Rat liver 3α-hydroxysteroid dehydrogenase (3α-HSD, EC 1.1.1.50) is an NAD(P)⁺-dependent oxidoreductase which will terminate androgens action by converting 5α-dihydrotestosterone to 3α-androstanediol. It is identical to dihydrodiol dehydrogenase and it can function as a 9-, 11-, and 15-hydroxyprostaglandin dehydrogenase. Its reactions are potently inhibited by the nonsteroidal anti-inflammatory drugs (NSAIDs). A cDNA (2.1 kilobases) for 3α-HSD was cloned from a rat liver cDNA expression library in agt11. Portions of the cDNA insert which contained an internal EcoRI site were subcloned into pGEM3, and dideoxysequencing revealed that the cDNA contains an open reading frame of 966 nucleotides which encode a protein of 322 amino acids with a monomer M₀ of 37,029. The identity of this clone was confirmed by two tryptic peptides and two endoproteinase Lys-C peptides from purified 3α-HSD within the nucleotide sequence. The amino acid sequence of rat liver 3α-HSD bears no significant homology with 3α-, 17β-, or 11β-hydroxysteroid dehydrogenases but has striking homology at the nucleotide level) which is a member of the aldehyde/aldolase reductase family. This sequence homology supports previous correlates which suggest that in rat 3α-HSD may represent an important target for NSAIDs. The nucleotide sequence also contains three peptides that have been identified by affinity labeling with either 3α-bromoacetoxyandrostenedione (substrate analog) or 11β-bromoacectoxyprogesterone (glucocorticoid analog) to comprise the active site (see accompanying article (Penning, T. M., Abrams, W. R., and Pawlowski, J. E. (1991) J. Biol. Chem. 266, 8826–8834). The sequence data presented suggests that 3α-HSD, prostaglandin F synthase, and aldehyde/aldolase reductases are members of a common gene family.

3α-Hydroxysteroid dehydrogenase (3α-HSD, EC 1.1.1.50) has been a focus of study because of its perceived role in the termination of androgen action. By catalyzing the conversion of 5α-dihydrotestosterone (a potent androgen) to 3α-androstanediol (a weak androgen), the levels of 5α-dihydrotestosterone and androgen-responsive tissues can be reduced (1–3).

The most thoroughly characterized 3α-HSD is the homogenous enzyme from rat liver cytosol (4–6). Rat liver 3α-HSD is a monomeric (M₀, 34,000) NAD(P)⁺-dependent oxidoreductase which is abundantly expressed. Immunotitration of data with polyclonal antisera suggest that it represents 1–3% of the cytosolic protein in this tissue (7). This high level of expression makes it possible to obtain milligram quantities of the purified enzyme at concentrations approaching 10 mg/ml for x-ray crystallographic studies (6).

The abundance of rat liver 3α-HSD has facilitated the study of the diverse functions of the enzyme. First, it can function as a 3α-hydroxysteroid dehydrogenase and can metabolize androgens (8) and glucocorticoids (9) and is involved in the biosynthesis of bile acids (10, 11). Second, it can function as a dihydrodiol dehydrogenase, and by oxidizing trans-dihydrodiols of polycyclic aromatic hydrocarbons it can suppress formation of the ultimate carcinogens, the anti-diol epoxides (12–14). Third, it can function as a 9-, 11-, and 15-hydroxyprostaglandin dehydrogenase (15) and may be involved in regulating levels of inflammatory prostaglandins.

Rat liver 3α-HSD also satisfies several criteria expected of a target enzyme for nonsteroidal anti-inflammatory drugs (NSAIDs) (4–6). The enzyme is potently inhibited at its active site by NSAIDs in rank order of their pharmacological potency (4). Concentrations of drugs required to inhibit 3α-HSD are either similar or lower than those required to inhibit cyclooxygenase. 3α-HSD also binds arachidonic acid and prostaglandins with affinity in the low micromolar range (5) and can transform prostaglandins through its hydroxyprostaglandin dehydrogenase activity (15). An indomethacin-sensitive 3α-HSD has also been shown to be widely distributed in rat tissues including prostate, spleen, heart, testis, small intestine, stomach, lung, and brain (16).

