METHODS AND COMPOSITIONS FOR TREATING CANCER

FIELD OF THE INVENTION

Methods and compositions for treating cancer are described herein. More particularly, the methods for treating cancer comprise administering a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, such as abiraterone acetate (i.e., 3β-acetoxy-17-(3-pyridyl) androsta-5,16-diene), in combination with at least one additional therapeutic agent, such as an anti-cancer agent or a steroid. Furthermore, disclosed are compositions comprising a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, and at least one additional therapeutic agent such as an anti-cancer agent or a steroid, e.g., a corticosteroid or, more specifically, a glucocorticoid.

BACKGROUND

The number of people diagnosed with cancer has significantly increased. Of special interest are individuals diagnosed with androgen-dependent disorders, such as prostate cancer, and estrogen-dependent disorders, such as breast cancer since such diagnoses are increasing in number at an alarming rate.

Prostate cancer is currently the most common non-skin cancer and the second leading cause of cancer-related death in men after lung cancer. The primary course of treatment for patients diagnosed with organ-confined prostate cancer is usually prostatectomy or radiotherapy. Not only are these treatments highly invasive and have undesirable side effects, such localized treatments are not effective on prostate cancer after it has metastasized. Moreover, a large percent of individuals who receive localized treatments will suffer from recurring cancer.

Additionally, breast cancer incidence in women has increased from one out of every 20 women in 1960 to one out of every eight women in 2005. Moreover, it is the most common cancer among white and African-American women. Similar to treating prostate cancer, most options for women diagnosed with breast cancer are highly invasive and have significant side-effects. Such treatments include surgery, radiation and chemotherapy.

Hormone therapy is another treatment option for individuals diagnosed with prostate or breast cancer. Hormone therapy is a form of systemic treatment for prostate or breast cancer wherein hormone ablation agents are used to suppress the production or block the effects of hormones, such as estrogen and progesterone in the body, which are believed to promote the growth of breast cancer, as well as testosterone and dihydrotestosterone, which are believed to promote the growth of prostate cancer. Moreover, hormone therapy is less invasive than surgery and does not have many of the side effects associated with chemotherapy or radiation. Hormone therapy can also be used by itself or in addition to localized therapy and has shown to be effective in individuals whose cancer has metastasized.

Even though hormone therapy is less invasive and can be used on more advanced stages of cancer, some individuals administered current hormone therapy treatments may not show a significant response or may not show any response at all to such treatments. Additionally, some patients treated with current hormone therapy treatments may also suffer from relapsing or recurring cancer. Currently, such refractory cancer patients are left with very few treatment options.

Despite the progress made in the treatment of cancer, there remains a need for more effective ways to treat cancer such as, but not limited to, prostate cancer and breast cancer. Additionally, there is a need for effective anti-cancer treatment options for patients who are not responding to current anti-cancer treatments. Also, there is a need for effective anti-cancer treatment options for patients whose cancer has recurred.

SUMMARY OF THE INVENTION

Described herein are methods for treating a cancer in which a therapeutically effective amount of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, such as abiraterone acetate (i.e. 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene), is administered to a patient, e.g., a patient in need thereof, in combination with a therapeutically effective amount of at least one additional therapeutic agent including, but not limited to, an anti-cancer agent or steroid. Such methods can also provide an effective treatment for individuals with a refractory cancer, including individuals who are currently undergoing a cancer treatment. Therefore, in certain embodiments, the method is directed to treating a refractory cancer in a patient, in which a therapeutically effective amount of 17α-hydroxylase/$C_{17,20}$-lyase inhibitor is administered to a patient currently receiving an anti-cancer agent.

For example, in certain embodiments, the method for the treatment of a cancer in a mammal comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 0.1 mg/m^2 to about 20 mg/m^2 of mitoxantrone.

In another embodiment, the method for the treatment of a cancer in a mammal comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/m^2 to about 175 mg/m^2 of paclitaxel.

In still other embodiments, the method for the treatment of a cancer in a mammal comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/m^2 to about 100 mg/m^2 of docetaxel.

Furthermore, described herein is a method for the treatment of a cancer in a mammal comprising administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate; and an amount of about 0.01 mg to about 200 mg of leuprolide, wherein the leuprolide is administered over a period of about 3 days to about 12 months.

