# PHYSIOLOGICAL DISPOSITION OF 4-$C^{14}$-CORTISOL DURING LATE PREGNANCY [1]

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The plasma levels of free 17-hydroxycorticosteroids (17-OH-CS) were found to rise progressively during pregnancy (1–5). In cases of vaginal delivery and "indicated cesarean section," labor and delivery had a tendency to further increase the maternal concentrations (6, 7), while in cases of "elective, repeat cesarean section," the levels were not as high as those observed in cases where labor had taken place (8, 7).

The levels of 17-OH-CS in cord blood were observed to be very low in cases of "elective, repeat cesarean section" (8, 7). In cases of vaginal delivery and indicated cesarean section the concentrations were as high or higher than those of normal adults (3, 6–9).

Studies involving the intravenous infusion of ACTH or cortisol to patients selected for "elective, repeat cesarean section" strongly suggested that 17-OH-CS could cross the "placental barrier" from the mother to the baby (7). In all cases, there was a direct relationship between maternal and cord levels, the latter values being one-half to one-fifth of those of the corresponding mother (6, 7).

These findings suggested that, near term, the fetal adrenals produce little 17-OH-CS and that the increase in cord levels observed in cases of either vaginal delivery or "indicated cesarean section" was the result of elevated maternal concentrations.

The present work is an attempt to elucidate further the metabolism of corticosteroids during pregnancy. It was thought that radioactive cortisol might be useful since it would permit a dynamic study without interfering with the endogeneous production of steroid.

## METHODS

*Preliminary study on newborn infants.* If, following the injection of radioactive material to a mother, a large part of the dose were to cross the placenta, we had to be assured that the newborn would dispose of the compound as rapidly as do normal adults (10–13). Therefore, in a first step of the present study, we measured the urinary excretion of radioactivity during the 48-hour period following the administration of a minimal dose of 4-$C^{14}$-cortisol ($1.5 \times 10^{-3}$ microcurie) to two newborns, 15 and 20 hours of age. Since, as shown in the Results section, the 48-hour excretion of radioactivity was within normal limits, it was considered safe to proceed with the injection of mothers shortly before delivery.

*Experimental subjects.* The other subjects of the study were nine pregnant females, 20 to 38 years of age. An "elective, repeat cesarean section" was performed approximately at term and before onset of labor in all cases. Sections were deemed advisable because of a previous "indicated section," but otherwise the pregnancies had been normal. The surgery was performed with local infiltration of Novocain® and intravenous Pentothal® anesthesia.

*Purity of 4-$C^{14}$-cortisol.* The 4-$C^{14}$-cortisol was provided by the Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda, Maryland. Its specific activity was 1.467 millicuries per millimole. The purity of the compound was tested as previously described (13), and 90 to 95 per cent of the radioactivity was found to move as cortisol on our paper chromatograms when using the Bush (14) system of benzene : methanol : water (100 : 55 : 45).

*Mode of administration of 4-$C^{14}$-cortisol.* The radioactive steroid was dissolved in re-distilled ethanol so that 1 ml. of the solution contained approximately 1 microcurie. For human administration, 1 ml. of this solution was carefully pipetted out and diluted with 10 ml. of 5 per cent dextrose in water. The method of intravenous injection and the mode of calculation of the dose actually administered were similar to those previously described (13). The dose given to each of the subjects of the present study is indicated in Table I.

Subject A. O. received the injection 24 hours, and subjects A. P. and A. Q., 48 and 46 hours, respectively, prior to delivery. In the six other cases the steroid was given from 17 to 69 minutes before delivery (see Table I).

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TABLE I

*Radioactivity readily extractable from plasma with chloroform, and that extractable following β-glucuronidase hydrolysis at various intervals following 4-C$^{14}$-cortisol administration (expressed as percentage of the administered dose per liter of plasma)*

| SUBJECTS | | TIME AFTER INJECTION | % DOSE PER LITER PLASMA | |
|---|---|---|---|---|
| | | | FREE FRACTION | GLUCURONOSIDE FRACTION |
| A.P. | MOTHER, 21 yrs., 82 kg. 779,600 c/m, 48 hrs. prior to delivery | 30' | 5.04 | 0.36 |
| | | 60' | 4.09 | 0.29 |
| | | 120' | 3.26 | 0.46 |
| | | 240' | 1.85 | 0.46 |
| | | 48 hrs. | 0. | 0. |
| | BABY ♀, 3670 g. | 48 hrs. | 0. | 0. |
| A.Q. | MOTHER, 24 yrs., 69 kg. 812,000 c/m, 46 hrs. prior to delivery | 20' | 6.20 | 0.40 |
| | | 60' | 4.51 | 0.44 |
| | | 120' | 3.05 | 0.39 |
| | | 240' | 1.75 | 0.43 |
| | | 46 hrs. | 0. | 0. |
| | BABY ♀, 3050 g. | 46 hrs. | 0. | 0. |
| A.O. | MOTHER, 26 yrs., 65 kg. 766,500 c/m, 24 hrs. prior to delivery | 30' | 4.75 | 0.35 |
| | | 60' | 3.55 | 0.56 |
| | | 140' | 2.47 | 0.48 |
| | | 250' | 1.45 | 0.54 |
| | | 24 hrs. | 0. | 0. |
| | BABY ♂, 2810 g. | 24 hrs. | 0. | 0. |
| A.G. | MOTHER, 23 yrs., 65 kg. 904,700 c/m, 17' prior to delivery | 17' | 7.70 | 0.46 |
| | | 27' | 5.86 | 0.33 |
| | | 150' | -- | 0.31 |
| | | 260' | 1.85 | 0.52 |
| | BABY ♂, 2690 g. | 17' | 1.61 | 0.13 |
| A.H. | MOTHER, 36 yrs., 70 kg. 872,500 c/m, 27' prior to delivery | 27' | 4.19 | 0.34 |
| | | 60' | 3.75 | 0.32 |
| | | 145' | 3.04 | 0.30 |
| | | 263' | 1.63 | 0.38 |
| | BABY ♀, 2680 g. | 27' | 1.46 | 0.09 |
| A.E. | MOTHER, 38 yrs., 76 kg. 907,100 c/m, 39' prior to delivery | 20' | 5.14 | 0.47 |
| | | 39' | 3.46 | 0.19 |
| | | 150' | 1.49 | 0.19 |
| | | 240' | 1.15 | 0.16 |
| | BABY ♀, 2800 g. | 39' | 1.21 | 0.09 |
| A.L. | MOTHER, 22 yrs., 76 kg. 936,500 c/m, 51' prior to delivery | 17' | 6.42 | 0.30 |
| | | 37' | 4.62 | 0.31 |
| | | 51' | 3.29 | 0.38 |
| | | 120' | 2.50 | 0.35 |
| | | 240' | 1.70 | 0.37 |
| | BABY ♂, 3200 g. | 51' | 1.57 | 0.08 |
| A.I. | MOTHER, 25 yrs., 93 kg. 814,200 c/m, 60' prior to delivery | 15' | 6.26 | 0.48 |
| | | 60' | 4.11 | 0.48 |
| | | 120' | 2.27 | 0.43 |
| | | 240' | 1.17 | 0.36 |
| | BABY ♂, 2090 g. | 60' | 0.80 | 0.13 |
| A.M. | MOTHER, 24 yrs., 50 kg. 900,000 c/m, 69' prior to delivery | 20' | 6.80 | 0.33 |
| | | 69' | 4.64 | 0.56 |
| | | 120' | 3.98 | 0.56 |
| | | 240' | 2.46 | 0.63 |
| | BABY ♂, 3200 g. | 69' | 1.70. | 0.24 |

