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Biochemistry

Deoxycorticosterone biosynthesis in human kidney: Potential for formation of a potent mineralocorticosteroid in its site of action

(steroid 21-hydroxylase/steroid 21-monooxygenase)

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ABSTRACT **The extra-adrenal formation of deoxycorticosterone (DOC) from plasma progesterone has been demonstrated in humans. In those studies it was shown that in some persons the volume of plasma cleared of progesterone by DOC formation was great, namely, 75 liters/24 hr. Because steroid 21-hydroxylase activity [steroid 21-monooxygenase; steroid, hydrogen-donor:oxygen oxidoreductase (21-hydroxylating), EC 1.14.99.10] could not be demonstrated in homogenates or microsome-enriched preparations of human lung or liver tissue, we speculated that the 21-hydroxylation of plasma progesterone might take place in the kidney. Employing whole tissue homogenates and microsome-enriched preparations of human kidney tissue, we demonstrated the formation of [^{3}H]DOC from [^{3}H]progesterone. The rate of formation of DOC from progesterone in microsomal preparations from kidney tissues of adult humans varied from 0 to 803 pmol per mg of microsomal protein per hr. The value computed for the apparent K_m of the enzyme for progesterone was 0.140 μM. On the basis of these findings, we conclude that steroid 21-hydroxylase activity is present in human kidney tissue and that the kidney may be an important site of DOC formation as well as a site of DOC action.**

The 21-hydroxylation of plasma progesterone has been demonstrated in pregnant, nonpregnant, and adrenalectomized women and in men (1). The fractional conversion of plasma progesterone to deoxycorticosterone (DOC) ($[\rho]_{BU}^{PDOC}$) computed from the $^3H/^{14}C$ ratio of urinary 3α,21-dihydroxy-5β-pregnan-20-one (tetrahydro-DOC) and that of the injected tracers, [^{3}H]progesterone and [^{14}C]DOC, varied from 0.002 to 0.03 [0.009 ± 0.001 (mean ± SEM, n = 32)] among the subjects of those studies (1, 2). In a given woman the $[\rho]_{BU}^{PDOC}$ was the same when she was and was not pregnant. These findings are indicative that 21-hydroxylation of plasma progesterone is not rate limiting over wide ranges of levels of progesterone in plasma. Thus, the rate of DOC formation from plasma progesterone is proportional to the concentration of progesterone in plasma (2). Because the fractional conversion of plasma progesterone to DOC in adrenalectomized persons was similar to that found in normal persons it was concluded that 21-hydroxylation of circulating progesterone is principally a nonadrenal phenomenon.

It can be computed that in some persons 75 liters of plasma are cleared of progesterone each day by DOC formation (1, 2). On the basis of this finding we deduced that steroid 21-hydroxylase [steroid 21-monooxygenase; steroid, hydrogen-donor:oxygen oxidoreductase (21-hydroxylating), EC 1.14.99.10] must be distributed among many tissues or else is present principally in an organ(s) with a large blood flow. To evaluate this latter possibility, we incubated homogenates and microsome-enriched fractions prepared from human liver, lung, and kidney tissues with radiolabeled progesterone. Radiolabeled DOC was formed in incubation mixtures containing human kidney tissue and [^{3}H]progesterone, but not in those containing human liver or lung tissue and [^{3}H]progesterone.

MATERIALS AND METHODS

Steroids. [1,2,6,7-^{3}H(N)]Progesterone (98 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), purchased from New England Nuclear, was purified by column chromatography on ethylene glycol/Celite (3). [4-^{14}C]DOC (40 mCi/mmol), also purchased from New England Nuclear, was purified by liquid–liquid partition chromatography on Celite, employing the solvent system isooctane/*tert*-butyl alcohol/methanol/water (25:10:9:6, vol/vol) (3). Nonradiolabeled progesterone, DOC, and DOC acetate were purchased from Steraloids (Wilton, NH).

