected to be higher than values for the ketonic fraction commonly reported. Neutral reducing lipids were estimated using the phosphomolybdate reaction according to Heard, Sobel, and Venning (12), and glycogenic corticoids by the bioassay technique of Venning, Kazmin, and Bell (13). Values reported as "pH_1 hydrolyzed" represent determinations on extracts of urine acidified with hydrochloric acid and kept at pH_1 for one hour. "Glucuronidase hydrolysis" signifies that the extracted material had initially been subjected to hydrolysis for 48 hours at pH 4.5 in the presence of β-glucuronidase (derived from spleen) in a concentration of 100 units per milliliter, and subsequently to acid hydrolysis as described above.

In the tables, excretion of urinary steroids is generally given both uncorrected and corrected for urinary creatinine excretion. The latter method of expression was thought preferable where many of the urine collections were made outside the hospital, as in the case of the data presented in Figure 1; in Figure 2, uncorrected data are charted because creatinine analyses were not available for all samples. Values for glycogenic corticoid excretion are uncorrected for urinary creatinine excretion, and represent cortisone equivalents.

Eosinophils were enumerated by the method of Manners (14).

Case reports

1. *S. C.*, H.U.P., Ob. 43769, was a 36-year-old colored female who had bilateral thoracolumbar sympathectomy and subtotal adrenalectomy performed for hypertension by Dr. Harold A. Zintel in three stages in June 1949 and March 1950. Her case has been reported in detail elsewhere (15). She became pregnant in August 1951. Gestation was uneventful, and she continued to take no steroid save for an occasional 12.5 or 25 milligrams of

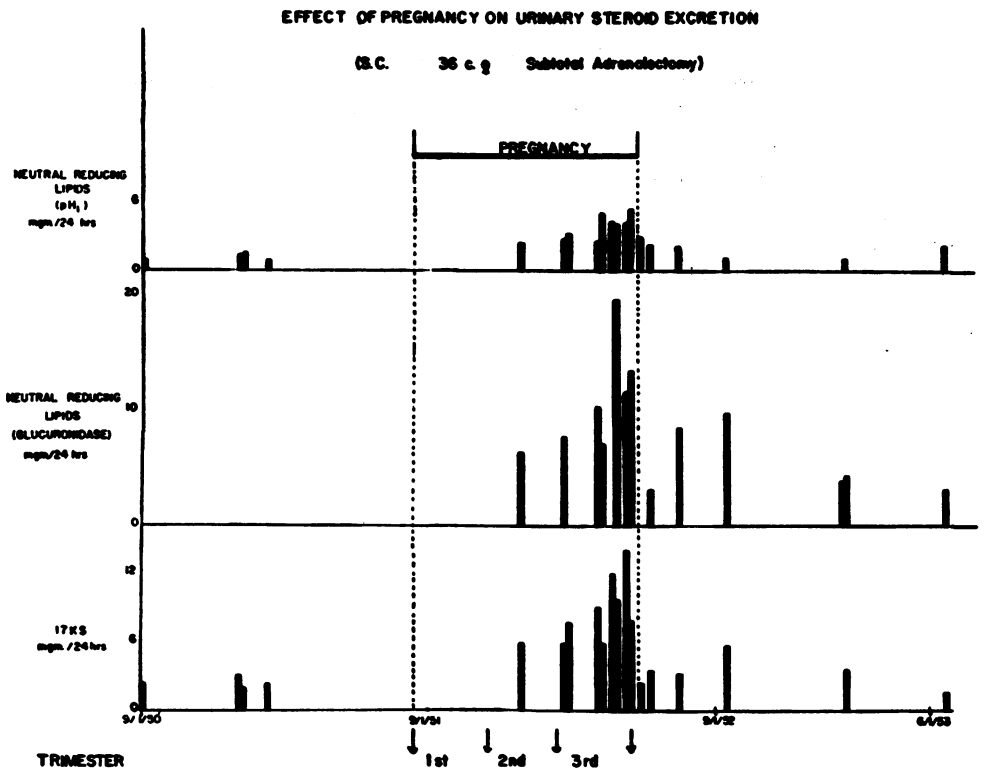

FIGURE 1.

cortisone. The blood pressure was little affected by pregnancy, remaining throughout in the range of 115–140/80–100. On May 19, 1952, she was delivered by Cesarean section, performed by Dr. George Hoffman, of a normal male infant weighing 1280 grams. Cortisone was administered at this time, and there were no post-partal complications. Mother and child remain in good health.

2. *B. L.*, H.U.P., Ob. 44812, was an 18-year-old white female followed by us since January 1951. Her complaint, at that time, was severe and progressive headache of six months' duration, accompanied by dizziness, blurring of vision, nausea, and vomiting. Blood pressure was 200–240/140–180, and retinopathy, Keith-Wagner Grade IV, was present. Phenolsulfonphthalein excretion was 15 per cent in 15 minutes, 70 per cent in two hours.

Bilateral subdiaphragmatic sympathectomy and splanchnicectomy, left 95 per cent adrenalectomy, and right total adrenalectomy were performed as a two-stage procedure by Dr. Zintel on January 27 and February 6, 1951. After operation, her symptoms were relieved and the retinopathy reverted to Grade I and II, but her blood pressure remained high (165–200/120–140). She required no replacement therapy, and metabolic studies revealed the presence of measurable adrenal cortical reserve.

The patient was married in 1952, and in early July 1952 became pregnant. She was admitted to the hospital in September 1952, at which time the site of the left adrenal was explored and an adrenal remnant weighing 1.1 Gm. was removed. She was hospialized throughout much of the remainder of her pregnancy and required a minimum of 25 mgm. of oral cortisone daily at all times. She felt well but her blood pressure remained elevated throughout her pregnancy. On January 20, 1953, in the 29th week of pregnancy, the patient went into spontaneous labor and was delivered by Dr. Michael Newton of a living female infant weighing 800 grams, which, however, died of prematurity after three days. The post-partal course was uneventful and the patient, at the present time, remains asymptomatic, though her blood pressure is high (210–230/140–155). She continues to require a minimum of 25 milligrams of oral cortisone daily.

COMMENT

It is clear that both our patients had gross adrenal deficiency.[8] B. L. may have had total deficiency, although we know of no practical method of ascertaining the complete absence of functioning adrenal cortical tissue. S. C. is certainly adrenal-deficient, as indicated by both melanosis and by her susceptibility to the precipitation

[8] For convenience the term *deficiency* as used in this communication, refers to lack of adrenal cortical tissue, of whatever degree; *insufficiency* indicates the physiologic consequences, of whatever gravity, of lack of available hormone in relation to need.

of adrenal insufficiency; but she probably has some functioning cortical tissue, since she requires little or no substitution therapy. Repeated study of 24-hour urinary sodium elimination during pregnancy in the two patients while they were taking self-selected diets indicated that the difference between the patients with respect to their need for substitution therapy is probably not attributable to any important difference in their salt consumption: S. C. excreted 228 ± 42 milliequivalents of sodium per 24 hours, and B. L. 186 ± 23 milliequivalents.