In other embodiments, the method for the treatment of a cancer in a mammal comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 0.01 mg to about 20 mg of goserelin, wherein the goserelin is administered over a period of about 28 days to about 3 months.

Additionally, in another embodiment, the method for the treatment of a cancer in a mammal comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 0.01 mg to about 20 mg of triptorelin, wherein the triptorelin is administered over a period of about 1 month.

The method for the treatment of a cancer in a mammal can also comprise administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 0.1 μg/day to about 500 μg/day of seocalcitol, such as about 100 μg/day of seocalcitol.

Also, the method for the treatment of a cancer in a mammal can comprise administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/day to about 300 mg/day of bicalutamide.

In yet another embodiment, the method for the treatment of a cancer in a mammal can comprise administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/day to about 2000 mg/day of flutamide.

Moreover, the method for the treatment of a cancer in a mammal can comprise administering an amount of about 50 mg/day to about 2000 mg/day of abiraterone acetate and an amount of about 0.01 mg/day to about 500 mg/day of a glucocorticoid including, but not limited to, hydrocortisone, prednisone or dexamethasone.

Also described herein are compositions for the treatment of cancer that comprise a combination of a therapeutically effective amount of at least one 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and a therapeutically effective amount of at least one additional anti-cancer agent, such as, but not limited to, mitoxantrone, paclitaxel, docetaxel, leuprolide, goserelin, triptorelin, seocalcitol, bicalutamide, flutamide, or a steroid including, but not limited to, hydrocortisone, prednisone, or dexamethasone.

Finally, single unit dosage forms comprising abiraterone acetate and a glucocorticoid, optionally with carriers, diluents or excipients, are contemplated. Also, kits comprising at least one 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and an additional anti cancer agent or steroid are contemplated. For example, the kit may include a vial containing abiraterone acetate and another vial containing a glucocorticoid.

DEFINITIONS

As used herein and unless otherwise defined the word "cancer," refers to the growth, division or proliferation of abnormal cells in the body. Cancers that can be treated with the methods and the compositions described herein include, but are not limited to, prostate cancer, breast cancer, adrenal cancer, leukemia, lymphoma, myeloma, Waldenström's macroglobulinemia, monoclonal gammopathy, benign monoclonal gammopathy, heavy chain disease, bone and connective tissue sarcoma, brain tumors, thyroid cancer, pancreatic cancer, pituitary cancer, eye cancer, vaginal cancer, vulvar cancer, cervical cancer, uterine cancer, ovarian cancer, esophageal cancer, stomach cancer, colon cancer, rectal cancer, liver cancer, gallbladder cancer, cholangiocarcinoma, lung cancer, testicular cancer, penal cancer, oral cancer, skin cancer, kidney cancers, Wilms' tumor and bladder cancer.

As used herein, and unless otherwise defined, the terms "treat," "treating" and "treatment" include the eradication, removal, modification, management or control of a tumor or primary, regional, or metastatic cancer cells or tissue and the minimization or delay of the spread of cancer.

As used herein, and unless otherwise defined, the term "patient" means an animal, including but not limited to an animal such as a human, monkey, cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, or guinea pig. In one embodiment, the patient is a mammal and in another embodiment the patient is a human. In certain embodiments, the patient can be an adult male or female. In some embodiments, the patient is a male of age about 30 years to about 85 years. In other embodiments, the patient is a female of age about 30 years to about 85 years. In a particular embodiment, the patient has or is susceptible to having (e.g., through genetic or environmental factors) cancer. In a further embodiment, the patient has or is susceptible to having (e.g., through genetic or environmental factors) a tumor. In other embodiments, the patient can be castrated or non-castrated.

The term "17α-hydroxylase/$C_{17,20}$-lyase inhibitor" as used herein refers to an inhibitor of 17α-hydroxylase/$C_{17,20}$-lyase, (which is an enzyme in testosterone synthesis), an analog thereof, derivative thereof, metabolite thereof or pharmaceutically acceptable salt thereof. Also, unless otherwise noted, reference to a particular 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can include analogs, derivatives, metabolites or pharmaceutically acceptable salts of such particular 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

The term "anti-cancer agent" as used herein refers to any therapeutic agent that directly or indirectly kills cancer cells or directly or indirectly prohibits stops or reduces the proliferation of cancer cells. It should be noted that even though throughout this specification and in the claims the phrase "anti-cancer agent" is written as a singular noun, for example: "an anti-cancer agent" or "the anti-cancer agent," the phrase "anti-cancer agent" should not be interpreted as being limited to the inclusion of a single anti-cancer agent.