*Collection of blood and urine samples.* Samples of maternal blood were drawn in heparinized syringes at various times after the injection. In all cases, a sample was collected at the exact time of delivery in addition to a specimen of cord blood. The plasma was immediately separated from the red blood cells. Urine samples were

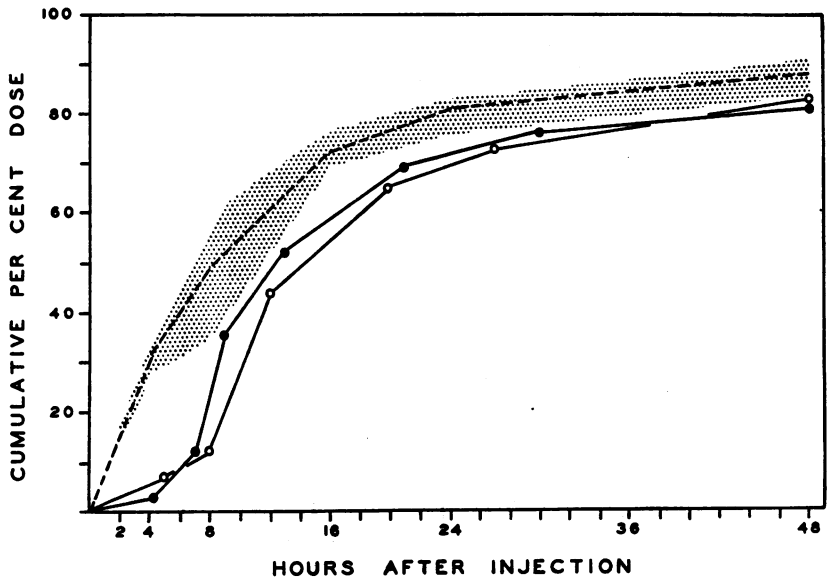


FIG. 1. CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY FOLLOWING 4-$C^{14}$-CORTISOL INJECTION IN TWO NORMAL NEWBORNS, 15 AND 20 HOURS OF AGE

The broken line is the average urinary excretion of seven normal young adults (13, 15). The shaded area shows the range of values for these normal individuals. The full lines join the values obtained in the two normal newborns.

collected from the mothers and their babies at specified times following cortisol administration (see Table I).

*Preparation and assay of the various plasma and urine extracts.* Each plasma sample was extracted three times with freshly re-distilled chloroform (40 ml. × 3) in order to extract the free steroids ("free fraction"), after which 100 ml. of absolute ethanol was added to the plasma residue. After shaking and centrifuging, the liquid phase was decanted and the remainder was washed with 50 ml. of ethanol. The pooled ethanol extracts were then evaporated to dryness. The ethanol residue was dissolved in 20 ml. of distilled water, incubated with β-glucuronidase (approximately 500 units per ml. of plasma), at pH 4.6 (acetate buffer), at 37° C. for 48 hours.[8] Extraction was then performed with chloroform (40 ml. × 3) to obtain the steroids formerly present as conjugates of glucuronic acid ("glucuronoside fraction").

A 25-ml. aliquot of each urine was extracted three times with an equal volume of chloroform in order to obtain the "free fraction." A β-glucuronidase hydrolysis was then carried out on the urine residue under the same conditions regarding temperature and pH as for the plasma ("glucuronoside fraction"), followed by 48-hour continuous extraction with ether at pH 0.8. Three ml. of concentrated hydrochloric acid was then added to the aqueous residue, the mixture boiled for 20 minutes and then extracted three times with its volume of chloroform.

A great discrepancy between the total urinary radioactivity and the sum of those found in the various urinary fractions was observed in all cases, and for this reason a count of the isotopic activity was made on the urine residues following the removal of "free," "glucuronoside" and "continuous ether extraction" fractions of patients A. M. and A. O. The urine residues of patient A. G. were extracted twice with equal volumes of butanol and the butanol extracts were assayed for radioactivity.

All extracts of plasma and urine, as well as whole urine were assayed for radioactivity in the manner previously described (13).

Some of the "free fractions" prepared from maternal and cord plasma were chromatographed on paper using the Bush (14) system, benzene: methanol: water (100: 55: 45). The following areas of the chromatogram were cut out and eluted with ethanol: 1) the origin, 2) the zone between the origin and the cortisol area, 3) the cortisol area, 4) the zone between the cortisol area and that of dihydrocortisone, the latter area being included in this zone, 5) the rest of the chromatogram with the front included. The ethanol extract was then assayed for radioactivity content.