RESULTS

Effect of pregnancy upon urinary steroid excretion

Neutral reducing lipids and 17-ketosteroids (Figures 1 and 2). The quantities of neutral 17-ketosteroids excreted following pregnancy by B. L., and of neutral reducing lipids and neutral ketosteroids excreted by S. C. in the non-pregnant state, were both distinctly lower than the mean values for normal women and for hypertensive women obtained in this laboratory. During pregnancy excretion of these substances by both women was augmented, the values for neutral reducing lipids falling within the range of values which have been observed at corresponding times in a small number of observations of normal pregnancy in this laboratory. The increases were unrelated to hormone therapy, for in one patient (S. C. [Figure 1]) no hormone was given within 48 hours prior to the beginning of any of the collections, while in the other (B. L. [Figure 2]) cortisone administration was kept substantially constant (25 to 37 milligrams daily) during and immediately prior to collections. The effect of pregnancy upon urinary steroid excretion in B. L. is somewhat more impressive when correction is made for urinary creatinine excretion, though by no means as striking as the influence of pregnancy upon urinary streoid excretion in S. C. However, the highest values in S. C. were observed

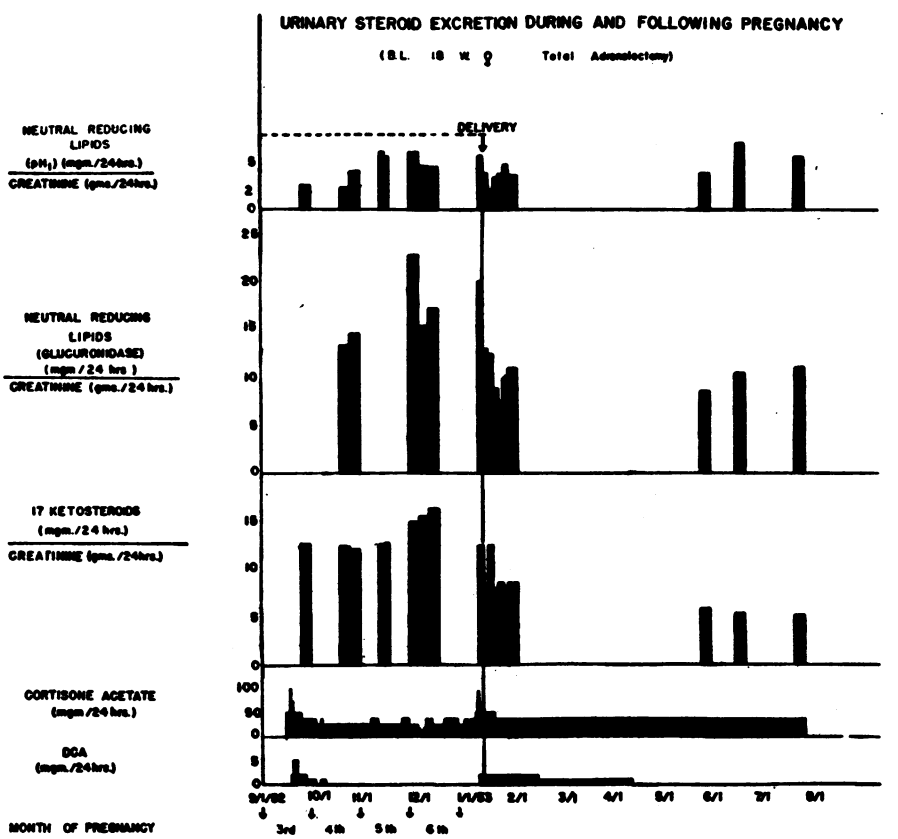

FIGURE 2.

TABLE I

Urinary excretion of glycogenic corticoids by adrenal-deficient women during and following pregnancy

Patient	Date	Medication* mgm./24 hr.	Month of pregnancy	Glycogenic corticoids μgm./24 hr.
S. C.	4/9–11/52	0	8th	114
	4/28–30/52	0	9th	130
	9/17–19/52	0	Post-partum	26
	2/9–11/53	0	Post-partum	41
	6/20–22/53	0	Post-partum	34
	6/22–24/53	ACTH 60	Post-partum	46
	6/24–26/53	ACTH 60	Post-partum	38
	6/26–28/53	ACTH 60	Post-partum	50
	6/28–30/53	ACTH 60	Post-partum	42
B. L.	10/22–24/52	E 25	4th	88
	12/2–4/52	E 25	6th	151
	5/31 to 6/2/53†	E 37	Post-partum	76
	6/2–10/53†	E 37 ACTH 60	Post-partum	42
	6/25–27/53	E 37	Post-partum	45
	7/27–29/53	E 37	Post-partum	23

* E: Cortisone acetate (oral), supplied by Dr. Elmer Alpert of Merck and Company. ACTH: H. P. Acthar Gel (intramuscular), supplied by Dr. C. J. O'Donovan of the Armour Laboratories.

† Hydrolyzed at pH_1 only. All other samples subjected to initial hydrolysis with β-glucuronidase followed by hydrolysis at pH_1.

during the last six weeks of pregnancy, and comparable specimens were not obtained in B. L. owing to her premature delivery. Whether or not the creatinine correction is applied, a progressive increase of urinary steroid excretion beginning as early as the second trimester is observable in both patients during pregnancy.

Glycogenic corticoids (*Table I*). Material capable of causing glycogen deposition in the liver of the fasted adrenalectomized mouse was excreted in the urine of both patients at all times. Following pregnancy, the values have been of the order of magnitude found in patients with Addison's disease on minimal replacement therapy, and in B. L. the excretion of this material may be attributable, in part at least, to oral cortisone ingestion. During the latter half of pregnancy both women were excreting three to four times this quantity, and at these periods the values were in the range found in patients with Cushing's disease. These values fall close to the mean of those observed in normal pregnancy at corresponding times as determined in the same laboratory (7).

Physiological studies during pregnancy

Response to intravenous corticotrophin (*Table II*). Repeated attempts were made to obtain evidence of an adrenal-like response to 24 or 48-hour corticotrophin tests. Either 10 or 20 U.S.P. units in glucose or saline solution were administered by intravenous infusion over an eight-hour period, in order to supply a near maximum stimulating dose (16). Adrenal-like response was evaluated in terms of 24 or 48-hour excretion of neutral reducing lipids (using two methods of hydrolysis), and of 17-ketosteroids, comparing the experimental periods (reckoned from the beginning of the 8-hour infusions) with control values, usually those for the period immediately preceding the test infusion; and also by comparing the eosinophil counts prior to and at the termination of the infusion.