Tissue Preparation. Kidney tissue was obtained from a pregnant woman and her stillborn term infant within 3 hr of death due to injuries sustained in an automobile accident, from five other adults within 4 hr of death, and from seven subjects 5 hr or more after death. Three kidneys that had been maintained (9–25 hr) on an infusion pump at 4°C in preparation for transplantation (but for various reasons were unsuitable for transplantation) also were used in this study. Human tissues were obtained in accordance with the Donor's Anatomical Gift Act of the State of Texas after consent in writing was obtained from the next of kin under the auspices of a protocol approved by the Human Research Review Committee of this institution. The kidney tissues were dissected free of capsule and adventitious tissue. In the case of the tissues obtained from the pregnant woman and the stillborn infant, 1 g of each tissue was homogenized in 5 ml of potassium phosphate buffer (50 mM, pH 7.4) containing $MgCl_2$ (3 mM). Samples of the homogenates were used as enzyme source. In addition, 1 g of each tissue obtained from the pregnant woman and her stillborn infant and 1 g of the tissues obtained from the other subjects were homogenized with a Teflon/glass homogenizer in 5 ml of sucrose (0.25 M), and these homogenates were centrifuged at 15,000 × g for 10 min at 4°C. The supernatant fluid was centrifuged at 100,000 × g for 1 hr at 4°C. The pellets were resuspended in 5 ml of sucrose (0.25 M) and centrifuged at 100,000 × g for 1 hr at 4°C. The pellets were resuspended in potassium phosphate buffer (50 mM, pH 7.4) containing $MgCl_2$ (3 mM). Samples (1 ml) of the microsome-enriched preparations were used as the enzyme source. Microsome-enriched fractions of human lung and liver tissues were prepared in a similar manner and were used as the enzyme source. The microsome-enriched preparations were added to vessels containing 1 ml of a NADPH-generating system, which consisted of $NADP^+$ (1.25

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Abbreviations: DOC, deoxycorticosterone; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography.

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mg/ml), glucose 6-phosphate (2.8 mg/ml), and glucose-6-phosphate dehydrogenase (1.2 units/ml) in potassium phosphate (50 mM, pH 7.4) buffer containing $MgCl_2$ (3 mM). [^{3}H]Progesterone (5×10^6 cpm), dissolved in ethanol (20 μl), and various quantities of nonradiolabeled progesterone (0–5 nmol), dissolved in ethanol (20 μl), were added to initiate the reaction. Incubations were conducted for various times at 37°C in an oscillating water bath in an atmosphere of room air. In most instances [^{14}C]DOC (5000–7000 cpm) was added to the incubation mixture at the end of the incubation period as an internal recovery standard and the reaction was terminated by the addition of ethyl acetate.

Samples (50–200 μl) of the homogenates or of the microsome-enriched suspensions were taken for measurement of protein according to the method of Lowry *et al.* (4), employing bovine serum albumin as the standard. Samples of tissue preparations (0.5–1.0 ml) also were taken for the determination of the endogenous tissue concentrations of progesterone and DOC. Progesterone and DOC in the tissues were quantified by radioimmunoassay (2, 5, 6).

