Cytochrome P450c17 catalyzes the 17α-hydroxylase activity required for glucocorticoid synthesis and the 17,20 lyase activity required for sex steroid synthesis. Most P450 enzymes have fixed ratios of their various activities, but the ratio of these two activities of P450c17 is regulated post-translationally. We have shown that serine phosphorylation of P450c17 and the allosteric action of cytochrome \( b_5 \) increase 17,20 lyase activity, but it has not been apparent whether these two post-translational mechanisms interact. Using purified enzyme systems, we now show that the actions of cytochrome \( b_5 \) are independent of the state of P450c17 phosphorylation. Suppressing cytochrome \( b_5 \) expression in human adrenal NCI-H295A cells by > 85% with RNA interference had no effect on 17α-hydroxylase activity but reduced 17,20 lyase activity by 30%. Increasing P450c17 phosphorylation could compensate for this reduced activity. When expressed in bacteria, human P450c17 required either cytochrome \( b_5 \) or phosphorylation for 17,20 lyase activity. The combination of cytochrome \( b_5 \) and phosphorylation was not additive. Cytochrome \( b_5 \) and phosphorylation enhance 17,20 lyase activity independently of each other, probably by increasing the interaction between P450c17 and NADPH-cytochrome P450 oxidoreductase.
now show that these two mechanisms of augmenting 17,20 lyase activity function independently.

MATERIALS AND METHODS

Cell Culture and Microsome Preparation—NCI-H295A cells (34), an adherent human adenocortical carcinoma NCI-H295 cells (35, 36), were grown in 150-mm Petri dishes as described (34). HepG2, JEG3 (37), and HEK293 (38) cells were grown as described. NCI-H295A cells from several plates were collected by scraping, washed with chilled phosphate-buffered saline (PBS), suspended in 50 mM sodium phosphate containing 150 mM KCl, and lysed by sonication. Unbroken cells and mitochondria were removed by centrifugation at 10,000 × g for 15 min, and microsomes were collected by ultracentrifugation at 100,000 × g for 90 min in a Beckman T-100 rotor. Microsomes were resuspended in 50 mM potassium phosphate buffer containing 20% glycerol.

Cytochrome b5 Expression Analysis—Expression of cytochrome b5 in various cell lines was analyzed by RT-PCR using GAPDH as control. The primer sequences used were for cytochrome b5 forward AGAGCAGTCGGACGA-3 and reverse GGCCATGT-3. Expression and Purification of Human P450c17—Human P450c17 was expressed in E. coli strain BL21(DE3) transformed with plasmid pCWH17-mod(His)4 containing the cDNA for N-terminally tagged P450c17, and purified as described (39). The recombinant plasmid was purified from bacterial cultures and digested with BglI and HindIII and ligated to the 64-mer oligonucleotide (IPTG) and 0.4 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) or 0.4 mM 3-aminolevulinic acid were added, and the culture was shaken at 125 rpm for 10 min at 4 °C. The bacteria were then washed with PBS, treated with lysozyme (0.1 mg/ml) and EDTA (0.1 mM, pH 8.0), and membranes were prepared as described for P450c17. Membranes were dissolved in 100 mM phosphate buffer containing 100 mM NaCl, 1.5 mM β-glycerolphosphate, and 0.4 mg/ml bovine serum albumin. The membranes were expressed using 1 mg/ml dithionite, and the spectra were recorded at 600 nm and 490 nm using a millimolar extinction coefficient of 9.0 and 8.5, respectively. The amount of phosphatase released into the supernatant was monitored by malachite green reaction (BioMol Laboratories).

Expression and Purification of Human POR—Human POR was expressed in bacteria by transforming E. coli BL21(DE3)pLysS cells with a pET22b vector (Novagen) containing human POR cDNA (17). Freshly transformed E. coli were selected on a LB agar plate with 100 μg/ml carbenicillin and IPTG. A single colony was selected, grown to 40% with 0.5–0.7, 0.4 mM IPTG and 0.4 mg/ml chloramphenicol, and purified as described (42). The catalytic activity of this POR preparation was assessed by assays using either cytochrome c or NADPH as substrates as described (17).

Expression and Purification of Cytochrome b5—Human cytochrome b5 was purified as described (43). A single colony was grown the next day and the process of induction and selection with carbenicillin and IPTG was repeated three more times. After four cycles of selection, the E. coli were transformed with plasmid pLysS Rare, which encodes lysozyme and six rare RNAs that assist the expression of mammalian proteins by complementing the codon bias of mammalian mRNAs (44). The resultant E. coli were used for expression of cytochrome b5. Expression and purification was performed as described previously (44).

