Specific accumulation of 17 α -hydroxyprogesterone in microsomal membranes during the process of cytochrome *P*-450(C-17)-catalysed androgen biosynthesis

A dynamic study of intermediate formation and turnover*

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A complete dynamic analysis of cytochrome *P*-450(C-17)-catalysed androgen biosynthesis from a single dose of progesterone and 17 α -hydroxyprogesterone in a double-label double-substrate experiment was performed in order to elucidate the controversial intermediacy of 17 α -hydroxyprogesterone. Label distribution within the steroid fractions as well as in the membrane and buffer compartments yields direct evidence that the endogenously formed 17 α -hydroxyprogesterone (which is in an 'intermediate state') accumulates to a higher degree in microsomal membranes than does the exogenously added 17 α -hydroxyprogesterone (which is in a 'substrate state') under certain conditions. It is also demonstrated that endogenously formed 17 α -hydroxyprogesterone may partly leave the membrane compartment (in terms of a 'leakage' or 'overflow' phenomenon) and is then able to equilibrate with the pool of exogenously added 17 α -hydroxyprogesterone. Since only the label distribution in the membrane-associated (but not always in the aqueous) 17 α -hydroxyprogesterone pool corresponds to the label distribution in the androgen fraction, it is concluded that only the membrane-associated 17 α -hydroxyprogesterone pool is directly accessible to cytochrome *P*-450(C-17)-catalysed conversion into androgens.

INTRODUCTION

Enzyme proteins catalysing the conversion of cholesterol to androgens in the testis are generally organized within subcellular membranes, i.e. the mitochondria and the smooth endoplasmic reticulum [1–3]. Two enzymes which are important targets for the hormonal regulation of the androgen biosynthetic process [4,5] are cytochrome *P*-450-dependent mono-oxygenases. The first system is the cytochrome *P*-450(csc)-dependent cholesterol mono-oxygenase (EC 1.14.15.6; csc = cholesterol side-chain cleavage) which is localized in the mitochondrial inner membranes [6,7]. This mono-oxygenase catalyses subsequent hydroxylation reactions at adjacent carbon atoms (C-22 and C-20) of the cholesterol molecule and C-20–C-22 scission yielding pregnenolone as the product [8,9]. The cholesterol pool utilized by this enzyme for acute androgen biosynthesis is fixed in the mitochondrial inner membranes [7]. The second system is the cytochrome *P*-450(C-17)-dependent steroid 17 α -mono-oxygenase/C-17,20-lyase complex (EC 1.14.99.9/EC 4.1.2.30) which is localized in the hydrophobic regions of the endoplasmic reticulum membranes and co-organized with other enzymes of the steroidogenic pathway into a heterogenous system [3,10–12]. Both the hydroxylation of progesterone at the C-17 α position (yielding 17 α -hydroxyprogesterone) and the subsequent C-17–C-20 scission yielding androstenedione as the product are catalysed in a hydrophobic milieu by this single protein,

as has been proved by subtle purification and analytical procedures [12–14]. Hormonal induction responses confirm this concept that both the 17 α -mono-oxygenation and C-17,20-lyase reactions are catalysed by the same gene product and at the same active site [15].

Though the intermediates of the cytochrome *P*-450(csc)-catalysed cholesterol conversion have been found to be present predominantly as enzyme-bound intermediates forming reactive complexes with the enzyme [9,12], the nature of the intermediate of the cytochrome *P*-450(C-17)-catalysed progesterone to androgen conversion is less clear. On the one hand, it has been postulated that 17 α -hydroxyprogesterone is not an obligatory intermediate in the androgen biosynthetic process [11,16,17], but on the other hand, 17 α -hydroxyprogesterone is secreted by testes or isolated Leydig cells during the androgen biosynthetic process [18–20].

Previous theoretical reports have considered subcellular structures in heterogenous systems (organized multienzyme sequences, membrane-bound enzymes) as microenvironments determining enzyme interactions with substrates or intermediates [21,22]. In particular, the role of microsomal membranes in establishing a microenvironment for cytochrome *P*-450-dependent mono-oxygenations has been emphasized [12,23]. In view of these models, the present study was initiated with the aim to test the hypothesis that only a certain portion of the 17 α -hydroxyprogesterone formed during cytochrome *P*-450(C-17)-catalysed androgen biosynthesis

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from precursor steroids would be secreted, whereas the other portion would specifically accumulate in microsomal membranes serving directly as the substrate of the C-17,20-lyase function of the cytochrome *P*-450(C-17) which is membrane-bound as well.

