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Research Article



# ON THE RATE OF HYDROCORTISONE CLEARANCE FROM PLASMA IN PREGNANT WOMEN AND IN PATIENTS WITH LAENNEC'S CIRRHOSIS *

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The question of whether or not there is an increase in adrenal cortical secretion during pregnancy remains unsettled. Morphologic studies of the adrenal during gestation have revealed enlargement of the gland in some species (1) and no enlargement in others (2). In the human female, several groups of investigators have observed a progressive rise in the plasma 17-hydroxycorticosteroid (17-OH-corticosteroid) level during pregnancy (3–8). Paper chromatographic studies (9), confirmed in this laboratory (10), have indicated that the elevated plasma 17-OH-corticosteroid level is due to an increase in circulating hydrocortisone, and not to a nonspecific chromogen. Measurement of urinary 17-OH-corticosteroids has given equivocal results. Jayle, Desgrez, Serpicelli and Rozeg (11), Devis (12), Mills (13), and Appleby and Norymberski (14) found that the rise in urinary 17-OH-corticosteroids and 17-ketogenic steroids during pregnancy was of only moderate degree. Contradictory results have been obtained from studies of urinary 11-oxygenated 17-ketosteroids, the principal C19 metabolites of hydrocortisone (15). Dobriner, Lieberman, Rhoads and Taylor (16) and Davis and Plotz (17) reported that urinary 11-oxygenated 17-ketosteroids did not increase with advancing gestation, while Huis in't Veld (18) and Birke, Gemzell, Plantin and Robbe (19) claimed that these steroids were present in increased quantity in the third trimester.

In an attempt to reconcile the apparent discrepancy between the finding of elevated plasma and of relatively normal urinary 17-OH-corticosteroid levels in pregnancy, Migeon, Bertrand and Wall investigated the rate of disappearance of injected 4-$C^{14}$-hydrocortisone from plasma of pregnant women near term and found it to be delayed (20). These workers also observed a reduced rate of appearance of plasma and urine 17-OH-corticosteroid conjugates (glucuronides) after the administration of labeled hydrocortisone. These data suggested that the co-existence of high plasma (unconjugated) 17-OH-corticosteroid values and normal urine (unconjugated plus conjugated) 17-OH-corticosteroid values might be partly explained by the presence in both plasma and urine of a greater proportion of free hydrocortisone and a smaller proportion of conjugated hydrocortisone metabolites than normal (20). Devis (12) and Gray (21) have reported increased amounts of free 17-OH-corticosteroid in the urine of pregnant women.

The present report describes studies upon the rate of hydrocortisone clearance from plasma in late pregnancy and in Laennec's cirrhosis, a condition in which hydrocortisone disposal rate is reportedly delayed (22–24). The data suggest that a delay in hydrocortisone clearance may not completely account for the elevated plasma 17-OH-corticosteroid levels observed in the pregnant state.

## MATERIALS AND METHODS

*Patients.* Subjects were pregnant women in the third trimester of pregnancy who were not acutely ill and who showed no evidence of rheumatic heart disease, diabetes mellitus or pre-eclampsia. The women were hospitalized for premature rupture of the membranes or in anticipation of elective Caesarian section. Patients with Laennec's cirrhosis were six men and one woman with the typical clinical and laboratory features of parenchy-

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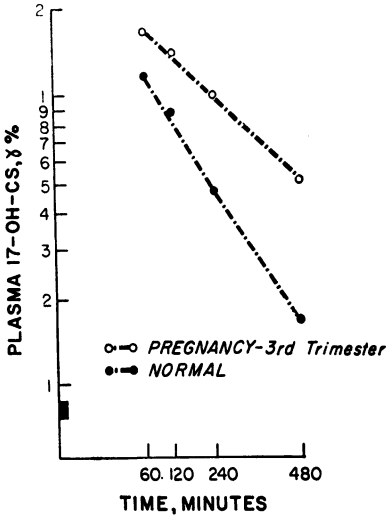


FIG. 1. SEMILOGARITHMIC PLOT OF THE RATE OF FALL OF PLASMA 17-OH-CORTICOSTEROID LEVELS AFTER INTRAVENOUS INJECTION OF 100 MG. HYDROCORTISONE

Average per cent decrease in level (per hour) for pregnant women (15 per cent) is significantly less than that for normal subjects (27 per cent) at the 1 per cent level.

mal liver disease who exhibited minimal jaundice and ascites at the time of study. Normal subjects were female hospital patients of child-bearing age who had no endocrine disease and who were not acutely ill, or presumably healthy female laboratory workers.