To gain insights into the possible connection between the hydroxysteroid, dihydrodiol, and hydroxyprostaglandin dehydrogenase activities of 3α-HSD, the enzyme structure is currently being analyzed in this laboratory. We now describe the isolation of a cDNA clone for 3α-HSD which has allowed us to deduce the amino acid sequence of this protein.

This research was supported by National Institutes of Health Grants GM33464 and CA39504, Research Career Development Award CA01335 (to T. M. P.), and a Pharmaceutical Manufacturers Association advanced predoctoral fellowship (to J. M.). The abbreviations and trivial names used are: 3α-HSD, 3α-hydroxysteroid dehydrogenase (3α-hydroxysteroid:NAD(P)⁺ oxidoreductase (EC 1.1.1.50); dihydrodiol dehydrogenase, trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (EC 1.1.1.20); NSAIDs, nonsteroidal anti-inflammatory drugs; RP-HPLC, reverse-phase high pressure liquid chromatography; PTH, phenylthiobutyraldoxime; TPCK, L-1-tosylamide-2-phenylethyl chloromethyl ketone; SDS, sodium dodecyl sulfate; kb, kilobases.

Cloning and Sequencing of the cDNA for Rat Liver 3α-Hydroxysteroid/Dihydrodiol Dehydrogenase*
sequence obtained bears striking homology with bovine lung prostaglandin F synthase and no significant homology with other hydroxysteroid dehydrogenases which have been recently cloned and sequenced.

**MATERIALS AND METHODS**

Homogeneous 3α-HSD was prepared from male Sprague-Dawley rat liver cytosol (5). Rabbit anti rat 3α-HSD sera were obtained as previously described (7). Goat anti-rabbit IgG horseradish peroxidase conjugate was purchased from Bio-Rad. A female Sprague-Dawley rat liver cDNA library was obtained from Clontech (Palo Alto, CA). Nitrocellulose and Nytran filters were purchased from Schleicher & Schuell. T4 DNA ligase was purchased from New England Biolabs. T4 DNA ligase was purchased from U. S. Biochemical Corp. Restriction endonucleases were obtained from U. S. Biochemical Corp., New England Biolabs, GIBCO/BRL, and Boehringer Mannheim. pGEM3, the GemSeq sequencing, Riboprobe Gemini System 11, and Erase-a-Base deletion kits were purchased from Promega Biotec (Madison, WI).

**Isolation and Confirmation**

In immunopositive clones were detected using goat anti-rabbit IgG horseradish peroxidase conjugate and 4-chloro-1-naphthol plus H2O2. Positive clones were plaque-purified and grown in Escherichia coli strain Y1090. Phage DNA was isolated using the plate lysate method (17). A full-length clone was obtained by rescreening approximately 300,000 plaques from the cDNA library using 32P-labeled 36-mer oligonucleotide probes (Fig. 4) directed against the 5' ends of two partial-length immunopositive clones. The probes were end-labeled using T4 polynucleotide kinase and γ-[32P]ATP. The filters were hybridized with oligonucleotide probes in 6X SSC, 0.1% SDS (30 min at 68 °C) and 0.1 SSC (15 min at 80 °C) and autoradiographed.

Subcloning—cDNA inserts were isolated from the phage vector DNA by EcoRI digestion. The inserts were sized and separated by gel electrophoresis (1X agarose, 1X TBE 90 mm Tris-borate plus 2 mm EDTA). Since all of the positive clones contained an internal EcoRI site, thus generating two fragments (except clone 3α-HSD-I), Southern analyses using the oligonucleotide probes were performed to determine the orientation of the fragments. For sequencing, cDNA fragments were subcloned into the EcoRI site of pGEM3 using T4 ligase (Promega Biotec). Following ampicillin resistance, and plasmid DNA was isolated (17). To facilitate sequencing, nested deletions were constructed using exonuclease III and S1 nuclease (Erase-A-Base, Promega Biotec) following the manufacturer's instructions.