Suppression of Cytochrome b5 by Short Hairpin RNA (shRNA) in NCI-H295A Cells—NCI-H295A cells were transfected with the retroviral vector pSUPERretro (45, 46) expressing shRNA targeted against both types 1 and 2 human cytochrome b5. The pSUPERretro vector was digested with BglII and HindIII and ligated to the 64-mer oligonucleotide duplexes (sense 5′-GATCCCCAAAAGCTGGAGTGACCGTATTCA-3′ and antisense 3′-GGGTTGACCTCCATCGGATCAGTTCTACGGATGGA-5′) encoding small interfering RNA (siRNA) against cytochrome b5, and a 9-nucleotide loop region. The recombinant plasmid was purified from bacterial cultures and digested with EcoRI and HindIII to check the inserts. Purified plasmid was transformed into NCI-H295A cells, and cells containing the virus were selected by puromycin and used for cytochrome b5 measurement and P450c17 enzyme assays.

Measurement of Cytochrome b5 Protein and mRNA—The cytochrome b5 content of NCI-H295A cells was measured by differential spectrophotometry. After drug treatment, cells were homogenized in potassium phosphate buffer (50 mM, pH 7.4) containing 150 mM KCl; the homogenate was cleared by centrifugation at 5000 × g for 10 min, placed into reference and sample cuvettes and baseline spectra were recorded between 400 and 500 nm at a speed of 120 nm/min. Cytochrome b5 in the sample cuvette was reduced with 1 mg sodium dithionite, and the spectra were recorded again. Cytochrome b5 content was estimated by the difference in absorbance at 423 and 490 nm using a millimolar extinction coefficient of 13266 Regulation of 17,20 Lyase Activity
181 nmol cm$^{-1}$ (47). Cytochrome $b_5$ mRNA was estimated by RT-PCR.

**In Vitro Phosphorylation of P450c17**—To prepare a cytoplasmic fraction enriched for the kinases that phosphorylate P450c17 and devoid of phosphatase activity, NCI-H295A cells were lysed, and cytosol was passed over a $\gamma$-ATP-Sepharose column (Upstate Biotechnologies); the column was washed with NAD, NADP, ADP, and AMP, and ATP-binding proteins were eluted with 10 mM ATP (29). Kinase activity was checked by phosphorylation of microsomes or purified bacterially expressed recombinant human P450c17 by incorporation of $\gamma$-32P-ATP. Purified P450c17 was phosphorylated in vitro using 6 $\mu$g of purified P450c17 incubated with 10 $\mu$g of kinase fraction in the presence of 50 mM HEPES buffer, 10 mM ATP, 60 mM MgCl$_2$, and 10 $\mu$M okadaic acid at 25 °C for 30 min and phosphorylated P450c17 was purified on mini Ni-NTA columns (29).

**P450c17 Enzyme Assays**—The 17α-hydroxylase and 17,20 lyase activities of P450c17 were assayed as described (13, 39). Purified P450c17 (10 pmol) and POR (20 pmol) were incubated with 100 mM potassium phosphate, 6 mM potassium acetate, and 10 $\mu$M reduced glutathione, 20% glycerol, 20 $\mu$g microsomal cytochrome $b_5$, 0.1 mM glucose-6-phosphate and radiolabeled substrate (1$\nu$C) for hydroxylase assay and [3H]17OH-Preg for lyase assay) with or without 10 pmol of cytochrome $b_5$ for 5 min at 25 °C in a total volume of 200 $\mu$L. The reaction was started by the addition of 20 $\mu$L of 10 mM NADPH and incubation at 37°C were carried out for various times and stopped by adding ethyl acetate/iso-octane (3:1) to extract the steroids. Steroids from different reactions were spotted on silica gel F 6254 thin layer chromatography plates (Merck) and developed with ethyl acetate/chloroform (3:1) (4). Plates were dried, and steroids were quantitated by autoradiography on a Storm 860 phosphorimager using Image Quant software. Kinetic behavior was approximated as a Michaelis-Menten system. Curve fitting and calculations of maximum velocity ($V_{max}$) and apparent Michaelis constant ($K_m$) values were performed using LEONORA (48).