MATERIALS AND METHODS

Testes from male Han:Wistar rats aged 12 weeks were homogenized in a 4-fold volume of TS buffer (250 mM-sucrose/20 mM-Tris, pH 7.4). Supernatants of a 10 min $\times$ 12000 *g* centrifugation were centrifuged at 150000 *g* for 45 min. The pellet was resuspended in TS buffer and subjected once again to the 12000 *g* and 150000 *g* centrifugation steps in order to minimize contamination with cytosol. This procedure appears to be necessary since it cannot be excluded that cytosol affects either steroid binding to microsomal enzymes or the activities of these enzymes [16]. The final pellet was resuspended in TS buffer to yield a concentrated microsomal preparation.

Microsomal suspensions were diluted with 50 mM-phosphate buffer, pH 7.4, and the simultaneous metabolism of 1 μ M-progesterone [including 500 Bq of [1,2-³H]progesterone/ml (2.0 TBq/mmol; New England Nuclear, Dreieich, Germany); steroids were added in ethanol-containing buffer, the final ethanol concentration being 0.7 %] and of 1 μ M-17 α -hydroxyprogesterone [including 500 Bq of 17 α -hydroxy[4-¹⁴C]progesterone/ml (1.8 GBq/mmol; New England Nuclear)] was followed in the presence of 280 μ M-MgCl₂ and 130 μ M-NADPH at 32 °C, as this is near the physiological scrotal temperature of the rat testis. The final concentration of microsomal protein was 1.0 mg/ml [24] corresponding to 0.5 mg of microsomal lipid/ml [25] and 30 nM-cytochrome *P*-450 [26]. In addition to the main incubation, aliquots were incubated under identical conditions in cuvettes in a Shimadzu UV-300 spectrophotometer in the single wavelength/double beam mode, and the loss of magnitude of steroid-induced type-I difference spectra was continuously recorded as described elsewhere [27]. This procedure allowed an estimation of the portion of cytochrome *P*-450-bound steroid, but is not able to differentiate between the steroids actually bound to the cytochrome *P*-450 since progesterone and 17 α -hydroxyprogesterone as well as androstenedione and testosterone all induce type-I difference spectra with molar absorption coefficients of 120, 110, 26 and 21 mm⁻¹ · cm⁻¹, respectively, for the 387 nm–420 nm absorption difference (N. Kühn-Velten *et al.*, unpublished work).

After the incubation times specified in the Results section, aliquots of the main incubation were removed and added to a concentrated NADP⁺ solution (final concentration 3.6 mM) in ice-cooled ultracentrifuge tubes. In accordance with previous findings [1], this has been found to be an effective method to stop completely and immediately further steroid metabolism. Membrane suspensions were either left on ice, or were centrifuged at 0 °C as described above. Aliquots of the original suspensions and of the aqueous supernatants were extracted with diethyl ether (93 % mean recovery), the total activities were counted, and the steroid fractions were separated by t.l.c. as described previously [28]. Steroid concentrations were then calculated from ³H label and ¹⁴C label distribution between steroid fractions and from the distribution of ³H label and ¹⁴C label

between the supernatant and the membrane (= total minus supernatant) fractions. Androstenedione and testosterone were combined to the androgen fraction since they are usually in equilibrium. Since it was not differentiated between protein-bound and lipid-associated steroid fractions, and since it could not be decided from the spectral effects at the cytochrome *P*-450(C-17) which relative portion of a certain steroid in the membrane-associated steroid mixture was really bound to the cytochrome *P*-450, steroid partition coefficients, *K*_p, were calculated for membrane/supernatant (and not for lipid/water) concentration ratios.