Purification of Radiolabeled DOC Isolated from the Incubation Mixtures. The radiolabeled DOC formed in incubations of the tissue preparations of the kidneys of the pregnant woman and her stillborn infant was purified by employing two techniques. First, duplicate samples of whole tissue homogenates were incubated with [^{3}H]progesterone for 2 hr at 37°C. In this instance the [^{14}C]DOC internal standard was not added to the incubation mixture, but at the end of the incubation period, nonradiolabeled DOC (100–200 μg) was added and the incubation mixtures were extracted twice with ethyl acetate. The solvent was evaporated and the residue was chromatographed by thin-layer chromatography (TLC) employing the solvent system methylene chloride/diethyl ether (9:1, vol/vol). The area of this chromatogram containing the steriod(s) that comigrated with authentic DOC was scraped from the plate and the steroid(s) were eluted with ethyl acetate. The solvent was evaporated and the residue was treated with a mixture of pyridine and acetic anhydride (1:1, vol/vol) for 2 hr at 37°C. The reaction product was chromatographed by TLC employing the solvent system isooctane/ethyl acetate (1:1, vol/vol). The area of this chromatogram containing steroid(s) that comigrated with authentic DOC acetate was scraped from the plate and the steroids were eluted with ethyl acetate. An aliquot of each sample was assayed for radioactivity. Counting efficiency was 31% for ^{3}H and 65% for ^{14}C. DOC acetate (approximately 60 mg) was added to each sample. Each sample was recrystallized in the solvent pair diethyl ether/petroleum ether (bp 20–40°C) and the specific activities of DOC acetate in successive mother liquors and final crystals were determined. The mass of DOC acetate was quantified by UV spectrophotometry.

Duplicate samples of the microsome-enriched preparations were incubated with [^{3}H]progesterone for 2 hr at 37°C. At the end of the incubation period, [^{14}C]DOC and nonradiolabeled DOC (200 μg) were added and the incubation mixtures were extracted twice with ethyl acetate. The solvent was evaporated and the residue was chromatographed by gradient-elution chromatography on ethylene glycol/Celite (3). The fractions of the chromatogram that contained DOC were combined. The solvent was evaporated and the residue was chromatographed by liquid–liquid partition chromatography on Celite employing the solvent system isooctane/*tert*-butyl alcohol/methanol/water (25:10:9:6) (7). The fractions containing DOC were combined, the solvent was evaporated, and the residue was chromatographed by TLC as described above. After TLC, half of the sample was evaporated, and the residue was treated with a mixture of pyridine and acetic anhydride (1:1, vol/vol) for 2 hr at 37°C. The DOC acetate was purified by TLC as described and, after TLC, authentic nonradiolabeled DOC acetate (40 mg) was added to each sample that was recrystallized. The remaining half of the sample was purified further by high-pressure liquid chromatography (HPLC) as described (2).

In subsequent studies, we established that an abbreviated purification procedure was sufficient to establish radiochemical homogeneity of the radiolabeled DOC formed from [^{3}H]progesterone in incubations with kidney tissue preparations. The abbreviated procedure consisted of chromatography of the residue of the extract of the incubation mixture by TLC [ethyl acetate/isooctane (7:3, vol/vol)], TLC [methylene chloride/diethyl ether (4:1, vol/vol)], HPLC, acetate formation, TLC of the DOC acetate [isooctane/ethyl acetate (1:1, vol/vol)], and recrystallization to constant ^{3}H/^{14}C ratios.

RESULTS

Tissue Concentrations of DOC and Progesterone. The quantities of both DOC and progesterone found in the tissue preparations before the addition of progesterone (zero time) uniformly were less than the lower limits of detectability of the assay (24 pg/ml and 100 pg/ml for DOC and progesterone, respectively).

Demonstration of the Presence of Steroid 21-Hydroxylase Activity in Human Kidney Tissue. The formation of [^{3}H]DOC from [^{3}H]progesterone in incubations of human kidney tissue preparations of a pregnant woman and her stillborn infant was established in two ways. First, [^{14}C]DOC was added to incu-

Table 1. Establishment of radiochemical homogeneity of [^{3}H]DOC formed from [^{3}H]progesterone by human kidney tissue by chromatography and recrystallization to constant ^{3}H/^{14}C ratios

Tissue sample			^{3}H/^{14}C ratio after each purification step									
			Chromatography									
			Gradient elution on ethylene glycol/Celite	Liquid–liquid partition on Celite	TLC		TLC	Crystallization (DOC-Ac)*				
Organ	Source	Preparation	(DOC)	(DOC)	(DOC)	HPLC	(DOC-Ac)	ML_1	ML_2	ML_3	ML_4	X
Kidney	Pregnant woman	Microsome	101	100†	115‡	113‡	112	114	114	114	116	109
Kidney	Newborn	Microsome		17.5†	19.2		19.1	18.7	19.1	19.3		19.0

* ML, mother liquor; X, final crystals.