### RESULTS

**Isoforms of Cytochrome $b_5$ in NCI-H295A Cells**—Three forms of cytochrome $b_5$ have been described: the 98-amino acid soluble and 134-amino acid microsomal form are encoded by one gene (49, 50) and a 146-amino acid form associated with the outer mitochondrial membrane (OM$b_5$) is encoded by a second gene (51–53). To identify the forms of cytochrome $b_5$ found in NCI-H295A cells, we performed RT-PCR with two pairs of oligonucleotide primers that will amplify the three products of the two genes. In human liver HepG2 cells, the mRNAs for microsomal cytochrome $b_5$ and for OM$b_5$ were found in approximately equal amounts, but in human kidney HK293, human placenta JEG-3 cells, and especially in human adrenal NCI-H295A cells, the mRNA for microsomal cytochrome $b_5$ was much more abundant than the mRNA for OM$b_5$ (Fig. 2A). The mRNA for the soluble form, generally associated with erythropoietic tissues, was not detected in the cell lines tested. As human P450c17 is a microsomal enzyme and associated with erythropoietic tissues, we focused attention on microsomal cytochrome $b_5$. To determine the effect of cytochrome $b_5$ on the catalytic activities of P450c17 in steroidogenic cells, we used RNA interference to knock down cytochrome $b_5$ mRNA in human adrenal NCI-H295A cells (Fig. 2B). An shRNA targeted against cytochrome $b_5$ was transfected into human adrenal NCI-H295A cells, and cells were harvested 24, 48, 72, and 96 h later. Expression of this shRNA caused a gradual decrease in spectrally assayable cytochrome $b_5$ over time: cytochrome $b_5$ was reduced by 15% after 24 h, 65% after 48 h, and 85% after 72 h (Fig. 2C). Knockdown of cytochrome $b_5$ had no effect on 17α-hydroxylase activity in NCI-H295A cells for up to 96 h (Fig. 2, D and E). There was no measurable change in the 17,20 lyase activity in the first 24 h, but 17,20 lyase activity was reduced 15% after 48 h and 30% after 72–96 h (Fig. 2, D and E). This decrease in 17,20 lyase activity was much less than the decrease in cytochrome $b_5$ protein, suggesting that another factor was promot-
with either alkaline phosphatase or PP2A (data not shown); by contrast, native human P450c17 isolated from human adrenal cells or NCI-H295A cells releases readily detected phosphate under these conditions. Second, mass spectrometric analysis of tryptic peptides of bacterially expressed human P450c17 under these conditions. Second, mass spectrometric analysis of tryptic peptides of bacterially expressed human P450c17 under these conditions. Second, mass spectrometric analysis of tryptic peptides of bacterially expressed human P450c17 under these conditions.

**Effect of Cytochrome b_{5} and Phosphorylation on Bacterially Expressed Human P450c17**—Using the purified, bacterially expressed human P450c17 and POR, we examined the effects of cytochrome b_{5} and serine phosphorylation as independent variables on 17a-hydroxylase and 17,20 lyase activities in vitro. The P450c17-POR-b_{5} system was reconstituted using phosphatidylcholine (to provide a membrane environment) and an NADPH regeneration system consisting of glucose-6-phosphate dehydrogenase. When recombinant human P450c17 was incubated with POR at a molar ratio of 1:2 and cytochrome b_{5} was added in molar ratios of cytochrome b_{5} to P450c17 ranging from 0.1 to 100, an effect of cytochrome b_{5} on 17,20 lyase activity was first seen at a ratio of 0.5. A 1:1 ratio doubled the 17,20 lyase activity and a 3:1 ratio tripled it, but the maximal effect was seen until a molar ratio of about 30:1 (Fig. 5A). This high ratio for a maximal effect is consistent with previous results (13). Although the molar ratios of cytochrome b_{5} to P450c17 in human androgen-producing cells are not known, it is clear that a substantial effect is achieved with low ratios.