*Control subjects for the present study.* The results obtained in a group of five normal young adult males of a previous study (13) and in two normal non-pregnant females (15) were used in the present work as control values. The two normal non-pregnant females (subjects A. R. and B. A.) were 28 and 31 years of age. The radioactive steroid was administered to subject A. R. 19 days and to subject B. A. 15 days after the beginning of the last period. Plasma and urine values of subject B. A. were identical to the various mean values for the group

[8] Ketodase, a beef liver preparation, from Warner-Chilcott Laboratories was used in the present study.

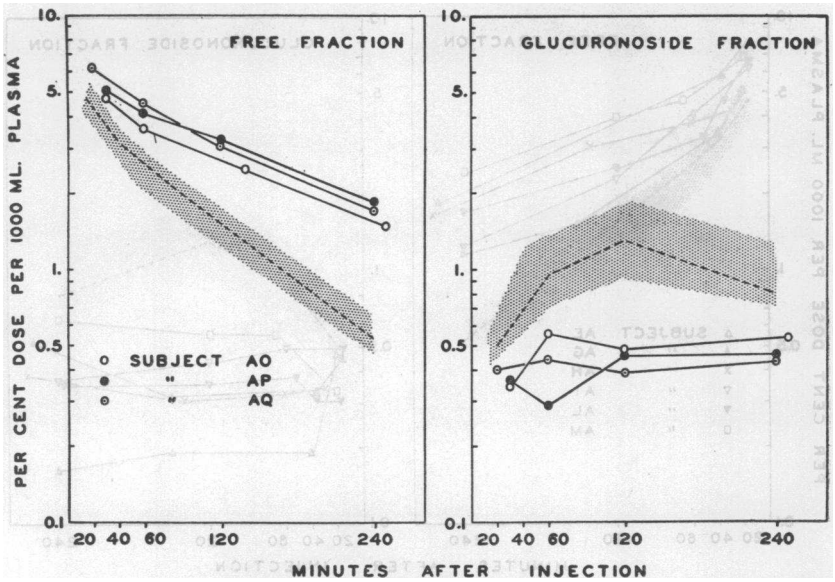


FIG. 2. PLASMA RADIOACTIVITY IN THE "FREE" AND "GLUCURONOSIDE" FRACTIONS OF MOTHERS WHO RECEIVED 4-C$^{14}$-CORTISOL 24 TO 48 HOURS PRIOR TO AN ELECTIVE, REPEAT CESAREAN SECTION

The broken line joins the average of the values obtained in five normal young adult males (13) and two normal young adult non-pregnant females (15). The shaded area shows the range of values for these normal individuals. The full lines join the values obtained in subjects A. O., A. P. and A. Q.

of five normal males. Some of the urinary levels of subject A. R. were slightly different from the corresponding mean of the normal males, but they were all in the range of variation of the male group. Furthermore, sex did not appear to influence the removal of non-isotopic cortisol in a control group of 26 young adults, comprising 12 non-pregnant females and 14 males. Consequently, it was thought possible to compare the 4-C$^{14}$-cortisol studies in pregnant women with those carried out in the two non-pregnant females as well as in the male group.

Our results, for control subjects, are similar to those reported by other authors (11). When Peterson and Wyngaarden (12) studied the specific activity of cortisol in plasma, instead of the total free radioactivity, they obtained a wider range of variance for the values of half-life. This might be due to the fact that the error of the chemical technique adds itself to that of the isotopic assay, while the latter one is only involved in the study of total free plasma radioactivity (11, 13).

## RESULTS

### *Rate of elimination of radioactivity by newborn infants*

The cumulative urinary excretion of radioactivity following 4-C$^{14}$-cortisol infusion to two newborns is shown in Figure 1 and is compared to the average excretion of five normal males (13) and two normal females (15). It can be seen that, after an initial lag period of 4 to 8 hours, the rate of elimination of radioactivity by the two babies was similar to that observed in adults, and 48 hours after the injection the cumulative excretions were 81.2 and 83 per cent of the dose.

### *Radioactivity in the "free" and "glucuronoside" fractions from maternal and cord plasma*

The amount of radioactivity readily extractable from maternal plasma by chloroform following 4-C$^{14}$-cortisol administration is shown in Table I. The results are expressed in per cent of the administered dose per liter of plasma. These values were plotted in Figures 2 and 3, and were compared to the average curve obtained from seven normal young adults (13, 15). In most cases the logarithms of the values between the first and fourth hours fell approximately on a straight line; during this period of time, equilibrium probably existed between the various compartments of the free steroid pool of the body. The average half-

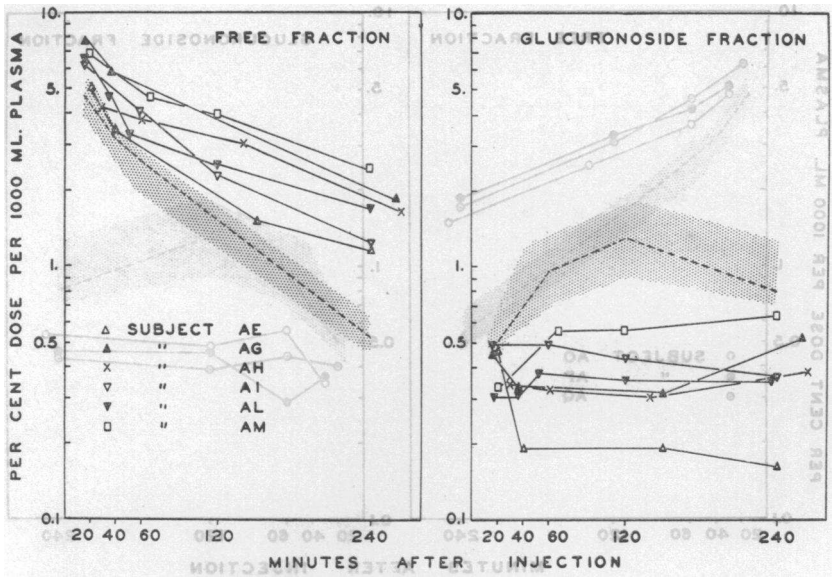


FIG. 3. PLASMA RADIOACTIVITY IN THE "FREE" AND THE "GLUCURONOSIDE" FRACTIONS OF MOTHERS WHO RECEIVED 4-$C^{14}$-CORTISOL 17 TO 69 MINUTES PRIOR TO ELECTIVE, REPEAT CESAREAN SECTION

life of the free fraction was then 140 minutes with a range of 100 to 180 minutes, compared to 80 minutes for the control group.