As used herein, and unless otherwise defined, the phrase "therapeutically effective amount" when used in connection with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor or therapeutic agent means an amount of the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor or therapeutic agent effective for treating a disease or disorder disclosed herein, such as cancer.

As used herein and unless otherwise defined the phrase "refractory cancer," means cancer that is not responding to an anti-cancer treatment or cancer that is not responding sufficiently to an anti-cancer treatment. Refractory cancer can also include recurring or relapsing cancer.

As used herein and unless otherwise defined the phrase "refractory patient," means a patient who has refractory cancer.

As used herein and unless otherwise defined the phrase "relapse cancer," means cancer that was at one time responsive to an anti-cancer treatment but has become no longer responsive to such treatment or is no longer responding sufficiently to such treatment.

As used herein and unless otherwise defined the phrase "recurring cancer," means cancer that has returned after a patient has been earlier diagnosed with cancer, under gone treatment or had been previously diagnosed as cancer-free.

As used herein and unless otherwise defined the term "derivative" refers to a chemically modified compound wherein the chemical modification takes place at one or more functional groups of the compound. The derivative may retain or improve the pharmacological activity of the compound from which it is derived.

As used herein and unless otherwise defined the term "analog" refers to a chemical compound that is structurally similar to another but differs slightly in composition (as in the replacement of one atom by an atom of a different element or in the presence of a particular functional group).

As used herein and unless otherwise defined the phrase "pharmaceutically acceptable salt" refers to any salt of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor which retains the biological effectiveness of the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor. Examples of pharmaceutically acceptable salts include, but are not limited to, acetates, sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, gamma-hydroxybutyrates,

glycollates, tartarates, alkanesulfonates (e.g. methane-sulfonate or mesylate), propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates. Several of the officially approved salts are listed in Remington: The Science and Practice of Pharmacy, Mack Publ. Co., Easton.

DETAILED DESCRIPTION OF THE INVENTION

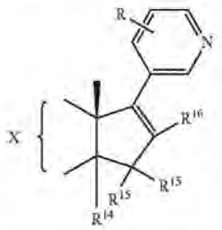
The methods described herein for treating cancer comprise administering to a mammal, preferably a human, a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor in addition to at least one therapeutic agent, such as an anti-cancer agent or steroid, particularly a glucocorticoid. The compositions described herein comprise a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and at least one additional therapeutic agent, such as an anti-cancer agent or steroid, particularly a corticosteroid or glucocorticoid. Other anti-cancer treatments such as, administration of yet another anti-cancer agent, radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy, can be used with the methods and compositions.

17α-Hydroxylase/$C_{17,20}$-Lyase Inhibitors

17α-hydroxylase/$C_{17,20}$-lyase inhibitors have been shown to be useful in the treatment of cancer, specifically hormone-dependent disorders such as, androgen-dependent and estrogen-dependent disorders like prostate cancer and breast cancer respectively, as described in U.S. Pat. No. 5,604,213 to Barrie et al., which is herein incorporated by reference in its entirety.

In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be 17-(3-pyridyl)androsta-5,16-dien-3βol; 17-(3-pyridyl)androsta-3,5,16-triene; 17-(3-pyridyl)androsta-4,16-dien-3-one; 17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol; 17-(3-pyridyl)-5α-androst-16-en-3α-ol; 17-(3-pyridyl)-5α-androst-16-en-3-one; 17-(3-pyridyl)-androsta-4,16-diene-3,11-dione; 17-(3-pyridyl)-androsta-3,5,16-trien-3-ol; 6α- and 6β-fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one; 17-(3-pyridyl)androsta-4,16-dien-3,6-dione; 3α-trifluoromethyl-17-(3-pyridyl)androst-16-en-3β-ol or their acid addition salts and 3-esters as well as metabolites, analogs, derivatives or a pharmaceutically acceptable salt thereof.