To examine the potential interplay of cytochrome b_{5} and phosphorylation of P450c17 on 17,20 lyase activity, bacterially expressed human P450c17 was phosphorylated in vitro using a kinase-enriched fraction from NCI-H295A cytosol that was devoid of 17a-hydroxylase or 17,20 lyase activity (29). P450c17 activities were assayed in reconstituted systems containing 10 pmol of P450c17, 20 pmol of POR, and 20 μg of phosphatidylcholine. In the absence of cytochrome b_{5} or serine phosphorylation, bacterially expressed human P450c17 had 17a-hydroxylase activity but very little 17,20 lyase activity. When cytochrome b_{5} was added to bacterially expressed human P450c17 in vitro at a cytochrome b_{5} to P450c17 ratio of 3:1, 17,20 lyase activity increased 3–4–fold. When bacterially expressed human P450c17 was phosphorylated with the kinase fraction from NCI-H295A cytosol, 17,20 lyase activity increased 4–5-fold in the absence of cytochrome b_{5} (Fig. 5B). Addition of cytochrome b_{5} to phosphorylated P450c17 at a cytochrome b_{5} to P450c17 ratio of 3:1, increased activity further, but the effects of cytochrome b_{5} and phosphorylation were neither additive nor cooperative (Fig. 5B). To explore these findings further, we performed kinetic analysis of P450c17 activities from non-phosphorylated and phosphorylated P450c17 with and without cytochrome b_{5} (Table I). Neither phosphorylation nor addition of cytochrome b_{5} affected the kinetic parameters of the 17a-hydroxylase reaction (p > 0.5). Addition of cytochrome b_{5} increased the catalytic efficiency (V_{max}/K_{m}) of the 17,20 lyase reaction 4-fold (p = 0.013), and P450c17 phosphorylation increased catalytic efficiency 6-fold (p = 0.003 compared with control). However, addition of cytochrome b_{5} to phosphorylated P450c17 increased the 17,20 lyase activity only 7-fold. This effect was not significantly different from the action of phosphorylation alone (p = 0.2). Thus the effects of cytochrome b_{5} and P450c17 phosphorylation are not additive. Most of the effect seems to come from increased reaction velocities, as changes in K_{m} were less than 2-fold.

**Effect of POR and Cytochrome b_{5} on 17,20 Lyase Activity**—POR is the obligate electron donor for both activities of P450c17, and high molar ratios of POR to P450c17 increase 17,20 lyase activity (14, 23). To examine the role of POR in modulating 17,20 lyase activity, we fixed the molar ratio of cytochrome b_{5}:P450c17 at 3:1 and varied the amount of POR. As expected, excess POR significantly increased 17,20 lyase activity (Fig. 6A). A dose response was seen for POR either in the absence or presence of cytochrome b_{5}, but 17,20 lyase activity was always greater when cytochrome b_{5} was present. Addition of POR increased the hydroxylase activity slightly but addition of cytochrome b_{5} had no effect on the hydroxylase activity. These data support our previous findings that cytochrome b_{5} augments 17,20 lyase activity by facilitating electron transfer from POR (13, 29).

**Effect of Cytochrome b_{5} and Phosphorylation on NCI-H295A Microsomes**—To determine whether the effects seen on recombinant human P450c17 in vitro also occurred in a native protein environment, we isolated microsomes from NCI-H295A cells and examined the effect of cytochrome b_{5} and of in vitro protein phosphorylation on 17a-hydroxylase and 17,20 lyase activities. Using the kinase preparation from NCI-H295A cytosol (29) we phosphorylated microsomes from NCI-H295A cells in vitro and assayed them for hydroxylase and lyase activities in the presence and absence of purified cytochrome b_{5} (Fig. 6B). NCI-H295A cells normally have a low level of 17,20
Fig. 5. **Dose response of cytochrome b5 on 17,20 lyase activity.** A, effect of increasing the ratio of cytochrome b5 to P450c17 using purified, bacterially expressed human b5 and P450c17 combined in vitro. Upper panel, maximal 17,20 lyase activity is achieved at a b5:P450 ratio of 30. Data are mean ± S.D. of three experiments. Lower panel, representative thin layer chromatogram of one of the experiments. B, effect of cytochrome b5 on purified, bacterially expressed human P450c17. In the presence of purified, bacterially expressed human POR (P450c17: POR: 1:2), P450c17 has a low level of 17,20 lyase activity. The 17,20 lyase activity (solid bars) and 17α-hydroxylase activity (open bars) under these control conditions are set at 100%. Addition of purified cytochrome b5 (b5) in a 1:1:2 ratio of b5:P450c17: POR increased 17,20 lyase activity 3-fold; phosphorylation of human P450c17 with the kinase fraction from NCI-H295H cell cytoplasm increased 17,20 lyase activity 4-fold (Kinase); and addition of cytochrome b5 in a 1:1:2 ratio of b5:P450c17: POR using the phosphorylated P450c17 increased 17,20 lyase activity 6-fold. The 17α-hydroxylase activity of P450c17 remained unaffected in all cases. Data are mean ± S.D. of three experiments.