*Analytic methods.* Free plasma 17-OH-corticosteroid levels were determined by the Silber-Porter method (25) as modified in this laboratory (26). Plasma 17-OH-corticosteroid glucuronides (presumed to be largely the glucuronides of 3 alpha, 17 alpha, 21-tri-hydroxypregnane-11,20-dione or tetrahydrocortisone, and of 3 alpha, 11 beta, 17 alpha, 21-tetrahydroxypregnan-20-one or tetrahydrocortisol) were measured as Porter-Silber chromogen following beta-glucuronidase hydrolysis for 48 hours according to a modified Bongiovanni-Eberlein procedure (22). Free hydrocortisone was first extracted exhaustively from plasma samples with dichloromethane (three times with fivefold volumes). The method of hydrolysis is quantitative, as verified by hydrolysis of a sample of authentic tetrahydrocortisone glucuronide.[1] Following hydrolysis, the samples were treated with trichloroacetic acid (2 ml. 10 per cent trichloroacetic acid added to 10 ml. of hydrolysate) and the precipitated protein removed by centrifugation. No appreciable steroid was lost as a result of this maneuver. After centrifugation, the clear supernatant fluid was extracted with dichloromethane. Reaction with the Porter-Silber phenylhydrazine-sulfuric acid reagent did not produce the pink chromogens reported by Migeon and coworkers (20). Urinary 17-OH-corticosteroids were also measured as Porter-Silber chromogen, with and without glucuronidase hydrolysis (total and free steroid, respectively) (25).

*Procedures.* Rate of hydrocortisone clearance was studied in 21 pregnant, 7 cirrhotic and 22 normal subjects in the following manner. Hydrocortisone (in the form of the water-soluble hemisuccinate or phosphate derivatives) in a dose of 100 mg. was injected intravenously over a period of two minutes. Serial blood specimens for free plasma 17-OH-corticosteroid determination were withdrawn at 40, 60, 120, 240 and 480 minutes, and in some instances, 20 minute samples were also taken. In certain of these subjects urine was also collected during the 480 minute period of study following hydrocortisone injection, and free and conjugated urinary 17-OH-corticosteroids were measured. In two pregnant and two normal individuals serial blood samples were withdrawn after hydrocortisone injection for the measurement of free and conjugated plasma 17-OH-corticosteroids.

Steroid disappearance rate was further studied by the administration of a reduced C21 metabolite of hydrocortisone. Tetrahydrocortisone, 75 mg. (approximately 1 mg. per Kg. body weight), was dissolved in 8 ml. of ethanol and administered intravenously in 50 to 75 ml. of isotonic saline over a period of five minutes. Subjects were five pregnant women and four normal women. Blood samples for measurement of free plasma 17-OH-corticosteroid levels were withdrawn at 20, 40, 60 and 90 minutes.

Response of plasma 17-OH-corticosteroids to corticotropin was studied in a manner previously described, in which steroid determinations were made on blood samples obtained before and after a four hour intravenous infusion of 25 I.U. adrenocorticotropic hormone (ACTH) (27). Subjects were nine pregnant women and six of the seven patients with Laennec's cirrhosis.

## RESULTS

### 1. *Rate of hydrocortisone clearance from plasma during pregnancy*

Figure 1 shows the comparative rates of hydrocortisone clearance in 21 pregnant women near term and 13 normal women. The rate of hydrocortisone clearance is significantly slower in the pregnant subjects (difference in calculated slopes, pregnant *vs.* normal, $p < 0.01$). Average rate of decline of free plasma 17-OH-corticosteroid value is 15 per cent per hour in pregnancy, 27 per cent per hour in normal individuals. In a small series of women studied in the postpartum period the delayed clearance of hydrocortisone was found to persist for about six days, a period during which plasma 17-OH-corticosteroids remain elevated, although falling gradually toward normal (7, 10).