In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can have the structure of formula (I):



(I)

wherein X represents the residue of the A, B and C rings of a steroid which can be, without limitation, androstan-3α- or 3β-ol; androst-5-en-3β- or 3β-ol; androst-4-en-3-one; androst-2-ene; androst-4-ene; androst-5-ene; androsta-5,7-dien-3α or 3β-ol; androsta-1,4-dien-3-one; androsta-3,5-diene; androsta-3,5-diene-3-ol; estra-1,3,5[10]-triene; estra-1,3,5[10]-trien-3-ol; 5α-androstan-3-one; androst-4-ene-3,11-dione; 6-fluoroandrost-4-ene-3-one; or androstan-4-ene-3,6-dione; each of which, where structurally permissible, can be further derivatized in one or more of the following ways, including, but not limited to, to form 3-esters; to have one or more carbon or carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions; as 3-oximes; as 3-methylenes; as 3-carboxylates; as 3-nitriles; as 3-nitros; as 3-desoxy derivatives; to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoro-methyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B, or C-ring; or to be 19-nor;

R represents a hydrogen atom or an alkyl group of 1-4 carbon atoms;

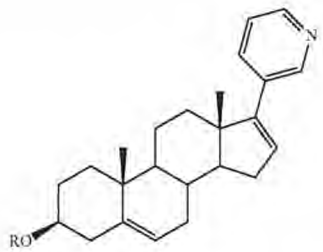
R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms;

each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms; and

R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts, but excluding 3β-acetoxy-17-(3-pyridyl)androsta-5,14,16-triene, 3β,15α- and 3β,15β-diacetoxy-17-(3-pyridyl) androsta-5,16-diene and 3β-methoxy-17-(3-pyridyl-5α-androst-16-ene.

Suitable inhibitors also include metabolites, derivatives, analogs, or pharmaceutically acceptable salts of formula (I).

In another embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can have the structure of formula (I):

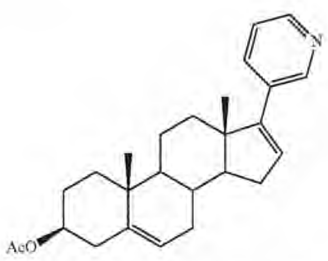


(I)

wherein R represents hydrogen or a lower acyl group having 1 to 4 carbons. Suitable inhibitors also include derivatives, analogs, or pharmaceutically acceptable salts of formula (I).

In still another embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be a 3β-alkanoyloxy-17-(3-pyridyl) androsta-5,16-diene in which the alkanoyloxy group has from 2 to 4 carbon atoms.

In a preferred embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor comprises abiraterone acetate or 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene which has the following structural formula:

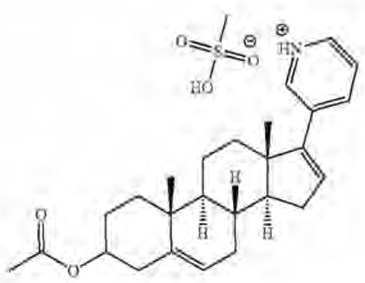


(II)

and pharmaceutically acceptable salts thereof.

Preferred salts of abiraterone acetate and methods of making such salts are also disclosed in U.S. Provisional Application No. 60/603,559 to Hunt, which is incorporated by reference in its entirety. Preferred salts include, but are not limited to, acetates, citrates, lactates, alkanesulfonates (e.g. methanesulfonate or mesylate) and tartarates. Of special interest is the abiraterone acetate mesylate salt (i.e. 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene mesylate salt) which has the following structural formula:

(III)



The 17α-hydroxylase/$C_{17,20}$-lyase inhibitors can be made according to any method known to one skilled in the art. For example, such inhibitors can be synthesized according to the method disclosed in U.S. Pat. Nos. 5,604,213 and 5,618,807 to Barrie et al., herein incorporated by reference. Another method of making 17α-hydroxlase/$C_{17,20}$-lyase inhibitors is disclosed in U.S. provisional application 60/603,558 to Bury, herein incorporated by reference.

The amount of 17α-hydroxylase/$C_{17,20}$-lyase inhibitor administered to a mammal having cancer is an amount that is sufficient to treat the cancer, whether the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor is administered alone or in combination with an additional anti-cancer treatment, such as an additional anti-cancer agent.