Responses to the infusions of corticotrophin uniformly were trivial or lacking in S. C. The normal eosinophil decrease at the end of such an 8-hour infusion amounts to a minimum of 90 per cent and the average increment in 24-hour urinary steroid excretion (*i.e.*, the mean increment of neutral reducing lipids determined following hydrolysis at pH_1 and by β-glucuronidase and of 17-ketosteroids) amounts to a minimum of 6 milligrams (17). A significant reduction (17) of circulating eosinophils was never seen in either patient. In two of the tests in B. L., steroid out-

TABLE II

Effect upon urinary steroid excretion and circulating eosinophils of 8-hour intravenous corticotrophin infusions during pregnancy in adrenal-deficient women

Pt.	Date	Day of pregnancy	ACTH* *USP units*	Creatinine *gm./24 hr.*	Steroids (*mgm./24 hr.*) Neutral reducing lipids pH1	Steroids (*mgm./24 hr.*) Neutral reducing lipids Glucuronidase	Steroids (*mgm./24 hr.*) 17-KS	Steroids (*mgm./24 hr.*) Creatinine (*gm./24 hr.*) Neutral reducing lipids pH1	Steroids (*mgm./24 hr.*) Creatinine (*gm./24 hr.*) Neutral reducing lipids Glucuronidase	Steroids (*mgm./24 hr.*) Creatinine (*gm./24 hr.*) 17-KS	Eosinophils Before ACTH *per mm.*³	Eosinophils After ACTH *per mm.*³	Eosinophils Change *per mm.*³	Eosinophils Change %
	1952													
S. C.	2/27–28	198	0	.97	3.3	X	7.6	3.4	X	7.8				
	2/29–3/1	199	10	.94	2.1	7.0	6.9	2.2	7.5	7.3	125	80	− 45	−36
	4/1–2	231	0	1.00	2.5	10.2	9.1	2.5	10.2	9.1				
	4/4–5	232	10	1.10	2.9	9.0	11.8	2.6	8.2	10.7	84	76	− 8	−10
	5/11–12	272	0	1.02	4.2	11.5	13.8	4.1	11.3	13.5				
	5/13–14	274	20	1.08	4.1†	19.6†	10.6†	3.8†	18.1†	9.8†	47	55	+ 8	+17
	5/14–15	275	20	1.27	4.0	15.7	10.9	3.1	12.4	8.6	41	34	− 7	−17
	7/15–16‡		0	.75	2.0	8.2	3.2	2.7	10.9	4.3				
	7/16–17‡		10	1.33	2.5	6.1	2.6	1.9	4.6	2.0	45	54	+ 9	+20
B. L.	9/25–26	117	0	.55	1.4	X	6.9	2.5	X	12.6				
	10/2–3	123	10	.68	4.8	13.6	6.0	7.1	20.0	8.8	242	166	− 76	−31
	10/3–4	124	10	.64	4.1	12.7	11.6	6.4	19.8	18.2	233	128	−105	−45
	10/29–30	150	0	.53	2.1	7.7	6.4	4.0	14.5	12.1				
	10/30–31	151	10	.56	3.0	11.7	7.3	5.4	20.8	13.0	179	230	+ 51	+28
	10/31–11/1	152	10	.55	3.7	10.6	7.5	6.7	19.3	13.6	165	175	+ 10	+ 6
	12/4–5	186	0	.79	3.5	13.5	12.9	4.4	17.1	16.3				
	12/8–9	190	10	.75	2.4	X	11.3	3.2	X	15.0	144	166	+ 22	+15

* Corticotrophin supplied by Dr. E. C. Vonder Heide of Parke, Davis and Company.
† Oral cortisone acetate 25 milligrams given on morning of 5/13.
‡ Two months post-partum.

put was somewhat increased on the days when corticotrophin was administered; but the increases are small and of questionable significance, and no effect of corticotrophin is observable on the occasions of the third test.

Precipitation of acute adrenal insufficiency (Figures 3, 4, and 5). Evidence of acute adrenal insufficiency was obtained in both patients during pregnancy. B. L. was hospitalized for much of the gestational period, and an effort was made, for therapeutic reasons, to supply no more than the minimum required replacement therapy. Symptoms of incipient acute adrenal insufficiency appeared promptly whenever an attempt was made to reduce the daily ration of cortisone acetate below 25 milligrams per day. In Figure 3 are shown data indicating that adrenal insufficiency promptly followed reduction of exogenous cortisone to 12.5 milligrams daily; immediately prior to hormone reduction she was excreting glycogenic corticoids in the urine in the amount of 151 micrograms per 24 hours (Table I). Frank symptomatic and chemical evidence of early acute adrenal insufficiency later appeared, in spite of the provision of additional exogenous hormone, in the wake of a dental infection followed by a tooth extraction (Figure 4).

S. C. was followed as an outpatient during most of her pregnancy. She was admitted to the Metabolic Unit during the seventh month of pregnancy, in the course of which evidence was obtained that acute adrenal insufficiency could readily be precipitated by dietary sodium restriction (Figure 5). Urinary glycogenic corticoid was first determined six weeks later, but the excretion of neutral reducing lipids and 17-ketosteroids was already elevated (Figure 1).

Response to corticotrophin following pregnancy (Tables I and III).

A daily injection of potent, long-acting corticotrophin was given for eight days to both patients well after the post-partal period in an attempt to ascertain whether they harbor residual

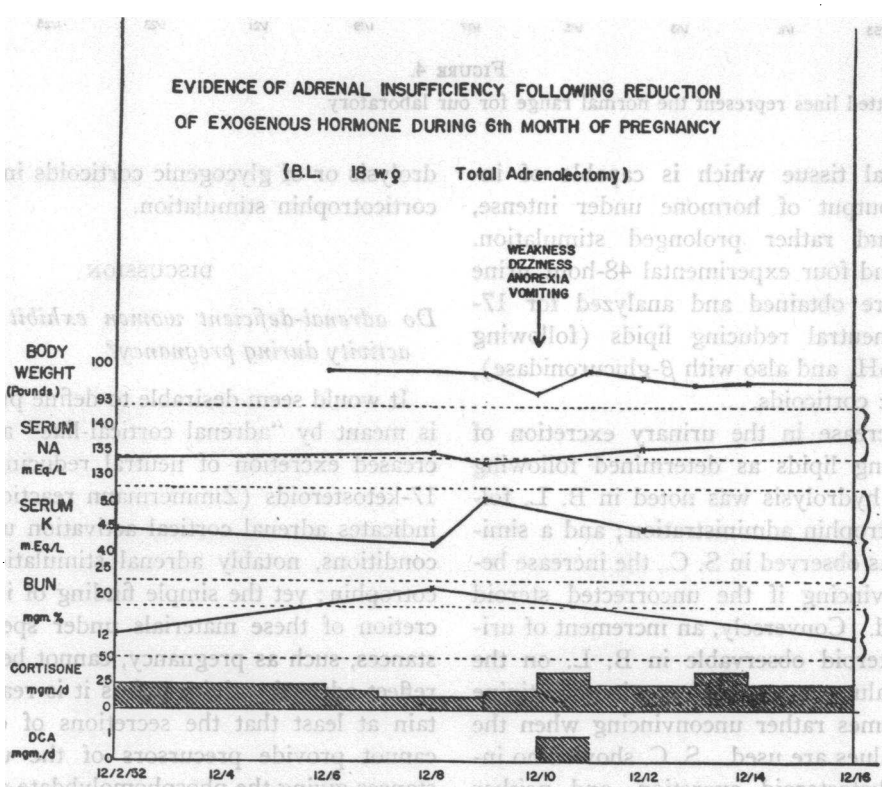

FIGURE 3.

Dotted lines represent the normal range for our laboratory.