† Different amounts (dpm) of [^{14}C]DOC were added as recovery standard prior to purification of the radiolabeled DOC and thus the ^{3}H/^{14}C ratios are different, whereas the fractional conversion of [^{3}H]progesterone to DOC was similar in the kidney tissues of the pregnant woman and her stillborn infant.

‡ Sample obtained from this experiment was divided in half after TLC (DOC). After purification of DOC by Celite chromatography and TLC, one half was acetylated and purified by TLC and crystallization and one half was chromatographed by HPLC.

Table 2. Establishment of radiochemical homogeneity of [^{3}H]DOC formed from [^{3}H]progesterone by human kidney tissue by recrystallization to constant specific activity

Tissue sample*			Radioactivity recovered as [^{3}H]DOC-Ac after TLC, acetylation, and TLC of DOC-Ac, dpm	Amount of nonradiolabeled DOC added, mg	Specific activity, dpm/mg						
							Crystallization†				
Organ	Source	Preparation			Computed	Measured	ML_1	ML_2	ML_3	ML_4	X
Kidney	Pregnant woman	Homogenate A	16,959	61.5	273	289	328	250	298	287	239
		Homogenate B	13,218	61.9	214	224	306	226	194	218	321
Kidney	Newborn	Homogenate A	26,418	60.3	438	507	430	596	408	432	474
		Homogenate B	20,862	60.8	342	373	357	348	281	333	376
Adrenal	Newborn	Homogenate	372	61.0	6	5.6‡					

* A and B represent duplicate experiments employing the same kidney tissue.
† ML, mother liquor; X, final crystals.
‡ The specific activity of the radiolabeled DOC was very low at this purification step. Therefore, crystallization was not performed.

bation mixtures of microsome-enriched preparations of human kidney tissues that had been incubated with [^{3}H]progesterone. The $^3H/^{14}C$ ratios of DOC isolated after each chromatographic step and after derivative formation and chromatography of the DOC acetate, as well as the $^3H/^{14}C$ ratios of DOC acetate in each mother liquor and in the final crystals, are presented in Table 1. It was found that the $^3H/^{14}C$ ratio of DOC isolated from the incubation mixture varied little after the first chromatographic step. This was also found to be the case in incubations of other kidney preparations that contained high steroid 21-hydroxylase activity. There was no steroid 21-hydroxylase activity in boiled kidney preparations. Second, [^{3}H]DOC isolated from incubations of kidney tissue homogenates incubated with [^{3}H]progesterone was isolated and acetylated. Then nonradiolabeled DOC acetate was added to the [^{3}H]DOC acetate. The DOC acetate was recrystallized. The calculated specific activity of the original mixture of DOC acetate, the determined specific activity of the original mixture, and the specific activities determined in successive mother liquors and final crystals were similar (Table 2). The fractional conversions of [^{3}H]progesterone to [^{3}H]DOC by whole homogenates of adult and newborn kidney tissues were 0.07 and 0.15, respectively. The fractional conversions of [^{3}H]progesterone to [^{3}H]DOC in microsome-enriched preparations of adult and neonatal tissues were 0.32 and 0.36, respectively.

Validation of the Reliability of the Short Method of Purification of Radiolabeled DOC Formed in Incubations of Human Kidney with [^{3}H]Progesterone. It was established that an abbreviated purification procedure could be employed to attain radiochemical homogeneity of the DOC isolated from incubations of kidney tissue. [^{14}C]DOC was added to duplicate incubation mixtures of microsome-enriched preparations of human kidney tissues incubated with [^{3}H]progesterone. The radiolabeled DOC in the two mixtures was then purified in two different ways. The $^3H/^{14}C$ ratios of DOC after each purification step of the abbreviated procedure were compared to those values after each purification step in the longer procedure (Table 3). The $^3H/^{14}C$ ratios of the purified DOC were nearly identical with the two procedures. Therefore, in most of the subsequent studies we employed the abbreviated procedure.