RESULTS

The comparison of Fig. 1 and Fig. 2 demonstrates that [³H]progesterone and 17 α -hydroxy[¹⁴C]progesterone are simultaneously metabolized by testis microsomes in the presence of NADPH. Progesterone conversion, however, occurs much faster (half-life period 1.5 min) than 17 α -hydroxyprogesterone conversion (half-life period 5.8 min). Both ³H-labelled (from progesterone possibly via 17 α -hydroxyprogesterone) and ¹⁴C-labelled (directly from 17 α -hydroxyprogesterone) androgens are obviously synthesized at identical rates, although the overall concentrations of 17 α -hydroxy[³H]progesterone are lower than those of 17 α -hydroxy[¹⁴C]progesterone during the first minutes of the incubation (but not from 5 min onwards). The rate of androgen formation from 17 α -hydroxy[³H]progesterone during this initial phase would therefore be expected to be lower than the rate of androgen formation from 17 α -hydroxy[¹⁴C]progesterone, provided that overall ³H-labelled and ¹⁴C-labelled 17 α -hydroxyprogesterone pools would be equally considered as a substrate pool by the C-17,20-lyase function of the cytochrome *P*-450(C-17). Our results are therefore inconsistent with the assumption of a true intermediacy of 17 α -hydroxyprogesterone during the first phase of the experiment.

It is further demonstrated in Fig. 1 and Fig. 2 that considerable amounts of the overall steroid pools accumulate in the microsomal membranes which make up only about 0.15 % of the total incubation volume. This steroid accumulation in the membrane compartment is highest for progesterone (Fig. 1) and least for the androgens. In the initial phase of the incubation (until the fifth minute), 17 α -hydroxy[³H]progesterone is obviously enriched in the membrane compartment to a higher percentage than 17 α -hydroxy[¹⁴C]progesterone (Figs. 1 and 2), but later on 17 α -hydroxy[³H]progesterone and 17 α -hydroxy[¹⁴C]progesterone distribute equally between buffer and membrane spaces. These patterns are confirmed by exact calculations, the results of which are given in Fig. 3.

A direct comparison of the development of steroid-induced type-I difference spectra at the cytochrome *P*-450(C-17) and of steroid distribution between the buffer and membrane spaces reveals that steroid binding to cytochrome *P*-450(C-17) contributes only to a minor extent (10 % at the maximum) to the steroid accumulation in the membrane fraction (results not shown). Therefore, most of the steroids accumulating in testicular microsomal membranes *in vitro* seem to be associated with the membrane lipid phase.

The discrepancy between [³H]androgen and [¹⁴C]-androgen production rates and overall 17 α -hydroxy[³H]-

Fig. 1. Metabolism of $[^3\text{H}]$ progesterone by testis microsomes

Changes with time of $[^3\text{H}]$ progesterone (hexagons), 17α -hydroxy $[^3\text{H}]$ progesterone (squares) and $[^3\text{H}]$ androgen (circles) amounts (related to 1 ml of incubation volume) during simultaneous metabolism of $1\ \mu\text{M}$ - $[^3\text{H}]$ progesterone and $1\ \mu\text{M}$ - 17α -hydroxy $[^{14}\text{C}]$ progesterone by testis microsomes. Filled symbols indicate overall steroid concentrations (means $\pm$ S.D. from single analyses of $n = 10$ preparations). Hatched areas depict that portion of the respective steroid fraction that is associated with the membrane compartment (means from triplicate analyses of $n = 2$ preparations).

progesterone and 17α -hydroxy $[^{14}\text{C}]$ progesterone concentrations as stated above (Figs. 1 and 2) can further be demonstrated by a comparison of the respective $^3\text{H}/^{14}\text{C}$ ratios: during the first half of the incubation period,

Fig. 2. Metabolism of 17α -hydroxy $[^{14}\text{C}]$ progesterone by testis microsomes

Changes with time of 17α -hydroxy $[^{14}\text{C}]$ progesterone (squares) and $[^{14}\text{C}]$ androgen (circles) amounts (related to 1 ml of incubation volume) during simultaneous metabolism of $1\ \mu\text{M}$ - $[^3\text{H}]$ progesterone and $1\ \mu\text{M}$ - 17α -hydroxy $[^{14}\text{C}]$ progesterone by testis microsomes. Open symbols indicate overall steroid concentrations (means $\pm$ S.D. from single analyses of $n = 10$ preparations). Hatched areas depict that portion of the respective steroid fraction that is associated with the membrane compartment (means from triplicate analyses of $n = 2$ preparations).