Additional Therapeutic Agents

Suitable compounds that can be used in addition to 17α-hydroxylase/$C_{17,20}$-lyase inhibitors as an anti-cancer agent include, but are not limited to, hormone ablation agents, anti-androgen agents, differentiating agents, anti-neoplastic agents, kinase inhibitors, anti-metabolite agents, alkylating agents, antibiotic agents, immunological agents, interferon-type agents, intercalating agents, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, mitotic inhibitors, matrix metalloprotease inhibitors, genetic therapeutics, and anti-androgens. The amount of the additional anti-cancer agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor. Below are lists of examples of some of the above classes of anti-cancer agents. The examples are not all inclusive and are for purposes of illustration and not for purposes of limitation. Many of the examples below could be listed in multiple classes of anti-cancer agents and are not restricted in any way to the class in which they are listed in.

Suitable hormonal ablation agents include, but are not limited to, androgen ablation agents and estrogen ablation agents. In preferred embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor is administered with a hormonal ablation agent, such as deslorelin, leuprolide, goserelin or triptorelin. Even though throughout this specification and in the claims the phrase "hormonal ablation agent" is written as a singular noun, for example; "a hormonal ablation agent" or "the hormonal ablation agent," the phrase "hormonal ablation agent" should not be interpreted as being limited to the inclusion of a single hormonal ablation agent. The amount of the hormonal ablation agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

Suitable anti-androgen agents include but are not limited to bicalutamide, flutamide and nilutamide. The amount of the anti-androgen agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In another embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor may be administered with a differentiating agent. Suitable differentiating agents include, but are not limited to, polyamine inhibitors; vitamin D and its analogs, such as, calcitriol, doxercalciferol and seocalcitol; metabolites of vitamin A, such as, ATRA, retinoic acid, retinoids; short-chain fatty acids; phenylbutyrate; and nonsteroidal anti-inflammatory agents. The amount of the differentiating agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In another preferred embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor may be administered with an anti-neoplastic agent, including, but not limited to, tubulin interacting agents, topoisomerase inhibitors and agents, acitretin, alstonine, amonafide, amphethinile, amsacrine, ankinomycin, anti-neoplaston, aphidicolin glycinate, asparaginase, baccharin, batracylin, benfluron, benzotript, bromofosfamide, caracemide, carmethizole hydrochloride, chlorsulfaquinoxalone, clanfenur, claviridenone, crisnatol, curaderm, cytarabine, cytocytin, dacarbazine, datelliptinium, dihaematoporphyrin ether, dihydrolenperone, dinaline, distamycin, docetaxel, elliprabin, elliptinium acetate, epothilones, ergotamine, etoposide, etretinate, fenretinide, gallium nitrate, genkwadaphnin, hexadecylphosphocholine, homoharringtonine, hydroxyurea, ilmofosine, isoglutamine, isotretinoin, leukoregulin, lonidamine, merbarone, merocyaulne derivatives, methylanilinoacridine, minactivin, mitonafide, mitoquidone, mitoxantrone, mopidamol, motretinide, N-(retinoyl)amino acids, N-acylated-dehydroalanines, nafazatrom, nocodazole derivative, ocreotide, oquizanocine, paclitaxel, pancratistatin, pazelliptine, piroxantrone, polyhaematoporphyrin, polypreic acid, probimane, procarbazine, proglumide, razoxane, retelliptine, spatol, spirocyclopropane derivatives, spirogermanium, strypoldinone, superoxide dismutase, teniposide, thaliblastine, tocotrienol, topotecan, ukrain, vinblastine sulfate, vincristine, vindesine, vinestramide, vinorelbine, vintriptol, vinzolidine, and withanolides. The amount of the anti-neoplastic agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

The 17α-hydroxylase/$C_{17,20}$-lyase inhibitors may also be used with a kinase inhibitor including p38 inhibitors and CDK inhibitors, TNF inhibitors, metallomatrix proteases inhibitors (MMP), COX-2 inhibitors including celecoxib, rofecoxib, parecoxib, valdecoxib, and etoricoxib, SOD mimics or $\alpha_v\beta_3$ inhibitors. The amount of the kinase inhibitor administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In another embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor may be administered with an anti-metabolite agent.