Rate of Formation of DOC from Progesterone by Human Kidney Tissue. In this study cortical and medullar tissues were separated by microdissection prior to homogenization. The rate of DOC formation was reasonably linear at progesterone concentrations between 0.18 and 0.37 μM (Fig. 1). When the progesterone concentration was greater than 1.0 μM there was no further significant increase in the rate of DOC formation. Interestingly, the specific activity of steroid 21-hydroxylase was considerably greater in cortical than in medullary tissue. Lineweaver–Burk analysis of these data was performed and the results are illustrated in Fig. 2. The value computed for the apparent K_m of the enzyme for progesterone was 0.140 μM and the V_{max} was 4.0 pmol per mg of microsomal tissue protein per hr in the cortical tissue used in this study. All subsequent experiments were performed at a progesterone concentration in the incubation mixture of 1 μM.

Experiments were performed with microsome-enriched preparations of kidney tissues obtained from an additional 14 nonpregnant subjects. In these studies cortex and medulla were not separated. The highest specific activity for steroid 21-hydroxylase activity (803 pmol per mg of microsomal protein per hr) was found in microsome-enriched preparations of kidney tissue obtained 2 hr after death of a 39-year-old woman who died of injuries sustained in an accident. In kidney tissue of a 65-year-old man who died of a myocardial infarction 3 hr prior to tissue preparation, the specific activity of the enzyme was 48 pmol per mg of microsomal protein per hr; and specific activities of 3.6 and 0.6 pmol per mg of microsomal protein per

Table 3. $^3H/^{14}C$ ratios of radiolabeled DOC after each chromatographic step and those of the successive mother liquors and of the final crystals: Comparison of the data obtained by utilizing two different chromatographic techniques

Sample*	Liquid–liquid partition chromatography	Chromatography†					Recrystallization‡				Fractional conversion of [^{3}H]progesterone to [^{3}H]DOC
		TLC_1	TLC_2	TLC_3	HPLC	TLC_4	ML_1	ML_2	ML_3	X	
A	17.5	19.2	19.2	19.1	—	—	18.7	19.1	19.3	19.0	0.37
B	—	19.1	18.2		17.4	16.6	17.8	18.1	18.2	18.2	0.35

* Kidney tissue from a stillborn infant was employed as the source of steroid 21-hydroxylase activity. The initial chromatographic step for the purification of radiolabeled DOC isolated from incubation mixture A was gradient elution chromatography on ethylene glycol/Celite (3).
† TLC systems employed: TLC_1, ethyl acetate/isooctane (7:3, vol/vol); TLC_2, methylene chloride/diethyl ether (4:1, vol/vol); TLC_3 and TLC_4, isooctane/ethylacetate (1:1, vol/vol). This last system was employed for TLC of DOC acetate. In the HPLC, methanol/water (85:15, vol/vol) was employed as the mobile phase.
‡ ML, mother liquor; X, final crystals.

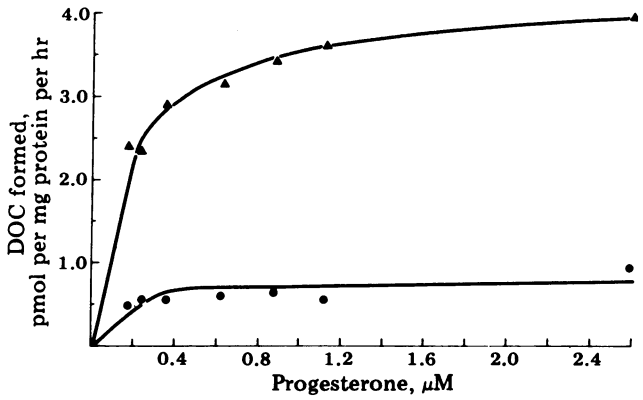

FIG. 1. Rate of formation of DOC from progesterone by human kidney tissue as a function of the progesterone concentration in the incubation mixture. The incubations were performed at 37°C for 2 hr in room air. Microsome-enriched fractions prepared from adult human kidney cortical (▲) and medullary (●) tissues were employed as the source of the 21-hydroxylase activity.