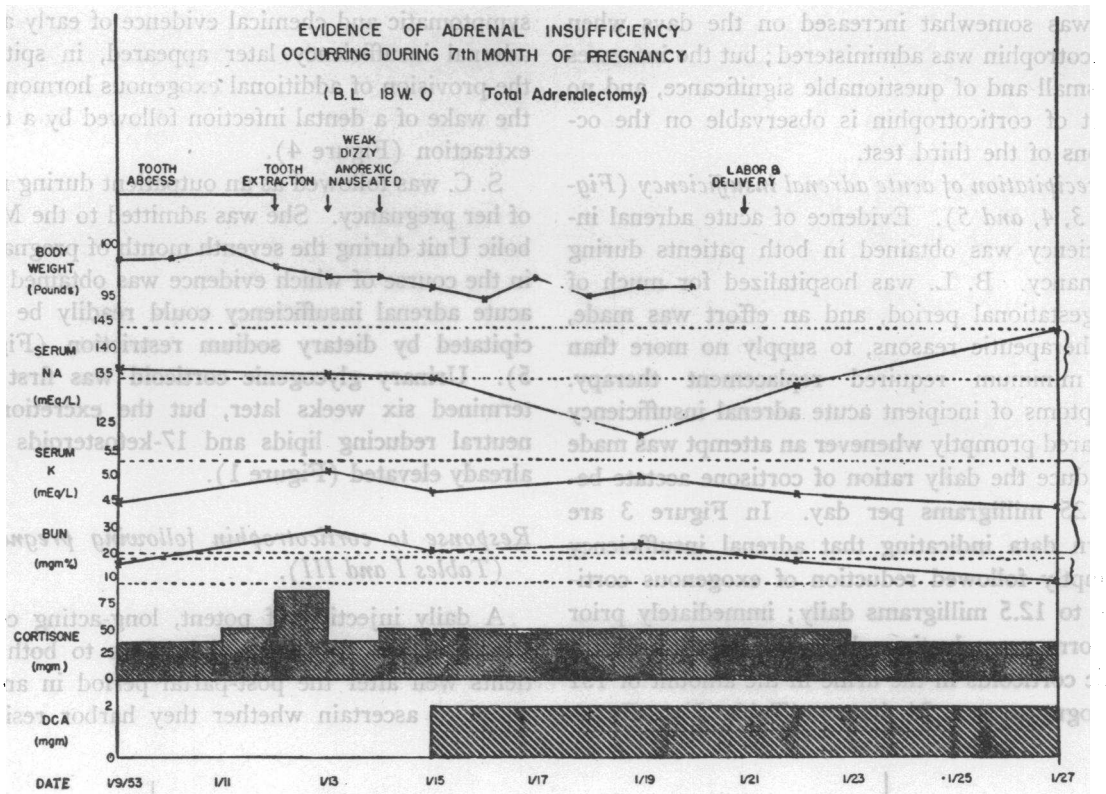

FIGURE 4.

Dotted lines represent the normal range for our laboratory.

adrenal cortical tissue which is capable of increasing its output of hormone under intense, continuous, and rather prolonged stimulation. One control and four experimental 48-hour urine collections were obtained and analyzed for 17-ketosteroids, neutral reducing lipids (following hydrolysis at pH_1 and also with β-glucuronidase), and glycogenic corticoids.

A small increase in the urinary excretion of neutral reducing lipids as determined following glucuronidase hydrolysis was noted in B. L. following corticotrophin administration; and a similar increase was observed in S. C., the increase being more convincing if the uncorrected steroid values are used. Conversely, an increment of urinary 17-ketosteroid observable in B. L. on the basis of the values corrected for urine creatinine excretion becomes rather unconvincing when the uncorrected values are used. S. C. showed no increase of 17-ketosteroid excretion, and neither patient increased her output either of neutral reducing lipids as determined following acid hydrolysis or of glycogenic corticoids in response to corticotrophin stimulation.

DISCUSSION

Do adrenal-deficient women exhibit adrenal-like activity during pregnancy?

It would seem desirable to define precisely what is meant by "adrenal cortical-like" activity. Increased excretion of neutral reducing lipids and 17-ketosteroids (Zimmermann reaction) certainly indicates adrenal cortical activation under certain conditions, notably adrenal stimulation by corticotrophin; yet the simple finding of increased excretion of these materials under special circumstances, such as pregnancy, cannot be assumed to reflect adrenal activity unless it is reasonably certain at least that the secretions of other glands cannot provide precursors of the urinary substances giving the phosphomolybdate and Zimmermann reactions. For example, an increase in the urinary excretion of 17-ketosteroids derived from

TABLE III

Effect of depot (intramuscular) corticotrophin upon urinary steroid excretion and circulating eosinophils in adrenal-deficient women following pregnancy

Pt.	Date	ACTH* USP units	Urine creatinine Gm./24 hr.	Steroid (mg./24 hr.) Neutral reducing lipids pH1	Steroid (mg./24 hr.) Neutral reducing lipids G'dase	Steroid (mg./24 hr.) 17-KS	Steroid (mg./24 hr.)/Creatinine (Gm./24 hr.) Neutral reducing lipids pH1	Steroid (mg./24 hr.)/Creatinine (Gm./24 hr.) Neutral reducing lipids G'dase	Steroid (mg./24 hr.)/Creatinine (Gm./24 hr.) 17-KS	Eosinophils per mm.³	Eosinophils Date
	1953										1953
S. C.†	6/20–22	0	.73	2.2	3.1	1.6	3.0	4.2	2.2	45	6/22
	6/22–24	60	1.08	2.0	4.5	3.8	1.9	4.2	3.5		
	6/24–26	60	.98	2.5	7.9	3.4	2.6	8.1	3.5		
	6/26–28	60	1.30	3.5	7.5	2.6	2.7	5.8	2.0	64	6/29
	6/28–30	60	1.25	3.0	6.0	2.2	2.4	4.8	1.8	55	6/30
B. L.‡	5/31–6/2	0	.98	3.7	8.4	5.6	3.8	8.6	5.7	155	6/2
	6/2–4	60	.85	3.4	11.6	5.1	4.0	13.7	6.0	255	6/4
	6/4–6	60	.74	2.9	10.8	4.7	3.9	14.6	6.4	171	6/5
	6/6–8	60	.92	3.2	13.2	7.0	3.5	14.3	7.6	137	6/6
	6/8–10	60	.80	2.7	12.6	6.4	3.4	15.8	8.0	160	6/10

* H. P. Acthar Gel, supplied by Dr. C. J. O'Donovan of the Armour Laboratories.
† Thirteen months post-partum.
‡ Four and one-half months post-partum. Patient received, throughout the period shown, 37 milligrams of oral cortisone acetate daily, supplied by Dr. Elmer Alpert of Merck and Company.

an interstitial cell tumor would neither reflect adrenal activity nor imply the secretion of compounds possessing the characteristic structural or biologic properties of the alpha-ketolic hormones of the adrenal cortex.