overall $^3\text{H}/^{14}\text{C}$ ratios are significantly higher in the androgen than in the 17α -hydroxyprogesterone fraction (Fig. 4). This result again seems to contradict the assumption that androgens are obligatorily formed from progesterone via 17α -hydroxyprogesterone. In the second phase of the incubation, overall $^3\text{H}/^{14}\text{C}$ ratios approach an identical value of 1 both in the 17α -hydroxyprogesterone and the androgen fractions. In the membrane compartment, $^3\text{H}/^{14}\text{C}$ ratios in the 17α -hydroxyprogesterone and androgen fractions are not different, whereas the difference is even greater in the buffer space than in the total incubation during the first phase of the experiment (Fig. 4). This pattern can be simply reduced to the fact that during the initial period of the incubation, a higher portion of the endogenously formed ^3H -labelled 17α -hydroxyprogesterone than of the exogenously added ^{14}C -labelled 17α -hydroxyprogesterone is accumulated in the microsomal membrane compartment, whereas this different pattern cannot be

Fig. 3. Partition coefficients K_p for steroid distribution between membrane and aqueous compartments during incubation

K_p values for ^3H -labelled (filled symbols) and ^{14}C -labelled (open symbols) progesterone (hexagons), 17α -hydroxyprogesterone (squares) and androgen (circles) fractions are derived from data presented in Figs. 1 and 2.

observed from 5 min of incubation onwards (Fig. 3). Consequently, $^3\text{H}/^{14}\text{C}$ ratios are initially higher in the 17α -hydroxyprogesterone fraction associated with the membrane compartment than in the 17α -hydroxyprogesterone fraction in the aqueous compartment (Fig. 4). In the main, $^3\text{H}/^{14}\text{C}$ ratios in the membrane-associated rather than those in the total 17α -hydroxyprogesterone fraction correspond to the $^3\text{H}/^{14}\text{C}$ ratios in the androgen fraction during the entire course of the incubation study (Fig. 4).

DISCUSSION

For many years, the question whether 17α -hydroxyprogesterone represents a true intermediate of the androgen biosynthetic process in the testis has been highly controversial. On the one hand, the fact that 17α -hydroxyprogesterone can be identified as an intermediate of androstenedione formation from progesterone by the purified steroid 17α -mono-oxygenase/C-17,20-lyase system has been used as argument in favour of the 'intermediate hypothesis' [19]. Furthermore, 17α -hydroxyprogesterone has been proved to be really secreted by perfused testes and by perfused Leydig cells [18,20]. On the other hand, it has been shown by our group that endogenously formed 17α -hydroxyprogesterone is metabolized with 6-fold lower apparent K_m values of the C-17,20-lyase reaction than exogenously added 17α -hydroxyprogesterone under steady-state conditions [20]. Further, endogenous 17α -hydroxyprogesterone is more effective in inhibiting the steroid 17α -mono-oxygenase reaction than is exogenous 17α -hydroxyprogesterone [29]. The hypothesis of different behaviour of endogenously formed and exogenously added 17α -hydroxyprogesterone, as developed from our previous experiments, is confirmed, differentiated and

Fig. 4. Changes with time of $^3\text{H}/^{14}\text{C}$ ratios in different steroid fractions in different compartments

Ratios $^3\text{H}/^{14}\text{C}$ (on a nmol basis related to 1 ml of incubation volume) for 17α -hydroxyprogesterone (squares) and androgens (circles) in the overall steroid pool (upper panel), in the membrane compartment (middle panel), and in the aqueous compartment (lower panel) are derived from data presented in Figs. 1 and 2.

partly explained by the observations made in the present study.