Suitable anti-metabolite agents may be selected from, but not limited to, 5-FU-fibrinogen, acanthifolic acid, aminothiadiazole, brequinar sodium, carmofur, cyclopentyl cytosine, cytarabine phosphate stearate, cytarabine conjugates, dezaguanine, dideoxycytidine, dideoxyguanosine, didox, doxifluridine, fazarabine, floxuridine, fludarabine phosphate, 5-fluorouracil, N-(2'-furanidyl)-5-fluorouracil, isopropyl pyrrolizine, methobenzaprim, methotrexate, norspermidine, pentostatin, piritrexim, plicamycin, thioguanine, tiazofurin, trimetrexate, tyrosine kinase inhibitors, and uricytin. The amount of the anti-metabolite agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In another embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor may be administered with an alkylating agent. Suitable alkylating agents may be selected from, but not limited to, aldo-phosphamide analogues, altretamine, anaxirone, bestrabucil, budotitane, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cyplatate, diphenylspiromustine, diplatinum cytostatic, elmustine, estramustine phosphate sodium, fotemustine, hepsul-fam, ifosfamide, iproplatin, lomustine, mafosfamide, mitolactol, oxaliplatin, prednimustine, ranimustine, semustine, spiromustine, tauromustine, temozolomide, teroxirone, tetraplatin and trimelamol. The amount of the alkylating agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In another preferred embodiment, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor may be administered with an antibiotic agent. Suitable antibiotic agents may be selected from, but not limited to, aclarubicin, actinomycin D, actinoplanone, adriamycin, aeroplysinin derivative, amrubicin, anthracycline, azino-mycin-A, bisucaberin, bleomycin sulfate, bryostatin-1, calichemycin, chromoximycin, dactinomycin, daunorubicin, ditrisarubicin B, dexamethasone, doxorubicin, doxorubicin-fibrinogen, elsamicin-A, epirubicin, erbstatin, esorubicin, esperamicin-A1, esperamicin-A1b, fostriecin, glidobactin, gregatin-A, grincamycin, herbimycin, corticosteroids such as hydrocortisone, idarubicin, illudins, kazusamycin, kesarirhodins, menogaril, mitomycin, neoenactin, oxalysine, oxaunomycin, peplomycin, pilatin, pirarubicin, porothramycin, prednisone, prednisolone, pyrindanycin A, rapamycin, rhizoxin, rodorubicin, sibanomicin, siwenmycin, sorangicin-A, sparsomycin, talisomycin, terpentecin, thrazine, tricrozarin A, and zorubicin. The amount of the antibiotic agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

Alternatively, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitors may also be used with other anti-cancer agents, including but not limited to, acemannan, aclarubicin, aldesleukin, alemtuzumab, alitretinoin, altretamine, amifostine, amsacrine, anagrelide, anastrozole, ancestim, bexarotene, broxuridine, capecitabine, celmoleukin, cetrorelix, cladribine, clotrimazole, daclizumab, dexrazoxane, dilazep, docosanol, doxifluridine, bromocriptine, carmustine, cytarabine, diclofenac, edelfosine, edrecolomab, eflornithine, emitefur, exemestane, exisulind, fadrozole, filgrastim, finasteride, fludarabine phosphate, formestane, fotemustine, gallium nitrate, gemcitabine, glycopine, heptaplatin, ibandronic acid, imiquimod, iobenguane, irinotecan, irsogladine, lanreotide, leflunomide, lenograstim, lentinan sulfate, letrozole, liarozole, lobaplatin, lonidamine, masoprocol, melarsoprol, metoclopramide, mifepristone, miltefosine, mirimostim, mitoguazone, mitolactol, molgramostim, nafarelin, nartograstim, nedaplatin, nilutamide, noscapine, oprelvekin, osaterone, oxaliplatin, pamidronic acid, pegaspargase, pentosan polysulfate sodium, pentostatin, picibanil, pirarubicin, porfimer sodium, raloxifene, raltitrexed, rasburicase, rituximab, romurtide, sargramostim, sizofiran, sobuzoxane, sonermin, suramin, tasonermin, tazarotene, tegafur, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, thalidomide, thymalfasin, thyrotropin alfa, topotecan, toremifene, trastuzumab, treosulfan, tretinoin, trilostane, trimetrexate, ubenimex, valrubicin, verteporfin, vinorelbine. The amount of the anti-cancer agent administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