**Peptide Sequencing**—Peptides were covalently attached to either an arylamine or diisothiocyanate Sequelon membrane via their free carboxyl and amino groups, respectively, for solid-phase sequencing (20). Automated Edman degradation was performed on a PTH-derivatives (Fig. 1A) and two tryptic peptides (T1α and T1β) were subsequently purified (Fig. 1, B and C) for sequencing. The sequence obtained for T1α corresponds to a peptide of 12 amino acids: NH2-Leu-Trp-Ser-Thr-Phe-His-Leu-Pro-Leu-Glu-CO2H (endoproteinase Lys-C), while the sequence of T1β, for 3α-HSD and the enzymes in the GenBank and EMBL data banks were described elsewhere (7).

**RESULTS AND DISCUSSION**

To confirm the identity of the cDNA clones, peptide sequence data were obtained from purified 3α-HSD. For these studies, homogeneous 3α-HSD was reduced, carboxymethylated, and subjected to trypsin digestion. Tryptic peptide maps were obtained by RP-HPLC (Fig. 1A) and two tryptic peptides (T1α and T1β) were subsequently purified (Fig. 1, B and C) for sequencing. The sequence obtained for T1α corresponds to a peptide of 12 amino acids: NH2-Leu-Trp-Ser-Thr-Phe-His-Arg-Pro-Glu-Leu-Val-Ara-CO2H, while the sequence of T1β corresponds to a peptide of 19 amino acids: NH2-His-Phe-Asp-Ser-Ala-Tyr-Leu-Tyr-Glu-Val-Glu-Glu-Glu-Glu-Glu-Glu-Arg-Ala-CO2H (Table I). Two peptides were also purified by RP-HPLC following endoproteinase Lys-C digestion of 3α-HSD (data not shown), which gave the following sequences: NH2-Ala-Leu-Leu-Asp-Gly-Leu-Leu-Arg-Asp-Ara-Phe-Arg-Tyr-Arg-Asn-Ala-Lys-CO2H (endoproteinase Lys-C), and NH2-Tyr-Phe-Asp-Asp-His-Pro-Asn-His-Pro-Ph-Thr-Asp-Glu-CO2H (endoproteinase Lys-C). Together these peptides comprise 59 amino acids of the protein. The amino acid composition predicts 311 amino acids in the whole protein (6), and these peptides therefore comprise 19% of the entire sequence.

**Isolation and Confirmation of the Identity of the cDNA Clone for Rat Liver 3α-HSD**—Two immunopositive clones were isolated from the cDNA library screened with rabbit anti-rat 3α-HSD serum. The first clone (3α-HSD-I) contained a...
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Peptides (T, and T2) were subjected to automated sequencing using Edman chemistry, and the PTH-derivatives released in each cycle were separated by RP-HPLC and detected by their absorbance at 269 and 313 nm. The PTH-derivatives were quantitated against a standard mixture containing 100 pmol of each PTH-derivative.

| Peptide | Cycle | PTH-derivative | Yield (pmol) |
|---------|-------|----------------|-------------|
| Leu     | 1     | His            | (32.0)      |
| Trp     | 2     | Phe            | 185.0       |
| Ser     | 3     | Asp            | 109.0       |
| Thr     | 4     | Ser            | 86.0        |
| Phe     | 5     | Ala            | 163.0       |
| His     | 6     | Tyr            | 125.6       |
| Arg     | 7     | Leu            | 142.4       |
| Pro     | 8     | Tyr            | 132.9       |
| Glu     | 9     | Glu            | 92.1        |
| Leu     | 10    | Val            | 107.1       |
| Val     | 11    | Glu            | 36.0        |
| Arg     | 12    | Glu            | 62.0        |
| Glu     | 13    | Glu            | 57.3        |
| Val     | 14    | Gly            | 70.4        |
| Gly     | 15    | Gly            | 57.3        |
| Glx     | 16    | Glx            | 64.9        |
| Ala     | 17    | Ala            | 62.1        |
| Ile     | 18    | Ile            | 52.4        |
| Arg     | 19    |                | 6.7         |

*The average repetitive yield was ≥94%. The yield is the net of the previous cycle except where presented in parentheses, and is then the gross yield.

**T2 was sequenced on two different occasions and gave identical sequence information.

PTH-dehydroalanine was detected in this cycle at 313 nm.