### Table I

**Kinetics of P450c17 activities with cytochrome b5 and kinase treatments**

|                | 17α-Hydroxylase |            | 17,20 Lyase |            |
|----------------|-----------------|------------|------------|------------|
|                | K_m<sup>a</sup> | V_max<sup>b</sup> | V_max/K_m<sup>b</sup> | % of WT | K_m<sup>a</sup> | V_max<sup>b</sup> | V_max/K_m<sup>b</sup> | % WT |
| Control        | 3.7 ± 0.11      | 0.124 ± 0.027 | 0.034 (100) |          | 1.21 ± 0.29 | 0.047 ± 0.012 | 0.039 (100) |      |
| Cytochrome b5  | 3.2 ± 0.05      | 0.117 ± 0.021 | 0.037 (109) |          | 0.73 ± 0.15 | 0.127 ± 0.022 | 0.173 (446) |      |
| Kinase         | 3.9 ± 0.07      | 0.138 ± 0.032 | 0.035 (103) |          | 0.66 ± 0.12 | 0.159 ± 0.019 | 0.241 (617)<sup>d</sup> |      |
| Kinase + cytochrome b5 | 3.1 ± 0.09 | 0.123 ± 0.039 | 0.040 (116) |          | 0.67 ± 0.19 | 0.187 ± 0.024 | 0.279 (716)<sup>d</sup> |      |

<sup>a</sup> K_m values are in μM progesterone for 17α-hydroxylase and μM 17OH-pregnenolone for 17,20 lyase.

<sup>b</sup> p > 0.5 for all paired comparisons.

<sup>c</sup> p < 0.015 for comparison with cytochrome b5.

<sup>d</sup> p = 0.003 for comparison with cytochrome b5.

<sup>‡</sup> p = 0.2 for comparison with kinase alone.

Dose response of cytochrome b5 on 17,20 lyase activity. Adding cytochrome b5 increased 17,20 lyase activity 2–3-fold but had no effect on 17α-hydroxylase activity. In vitro phosphorylation of microsomal P450c17 using the kinase fraction from NCI-H295A cells increased 17,20 lyase activity 3.5-fold but had no effect on 17α-hydroxylase activity. Adding cytochrome b5 to microsomes that had been phosphorylated in vitro increased 17,20 lyase activity 5-fold, but had no effect on 17α-hydroxylase activity. Thus both protein phosphorylation and cytochrome b5 can enhance 17,20 lyase activity independently of each other, but their effects are neither additive nor cooperative.

### Discussion

Steroidogenesis in the primate adrenal is divided into three morphologically and functionally distinct zones (54). The zona glomerulosa, located just below the adrenal capsule, does not express 17α-hydroxylase and 17,20 lyase activities and produces 17-hydroxy 19-carbon precursors of sex steroids under ill-defined regulation. The event(s) triggering adrenarche remain unknown (21). Understanding the reticularis-specific activation of the 17,20 lyase activity of P450c17 has been a major challenge as there are no non-primate systems that recapitulate this biology (22). Several mechanisms contribute to the developmental and tissue-specific differential regulation of P450c17 activities (54). Serine phosphorylation of P450c17 increases 17,20 lyase activity without significantly affecting 17α-hydroxylase activity (28) and treatment with alkaline phosphatase or PP2A eliminates almost all 17,20 lyase activity without changing 17α-hydroxylase activity (28, 29). Thus the activities of P450c17 can be differentially regulated by protein phosphorylation based on differential expression of protein kinases and/or phosphatases in different cell types or times in development, thus determining the pattern of steroid hormones produced.