Six hours after the injection of 4-$C^{14}$-cortisol, the free fraction was still 1.25 per cent of the dose per liter of plasma in patient A. Q., while it was only 0.34 per cent (subject B. A.) and 0.20 per cent (normal male) in two control individuals.

Table I and Figures 2 and 3 also show the radioactivity extractable from plasma after β-glucuronidase hydrolysis. Two hours after the injection this fraction was approximately one-half or less of that found in normal subjects.

The simultaneous values for maternal and cord plasma are shown in Table I. Their ratio was from 2.09 (subject A. L.) to 5.14 (subject A. I.)

TABLE II

*Paper chromatographic study of the radioactivity readily extractable from plasma with chloroform*

| Plasma samples | | | Radioactivity in per cent of total c.p.m. applied on chromatogram | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Chromatogram area | | | | | |
| Subjects | | Minutes after injection | No. 1 (Origin) | No. 2 | No. 3 (Cortisol) | No. 4 | No. 5 (Front) | Per cent recovery |
| B. A. | Control—Non-pregnant young adult female | 20 | 1.8 | 16.4 | 48.2 | 24.0 | 0.2 | 89.6 |
| | | 60 | 2.5 | 18.3 | 45.0 | 30.2 | 0 | 96.0 |
| | | 120 | 3.3 | 21.0 | 46.7 | | | |
| | | 240 | 2.4 | 17.6 | 50.2 | 28.1 | 0 | 98.3 |
| A. E., A. G., A. I. (Pool)* | Maternal | 15–20 | 2.8 | 12.6 | 53.5 | 24.5 | 0 | 93.4 |
| | | 20–60 (Deliv.) | 2.0 | 20.5 | 57.0 | 35.0 | 0 | 114.5 |
| | | 120–240 | 4.3 | 27.0 | 51.0 | 20.4 | 0.1 | 102.8 |
| | Cord | 20–60 | 0 | 16.0 | 42.5 | 30.6 | 0 | 89.1 |
| A. O., A. P. (Pool)† | Maternal | 30 | 4.8 | 22.0 | 50.7 | 18.0 | 0 | 95.5 |
| | | 60 | 3.2 | 24.4 | 46.9 | 24.1 | 0 | 98.6 |
| | | 120–140 | 2.9 | 20.2 | 50.5 | 19.3 | 0 | 92.9 |
| | | 240 | 4.6 | 26.0 | 55.1 | 16.6 | 0 | 102.3 |

* 4-$C^{14}$-cortisol administered 20 to 60 minutes prior to delivery.
† 4-$C^{14}$-cortisol administered 24 and 48 hours prior to delivery.

TABLE III

*Cumulative urinary radioactivity following injection of 4-C$^{14}$-cortisol (expressed as percentage of the administered dose)*

| PATIENTS | | HRS. AFTER INJECT. | TOTAL | FREE | GLUCURONOSIDE | CONT. EXTRACT. pH 0.8 | STRONG ACID HYDROL. | UNACCOUNTED FOR |
|---|---|---|---|---|---|---|---|---|
| A.P. | Mother | 4 | 11.15 | 0.96 | 3.04 | 2.82 | | |
| | | 8 | 36.50 | 2.77 | 9.82 | 7.85 | | |
| | | 12 | 55.40 | 3.64 | 14.60 | 10.42 | | |
| | | 24 | 68.00 | 4.49 | 18.10 | 13.85 | | |
| | | 48 | 71.90 | 4.62 | 19.09 | 14.62 | | |
| | | 72 | 72.30 | 4.62 | 19.25 | 14.62 | | |
| | Baby | 24 | 0.045 | 0.(?) | 0.009 | 0.013 | | |
| A.O. | Mother | 4 | 16.80 | 1.09 | 5.02 | 3.69 | 0.92 | 6.08 |
| | | 8 | 43.40 | 2.68 | 12.50 | 9.51 | 1.97 | 16.74 |
| | | 12 | 59.50 | 3.08 | 17.50 | 12.85 | 2.70 | 23.37 |
| | | 24 | 73.50 | 4.05 | 22.50 | 15.82 | 3.65 | 27.48 |
| | | 48 | 74.80 | 4.19 | 23.40 | 16.35 | 3.95 | 26.91 |
| | Baby | 24 | 0.406 | 0.012 | 0.058 | 0.076 | 0.022 | 0.238 |
| A.G. | Mother | 4 | 11.50 | 0.90 | 3.39 | 1.48 | | |
| | | 8 | 36.40 | 5.60 | 9.75 | 6.64 | | |
| | | 12 | 48.3 | 6.21 | 13.82 | 8.71 | | |
| | | 24 | 61.2 | 6.70 | 17.50 | 11.00 | | |
| | | 48 | 64.4 | 6.75 | 18.22 | 11.24 | | |
| | Baby | 24 | 1.037 | 0.035 | 0.102 | 0.150 | | |
| | | 48 | 1.626 | 0.044 | 0.141 | 0.217 | | |
| A.H. | Mother | 4 | 11.38 | 0.83 | 3.60 | 2.30 | 0.58 | 4.07 |
| | | 8 | 32.60 | 2.12 | 9.67 | 7.48 | 1.48 | 11.85 |
| | | 12 | 42.60 | 2.65 | 12.50 | 9.28 | 3.85 | 14.32 |
| | | 24 | 52.80 | 2.99 | 15.60 | 11.45 | 4.95 | 17.81 |
| | | 48 | 57.0 | 3.14 | 17.7 | 12.55 | 5.07 | 18.54 |
| | Baby | 24 | — | — | — | — | — | — |
| | | 48 | 0.409 | 0.014 | 0.025 | 0.049 | 0.021 | 0.300 |
| A.E. | Mother | 4 | 12.60 | 0.87 | 3.39 | 1.69 | 0.70 | 5.95 |
| | | 8 | 33.90 | 3.39 | 7.84 | 5.71 | 1.34 | 15.62 |
| | | 12 | 59.20 | 4.94 | 13.45 | 7.75 | 3.78 | 29.28 |
| | | 24 | 75.20 | 5.85 | 16.45 | 9.48 | 5.21 | 38.21 |
| | | 48 | 78.80 | 5.98 | 17.0 | 9.87 | 5.21 | 40.74 |
| | Baby | 24 | 1.220 | 0.024 | 0.048 | 0.096 | 0.096 | 0.956 |
| A.M. | Mother | 4 | 12.55 | 1.34 | 3.00 | 2.16 | | |
| | | 8 | 36.8 | 2.94 | 9.09 | 8.28 | | |
| | | 12 | 57.2 | 4.25 | 14.82 | 11.20 | | |
| | | 24 | 68.2 | 4.55 | 18.30 | 13.90 | | |
| | | 48 | 71.6 | 4.58 | 19.20 | 14.80 | | |
| | Baby | 24 | 2.022 | 0.049 | 0.296 | 0.390 | | |
| | | 48 | 2.435 | 0.053 | 0.330 | 0.481 | | |