[1] Authentic tetrahydro E glucuronide was made available through the courtesy of Dr. Seymour Lieberman.

TABLE I

*Free and conjugated plasma 17-OH-corticosteroid levels following the I.V. injection of 100 mg. hydrocortisone*

| Patients | Time (*mins.*) | Plasma 17-OH-corticosteroids, μg. % 20 | 40 | 60 | 120 | 240 | 480 |
|---|---|---|---|---|---|---|---|
| Normal subjects (average of 2) | Free | 195 | 155 | 152 | 94 | 52 | 22 |
| | Conjugated* | 22 | 27 | 15 | 14 | 12 | 0 |
| Pregnant women (average of 2) | Free | 204 | 170 | 167 | 139 | 107 | 74 |
| | Conjugated | 8 | 6 | 11 | 10 | 0 | 0 |

* In normal subjects, previous studies have tended to show higher values of 17-OH-corticosteroid glucuronides than are found in this study (9, 20, 23, 24). The discrepancy cannot be entirely accounted for. A possible explanation is the fact that in this investigation the injected steroid was the succinate or phosphate derivative of hydrocortisone. The earlier studies were carried out with hydrocortisone alcohol (labeled or unlabeled) (9, 20, 23, 24). The derivatives may be metabolized differently than the free steroid. Some support for this supposition may be derived from the fact that in the present study a greater absolute and relative amount of free urinary corticosteroid was observed after hydrocortisone hemisuccinate or phosphate administration than was reported by Migeon and associates, who employed isotopic hydrocortisone alcohol (9) (see Table II).

Table I shows hydrocortisone clearance and the appearance in plasma of conjugated 17-OH-corticosteroids in two normal and two pregnant subjects. The delayed clearance of hydrocortisone in the pregnant women is accompanied by subnormal levels of 17-OH-corticosteroid glucuronides in simultaneous blood samples. The data confirm the observations of Migeon and associates (9, 20).

Table II shows the results of steroid measurements in urine following hydrocortisone injection. It will be seen that the average quantity of free 17-OH-corticosteroid found in the urine of six pregnant women (8.3 mg. per eight hours) is much larger than that found in urine of six normal subjects (4.7 mg. per eight hours). This finding also confirms the earlier studies (9, 20). The nearly equal values for conjugated urinary 17-OH-corticosteroids (16.3 and 14.6 mg. per eight hours) in pregnant and normal subjects may be in part due to measurement of a nonspecific chromogen which appears in the urine of pregnant women after enzymatic hydrolysis. Conjugated 17-OH-corticosteroids in pregnancy urine are somewhat higher than normal by this technique (10). It is of interest that Migeon and co-workers, using 4-$C^{14}$-cortisol, found only moderate reductions in urinary excretion of corticosteroid glucuronides during pregnancy (9).

TABLE II

*Free and conjugated urinary 17-OH-corticosteroids during the eight hour period following the I.V. injection of 100 mg. hydrocortisone*

| | No. of subjects | Average value of urinary steroids: Conjugated | Free | Ratio of conjugated to free steroid |
|---|---|---|---|---|
| | | *mg./8 hrs.* | | |
| Normal subjects | 6 | 14.6 | 4.7 | 3.1 : 1 |
| Pregnant women | 6 | 16.3 | 8.3 | 1.97 : 1 |

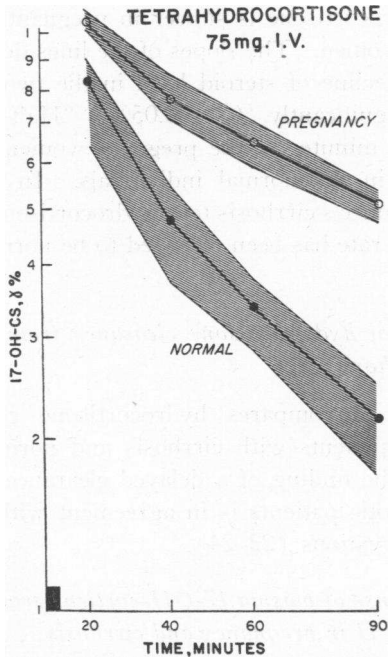


FIG. 2. SEMILOGARITHMIC PLOT OF THE RATE OF FALL OF PLASMA 17-OH-CORTICOSTEROID LEVELS AFTER INTRAVENOUS INJECTION OF TETRAHYDROCORTISONE, 75 MG.