A significant α-aminobutyric acid peak was detected in this cycle at 313 nm, which is a degradation product of threonine.

3α-HSD-I

| Restriction Map |
|----------------|
| Eco RI |
| 200 bp |
| 3α-HSD-I |

3α-HSD-II

| Restriction Map |
|----------------|
| Eco RI |
| 3α-HSD-II |

3α-HSD-III

| Restriction Map |
|----------------|
| Eco RI |
| 3α-HSD-III |

Fig. 2. Restriction maps of the cDNA clones for 3α-HSD. The cDNA inserts isolated from the λgt11 library are aligned with reference to their internal EcoRI sites. Except for the 5' EcoRI site of 3α-HSD-I, the flanking EcoRI sites are due to the linkers added to the cDNA during library construction. The dark boxes represent the coding region for 3α-HSD, and the hatched regions represent noncoding regions. bp, base pairs.

The peptide map shown in A and reapplied to the same column which was now eluted with a gradient of 20–60% solvent A over 100 min at a flow rate of 1.0 ml/min (Solvent A = 95% acetonitrile in 0.1% trifluoroacetic acid). Elution of the peptides from the column was monitored by measuring absorbance at 214 nm (→), and 1.0-ml fractions were collected (A). Peptide T2 was isolated from the peptide map and reapplied to the same column which was now eluted with a gradient of 20–60% solvent A over 100 min at a flow rate of 1.0 ml/min. Elution of the peptides from the column was monitored by measuring absorbance at 214 nm (→), and 1.0-ml fractions were collected. At this stage peptide T2 was judged to be pure (C).

Fig. 1. Isolation of tryptic peptides of 3α-HSD. Homogeneous 3α-HSD (1 mg) was reduced and carboxymethylated (see "Materials and Methods") and digested with TPCK-treated trypsin at a final concentration of 3% (w/w) for 18 h at 37 °C. Following digestion, the peptides were separated using RP-HPLC on a C18 column (Waters) and eluted with a gradient of 5–80% solvent A over 100 min at a flow rate of 1.0 ml/min (Solvent A = 95% acetonitrile in 0.1% trifluoroacetic acid). Elution of the peptides from the column was monitored by measuring absorbance at 214 nm (→), and 1.0-ml fractions were collected (A). Peptide T2 was isolated from the peptide map and reapplied to the same column which was now eluted with a gradient of 20–60% solvent A over 100 min at a flow rate of 1.0 ml/min. Elution of the peptides from the column was monitored by measuring absorbance at 214 nm (→), and 1.0-ml fractions were collected. At this stage peptide T2 was judged to be pure (C).

cDNA insert of 1.8 kb (25) and the second clone (3α-HSD-II) contained an internal EcoRI restriction site and yielded two fragments of 0.5 kb (3α-HSD-IIa) and 0.8 kb (3α-HSD-IIb) following EcoRI digestion. DNA fragments were subcloned into pGEM3 and subjected to dideoxysequencing. Sequence analysis showed that 3α-HSD-I and 3α-HSD-IIb contained only 108 base pairs of open reading frame at their 5' ends followed by an in-frame stop codon. Translation of the open reading frame revealed the presence of the amino acid se-
isolated and yielded fragments of 1.1 and 1.0 kb in length following EcoRI digestion. Both fragments were subcloned into pGEM3 for sequence analysis, and it was determined that 3α-HSD-III contained the entire coding region for 3α-HSD. The alignment of the clones based on their restriction maps and sequence data is shown in Fig. 2.

Northern Analysis and Detection of 3α-HSD mRNA—Using clone 3α-HSD-II, cRNA probes were synthesized using the SP6 and T7 promoters of pGEM3 and the appropriate RNA polymerases to generate sense and anti-sense probes, respectively. Northern analysis of total rat liver RNA revealed the presence of a single mRNA species of 2.7 kb which could only be detected with the anti-sense probe. These results confirmed the identity of the coding strand. The ability to detect 3α-HSD mRNA in extracts of total RNA supports the view that 3α-HSD and its message are abundantly expressed. The size of the 3α-HSD mRNA is significantly larger than the cDNA (2.1 kb) and indicates that portions of either the 5′- or 3′-untranslated regions are absent from the cDNA.