for the free fraction, and from 2.11 (subject A. E.) to 4.75 (subject A. L.) for the glucuronoside fraction.

It was previously noted in control individuals that only a fraction of the total free radioactivity moved on paper chromatograms in the cortisol area (11, 13). The same phenomenon was observed during late pregnancy, as shown in Table II; the percentage of activity corresponding to cortisol was rather constant throughout the experiment and similar to that observed in a non-pregnant female (subject B. A.) and in normal young adult males (13). Even though no attempt was made to characterize the extra-cortisol radioactivity, it is reasonable to believe that area No. 4 of the chromatograms would correspond to

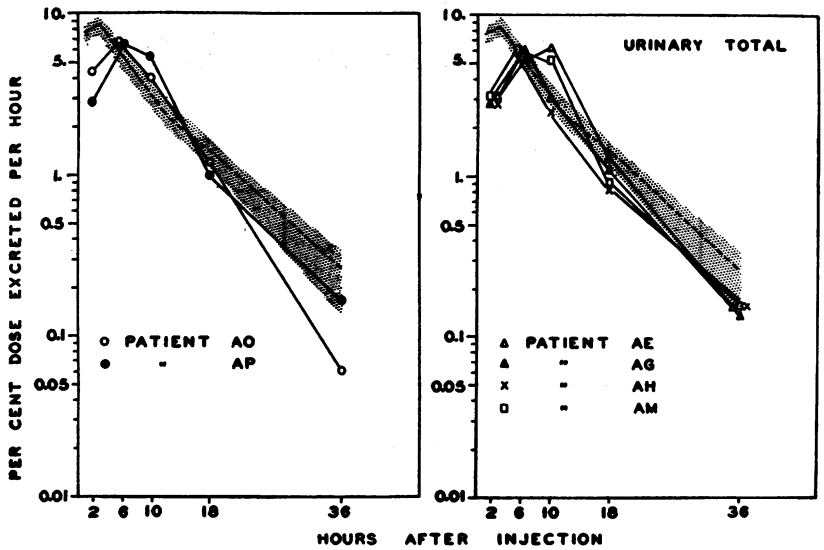


FIG. 4. RATES OF TOTAL URINARY EXCRETION OF RADIOACTIVITY IN MOTHERS WHO RECEIVED 4-$C^{14}$-CORTISOL 24 AND 48 HOURS PRIOR TO AN ELECTIVE, REPEAT CESAREAN SECTION (LEFT) AND IN MOTHERS WHO RECEIVED 4-$C^{14}$-CORTISOL 17 TO 69 MINUTES PRIOR TO SECTION (RIGHT)

The broken line joins the average of the rates obtained in five normal males (13) and two normal non-pregnant females (15). The shaded area shows the range of values for these normal individuals. The full lines join the values obtained in the patients studied.

the dihydro-compounds, while areas Nos. 1 and 2 would correspond to the 20-hydroxylated metabolites of cortisol as well as the tetrahydro-compounds.

*Radioactivity in the urine of mothers and their babies*

The activities of the various urinary fractions are recorded in Table III. The rate of excretion of radioactivity per hour was calculated for the individual fractions covering different periods of urinary collection. These values were plotted on semi-logarithmic paper and the results are shown in Figures 4 to 7. These figures show also the average values obtained in seven normal subjects (13, 15) with the range of variation of these values.

The total 48-hour excretion of radioactivity by the various patients was smaller than that of the normal subjects of previous studies (13, 15) who excreted an average of 87 per cent of the dose. This was mainly due to a decreased rate of excretion during the first 4 hours following 4-$C^{14}$-cortisol administration (Figure 4). Excretion from the fourth to the twelfth hour was equal to, and in some cases higher than, that seen in controls. After the twelfth hour the values were at the lower limit of normal.

The urinary "free fraction" was within the normal range during the first 4 hours (Figure 5), but from the fourth to the twelfth hour, the rate of excretion in patients studied was three to five times higher than found in control subjects.

Conversely, the amount of radioactivity in the urinary "glucuronoside fraction" was lower than average during the first 8 hours after 4-$C^{14}$-cortisol injection (Figure 6), following which the values approached the lower limits of the control range.

The amount of radioactivity obtained after 48-hour continuous ether extraction at pH 0.8 was somewhat larger than the normal values during the 4 to 8 hour collection period (Figure 7). Thereafter, the rate of excretion was variable.