The 17α-hydroxylase/$C_{17,20}$-lyase inhibitors may also be administered or combined with steroids, such as corticosteroids or glucocorticoids. The 17α-hydroxylase/$C_{17,20}$-lyase inhibitors and the steroid may be administered in the same or in different compositions. Non-limiting examples of suitable steroids include hydrocortisone, prednisone, or dexamethasone. The amount of the steroid administered to a mammal having cancer is an amount that is sufficient to treat the cancer whether administered alone or in combination with a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

In one embodiment, provided herein are methods and compositions comprising both abiraterone acetate and a steroid particularly a corticosteroid, or more particularly a glucocorticoid. Steroids within the scope of the disclosure include, but are not limited to, (1) hydrocortisone (cortisol; cyprionate (e.g., CORTEF), oral; sodium phosphate injection (HYDROCORTONE PHOSPHATE); sodium succinate (e.g., A-HYDROCORT, Solu-CORTEF); cortisone acetate oral or injection forms, etc.), (2) dexamethasone (e.g., Decadron, oral; Decadron-LA injection, etc.), (3) prednisolone (e.g., Delta-CORTEF, prednisolone acetate (ECONOPRED), prednisolone sodium phosphate (HYDELTRASOL), prednisolone tebutate (HYDELTRA-TBA, etc.)), or (4) prednisone DELTASONE, etc.) and combinations thereof. See, e.g., GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 10TH EDITION 2001.

In a specific embodiment, single unit solid oral dosage forms which comprise an amount from about 50 mg to about 300 mg of abiraterone acetate and an amount from about 0.5 mg to about 3.0 mg of a steroid, e.g., glucocorticoid in a single composition, optionally with excipients, carriers, diluents, etc. is contemplated. For instance, the single unit dosage form can comprise about 250 mg of abiraterone acetate and about 1.0 mg, 1.25 mg, 1.5 mg, or 2.0 mg of a steroid, such as but not limited to corticosteroids or glucocorticoids.

Administration of the 17α-Hydroxylase/$C_{17,20}$-Lyase Inhibitor and an Additional Therapeutic Agent

The 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the additional therapeutic agent, such as an anti-cancer agent or a steroid can be administered by any method known to one skilled in the art. In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the additional therapeutic agent can be in separate compositions prior to administration. In the alternative, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the additional therapeutic agent can be combined into a single composition for administration.

The 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the additional therapeutic agent can be administered sequentially or simultaneously. If administered sequentially, the order of administration is flexible. For instance, 17α-hydroxylase/$C_{17,20}$-lyase inhibitor acetate can be administered prior to administration of the additional therapeutic agent. Alterna-

tively, administration of the additional therapeutic agent can precede administration of 17α-hydroxylase/$C_{17,20}$-lyase inhibitor.

Whether they are administered as separate compositions or in one composition, each composition is preferably pharmaceutically suitable for administration. Moreover, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the therapeutic agent, if administered separately, can be administered by the same or different modes of administration. Examples of modes of administration include parenteral (e.g., subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intravenous, intradetinal, intraperitoneal, intraportal, intraarterial, intrathecal, transmucosal, intra-articular, and intrapleural), transdermal (e.g., topical), epidural, and mucosal (e.g., intranasal) injection or infusion, as well as oral, inhalation, pulmonary, and rectal administration. In specific embodiments, both are oral.

For example, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be administered transdermally and the additional therapeutic agent can be administered parenterally. Alternatively, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be administered orally, such as in a tablet, caplet or capsule, while the additional therapeutic agent can be administered intravenously. Such intravenous administered therapeutic agents include, but are not limited to, docetaxel injections, such as Taxotere®; paclitaxel injections, such as Paclitaxel® and mitoxantrone injections, such as Novantrone®. Also, the additional therapeutic agent can be in the form of depots or implants such as leuprolide depots and implants, e.g. Viadur® and Lupron Depot®; triptorelin depots, e.g. Trelstar®; goserelin implants, e.g. Zoladex®.

The suitable daily dosage of the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor depends upon a number of factors, including, the nature of the severity of the condition to be treated, the particular inhibitor, the route of administration and the age, weight, and response of the individual patient. Suitable daily dosages of 17α-hydroxylase/$C_{17,20}$-lyase inhibitors can generally range from about 0.0001 mg/kg/day to about 1000 mg/kg/day, or from about 0.001 mg/kg/day to about 200 mg/kg/day, or from about 0.01 mg/kg/day to about 200 mg/kg/day, or from about 0.01 mg/kg/day to about 100 mg/kg/day in single or multiple doses.