hr were found in kidney tissues of a 56-year-old man and of a 31-year-old man, respectively. These tissues were obtained 4 hr after death. No steroid 21-hydroxylase activity was found in three kidneys that had been maintained for 9.5–25 hr on an infusion pump at 4°C. Steroid 21-hydroxylase activity could not be demonstrated in kidney tissue obtained 6 and 19 hr after death from two men ages 49 and 50. The enzyme activity was not demonstrable in kidney tissues of four women and one girl (ages 6–53 years) who had died 5 or more hr prior to the time of tissue preparation. The variations in the specific activities of steroid 21-hydroxylase among kidney tissues may be physiologically significant or else may represent only length of time from death until completion of kidney tissue microsome preparation.

Rate of Formation of DOC from Progesterone by Human Kidney Tissue as a Function of Protein Concentration and Time of Incubation. The rate of DOC formation from progesterone by microsome-enriched preparations of human kidney tissue was reasonably linear with protein concentration from 0.05 to 0.40 mg per ml (Fig. 3).

The results of studies of DOC formation from progesterone by microsome-enriched preparations of human kidney tissue as a function of incubation time are presented in Fig. 4. The rate of formation of DOC from progesterone was linear for 2 hr,

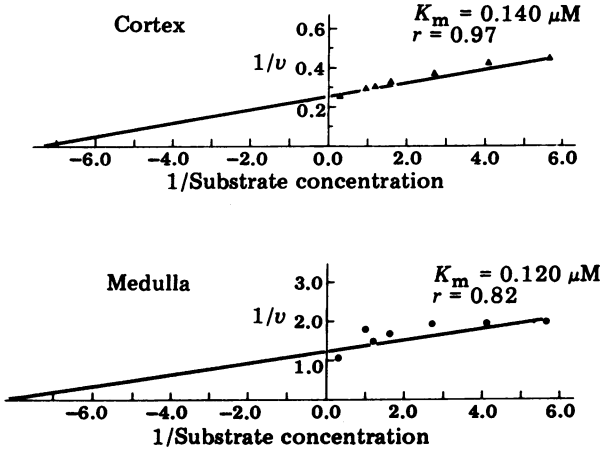

FIG. 2. Lineweaver–Burk analysis of the data presented in Fig. 1, in the same units. *r*, Correlation coefficient.

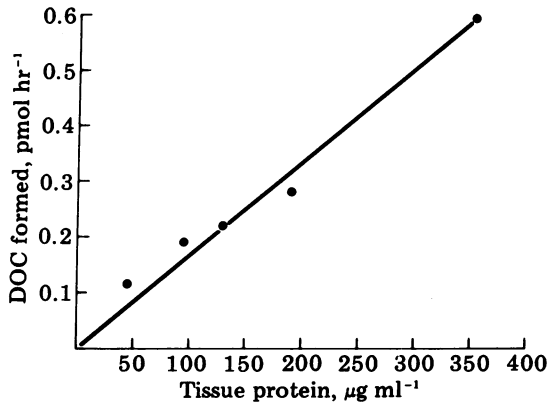

FIG. 3. Rate of formation of DOC from progesterone by adult human kidney tissue as a function of protein concentration. The incubations were performed at 37°C for 2 hr in room air. The progesterone concentration in the incubation mixture was 1 μM. Microsome-enriched fractions prepared from adult human kidney tissue were employed as the source of the 21-hydroxylase activity.

after which the activity appeared to decrease. DOC formation was also found to be linear with time of incubation up to 2 hr in various tissue preparations with widely different steroid 21-hydroxylase specific activities.