Table IV shows the difference between the total urinary radioactivity and the sum of the activities found in the "free," "glucuronoside" and "continuous extraction" fractions. Following the removal of these various fractions, the urine residue

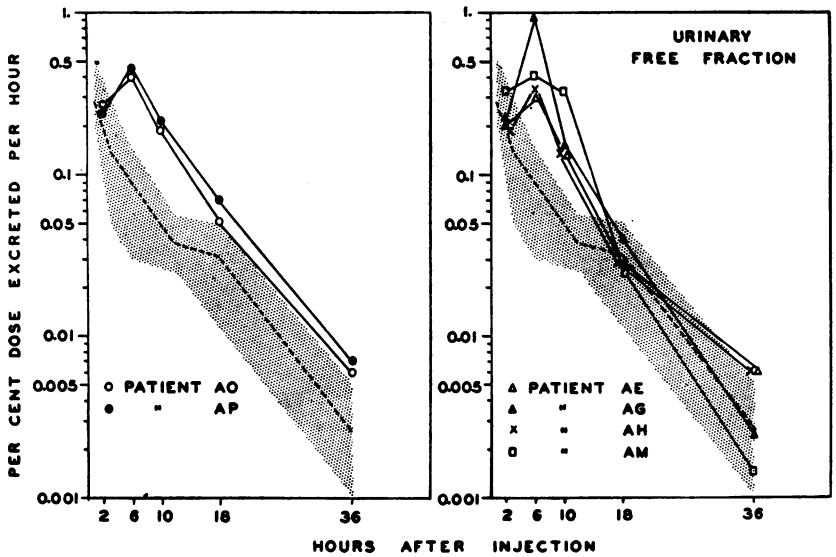


FIG. 5. RATES OF URINARY EXCRETION OF RADIOACTIVITY READILY EXTRACTABLE BY CHLOROFORM IN MOTHERS WHO RECEIVED 4-C$^{14}$-CORTISOL 24 AND 48 HOURS PRIOR TO ELECTIVE, REPEAT CESAREAN SECTION (LEFT) AND IN MOTHERS WHO RECEIVED 4-C$^{14}$-CORTISOL 17 TO 69 MINUTES PRIOR TO SECTION (RIGHT)

See legend for Figure 4.

of patients A. M. and A. O. still contained 21.27 and 20.71 per cent of the dose, respectively. The difference between these two values and the total radioactivity unaccounted for (approximately 10 per cent of dose) probably represents the sum of the experimental errors (handling and counting

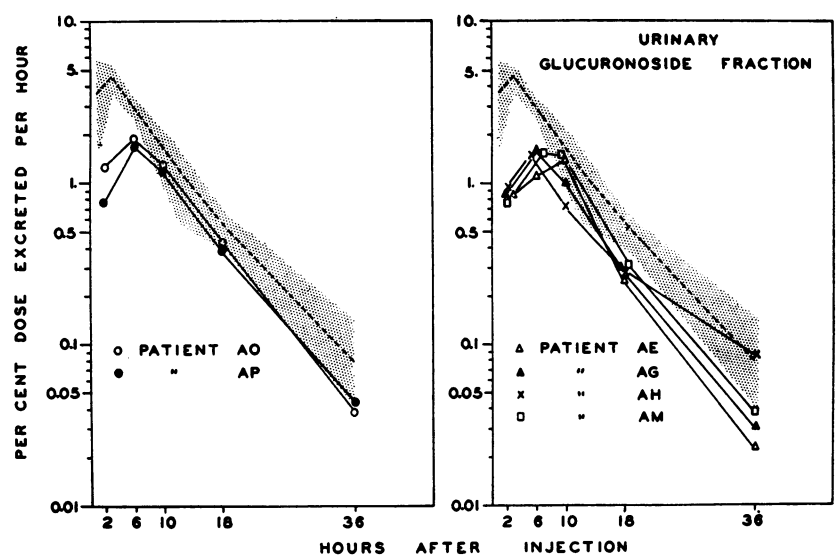


FIG. 6. RATES OF URINARY EXCRETION OF RADIOACTIVITY EXTRACTABLE BY CHLOROFORM FOLLOWING β-GLUCURONIDASE HYDROLYSIS IN MOTHERS WHO RECEIVED 4-C$^{14}$-CORTISOL 24 AND 48 HOURS PRIOR TO ELECTIVE, REPEAT CESAREAN SECTION (LEFT) AND IN MOTHERS WHO RECEIVED 4-C$^{14}$-CORTISOL 17 TO 69 MINUTES PRIOR TO SECTION (RIGHT)

See legend for Figure 4.

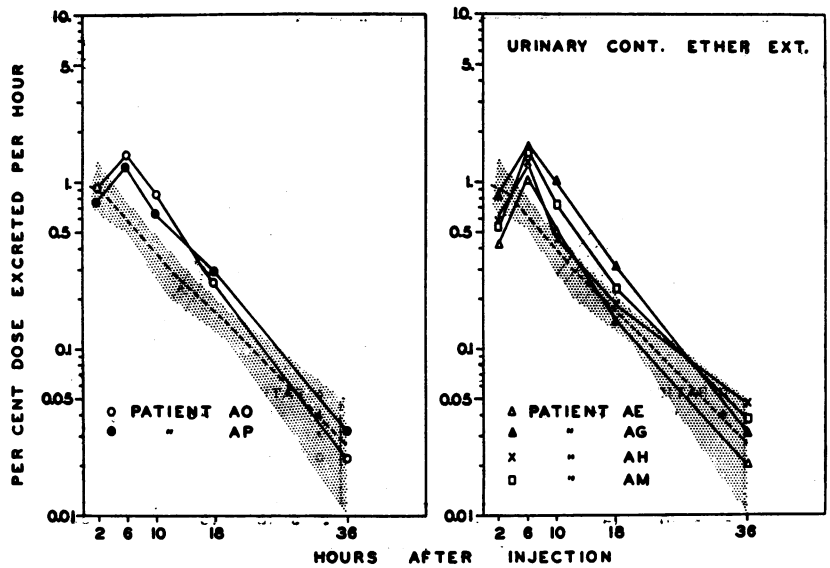


FIG. 7. RATES OF URINARY EXCRETION OF RADIOACTIVITY OBTAINED BY 48-HOUR CONTINUOUS ETHER EXTRACTION AT pH 0.8 IN MOTHERS WHO RECEIVED 4-$C^{14}$-CORTISOL 24 AND 48 HOURS PRIOR TO ELECTIVE, REPEAT CESAREAN SECTION (LEFT) AND IN MOTHERS WHO RECEIVED 4-$C^{14}$-CORTISOL 17 TO 69 MINUTES PRIOR TO SECTION (RIGHT)

See legend for Figure 4.

of each individual urine sample) made up to that point. In patient A. G., the butanol extract of the urine residue contained only 10.1 per cent of the dose and it seems that butanol does not extract all the activity left over in the urine after removal of "free," "glucuronoside" and "continuous extraction" fractions.