It is proposed, therefore, to understand by "adrenal-like" activity the secretion of compounds with physiological properties supposed to be possessed exclusively or at least predominantly by the C-21 alpha-ketolic steroid hormones of the adrenal

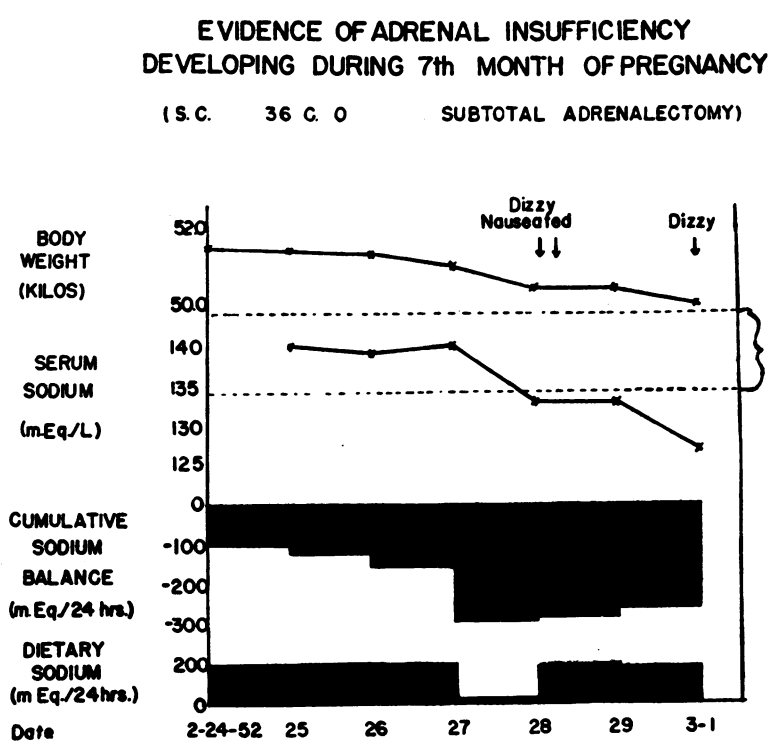

FIGURE 5.

Dotted lines represent the normal range for our laboratory.

cortex. It would appear that any attempt to establish the development of adrenal cortical-like activity during pregnancy in adrenal-deficient women, in this sense, must start with one or more of the three following findings: 1) augmented recovery from biological fluids, during pregnancy in such women, of compounds which can be shown to have the chemical structure or characteristic physiologic activity of C-21 alpha-ketolic steroids, or else of compounds which can be derived from such steroids and from no others; 2) demonstration of a temporary capacity to respond during pregnancy to corticotrophin administration in terms of acceptable indices of augmented excretion of C-21 alpha-ketolic steroids; 3) demonstration of conspicuous protection, during the pregnant state, against the development of acute adrenal insufficiency.

1) The data (Figures 1 and 2) confirm the observation of others that adrenal-deficient women excrete an increased quantity of 17-ketosteroids and of neutral reducing lipids in the urine during pregnancy. However, in view of the non-specificity of the Zimmermann and phosphomolybdate reactions, and of the augmentation and diversification of the function of at least several of the endocrine organs during pregnancy, it is exceedingly difficult to exclude the possibility that hormones other than C-21 alpha-ketolic steroids elaborated by the ovary or placenta might serve as the precursors for urinary compounds giving these reactions. One of us (7) has specifically pointed out that the Zimmermann reaction, by which 17-ketosteroids are usually estimated, is given by the pregnanolones, which derive from progesterone and appear in greatly augmented quantity in pregnancy urine, as well as by other 3- and 20-ketosteroids. It is therefore open to question whether the increased urinary elimination of substances giving the Zimmermann reaction necessarily indicates the development of adrenal-like activity in pregnancy. Indeed, no increase in 17-ketosteroid excretion is commonly found in normal pregnancy when the more specific antimony trichloride reaction is used to estimate 17-ketosteroids (7), though in one Addisonian who became pregnant both methods indicated increased 17-ketosteroid elimination (6).

If the increased urinary excretion of neutral reducing lipids during pregnancy reflects an increased secretion of C-21 alpha-ketolic steroids, one might expect that the urinary values for neutral reducing lipids would parallel those for glycogenic corticoids throughout pregnancy; but the parallelism is not impressive in normal pregnant women (7). Furthermore, the phosphomolybdate reaction, which was used for the determination of neutral reducing lipids in our studies as well as in those of Jailer and Knowlton, is not specific for C-21 alpha-ketolic steroids; it is given, for example, by any substance possessing an alpha-beta unsaturated 3-ketone group (18), which is present in progesterone. It is true that progesterone has never been found in urine, and that the ketone function has been reduced in its known urinary derivatives. These, however, represent less than 20 per cent of its degradation products (19), and it is entirely possible that hitherto unidentified metabolites of progesterone could be responsible, in some part, for the increase in the excretion of neutral reducing lipids observed in these patients.

The glycogenic corticoids, however, can scarcely be supposed to derive from any source other than the 11-oxygenated C-21 alpha-ketolic steroids characteristically present in the secretion of the adrenal cortex. The increased elimination of these substances in the urine of our patients during pregnancy (Table I) therefore constitutes convincing evidence for increased endogenous production of such compounds, unless it can be supposed that a higher proportion of secreted cortical hormone reaches the urine in active form during pregnancy, owing to some alteration during that state of the usual pathways of steroid metabolism.

2) Jailer and Knowlton (1) observed a 40 per cent decrease in circulating eosinophils on three occasions four hours following a test injection of epinephrin (twice, subcutaneously) and corticotrophin (once, intramuscularly) during the latter part of pregnancy in an adrenal deficient woman; on repetition of the tests following pregnancy, no similar decrease was observed. These findings were interpreted as indicating a temporary capacity in the pregnant adrenal-deficient woman to exhibit adrenal or adrenal-like response to agents capable of stimulating the adrenal cortex.

A 50 per cent reduction of circulating eosinophils (20) has generally been taken to represent

the minimum decrease constituting a positive response to the four-hour ACTH test; and a recent statistical study (21) locates the minimum normal decrease at 60 per cent. An eosinophil decrease of 40 per cent in the four-hour ACTH test may well fall within the range of changes which can be ascribed to the combined effects of random fluctuations of the eosinophil count and of enumeration error. A similar objection may also be urged against the significance attached to the eosinophil responses to epinephrin; moreover, subsequent work has indicated that epinephrin may depress the eosinophil count by a mechanism other than adrenal cortical activation (22). In both of our patients, near maximal stimulation with intravenous corticotrophin failed to elicit evidence of any consequential adrenal-like response during pregnancy (Table II).

3) Available reports of the effect of pregnancy upon the survival of adrenalectomized animals (2–4, 23–25) throw little light upon the possible development of adrenal-like activity in human pregnancy. Any prolongation of survival which might be observed as a result of pregnancy in the adrenalectomized animal might perhaps be attributed to the beneficial effects of certain sex hormones rather than to adrenal-like activity; for estrus in dogs and cats (26, 27) and progesterone in rats and ferrets (28–31) have been reported to prolong the survival of adrenal-deficient animals. Actually, however, while the survival of adrenalectomized bitches is reported (3) to be prolonged by pregnancy, a similar report for cats (2) could not be confirmed by other investigators (23, 24); and experiments in the white rat (4, 25) are also conflicting. The lack of agreement on this subject among physiologists, and the possibility of differences between species, appear to compel reliance upon clinical studies for the elucidation of the effect of pregnancy upon the physiology of human adrenal deficiency.