DISCUSSION

The importance of the conversion of circulating prehormones to biologically active hormones has been demonstrated in the case of 17β-estradiol and esterone formation from plasma C_{19}-steroids, testosterone formation from circulating androstenedione, and 5α-dihydrotestosterone formation from plasma testosterone (7–13). Other investigators have demonstrated steroid 21-hydroxylase activity in human fetal testicular tissue (14) and in abnormal human ovarian tissue *in vitro*†. However, to our knowledge, steroid 21-hydroxylase activity has not been found previously in human kidney tissue. The specific activity of steroid 21-hydroxylase was considerably greater in cortical than in medullary tissue of the human kidney. The rate of DOC

† Mahesh, V. B. & Greenblat, R. B. (1965) *Proceedings of the 47th Annual Meeting of the Endocrine Society*, New York, June 17–19, p. 25 (abstr.).

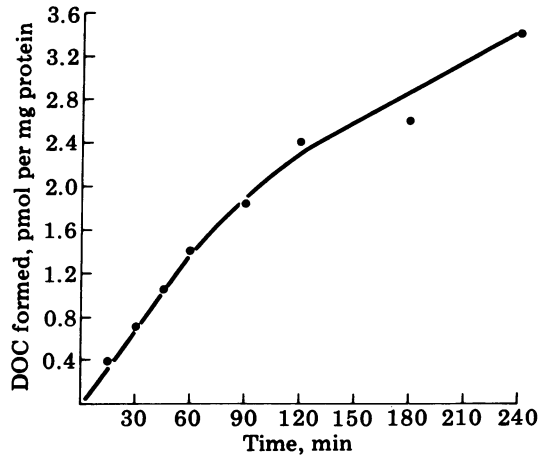

FIG. 4. Formation of DOC from progesterone by adult human kidney tissue as a function of incubation time. The incubations were performed at 37°C in room air. The progesterone concentration in the incubation mixtures was 1 μM. Microsome-enriched fractions prepared from adult human kidney tissue were employed as the source of the 21-hydroxylase activity.

formation, up to 800 pmol per mg of microsomal protein per hr, was great in some kidney tissues and may be sufficient to account for the demonstrated conversion of plasma progesterone to DOC in human subjects. Steroid 21-hydroxylase activity could not be demonstrated in microsome-enriched preparations of human liver or lung tissue (data not shown).

Interestingly, the fractional conversion of plasma progesterone to DOC varies widely among individuals (0.002–0.03), but in the same person the fractional conversion remains reasonably constant even with wide fluctuations in the plasma concentration of progesterone (1, 2). Ordinarily, the fractional conversion of a circulating steroid to its product(s) is similar among normal persons (10–13). The finding of strikingly different values for the fractional conversion of plasma progesterone to DOC among normal subjects is suggestive of the possibility of a genetic determination for the capacity of extra-adrenal 21-hydroxylation. If this were true, and if extraadrenal 21-hydroxylation were confined principally to the kidney, it would be possible that the *in situ* formation of relatively large amounts of DOC in the tissue site of mineralocorticosteroid action may be instrumental in the development of sodium retention and hypertension.