Sequencing of 3α-HSD cDNA—Clones 3α-HSD-I–III were subjected to dideoxysequencing using several strategies. First, restriction sites were utilized to generate a series of deletion subclones. Second, nested deletions were created using exonuclease III and 5′ nucleases. Third, oligonucleotides complementary to either strand were synthesized as sequencing primers. The different regions of the cDNA sequence are shown in Fig. 3. Using these strategies, the nucleotides spanning the open reading frame in both strands were sequenced. Sequencing revealed that the open reading frame is 966 nucleotides long and encodes a protein of 322 amino acids in length (Fig. 4). Although the start and stop codons are clearly evident, a polyadenylation signal (AATAAA) was not found in the 3′-untranslated region. The initiation codon was determined to be the ATG at position 1, since there are three in-frame stop codons between this initiation codon and the next in-frame upstream ATG codon. The assigned initiation codon is located within a potential eukaryotic translation initiation consensus sequence (26). The molecular weight predicted for the protein from the cDNA is 37,029 and is 9% higher than that determined by SDS-polyacrylamide gel electrophoresis.

The identity of the clone was confirmed by locating the sequences of the two tryptic peptides (T9 and T30) and the two endoproteinase Lys-C peptides (endoproteinase Lys-C 5 and endoproteinase Lys-C 9) from 3α-HSD within the nucleotide sequence. The amino acid composition predicted for 3α-HSD from its cDNA (Leu133, Lys98, Asp58, Ala114, Glu202, Val263, Ile38, Ala198, Phe187, Ser185, Arg186, Thr184, Gly183, Pro184, Tyr183, Cys182, His181, Met180, and Trp185) is in close agreement with the amino acid composition determined from complete amino acid hydrolysis of 3α-HSD (Leu132, Lys99, Glx205, Val264, Ile39, Ala199, Phe186, Ser184, Arg187, Thr185, Gly180, Pro185, Tyr184, Cys183, His182, Met181) (6). Since the NH2-terminus of 3α-HSD is not amenable to peptide sequencing, presumably due to a blocked amino acid, it is of interest that the chemically determined composition...
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Fig. 5. Sequence homology between 3α-HSD, prostaglandin F synthase, and members of the aldehyde reductase family. Entries in the GenBank and EMBL data banks which showed the highest degree of sequence homology with 3α-HSD are shown. In conducting this analysis, the Wilbur and Lipman similarity search program of the IntelliGenetics software package was used. Sequences are aligned using the ALIGN algorithm.

The regions of greatest homology including conservative substitutions (3 amino acid residues or more) are highlighted in boxes. Exact matches are signified with a 1, and conserved substitutions are shown with a :. The sequence highlighted in boldface indicates the peptide tagged with 3α-bromoacetoxysteroid.

The deduced amino acid sequence also contains three peptides that have been identified by affinity-labeling studies with bromoacetoxysteroids to comprise the active site, and these are located in the COOH-terminal portion of the open-reading frame (see accompanying paper (45)). Sequence Analysis—3α-HSD bears no significant sequence homology with other HSDs that have been recently cloned and sequenced, including human placental 3β-hydroxysteroid dehydrogenase (27), rat liver 11β-hydroxysteroid dehydrogenase (28), and human placental 17β-hydroxysteroid dehydrogenase (29, 30). Comparison of the homology between 3α-HSD and entries in the GenBank and EMBL data banks identifies the greatest degree of homology with prostaglandin F synthase from bovine lung (69% at the amino acid level and 74% at the nucleotide level) (31) which is a member of the aldehyde reductase family. Homology is also seen between 3α-HSD and rho-crystallin from the European common frog (35) (Fig. 5). This homology is worthy of mention since the crystallins represent structural proteins of the lens, they are abundantly expressed, and one of the crystallins is known to display lactate dehydrogenase activity (36). Sequence comparison with other trans-dihydrodiol dehydrogenases is not possible since our data represent the first complete sequence for an enzyme with this function. It is noteworthy that minor forms of mouse and guinea pig liver dihydrodiol dehydrogenase, which display 17β-HSD activity, co-purify with aldehyde reductase (37, 38). In addition, partial amino acid sequence data obtained by fast atom bombardment and plasma desorption mass spectrometry of peptides isolated from various isoforms of rabbit dihydrodiol dehydrogenase give sequences that are highly conserved both within our primary structure and the primary structure of mammalian aldehyde reductases (39).