It has been previously speculated that secreted intermediates of the androgen biosynthesis would be (possibly stabilized) byproducts of the true (possibly labile) enzyme-bound intermediates [18]; alternatively, secretion of steroid intermediates could reflect a 'leaky pathway' resulting from steroid transfer between enzymes [18]. Such a net 'leakage' of 17α -hydroxy[^3H]progesterone in the 'intermediate state' from the membrane into the aqueous compartment is observed in our study as a

Fig. 5. Fate of endogenously formed 17α -hydroxy $[^3\text{H}]$ progesterone

Net transfer rates of 17α -hydroxy $[^3\text{H}]$ progesterone (formed from $[^3\text{H}]$ progesterone) from the membrane compartment into the buffer compartment (hatched areas) and from the membrane compartment to the $[^3\text{H}]$ androgen fraction (open areas) are derived from data presented in Fig. 1.

transient phenomenon during the first phase of the model incubation (Fig. 5). This net 'leakage' of endogenous 17α -hydroxyprogesterone, however, should be interpreted to result mainly from the fact that during the first minutes, there may be a limitation of 17α -hydroxyprogesterone to androgen conversion. Within this period, rather large amounts of 17α -hydroxyprogesterone are formed from progesterone which competes with 17α -hydroxyprogesterone at the cytochrome *P*-450(C-17) active site in the membrane compartment [12]. During the second phase of the model incubation used in the present study, however, there is a net influx of 17α -hydroxy $[^3\text{H}]$ progesterone from the buffer into the membrane compartment that equals the rate of $[^3\text{H}]$ androgen formation from 6 min onwards (Fig. 5).

Chasalow has even denied an obligatory intermediate role of free 17α -hydroxyprogesterone in androgen biosynthesis because some androstenedione could be formed from progesterone without 17α -hydroxyprogesterone appearing [16]. More recently, it has also been found that androstenedione can not be formed from "freely diffusible 17α -hydroxyprogesterone" [17], and

that intermediates of the androgen biosynthesis do not equilibrate with the medium [17]. The critical disadvantage of these studies [16,17] is the investigation of only a short phase of the steroid metabolism *in vitro*. Since this phase corresponds roughly to the first 3 min of steroid turnover in the present study, those data are not contradictory to ours. The present study, in contrast, presents for the first time a complete dynamic analysis of the entire process of androgen biosynthesis from progesterone and 17α -hydroxyprogesterone. Only by this approach can it be shown that exogenous and endogenous 17α -hydroxyprogesterone are really able to equilibrate, depending on the incubation period considered.

Further, the present study for the first time considers quantitatively the possible accumulation of intermediates in the membrane compartment. Assuming that steroid substrates and intermediates interact with the hydrophobic substrate binding site of the cytochrome *P*-450 from the membrane phase and not from the aqueous phase [7,12,23,30–32], the actual concentration of a certain intermediate in the membrane compartment should be relevant for the 'handling' of that intermediate by the cytochrome *P*-450. In the case of the experiment described herein, the congruity of $^3\text{H}/^{14}\text{C}$ ratios in the membrane-associated 17α -hydroxyprogesterone pool and the androgen pool is indeed a clear indication that only the membrane-associated 17α -hydroxyprogesterone pool is accessible to the C-17,20-lyase function of the cytochrome *P*-450(C-17) and therefore determines androgen formation (Fig. 4). This result discloses an interesting parallelism to the well-accepted observation that only membrane-bound cholesterol can be metabolized to pregnenolone by the cytochrome *P*-450(csc) in the mitochondrial inner membranes in steroidogenic tissues, whereas other cholesterol pools cannot be reached by this enzyme [7]. As already indicated in previous studies emphasizing the role of a specific microenvironment for enzyme–substrate interactions [21–23], our study likewise demonstrates the significance of determining local substrate concentrations as close to the catalytic site as possible. In this context, we are aware of the possibility that there may be specific membrane domains (possibly in close proximity to the enzyme molecules [30,33]) which accumulate specific ligands to a higher degree than do other domains. However, this possible limitation apparently does not generally affect our model of a true intermediacy of the 17α -hydroxyprogesterone in the membrane compartment only.

On the basis of the concept that local steroid concentrations and therefore steroid accumulation in membranes determine steroid binding to and steroid metabolism by membrane-associated enzymes, the old problem of whether 17α -hydroxyprogesterone is an intermediate of androgen biosynthesis can now be solved as follows (Fig. 6).