## DISCUSSION

During pregnancy, near term, and during delivery by "elective, repeat cesarean section," the intravenously administered cortisol disappeared rapidly from the circulation as it did for the group of control subjects. The first 30 to 60 minutes appeared to represent the period of time required for the radioactive steroid to come into equilibrium with the non-radioactive cortisol in the various body compartments. Prior to the onset of labor, the chloroform-soluble radioactivity in the plasma disappeared much more slowly than in the control subjects despite the increase in blood volume observed in pregnant women (16). These findings demonstrated a rather marked modification in the metabolism of 4-$C^{14}$-cortisol. When the surgical stress associated with the cesarean section intervened shortly after the administration of radioactive steroid (69 minutes or less), the curves of the "free" and "glucuronoside" fractions in plasma were similar to those found in patients who had received 4-$C^{14}$-cortisol 24 to 48 hours prior to delivery; the rise in plasma 17-OH-CS levels observed following this surgical procedure (6) would, therefore, appear to be due to a true increase in adrenal function.

In the maternal plasma, the amount of radioactivity freed by $\beta$-glucuronidase hydrolysis was smaller than that found in the controls but not absolutely nil. However, we have been unable, up to this time, to measure the Porter-Silber chromogens in the plasma of pregnant women when using the method described by Bongiovanni (17) for the estimation of conjugated 17-OH-CS. A pink color was observed during the development of the reaction which did not appear to be due to the presence of an increased amount of estrone, estradiol, estriol or pregnane-3$\alpha$, 20$\alpha$-diol in the plasma extract obtained after $\beta$-glucuronidase hydrolysis. Our inability to measure the conjugated 17-OH-CS by the Porter-Silber reaction must be due, therefore, to interference by some other material rather than the total absence of the glucuronoside of the 17-OH-CS.

TABLE IV

*Comparison of unaccounted for urinary radioactivity following removal of "free," "glucuronoside" and "continuous extraction" fractions and radioactivity found in urine residue*

| | HOURS AFTER INJECTION | PATIENT A.M. | | PATIENT A.O. | | PATIENT A.O. | |
|---|---|---|---|---|---|---|---|
| | | I* | II** | I* | II** | I* | III*** |
| | | PER CENT OF THE DOSE | | | | | |
| MOTHER | 4 | 6.05 | 3.48 | 7.00 | 3.76 | 5.73 | 2.45 |
| | 8 | 10.44 | 6.62 | 11.71 | 7.78 | 8.68 | 3.84 |
| | 12 | 10.44 | 7.10 | 7.36 | 4.25 | 6.15 | 2.32 |
| | 24 | 4.52 | 4.05 | 5.06 | 4.92 | 5.44 | 1.18 |
| | 48 | 0.57 | 0. | 0. | 0. | 2.19 | 0.31 |
| | Total | 32.02 | 21.27 | 31.13 | 20.71 | 28.19 | 10.10 |
| CORD | 24 | 1.287 | 1.033 | 0.260 | 0.139 | 0.750 | 0.24 |
| | 48 | 0.284 | 0.200 | — | — | 0.477 | 0.14 |
| | Total | 1.571 | 1.233 | 0.260 | 0.139 | 1.227 | 0.38 |

* Difference between total urinary radioactivity and the sum of activities in "free," "glucuronoside" and "continuous extraction" fractions.

** Radioactivity in urine residue following removal of "free," "glucuronoside" and "continuous extraction" fractions.

*** Radioactivity in butanol extract of urine residue following removal of "free," "glucuronoside" and "continuous extraction" fractions.

In patients with cirrhosis of the liver, it has been shown that following cortisol administration the rate of disappearance of free 17-OH-CS was much slower than normal, due to an impairment of the rate of conjugation of the metabolites of cortisol (18, 19). A similar situation is observed during late pregnancy. However, in the cirrhotic patients, the resting levels of free plasma 17-OH-CS have been reported to be normal, while in pregnant women they are elevated. It would appear that during pregnancy the control mechanism of homeostasis is impaired, as it appears to be in dying patients (20). It is not possible at present to explain the reasons for such an impairment.

Chloroform extractable radioactivity was detected in cord blood, confirming the fact that cortisol and/or its free metabolites can cross the "placental barrier" (7). The radioactive steroids appeared to reach the fetus very rapidly since they were found in cord plasma of baby A. G. 17 minutes after the injection of 4-C$^{14}$-cortisol into the mother. It was interesting to note that the ratio of maternal to cord free radioactivity was in the same range as that observed after the administration of non-isotopic cortisol to the mother. When the steroid was administered 24 to 48 hours prior to delivery, no radioactivity was measurable in either maternal or cord blood at delivery time.

The amount of radioactivity liberated from cord plasma by $\beta$-glucuronidase hydrolysis was small but significant (Table I). The ratio of maternal to cord values of conjugated steroids was in the same range as that observed for the free compounds but there was no direct relationship between the two; that is, the highest values for the ratio of the free compounds did not necessarily correspond to the highest values for the conjugates. It is not clear whether the conjugated steroids found in cord blood came from the mother as such or if they were the result of the fetal metabolism of the free steroids which crossed the "placental barrier."

The fact that no radioactivity, free or conjugated with glucuronic acid, was found in the cord blood 24 to 48 hours after the administration of 4-C$^{14}$-cortisol to the mother would suggest that

the fetus has means of disposing of cortisol and its metabolites. They could either be excreted into the amniotic fluid or returned to the maternal circulation.

In the present study it was observed that the total amount of urinary radioactivity excreted by the mothers was significantly smaller than that eliminated by normal individuals. Urine collections were completed prior to the surgical procedure only in subject A. P. In subject A. O., only the first 24-hour collection was terminated prior to surgery. From these limited data, it would appear that during pregnancy, near term, the rate of excretion of total radioactivity following 4-$C^{14}$-cortisol administration is somewhat slower than in normal individuals.[4] However, the total urinary excretion represents the sum of various groups of metabolites and it was found that the "glucuronoside fraction" only was markedly diminished while the "free fraction" was increased; since the glucuronoside fraction constitutes a much larger percentage of the total, it is understandable that the total urinary radioactivity would be decreased.