Solid lines indicate averages for the five pregnant and four normal subjects; shaded areas indicate the extreme limits of variation for the two groups. Average per cent decrease of plasma 17-OH-corticosteroid level (per hour) for pregnant women is significantly less than that for normal subjects at the 5 per cent level.

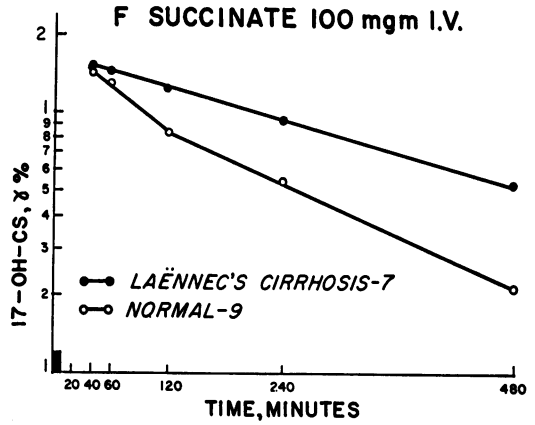


FIG. 3. SEMILOGARITHMIC PLOT OF RATE OF FALL OF PLASMA 17-OH-CORTICOSTEROID LEVELS IN SEVEN PATIENTS WITH CIRRHOSIS AND NINE NORMAL SUBJECTS

Lines represent average rates of decline.

*2. Rate of tetrahydrocortisone clearance from plasma in pregnancy*

Figure 2 shows that the average clearance of tetrahydrocortisone is slower in pregnant than in normal women. The slopes of the lines describing rate of decline of steroid level in the two groups differ significantly ($p < 0.05$). "Half-life" is about 75 minutes in the pregnant women and 48 minutes in the normal individuals. In patients with Laennec's cirrhosis tetrahydrocortisone disappearance rate has been reported to be normal (23, 24).

*3. Rate of hydrocortisone clearance from plasma in cirrhosis*

Figure 3 compares hydrocortisone clearance rates in patients with cirrhosis and normal subjects. The finding of a delayed clearance rate in the cirrhotic patients is in agreement with previous observations (22–24).

*4. Response of plasma 17-OH-corticosteroid levels to ACTH in pregnancy and cirrhosis*

Figure 4 shows the apparently exaggerated response to ACTH of plasma (free) 17-OH-corticosteroid levels in the third trimester of pregnancy (27). Plasma steroid levels are above normal before and after ACTH administration, and the average increment is significantly greater than normal ($p < 0.01$) (28). This finding is not in agreement with the observation of Migeon and associates who found essentially normal plasma 17-OH-corticosteroid response to ACTH near term (9).

Figure 5 shows that the plasma steroid response to corticotropin in the six patients with cirrhosis is normal or only slightly exaggerated. Average pre- and post-ACTH levels, 18 and 47 μg. per 100 ml., respectively, are within the normal limits (29).

## DISCUSSION

A delay in the rate of clearance of hydrocortisone from plasma of pregnant women near term appears to be well established by earlier work (9, 20) and by the present study. This observation raises two points for discussion: *1*) the problem of whether or not the slower rate of hydrocortisone clearance completely explains the elevated plasma hydrocortisone levels found in late pregnancy, and *2*) the mechanism responsible for the delay in hydrocortisone clearance.