Death from adrenal insufficiency occurring in association with pregnancy was frequent in Addisonians before salt and hormone therapy were developed; and while the fatalities commonly followed the stress of delivery or abortion, it appears that the condition of the untreated Addisonian may deteriorate progressively throughout gestation (32). The literature dealing with pregnancy complicated by Addison's disease, which has been reviewed to 1950 (6, 33), furnishes no evidence that hormone requirement regularly decreases at any time during pregnancy, substitution therapy having been considered necessary or desirable throughout gestation in most cases. The first trimester with its associated vomiting is especially hazardous even to the steroid-treated Addisonian (34, 35), but also in the third trimester extra therapy may be required (6), and adrenal crisis has been reported to follow dietary salt reduction and feeding of potassium in the seventh month (36). The vulnerability of our patients to adrenal insufficiency during the latter part of pregnancy (Figures 3, 4, and 5) is thus in accord with the experience of others.

Evidence to suggest the development of adrenal-like activity during pregnancy in adrenal-deficient women, then, is confined essentially to the finding that the urinary excretion of steroids commonly used as indices of adrenal cortical secretion, and especially of steroids exhibiting the biological activity of 11-oxygenated alpha-ketolic steroids, is enhanced during pregnancy in such women. The central problem in interpreting the data is the reconciliation of the vulnerability to adrenal insufficiency of the adrenal-deficient pregnant women with the concurrent high values for urinary excretion of glycogenic corticoids associated with pregnancy in such women. Since adrenal insufficiency in the physiologic sense results from inadequacy of hormone in relation to need, its appearance in our patients means only that the quantity of hormone available to them at the time (endogenous plus exogenous) was inadequate to their needs. If pregnancy itself very greatly increases the requirement for adrenal hormone, the augmented urinary steroid excretion of glycogenic corticoids might faithfully reflect an increased secretion of C-21 alpha-ketolic steroids, the adrenal insufficiency representing an increase of need in excess of increase of supply of hormone. On the other hand, if pregnancy does not strikingly increase basal hormone requirement, one would have to infer that the urinary steroid excretion of glycogenic corticoids by these patients does not reliably reflect the availability of alpha-ketolic steroids, but rather an increase in the proportion of available (secreted plus exogenous) hormone recovered in the urine; for it is difficult to suppose that a secretion rate corresponding to that

found in Cushing's disease would not be more than adequate to protect against the mild stress of salt withdrawal in S. C.,[4] or the more considerable stress of dental infection and tooth extraction in B. L.; nor could one otherwise account at all for the need for exogenous hormone exhibited by B. L. during her pregnancy.

A definitive choice between these alternatives is probably not warranted at present; but some evidence can be urged in support of the view that increased excretion of urinary steroids during pregnancy in these women may be attributable to alterations of the metabolism of secreted or administered steroids rather than an increased rate of secretion. Dobriner, Lieberman, Rhoads, and Taylor (37) found that the two 11-oxygenated 17-ketosteroids normally present in the urine of nongravid women are excreted in much smaller quantity as pregnancy progresses. As these compounds are indubitably derived from 11-oxygenated alpha-ketols ("glucocorticoids") secreted by the adrenal cortex (38) their near disappearance from the urine in normal pregnancy would appear to suggest that pregnancy is associated with hindrance to the oxidative removal of the alpha-ketol side chain of adrenal steroids. A decrease in the proportion of available alpha-ketolic steroid disposed of in this fashion would be consistent with an enhancement of the proportion of available alpha-ketol appearing as such (*i.e.*, as neutral reducing lipids and glycogenic corticoids) in the urine.

Moreover clinical experience with B. L. is somewhat difficult to reconcile with the view that very large quantities of adrenal steroids were being secreted endogenously by this patient. As shown in Figure 3, reduction of exogenous cortisone from 25 to 12.5 milligrams daily resulted in the prompt appearance of manifestations of adrenal insufficiency. If her total basal hormone requirement amounted to about 25 milligrams per day, a reduction of 12.5 milligrams per day would be expected to result promptly in the appearance of adrenal insufficiency; but if her requirement (and supply) of cortisone equivalent were many times greater, it seems surprising that withdrawal of so relatively small a quantity would be followed so promptly by symptomatic and chemical signs of adrenal insufficiency.

It is true that normal pregnant women are reported (39) to exhibit an increased concentration of "17-hydroxycorticoids" in the blood. Whether a similar increase is observable in pregnant adrenal-deficient women is not known; but it may be pointed out that the finding does not in any case afford conclusive proof of hyperadrenocorticism during pregnancy. If, as seems possible, pregnancy results in inhibition of the oxidative removal of the alpha-ketolic side chain, secreted 17-hydroxy alpha-ketolic steroids might be expected to survive for a longer period (and, hence, to be present in higher concentration in the blood) in a form detectable by the Porter-Silber reaction, though loss of biologic activity might well result from reduction of ring A or changes elsewhere in the steroid molecule.

There is thus nothing to disprove, and a good deal of circumstantial evidence to justify the suggestion, that the urinary steroid excretion in adrenal-deficient pregnant women may not reliably establish that any consequential increase in the supply of adrenal-like hormone occurs in such women during pregnancy.

With regard to the urinary steroid data in our patients there appear, therefore, to be two possible alternative interpretations neither of which can be definitely ruled out[5] at present: 1) The

[4] We have forborne to interject into the discussion the further complication of a distinction between the "glucocorticoids" (11-oxygenated alpha-ketolic steroids), whose secretion alone is reflected in the glycogenic corticoids, and the "mineralocorticoids." Yet in the instance of adrenal insufficiency precipitated by curtailment of sodium intake (Figure 5) one might raise the objection that a very large secretion of glucocorticoids might fail to protect against salt deprivation. A need for "mineralocorticoids" cannot, however, be invoked to account for B. L.'s requirement for small quantities of *cortisone* during her pregnancy. Nor can a need for mineralocorticoids very plausibly explain the precipitation of adrenal insufficiency by infection and surgery in a patient receiving both desoxycorticosterone and cortisone (Figure 4); for the 11-17-oxygenated steroids are presumably the principal agents conferring protection against stresses of this kind.

[5] Some guidance in selecting the correct explanation might possibly be anticipated from a quantitative comparison of the steroid excretion data from normal pregnant women with those from adrenal-deficient pregnant women. Any such hope is disappointed, for the individual variation in the excretion of glycogenic corticoids (7) and of neutral reducing lipids at any time during

urinary steroid values reflect a greatly increased rate of secretion of adrenal or adrenal-like steroids during pregnancy, the increased availability of such steroids being ineffective in protecting against adrenal insufficiency because of a concomitant marked increase of the requirement for adrenal hormone; or 2) the urinary steroid values are misleading when accepted at face value as indicators of the rate of adrenal secretion during pregnancy; the high values for the chemically determined steroids may be partly ascribable to nonspecific chromogens, and the excretion of neutral reducing lipids and glycogenic corticoids may reflect an increase in the proportion of available hormone eliminated in the urine as alpha-ketols.

Are any adrenal-like steroids which may be secreted during pregnancy by adrenal-deficient women necessarily of extra-adrenal origin?