Cytochrome b5 also enhances the 17,20 lyase activity of human P450c17 (24, 55–57) by allosteric action that does not involve electron donation (13). Whereas P450c17 is expressed in both the human zona fasciculata and zona reticularis and shows little change as a function of age, the expression of cytochrome b5 increases in the adrenal zona reticularis at the onset of adrenarche (58, 59) when adrenal 17,20 lyase activity and secretion of 19-carbon steroids (DHEA, androstenedione)
Regulation of 17,20 Lyase Activity

**FIG. 6. Effects of cytochrome b5 on the 17,20 lyase activity promoted by POR and by P450c17 phosphorylation.** A, increased POR favors 17,20 lyase activity. Bacterially expressed P450c17, in the absence (open bars) and presence (solid bars) of a 3-fold molar excess of cytochrome b5, was assayed for 17,20 lyase activity in the presence of 2-, 10-, 20-, or 50-fold molar excess of POR over P450c17. Data are mean ± S.D. of three experiments. B, effect of cytochrome b5 on human P450c17 in microsomes from NCI-H295A cells. The microsomes, which contain POR and P450c17 (with an unknown level of phosphorylation), have a low level of 17,20 lyase activity (solid bars) and substantially higher levels of 17α-hydroxylase activity (open bars); the level of these activities in the absence of cytochrome b5 is set at 100%. Exogenous addition of purified cytochrome b5 increased 17,20 lyase activity 2.5-fold but had no effect on 17α-hydroxylase activity. Phosphorylation of microsomal proteins with the kinase fraction from NCI-H295A cell cytoplasm increased 17,20 lyase activity 3.5-fold, and addition of purified cytochrome b5 to the phosphorylated microsomes increased 17,20 lyase activity 5-fold without affecting 17α-hydroxylase activity. Data are mean ± S.D. of three experiments.

increases. The adult human (60) and rhesus monkey (61) zona reticularis contains abundant cytochrome b5 as well as P450c17, and adrenal adenomas that produce high levels of DHEA also contain large amounts of cytochrome P450c17 and adrenal adenomas that produce high levels of sex steroids, including testicular Leydig cells, follicular theca cells, theca lutein cells, and ovarian stroma (60). Thus there is a strong association between the presence of 17,20 lyase activity and expression of cytochrome b5.

There are three forms of cytochrome b5 expressed from two genes. A gene on chromosome 18q23 consists of 6 exons encoding two alternatively spliced mRNAs: exons 1–4 encode the 98-amino acid soluble or endoplasmic reticulum form of cytochrome b5, and as OMb5 fosters 17α-hydroxylation and expression of cytochrome b5, it is likely that the major effects of cytochrome b5 on human adrenal synthesis of 19-carbon precursors of sex steroids is mediated by the microsomal form.

Overexpression of cytochrome b5 in non-steroidogenic HEK-293 cells co-transfected with P450c17 and POR dramatically enhances 17,20 lyase activity (64). However, reducing cytochrome b5 expression in NCI-H295A cells by 60% using RNA interference did not affect 17,20 lyase activity, and an 85% reduction in cytochrome b5 only decreased 17,20 lyase activity by 30%. Similarly, overexpression of cytochrome b5 in NCI-H295A cells increased 17,20 lyase activity only modestly. Consistent with this we have found that NCI-H295A cells contain about 5-fold more cytochrome b5 protein than do HEK-293 cells or other non-steroidogenic cell lines. Our data indicate that cytochrome b5 and protein phosphorylation enhance 17,20 lyase activity independently and that each mechanism is sufficient to achieve nearly maximal induction on its own. Their effects do not seem to be cooperative since we did not observe enhanced DHEA production in samples that were both phosphorylated and contained cytochrome b5 when compared with the effect of each factor individually. These results also suggest that endogenous cytochrome b5 in NCI-H295A cells is sufficient to maintain the 17,20 lyase activity and perhaps outer mitochondrial cytochrome b5 supports some of the 17,20 lyase activity that could not be reduced even after knockdown of more than 85% cytochrome b5.

Irrespective of P450c17 phosphorylation or the presence of cytochrome b5, increasing the ratio of POR to P450c17 increases 17,20 lyase activity (14, 23). As both the 17α-hydroxylase and 17,20 lyase activities require the donation of electrons from P450 oxidoreductase, this observation thus suggests that both the presence of cytochrome b5 and phosphorylation of P450c17 increase 17,20 lyase activity by facilitating the association of POR with P450c17, speeding the lyase reaction by increasing the efficiency of electron transfer. Thus the combination of these factors regulates the activities of P450c17 (Fig. 7). It is likely that the direction of steroid biosynthesis in a particular tissue or at a particular age is regulated by one or more of the factors involved in P450c17 activities. A change in 17,20 lyase activity may come from changes in cytochrome b5 levels, extent and pattern of P450c17 phosphorylation or presence of other enzymes like 3β-hydroxysteroid dehydrogenase. In a disease like polycystic ovary syndrome one or more of these factors might contribute to the overall change in DHEA production.

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