In the first phase of a complete incubation proving simultaneous metabolism of $[^3\text{H}]$ progesterone and 17α -hydroxy $[^{14}\text{C}]$ progesterone by testis microsomes, when the invasion from the progesterone pool into the overall 17α -hydroxyprogesterone pool is greater than the elimination from this pool into the androgen pool (Fig. 6a):

$[^3\text{H}]$ Progesterone is accumulated in the membrane to a high degree [11].

17α -Hydroxy $[^3\text{H}]$ progesterone is formed by the

Fig. 6. Dynamic model of steroid turnover and partition in microsomal membranes

Relative distribution of steroids (hexagons, progesterone; squares, 17α-hydroxyprogesterone; circles, androgens) and label (black areas, ^3H ; white areas, ^{14}C) between membrane (m) and buffer compartments is roughly indicated by the relative area of the symbols. Thick arrows indicate cytochrome *P*-450(C-17)-catalysed conversion of progesterone and 17α-hydroxyprogesterone; thin arrows indicate net transfer of steroids between compartments. (a) represents the situation at about 1 min; (b) is the situation at about 8 min after start of incubation as described in Figs. 1 and 2.

steroid 17α-mono-oxygenase function of the cytochrome *P*-450(C-17) within the membrane compartment. (Alternatively, an unknown labile intermediate may be initially synthesized which is rapidly stabilized into 17α-hydroxyprogesterone when leaving the hydrophobic pocket of the active site of this enzyme.) Part of the 17α-hydroxy[^3H]progesterone accumulates in the membranes ('intermediate pool') and is converted to androgens by the C-17,20-lyase function of the cytochrome *P*-450(C-17) within the membrane compartment. Another part is transferred (in terms of an 'overflow phenomenon') to the buffer space where it equilibrates with the 17α-hydroxy[^{14}C]progesterone pool ('substrate pool').

17α-Hydroxy[^{14}C]progesterone primarily present in the buffer compartment is continuously transferred into the membrane compartment where it equilibrates with the endogenously formed 17α-hydroxy[^3H]progesterone pool.

As a consequence, a relatively great portion of

17α-hydroxy[^3H]progesterone and a relatively small portion of 17α-hydroxy[^{14}C]progesterone become available for membrane-associated androstenedione synthesis catalysed by the C-17,20-lyase. Within the membrane phase, both ^3H -labelled and ^{14}C -labelled 17α-hydroxyprogesterone are equally accepted as substrates of this enzyme.

In the second phase of this model incubation, when the elimination from the 17α-hydroxyprogesterone pool is greater than the invasion into this pool (Fig. 6b):

^3H -labelled and ^{14}C -labelled 17α-hydroxyprogesterone have been equilibrated inside as well as outside the membrane, and $^3\text{H}/^{14}\text{C}$ ratios are identical in the 17α-hydroxyprogesterone and androgen fractions.

Therefore, this study reveals that 17α-hydroxyprogesterone represents a steroidogenic intermediate which accumulates in the microsomal membranes during the catalytic sequence [possibly in the vicinity of the cytochrome *P*-450(C-17)-dependent enzyme complex that both produces and converts this intermediate], but which is likewise able to equilibrate to a certain extent with the aqueous compartment outside the membrane. In general, we get the impression that this interpretation of 'intermediate handling' by this model of a membrane-bound, highly co-ordinated enzyme system is consistent with all the previous observations made on this process [9,13–20,29]. It is obvious and first demonstrated by the presented study that under experimental conditions *in vitro*, the nature of an intermediate of a membrane-associated metabolic pathway can only be understood on a dynamic basis. This concept may also be applicable for other membrane-bound enzyme complexes or other highly organized metabolic systems [22,34].