Even if one were to adopt the view that the augmented excretion of urinary steroid during pregnancy indicates increased secretion of alpha-ketolic steroids, the inference that such compounds must be secreted by some organ other than the maternal adrenal cortex will still not be firmly established until it is wholly clear that the latter organ cannot be their source. Even in the "totally adrenalectomized" patient the possibility of residual adrenal cortical tissue cannot be wholly dismissed; and in the individual rendered adrenal-deficient by subtotal adrenalectomy (17) as well as in the patient with Addison's disease (40) there is evidence that measurable though subnormal cortical function can be present.

We know of no evidence which can exclude the possibility that an adrenal remnant or rest might not be affected by pregnancy in such a fashion as to increase its output of adrenal hormone. It is true that a barely adequate adrenal remnant should be at all times under near-maximal stimulation by endogenous corticotrophin, according to current concepts of the homeostatic mechanism regulating adrenal cortical secretion (41), and our two patients exhibited little evidence of adrenal response to 24 and 48-hour corticotrophin tests during pregnancy (Table II). It is, however, conceivable that stimulation over months by corticotrophin hypothetically elaborated in large excess during pregnancy might result in actual hypertrophy or an adrenal remnant with consequent increase in the secretion of adrenal hormone. An attempt (Tables I and III) to simulate such a putative effect of pregnancy, conducted in our patients in the post-partal state by continuous stimulation over an 8-day period with depot corticotrophin, is at best suggestive of a modest response of the adrenal cortex to corticotrophin; a large increase of steroid excretion, such as had accompanied pregnancy, could not be reproduced. Certainly, however, the possibility of such activation during pregnancy under the influence of more prolonged stimulation by corticotrophin, or perhaps by some separate adrenal-growth-promoting factor (42), has not been excluded.

It seems most unlikely that the fetal adrenal makes any appreciable contribution of adrenal cortical hormone to the maternal organism during pregnancy. The newborn infant, whether born of a normal (43) or an adrenal-deficient mother (1), excretes only very small quantities of urinary steroids, including biocorticoids, and is resistant to corticotrophin stimulation (44). Jailer and Knowlton (1) suggested that the placenta might constitute an extra-adrenal source of "adrenal-like" hormone; and placentas of animal and of human origin have subsequently been reported to contain biologically active corticoid material (45, 46). However, there is no evidence to exclude the possibility that the material is simply stored there.

Since it is not possible to state with assurance that pregnancy causes increased secretion of C-21 alpha-ketolic steroids in adrenal-deficient women, nor that such secreted steroids cannot originate in maternal adrenal tissue, satisfactory proof of any extra-adrenal source of adrenal hormone is lacking at present.

From the point of view of practical management of the adrenal-deficient woman who becomes pregnant, it is imperative to emphasize the fact that gestation does not decrease the hazard of

pregnancy is very large, and the data are few; in consequence no significant comparison can be made. One can state only that available data do not permit a demonstration that the excretion of neutral reducing lipids and of glycogenic corticoids differ significantly in pregnant adrenal-deficient patients as compared with normal pregnant women. No data are available in our laboratory concerning the excretion of 17-ketosteroids during normal pregnancy.

acute adrenal insufficiency. The physiological studies conducted in our patients, as well as numerous reports in the literature, necessitate the conclusion that any extra C-21 alpha-ketolic steroids, of whatever source, which may become available to the severely adrenal-deficient woman in pregnancy are unable to prevent the prompt appearance of acute adrenal insufficiency at any time during pregnancy when specific therapy is withheld. While pregnancy of itself appears to have little effect upon hormone requirement, its complications and the stress of delivery will ordinarily require additional replacement therapy exactly as will any stress in a non-pregnant adrenal-deficient person.

SUMMARY

1. Increased urinary elimination of 17-ketosteroids (Zimmermann reaction) and of neutral reducing lipids is regularly found during pregnancy in adrenal-deficient women. The increase is observable as early as the beginning of the second trimester, and is progressive to term.

2. Urinary excretion of glycogenic corticoids was increased three- to four-fold during the latter half of pregnancy in two adrenal-deficient women.

3. Neither patient was protected by the pregnant state against the development of acute adrenal cortical insufficiency, and no decrease in hormone requirement was noted during pregnancy in the one patient requiring substitution therapy.

4. Neither patient developed during pregnancy any striking capacity to respond to near-maximal stimulation by intravenous corticotrophin over a 24- or 48-hour test period.

5. More prolonged (8-day) stimulation with depot corticotrophin following pregnancy failed in both subjects to reproduce the large increases of urinary steroids which accompanied pregnancy.

6. The values for the urinary excretion of 17-ketosteroids, neutral reducing lipids and particularly glycogenic corticoids observed in adrenal-deficient pregnant women suggest that such women acquire during pregnancy an endogenous source of large quantities of C-21 alpha-ketolic steroid hormones; but it is possible, for reasons which have been discussed, that these commonly used indices of adrenal secretory activity become misleading during pregnancy, and that little or no increase of the secretion of alpha-ketolic steroids accompanies pregnancy in adrenal-deficient women.

7. If adrenal-deficient women do develop an enhanced endogenous supply of adrenal-like alpha-ketolic steroids when they become pregnant, pregnancy must increase their requirement for hormone together with the supply since such women remain vulnerable to adrenal insufficiency during gestation. The source of supply of any such steroids is apparently not the fetal adrenal; that any organ other than residual material adrenal tissue can be implicated awaits demonstration.

ACKNOWLEDGMENTS

The authors wish to acknowledge the aid and support of Drs. William A. Jeffers and Carl Bachman in the conduct of these studies, and their kindness in reviewing the manuscript.

Technical assistance was rendered by Mrs. Irmgaard Landolt, Mr. Walter Applin, Mrs. Helen Bulaschenko, Mrs. Hana Conover, and Miss Katherine Macatsoris.

Through the kindness of Dr. Elmer Alpert, the Merck Company sustained hospitalization costs for B. L. throughout much of her pregnancy.

We are indebted to Dr. James Walker for the sodium analyses used for the construction of Figure 5.

REFERENCES

1. Jailer, J. W., and Knowlton, A. I., Simulated adrenocortical activity during pregnancy in an Addisonian patient. J. Clin. Invest., 1950, **29**, 1430.
2. Stewart, H. A., On certain relations between lipoid substances and the adrenals. Tr. 17th Internatl. Med. Congress, London, 1913, Section III, pt. 2, p. 173.
3. Rogoff, J. M., and Stewart, G. N., Studies on adrenal insufficiency. III. The influence of pregnancy upon the survival period in adrenalectomized dogs. Am. J. Physiol., 1927, **79**, 508.
4. Frior, W. M., and Grollman, A., Studies on the adrenal. I. Adrenalectomy in mammals with particular reference to the white rat (Mus norvegicus). Am. J. Physiol., 1933, **103**, 686.
5. Samuels, L. T., Evans, G. T., and McKelvey, J. L., Ovarian and placental function in Addison's disease. Endocrinology, 1943, **32**, 422.
6. Knowlton, A. I., Mudge, G. H., and Jailer, J. W., Pregnancy in Addison's disease. Report of four patients. J. Clin. Endocrinol., 1949, **9**, 514.
7. Venning, E. H., Adrenal function in pregnancy. Endocrinology, 1946, **39**, 203.
8. Wallace, W. M., Holliday, M., Cushman, M., and Elkinton, J. R., The application of the external