If the assumption is made that the elevated plasma hydrocortisone level of late pregnancy is due entirely to its slower rate of disappearance from plasma, it might be reasonable to expect elevated plasma hydrocortisone levels in other clinical conditions in which hydrocortisone clearance from plasma is delayed. The data of these and earlier studies (22–24) indicate that the levels of plasma 17-OH-corticosteroids in one such condition, Laennec's cirrhosis, are normal despite a definitely reduced rate of hydrocortisone clearance. Furthermore, in contrast to the apparently excessive response of free plasma 17-OH-corticosteroid levels to ACTH in pregnancy [found to be normal by Migeon and co-workers (9)], steroid response to corticotropin appears to be essentially normal in cirrhosis. Several explanations might be offered for these differences between the findings in pregnancy and cirrhosis. First, one might suppose that adrenal secretory rate is reduced in cirrhosis, and that a moderate decrease in hydrocortisone production is obscured by a normal plasma level which in turn depends upon a delay in clearance of hydrocortisone from plasma. Such an idea receives support from the work of Samuels, Brown, Eik-Nes, Tyler and Dominguez (30). The conclusion drawn by these workers that adrenal secretory rate of hydrocortisone is reduced by about 40 per cent in cirrhosis was based on calculations derived from the slope of plasma hydrocortisone response to ACTH and

the slope of hydrocortisone disappearance rate from plasma after a loading dose of this steroid. While further experiments may prove this conclusion to be correct, it is fair to state that the method of computation employed constitutes an indirect approach to the question which can perhaps be more directly attacked by newer methods using isotopically labeled steroids (31).

Second, it might be assumed that hydrocortisone is more firmly bound to plasma proteins in pregnant women than in normal or cirrhotic subjects. This assumption could explain the elevated plasma hydrocortisone level of pregnancy and the delayed hydrocortisone disappearance rate. However, Daughaday has reported that there is no increase in corticosteroid binding during gestation (32).

Third, it is conceivable that there is a derangement in one of the homeostatic mechanisms which normally regulates the rate of ACTH release from the anterior pituitary. It is well known that the level of circulating hydrocortisone is inversely proportional to the rate of corticotropin release (33). If this regulatory mechanism were normal in pregnancy, one would expect that a higher plasma hydrocortisone level would act to inhibit ACTH release, and that, lacking the stimulation of corticotropin, the adrenal cortex would secrete less hydrocortisone, resulting finally in a normal level of plasma hydrocortisone. Such a sequence of events might explain the findings in cirrhosis.

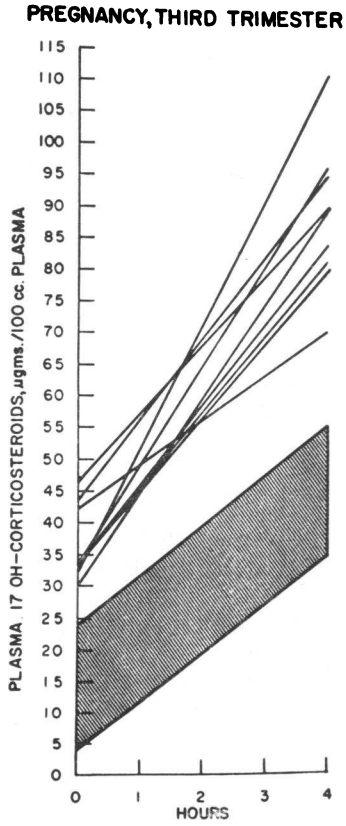


FIG. 4. RESPONSE OF PLASMA 17-OH-CORTICOSTEROID LEVELS TO ACTH IN PREGNANCY (NINE WOMEN)

Solid lines indicate responses of the pregnant women, shaded area shows extreme limits of variation in 40 normal subjects (29).

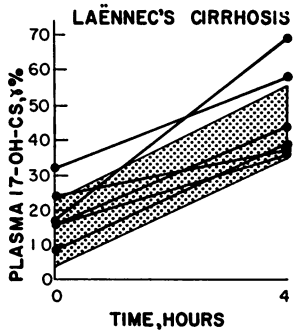


FIG. 5. RESPONSE OF PLASMA 17-OH-CORTICOSTEROID LEVELS IN SIX PATIENTS WITH CIRRHOSIS (SOLID LINES) AS COMPARED TO NORMAL RESPONSE (SHADED AREA)

The postulate of an altered homeostatic control of ACTH release in pregnancy may present a problem susceptible to investigation. Previous studies have shown that administration of 17 alpha, 21 - dihydroxy - delta$^{1,4}$ - pregnadiene - 3,20 - dione (prednisone) is capable of suppressing the apparently exaggerated response of plasma 17-OH-corticosteroids to ACTH in pregnancy (29), but the degree of suppression is less than that brought about by prednisone in normal subjects. These findings suggest that the pituitary during pregnancy can be influenced by circulating glucocorticoid levels, but that it is perhaps less sensitive than normal to this influence.