REFERENCES

1. Tamaoki, B. I. & Shikita, M. (1966) in *Steroid Dynamics* (Pincus, G., Nakao, T. & Tait, J. F., eds.), pp. 493–528, Academic Press, New York
2. Samuels, L. T., Busmann, L., Matsumoto, K. & Huseby, R. A. (1975) *J. Steroid Biochem.* **6**, 291–296
3. Yoshida, K. I., Takahashi, J., Winters, S. J., Oshima, H. & Troen, P. (1984) *J. Steroid Biochem.* **21**, 49–58
4. Fevold, H. R. (1983) *Annu. Rev. Physiol.* **45**, 19–36
5. Kühn-Velten, N., Bos, D. & Staib, W. (1986) *Biosci. Rep.* **6**, 451–457
6. Seybert, D. W., Lancaster, J. R., Lambeth, J. D. & Kamin, H. (1979) *J. Biol. Chem.* **254**, 12088–12098
7. Cheng, B., Hsu, D. K. & Kimura, T. (1985) *Mol. Cell. Endocrinol.* **40**, 233–243
8. Hume, R., Kelly, R. W., Taylor, P. L. & Boyd, G. S. (1984) *Eur. J. Biochem.* **140**, 583–591
9. Hochberg, R. B., McDonald, P. D., Ladany, S. & Lieberman, S. (1975) *J. Steroid Biochem.* **6**, 323–327
10. Menard, R. H. & Purvis, J. L. (1973) *Arch. Biochem. Biophys.* **154**, 8–18
11. Samuels, L. T. & Matsumoto, K. (1974) *Endocrinology* (Baltimore) **94**, 55–60
12. Hall, P. F. (1986) *Steroids* **48**, 131–196
13. Nakajin, S., Hall, P. F. & Onoda, M. (1981) *J. Biol. Chem.* **256**, 6134–6139
14. Suhara, K., Fujimura, Y., Shiroo, M. & Katagiri, M. (1984) *J. Biol. Chem.* **259**, 8729–8736
15. McCarthy, J. L. & Waterman, M. R. (1988) *J. Steroid Biochem.* **29**, 307–312

16. Chasalow, F. (1979) *J. Biol. Chem.* **254**, 3000–3005
17. Eckstein, B., Borut, A. & Cohen, S. (1987) *Eur. J. Biochem.* **166**, 425–429
18. Becker, S., Chubb, C. & Ewing, L. (1980) *Am. J. Physiol.* **239**, R184–R195
19. Nakajin, S. & Hall, P. F. (1981) *J. Steroid Biochem.* **14**, 1249–1252
20. Kühn-Velten, N., Wolff, J. & Staib, W. (1984) *Biosci. Rep.* **4**, 483–488
21. Welsh, G. R. (1983) *J. Theoret. Biol.* **100**, 211–238
22. Welsh, G. R., Keleti, T. & Vertessy, B. (1988) *J. Theoret. Biol.* **130**, 407–422
23. Peterson, J. A., O’Keeffe, D. H., Werringloer, J., Ebel, R. E. & Estabrook, R. W. (1978) in *Microenvironments and Metabolic Compartmentation* (Srere, P. A. & Estabrook, R. W., eds.), pp. 433–447, Academic Press, New York
24. Markwell, M. A. K., Haas, S. M., Tolbert, N. E. & Bieber, L. L. (1981) *Methods Enzymol.* **72**, 296–303
25. Folch, J., Lees, M. & Sloane-Stanley, J. H. (1957) *J. Biol. Chem.* **226**, 497–509
26. Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239**, 2370–2378
27. Estabrook, R. W., Martinez-Zedillo, G., Young, S., Peterson, J. A. & McCarthy, J. (1975) *J. Steroid Biochem.* **6**, 419–425
28. Kühn-Velten, N. & Staib, W. (1983) *FEBS Lett.* **154**, 70–74
29. Kühn-Velten, N. & Staib, W. (1984) *J. Steroid Biochem.* **21**, 697–700
30. Sheets, J. J. & Estabrook, R. W. (1985) *Biochemistry* **24**, 6591–6597
31. Krainev, A. G., Weiner, L. M., Alferyev, I. S. & Slynko, N. M. (1985) *Biochim. Biophys. Acta* **818**, 96–104
32. Kominami, S., Itoh, Y. & Takemori, S. (1986) *J. Biol. Chem.* **261**, 2077–2083
33. Jost, P., Griffith, O. H., Capaldi, R. A. & Vanderkooi, G. (1973) *Biochim. Biophys. Acta* **311**, 141–152
34. Clegg, J. S. (1984) *Am. J. Physiol.* **246**, R133–R151

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