In men and in nonpregnant women during the follicular phase of the ovarian cycle the production rate of progesterone has been estimated to be 1–3 mg/24 hr (15, 16); during the midluteal phase of the ovulatory cycle the rate of secretion of progesterone is approximately 40 mg/24 hr (15, 16); and in pregnant women near term, 250–600 mg of progesterone enter the maternal circulation daily (15, 17). Assuming renal plasma flow in the nonpregnant adult to be 600 ml/min (18) and the plasma DOC concentration (arising from adrenal secretion of DOC) to be 0.05 μg/liter (19), the rate of DOC delivery to the kidneys through the blood would be 30 μg/24 hr. If all DOC formed from plasma progesterone were synthesized in renal tissue in men and in women during the follicular phase of the ovarian cycle, DOC formation from plasma progesterone in the kidneys of a person whose $[\rho]_{BU}^{PDOC}$ was 0.02, would be 20–60 μg/24 hr. However, during the luteal phase of the ovulatory cycle, when ovarian progesterone secretion is 40 mg/24 hr, extra-adrenal DOC formation in a person in whom the fractional conversion of progesterone to DOC was 0.02 would be 0.8 mg/24 hr, an amount which is at least 20 times that delivered to the kidneys from the adrenal gland. Moreover, during pregnancy, when progesterone production is 250–600 mg/24 hr, DOC formation from plasma progesterone would be 5–12 mg/24 hr, an amount many-fold greater than that delivered to the kidneys from the adrenal gland. If the kidney is the principal site of extra-adrenal DOC formation, the quantity of DOC formed *in situ* in the kidney would be considerably greater than that delivered to the kidney by adrenal secretion. Thus, the concentration of DOC in plasma may not represent an accurate estimation of the DOC available to the kidney.

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1. Winkel, C. A., Milewich, L., Parker, C. R., Jr., Gant, N. F., Simpson, E. R. & MacDonald, P. C. (1979) *Proc. Soc. Gynecol. Invest.* **26,** 140 (abstr.).
2. Winkel, C. A., Milewich, L., Parker, C. R., Jr., Gant, N. F., Simpson, E. R. & MacDonald, P. C. (1980) *J. Clin. Invest.* **66,** 803.
3. Siiteri, P. K. (1963) *Steroids* **2,** 687–712.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
5. Milewich, L., Gomez-Sanchez, C., Madden, J. D. & MacDonald, P. C. (1975) *Gynecol. Invest.* **6,** 291–306.
6. Gomez-Sanchez, C., Milewich, L. & Holland, O. B. (1977) *J. Lab. Clin. Med.* **89,** 902–909.
7. Siiteri, P. K. & MacDonald, P. C. (1963) *Steroids* **2,** 713–730.
8. Bolte, E., Mancuso, S., Ericksson, G., Wiqvist, N. & Diczfalusy, E. (1964) *Acta Endocrinol.* **45,** 576–599.
9. Siiteri, P. K. & MacDonald, P. C. (1966) *J. Clin. Endocrinol. Metab.* **26,** 751–761.
10. Horton, R. & Tait, J. F. (1966) *J. Clin. Invest.* **45,** 301–313.
11. MacDonald, P. C., Rombaut, R. P. & Siiteri, P. K. (1967) *J. Clin. Endocrinol. Metab.* **27,** 1103–1111.
12. Siiteri, P. K. & MacDonald, P. C. (1973) in *Handbook of Physiology,* ed. Geiger, S. R. (Am. Physiol. Soc., Washington, DC), pp. 615–629.
13. Baird, D. T., Horton, R., Longcope, C. & Tait, J. F. (1969) *Rec. Prog. Horm. Res.* **25,** 611–664.
14. Acevedo, H. F., Axelrod, L. R., Ishikawa, E. & Takaki, F. (1963) *J. Clin. Endocrinol. Metab.* **13,** 885–890.
15. Dorfman, R. I. & Ungar, F. (1965) *Metabolism of Steroid Hormones* (Academic, New York), p. 617.
16. Arcos, M., Gurpide, E., VandeWiele, R. L. & Lieberman, S. (1964) *J. Clin. Endocrinol. Metab.* **24,** 237–245.
17. Lin, T. J., Lin, S. L., Erlenmeyer, F., Kline, I. T., Underwood, R., Billiar, R. B. & Little, B. (1972) *J. Clin. Endocrinol. Metab.* **34,** 287–297.
18. Chesley, L. C. (1978) in *Hypertensive Disorders in Pregnancy* (Appleton, New York), pp. 154–198.
19. Shöneshöfer, M. & Wagner, G. G. (1977) *J. Clin. Endocrinol. Metab.* **45,** 814–817.