Concerning the mechanism of the delayed hydrocortisone clearance rate in pregnancy, the data on tetrahydrocortisone clearance seem pertinent. Peterson and Englert and their respective associates (23, 24) have reported that the tetrahydrocortisone clearance rate from plasma is normal in patients with cirrhosis. On the basis of these data, it has been postulated that the delayed disappearance rate of hydrocortisone in cirrhosis is due to a defect in hepatic reduction of the steroid nucleus (*i.e.*, reduction of hydrocortisone to tetrahydrocortisone and tetrahydrocortisol) and not due

to a defect in hepatic conjugation of the reduced (tetrahydro) compounds with glucuronic acid (24).[2] In this connection, Isselbacher and Axelrod showed that reduction of ring A of the steroid nucleus is an essential prerequisite for steroid glucuronide formation in liver (35). The finding in the present study of a delayed disappearance rate of injected tetrahydrocortisone in pregnancy differs from the observations in cirrhosis, but is similar to the finding of delayed tetrahydrocortisone clearance in a single patient with congenital nonobstructive, nonhemolytic jaundice (36). This condition, in which resting free plasma 17-OH-corticosteroid levels are normal, is presumably characterized by defective hepatic formation of various glucuronides (36). The delayed tetrahydrocortisone clearance observed in pregnancy might be explained in a number of ways, but whatever the correct explanation, the mechanism would appear to be different from that operative in cirrhosis. Although several pathways of hydrocortisone metabolism may be involved [*e.g.,* hydroxylation at the C20 position, as demonstrated by Fukushima, Leeds, Bradlow, Kritchevsky, Stokem and Gallagher (37)], it is possible that the delayed hydrocortisone clearance rate in pregnancy may be related to defective conjugation of tetrahydrocortisone (and by extension, of tetrahydrocortisol) with glucuronic acid rather than altered reduction of hydrocortisone to its tetrahydro derivatives. Whether or not the delayed tetrahydrocortisone clearance rate can be explained by changes in binding of this steroid by plasma proteins is a question that awaits investigation.

In conclusion, it seems likely that delayed hydrocortisone clearance may partially explain the high plasma hydrocortisone level of late pregnancy. It seems improbable that this delayed hydrocortisone disappearance rate is the complete explanation, since delayed hydrocortisone clearance in cirrhosis is not accompanied by high plasma 17-OH-corticosteroid levels. It is possible that in pregnancy there exists a change in the homeostatic mechanism whereby hydrocortisone and corticotropin influence the rate of release of one another.

[2] Cohn and Bondy were able to detect only trace quantities of tetrahydrocortisone and tetrahydrocortisol glucuronides in this disease, but found significant amounts of unconjugated tetrahydrocortisone and tetrahydrocortisol (34). These authors suggested that in cirrhosis there may be a delayed formation of steroid glucuronide in addition to a reduced rate of reduction of the delta$^4$-3-ketone in ring A (34).

## SUMMARY

1. A delay in the clearance rate of hydrocortisone from plasma in late pregnancy and in cirrhosis of the liver is confirmed.

2. Plasma 17-OH-corticosteroid levels and their response to adrenocorticotropic hormone (ACTH) are greater than normal in late pregnancy, but normal in cirrhosis.

3. Tetrahydrocortisone clearance rate is delayed in pregnancy and reportedly normal in cirrhosis, suggesting that the delay in hydrocortisone clearance may have a different basis in the two conditions.

4. It is suggested that the elevated plasma 17-OH-corticosteroid level in late pregnancy cannot be entirely accounted for by a delay in the rate of hydrocortisone clearance from plasma.

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