Despite sequence homology with the aldehyde reductases, 3α-HSD has several properties which distinguish it from (32), bovine lens (33), and rat lens (34) share at least 58% homology with 3α-HSD at the amino acid level. Homology is also seen between 3α-HSD and rho-crystallin from the European common frog (35) (Fig. 5).
members of this family. These include its dual pyridine nucleotide specificity, its ability to catalyze both oxidation and reduction reactions at physiological pH, and its ability to reduce aromatic ketones.

In analyzing the primary structures of short chain alcohol dehydrogenases, Jornvall and co-workers (40) have described a motif Tyr-X-X-X-Lys which is completely conserved. This consensus sequence is found in 3α-HSD at amino acid residues 205–209 as Tyr-Cys-Lys-Ser-Lys and is also found in 3β-, 11β-, 17β-, and prokaryotic 20β-hydroxysteroid dehydrogenases (41) in the motif Tyr-X-X-X-Lys. It is also present in 15-hydroxyprostaglandin dehydrogenase from human placenta (40). This consensus sequence is also present in the aldol reductases and rhod-crystallin which bear sequence homology with 3α-HSD. However, the conserved Tyr and Lys residues are not found in prostaglandin F synthase, the protein with the most homology with 3α-HSD, suggesting that this sequence may contribute to the differences in the activities of these two enzymes.

Many pyridine nucleotide-dependent oxidoreductases contain a structural domain which is responsible for binding the co-factor (Rossmann-fold (42)) which is characterized by a pattern of secondary structure corresponding to (β-α-β-α-β-β). Chou-Fasman predictions of the secondary structure of 3α-HSD reveal that this structural domain may reside in the NH2-terminal half of the protein. Wierenga et al. (43) have identified a “fingerprint” of amino acids present in NAD(P)+-binding proteins, and 3α-HSD contains 2 glycine residues (Gly-20 and Gly-22) which are completely conserved in their model. This further supports the view that the co-factor binding site is located at the NH2 terminus of 3α-HSD.

Analysis of the deduced amino acid sequence described in this paper and the isolation of the active site peptides in the accompanying paper (45) indicate that the pyridine nucleotide-binding site may reside at the NH2 terminus while the steroid-binding site may reside at the COOH terminus of 3α-HSD. We have recently completed a detailed analysis of the kinetic mechanism for 3α-HSD which predicts an Ordered Bi mechanism in which the pyridine nucleotide binds before the substrate (44). These findings imply that during catalysis the pyridine nucleotide binds first to the NH2 terminus, the steroid substrate binds to the COOH terminus, and the two structural domains are brought together to form the central complex so that dehydrogenation can proceed. Confirmation of these predictions will have to await the elucidation of the x-ray crystallographic structure of the enzyme.