We have already mentioned above that, following 4-$C^{14}$-cortisol administration to the mothers, the half-life of free radioactivity in plasma was almost twice that of normal, and the total amount of urinary radioactivity was probably smaller than normal. At the same time, the plasma levels of free 17-OH-CS during pregnancy, near term, and prior to the onset of labor were found to be not more than twice the 8 to 9 A.M. levels of control subjects. It would appear, therefore, that there is no real increase in the production of adrenocortical steroids in pregnancy, but rather a decreased rate of catabolism of the corticosteroids produced.[5] It would seem to follow that the urinary excretion of corticosteroids during pregnancy, near term, should not be different from that of non-pregnant adult females; this is in contradiction to the interpretation of most results reported to date in the literature. An increased urinary excretion of glucocorticoids during pregnancy has been reported by Venning (22). Heard, Sobel and Venning (23), Jailer (24), and Devis (25) have noted an increased excretion of neutral reducing lipids. An elevation in the urinary levels of formaldehydrogenic substances and the steroids reacting with dinitrophenylhydrazine has also been reported by Tobian (26), and by Jayle, Desgrez, Serpicelli, and Rozeg (27), respectively. However, Gray (28) found that free cortisone excretions were higher than normal while the concentration of tetrahydrocortisone, obtained only after hydrolysis, was in the control range or perhaps slightly lower. Our data tend to support his findings and those of Devis (25) who reported a marked increase in the free 17-hydroxycorticoids in the urine of pregnant women. In the bioassay employed by Venning, Kazmin, and Bell (29), the free corticosteroids are not removed prior to pH 1.0 hydrolysis and it is probable that cortisol and cortisone are biologically more potent than their tetrahydro derivatives. It is conceivable, therefore, that the increase in glucocorticoid concentration observed by Venning (22) might be related to the increased proportion of free compounds excreted in the terminal phase of pregnancy. The methods which measure neutral reducing lipids or formaldehydogenic steroids are known to be relatively nonspecific. It is possible that some of the other steroids and/or their metabolites which are produced and excreted in large amounts during pregnancy might interfere with the measurement of urinary corticosteroids by these techniques. On the other hand, the determination of urinary 17,21-dihydroxy-20-ketones by the reaction of Porter and Silber (30) is probably somewhat more specific; little or no increase in urinary corticoids was observed during pregnancy when using this technique (25, 27). Little change in 17-ketogenic steroid excretion was also reported (31).

If we assume that the newborn disposes of the radioactive steroids received from the mother in the same manner that he does following the intravenous administration of a given dose of 4-$C^{14}$-cortisol, the total amount of urinary radioactivity excreted in the first 48 hours following birth would represent approximately 80 per cent of the total radioactivity which crossed the placental barrier; that is, 2 to 3 per cent of the dose administered to the mother. However, this assumption might

[4] Similar results have been obtained in two other patients (subjects A. T. and A. U.) who received 4-$C^{14}$-cortisol 48 hours prior to surgery (21).

[5] The same conclusion was drawn by Mills (31) from his work on plasma free cortisol levels and 17-ketogenic steroid excretion during pregnancy.

not be entirely true since it is possible that maternal conjugated steroids cross the "placental barrier," and because it is also probable that free and/or conjugated steroids are returned from the fetus to the mother, the entire process being a dynamic phenomenon.

When each urinary fraction excreted by the baby was expressed in percentage of the total output of radioactivity, the values for the glucuronoside fraction ranged from 3.9 to 20 per cent of the total with an average of 12 per cent, while the average for 7 normal adults was 51.6 per cent and that of the mothers studied was 27.5 per cent. The newborn appeared to have a diminished ability to conjugate cortisol metabolites with glucuronic acid. The pattern of the other urinary fractions did not differ greatly from that observed in normal adults, and consequently the amount of urinary radioactivity unaccounted for following the various hydrolyses was very large. As seen in Table III, a large amount of this activity was left in the urine and even butanol did not appear to remove it completely. The same phenomenon was observed in normal adults but to a lesser degree. It would seem that there are modes of conjugation of steroids with compounds other than sulfuric acid, and it is possible that the newborn, because of his partial inability to conjugate with glucuronic acid, would use these other modes of detoxification to a larger extent than does the normal adult.

The sum of the amounts of radioactivity excreted by the mother and her baby appeared to be somewhat smaller than the total urinary excretion observed in control subjects. We have some indication that some of this activity was trapped in the placenta and that some of it could be detected in amniotic fluid. It must also be noted that when 4-C$^{14}$-cortisol was administered shortly prior to the surgical procedure, a certain amount of the hormone was contained in the 500 to 750 ml. of blood lost by the mother at the time of delivery. Another possibility is that the mother might excrete amounts of fecal radioactivity larger than normal. Further work is in progress to study this problem.

## SUMMARY

The metabolism of 4-C$^{14}$-cortisol during pregnancy, near term, was found to be markedly altered. In plasma, the half-life of free radioactivity was twice that observed in control subjects and the amount of radioactivity released by $\beta$-glucuronidase hydrolysis was smaller than normal. The total amount of urinary radioactivity excreted was diminished, with an increase in the "free fraction" and a decrease in the "glucuronoside fraction." These data suggest that the increase in plasma free 17-hydroxycorticosteroids observed near term is related to a slower rate of catabolism of cortisol rather than to a more rapid rate of its production.

Similar results were obtained when the surgical procedure of elective, repeat cesarean section was performed 17 to 69 minutes after the administration of the radioactive steroid. The increase in plasma free 17-hydroxycorticosteroids observed following this procedure would then appear to be related to a real increase in cortisol production rather than solely to an interference in its catabolism.

In cord plasma, the amounts of free radioactivity and that released by $\beta$-glucuronidase hydrolysis were one-half to one-fifth those of the corresponding mother; this confirmed the observation that cortisol and/or its free metabolites can cross the "placental barrier."

No radioactivity, free or conjugated with glucuronic acid, was found in cord plasma 24 to 48 hours after 4-C$^{14}$-cortisol administration to the mother; this suggested that the fetus has means of disposing of cortisol and its metabolites.

The total amount of radioactive steroid crossing the placenta appeared to represent 2 to 3 per cent of the dose administered to the mother. However, this might be too low a figure if some of the steroids are returned from the fetus to the mother.

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