standard flame photometer to the analysis of biologic material. J. Lab. & Clin. Med., 1951, 37, 621.
9. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, 158, 581.
10. Karr, W. G., A method for the determination of blood urea nitrogen. J. Lab. & Clin. Med., 1924, 9, 329.
11. Holtorff, A. F., and Koch, F. C., The colorimetric estimation of 17-ketosteroids and their application to urine extracts. J. Biol. Chem., 1940, 135, 377.
12. Heard, R. D. H., Sobel, H., and Venning, E. H., The neutral lipide-soluble reducing substances of urine as an index of adrenal cortical function. J. Biol. Chem., 1946, 165, 699.
13. Venning, E. H., Kazmin, V. E., and Bell, J. C., Biological assay of adrenal corticoids. Endocrinology, 1946, 38, 79.
14. Manners, T., A new diluting fluid for the eosinophil count. Brit. M. J., 1951, 1, 1429.
15. Zintel, H. A., Wolferth, C. C., Jeffers, W. A., Hafkenschiel, J. H., and Lukens, F. D. W., Ninety-five percent subtotal adrenalectomy for essential hypertension. Surgical Forum. (Proc. 36th Clin. Congress Am. Coll. Surg.) Philadelphia, W. B. Saunders Company, 1950, p. 556.
16. Renold, A. E., Jenkins, D., Forsham, P. H., and Thorn, G. W., The use of intravenous ACTH: A study in quantitative adrenocortical stimulation. J. Clin. Endocrinol. & Metab., 1952, 12, 763.
17. Hills, A. G., Webster, G. D., Rosenthal, O., Dohan, F. C., Richardson, E. M., Zintel, H. A., and Jeffers, W. A., Quantitative evaluation of primary adrenal cortical deficiency in man. Am. J. Med., 1954, 16, 328.
18. Heard, R. D. H., and Sobel, H., Steroids. VIII. A colorimetric method for the estimation of reducing steroids. J. Biol. Chem., 1946, 165, 687.
19. Marrian, G. F., Some aspects of progesterone metabolism. Recent Progress in Hormone Research, New York, Academic Press, 1949, vol. IV, p. 3.
20. Thorn, C. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., A test for adrenal cortical insufficiency. The response to pituitary adrenocorticotrophic hormone. J. A. M. A., 1948, 137, 1005.
21. Shands, H. C., and Bartter, F. C., A statistical analysis of the "ACTH test": Changes in the eosinophil count in normal and in psychoneurotic subjects. J. Clin. Endocrinol. & Metab., 1952, 12, 178.
22. Thorn, G. W., The eosinophil, ACTH, epinephrin, and stress. Am. J. Med., 1953, 14, 139.
23. Corey, E. L., Survival period in the pregnant and lactating cat following adrenal extirpation. Proc. Soc. Exper. Biol. & Med., 1928, 25, 167.
24. Rogoff, J. M., and Stewart, G. N., Studies on adrenal insufficiency. VIII. The survival period of untreated adrenalectomised cats. Am. J. Physiol., 1929, 88, 162.
25. Carr, J. L., Effects of ovaries in various stages of activity and of pregnancy upon adrenalectomized rats. Proc. Soc. Exper. Biol. & Med., 1931, 29, 128.
26. Rogoff, J. M., and Stewart, G. N., Studies on adrenal insufficiency. VI. The influence of "heat" on the survival period of dogs after adrenalectomy. Am. J. Physiol., 1928, 86, 20.
27. Swingle, W. W., Parkins, W., Taylor, A. R., Hays, H. W., and Morrell, J. A., Effect of estrus (pseudopregnancy) and certain pituitary hormones on the life-span of adrenalectomized animals. Am. J. Physiol., 1937, 119, 675.
28. Gaunt, R., and Hays, H. W., The life-maintaining effect of crystalline progesterone in adrenalectomized ferrets. Science, 1938, 88, 576.
29. Gaunt, R., Nelson, W. O., and Loomis, E., Cortical hormone-like action of progesterone and non-effect of sex hormones on "water intoxication." Proc. Soc. Exper. Biol. & Med., 1938, 39, 319.
30. Greene, R. R., Wells, J. A., and Ivy, A. C., Progesterone will maintain adrenalectomized rats. Proc. Soc. Exper. Biol. & Med., 1939, 40, 83.
31. Schwabe, E. L., and Emery, F. E., Progesterone in adrenalectomized rats. Proc. Soc. Exper. Biol. & Med., 1939, 40, 383.
32. Fitz-Patrick, G., Addison's disease complicating pregnancy, labor, or the puerperium. Surg., Gynec. & Obst., 1922, 35, 72.
33. Brent, F., Addison's disease and pregnancy. Am. J. Surg., 1950, 79, 645.
34. Thorn, G. W., Dorrance, S. S., and Day, E., Addison's disease. Evaluation of synthetic desoxycorticosterone acetate therapy in 158 patients. Ann. Int. Med., 1942, 16, 1053.
35. Wells, C. N., and Wesp, J. E., Management of Addison's disease during pregnancy with cortisone and desoxycorticosterone. Am. J. Obst. & Gynec., 1952, 63, 925.
36. Galloway, C. E., Sutton, D. C., and Ashworth, J., An acute crisis of suprarenal insufficiency complicating pregnancy. Am. J. Obst. & Gynec., 1940, 40, 148.
37. Dobriner, K., Lieberman, S., Rhoads, C. P., and Taylor, H. C., Jr., The urinary excretion of 17-ketosteroids in pregnancy. Obst. & Gynec. Surv., 1948, 3, 677.
38. Dobriner, K., Lieberman, S., Wilson, H., Dunham, M., Sommerville, I. F., and Rhoads, C. P., Adrenal function and steroid excretion in disease. Proc. Second Clin. ACTH Conf., Blakiston, 1951, vol. I, p. 65.
39. Gemzell, C. A., Blood levels of 17-hydroxycorticosteroids in normal pregnancy. J. Clin. Endocrinol. & Metab., 1953, 13, 898.
40. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., Clinical studies with pituitary adrenocorticotropin. J. Clin. Endocrinol., 1948, 8, 15.

41. Sayers, G., The adrenal cortex and homeostasis. Physiol. Rev., 1950, **30**, 241.
42. Liddle, G. W., Rinfret, A. P., Richard, J., and Forsham, P. H., Functional evidence in man for the existence of an adrenal growth factor distinct from ACTH. J. Clin. Endocrinol. & Metab., 1953, **13**, 842.
43. Venning, E. H., Randall, J. P., and Gyorgy, P., Excretion of glucocorticoids in the newborn. Endocrinology, 1949, **45**, 430.
44. Read, C. N., Venning, E. H., and Ripstein, M. P., Adrenal cortical function in newly-born infants. J. Clin. Endocrinol., 1950, **10**, 845.
45. Johnson, R. H., and Haines, W. J., Extraction of adrenal cortex hormone activity from placental tissue. Science, 1952, **116**, 456.
46. DeCourcy, C., Gray, C. H., and Lunnon, J. B., Adrenal cortical hormones in human placenta. Nature, 1952, **170**, 494.