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REFERENCES

1. Dorfman, R. I., and Shipley, R. A. (1956) Androsterone, pp. 122–126, John Wiley & Sons, Inc., New York.
2. Liao, S., Liang, J., Fang, S., Castañeda, E., and Shao, T.-C. (1973) J. Biol. Chem. 248, 6154–6162.
3. Teuwen, J. D., Moore, R. J., and Wilson, J. D. (1975) Biochemistry 14, 810–817.
4. Penning, T. M., and Talalay, P. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 4504–4508.
5. Penning, T. M., Mukharji, I., Barroso, S., and Talalay, P. (1984) Biochem. J. 222, 601–611.
6. Penning, T. M., Smithgall, T. E., Astkonas, L. J., and Sharp, R. B. (1986) Steroids 47, 221–247.
7. Smithgall, T. E., and Penning, T. M. (1988) Biochem. J. 254, 715–721.
8. Hoff, H. G., and Schriefers, H. (1984) Hoppe-Seyler’s Z. Physiol. Chem. 365, 377–391.
9. Tomkina, G. (1956) J. Biol. Chem. 218, 437–447.
10. Stolz, A., Takakawa, H., Ogawara, Y., Kuhlenkamp, J., and Kaplowitz, N. (1987) J. Clin. Invest. 79, 427–432.
11. Takikawa, H., Stolz, A., and Kaplowitz, N. (1987) J. Clin. Invest. 80, 852–860.
12. Glatt, H. R., Vogel, K., Bentley, P., and Oesch, F. (1979) Nature 277, 319–320.
13. Voger, K., Bentley, P., Platt, K.L., and Oesch, F. (1980) J. Biol. Chem. 255, 9621–9625.
14. Smithgall, T. E., Harvey, R. G., and Penning, T. M. (1986) J. Biol. Chem. 261, 6142–6148.
15. Penning, T. M., and Sharp, R. B. (1987) Biochem. Biophys. Res. Commun. 148, 646–652.
16. Smithgall, T. E., and Penning, T. M. (1988) Biochem. Pharmacol. 44, 831–835.
17. Schembrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 121–122, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
18. Smithgall, T. E., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467.
19. Kom, B., Frankel, F. R., Myers, J. C., and Lyttle, C. R. (1987) Proc. Natl. Acad. Sci. U.S.A. 120, 1402–1410.
20. Rave, N., Crkvenjakov, R., and Boedtker, H. (1979) Nucleic Acids Res. 6, 3559–3567.
21. DeLeon, D. V., Cox, K. H., Angerer, L. M., and Angerer, R. C. (1983) Deu. Biol. 100, 197–206.
22. Cramer, A. N., Delange, R. J., and Sigman, D. S. (1975) in Chemical Modification of Proteins: Selected Methods and Analytical Procedures (Work, T. S., and Work, E., eds) p. 103, Elsevier Biomedical Press, Amsterdam.
23. Pappin, D. J. C., Coui, J. M., and Koester, M. (1990) in Current Research in Protein Chemistry: Techniques, Structure and Function (Vilaininan, I., ed) pp. 191–203, Academic Press, New York.
24. Chow, P. Y., and Fasman, G. D. (1976) Adv. Enzymol. Relat. Areas Mol. Biol. 47, 45–147.
25. Issacson, K., Smithgall, T. E., Penning, T. M., and Lyttle, C. R. (1988) 70th Annual Meeting of the Endocrine Society, New Orleans, LA, Abstr. 1316 The Endocrine Society, Bethesda, MD.
26. Kosak, M. (1989) J. Cell Biol. 108, 229–241.
27. The V. L., Lachance, Y., Labrie, C., Leblanc, G., Thomas, J. L., Strickler, R. D., and Labrie, F. (1989) Mol. Endocrinol. 3, 1310–1312.
28. Agawala, A. K., Monder, C., Eckstein, B., and White, P. C. (1989) J. Biol. Chem. 264, 19389–19394.
29. The V. L., Labrie, C., Zhao, H. F., Coust, J., Lachance, Y., Simard, J., Leblanc, G., Cote, J., Berube, D., Gagne, R., and Labrie, F. (1989) Mol. Endocrinol. 3, 1301–1309.
30. Petiotkoto, H., Iacomou, V., Maentausta, O., and Vihko, R. (1986) FEBS Lett. 239, 73–77.
31. Watanabe, K., Fujii, Y., Nakayama, K., Okubo, H., Kuramitsu, S., Kagamiyama, H., Nakashima, S., and Haysbin, O. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 11–15.
32. Long, S., and LaMendola, J. (1989) J. Biol. Chem. 264, 17475–17477.
33. Petrash, J. M., and Favello, A. D. (1989) Curr. Eye Res. 8, 1021–1027.
34. Carper, D., Nishimura, K., Shinohara, T., Dietzchold, B., Wistow, G., Craft, J., Kawar, P., and Kimishita, J. H. (1987) FEBS Lett. 220, 259–265.
35. Tomarev, S. I., Zinovieva, R. D., Dolgilevich, S. M., Luchin, S. V., Krayev, A. S., Skryanbin, K. G., and Guse, G. J., Jr. (1984) FEBS Lett. 171, 297–302.
36. Wistow, G. J., Mulder, J. W. M., and de Jong, W. W. (1987) Nature 326, 524–524.