In some embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be administered in an amount from about 0.004 mg/day to about 5,000 mg/day, or from about 0.04 mg/day to about 3,000 mg/day, or from about 0.4 mg/day to about 1500 mg/day. In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor can be administered in an amount from about 0.1 mg/day to about 2000 mg/day or from about 1 mg/day to about 2000 mg/day or from about 50 mg/day to about 2000 mg/day or from about 100 mg/day to about 1500 mg/day or from about 5 mg/day to about 1,000 mg/day or from about 5 mg/day to about 900 mg/day or from about 10 mg/day to about 800 mg/day or from about 15 mg/day to about 700 mg/day or from about 20 mg/day to about 600 mg/day or from about 25 mg/day to about 500 mg/day in single or multiple doses.

In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor is co-administered with an additional anti-cancer agent such as mitoxantrone, paclitaxel or docetaxel. For example, a method for the treatment of a cancer in a mammal comprises administering an amount of abiraterone acetate and an amount of mitoxantrone. For example, the abiraterone acetate can be administered in an amount of about 0.01 mg/kg/day to about 100 mg/kg/day and the mitoxantrone can be administered in an amount of about 0.1 mg/m^2 to about 20 mg/m^2. Preferably, the mitoxantrone is administered over a period of between about 10 to about 20 minutes once every 21 days.

Also, a method for the treatment of a cancer in a mammal can comprise administering an amount of abiraterone acetate and an amount of paclitaxel. In one embodiment, the abiraterone acetate can be administered in an amount of about 0.01 mg/kg/day to about 100 mg/kg/day and the paclitaxel can be administered in the amount of about 1 mg/m^2 to about 175 mg/m^2. Preferably, the paclitaxel is administered over a period of between about 2 to about 5 hours once every three months.

Additionally, a method for the treatment of a cancer in a mammal comprises administering an amount of abiraterone acetate and an amount of docetaxel. For example, the abiraterone acetate can be administered in an amount of about 0.01 mg/kg/day to about 100 mg/kg/day and the docetaxel can be administered in an amount of about 1 mg/m^2 to about 100 mg/m^2. Preferably, the docetaxel is administered over a period of between about 1 to about 2 hours once every three weeks.

In certain embodiments, the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor is administered along with an anti-cancer agent that comprises a hormonal ablation agent, including, but not limited to, leuprolide, goserelin, or triptorelin. For example, one method for the treatment of a cancer in a mammal also comprises administering an amount of abiraterone acetate and an amount of leuprolide. The amount of abiraterone acetate can be about 0.01 mg/kg/day to about 100 mg/kg/day and the amount of leuprolide can be about 0.01 mg to about 200 mg over a period of about 3 days to about 12 months. Preferably, the leuprolide is administered in the amount of about 3.6 mg of leuprolide over a period of about 3 days to about 12 months.

Additionally, the methods for the treatment of cancer in a mammal include administering an amount of abiraterone acetate and an amount of goserelin. For example, the amount of abiraterone acetate can be about 0.01 mg/kg/day to about 100 mg/kg/day and the amount of goserelin can be about 0.01 mg to about 20 mg over a period of about 28 days to about 3 months. Preferably, the goserelin is administered in the amount of about 3.6 mg to about 10.8 mg over a period of about 28 days to about 3 months.

In certain embodiments the methods for the treatment of cancer in a mammal comprises administering an amount of abiraterone acetate and an amount of triptorelin. For example, the amount of abiraterone acetate can be about 0.01 mg/kg/day to about 100 mg/kg/day and the amount of triptorelin can be about 0.01 mg to about 20 mg, over a period of about 1 month, preferably the triptorelin is administered in the amount of about 3.75 mg over a period of about 1 month.

Also, in one embodiment, the method for the treatment of a cancer in a mammal comprises administering an amount of abiraterone acetate and an amount of seocalcitol. For instance, the method involves administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 0.1 μg/day to about 500 μg/day of seocalcitol, such as about 100 μg/day of seocalcitol.

In another embodiment, the method for the treatment of a cancer in a mammal comprises administering an amount of abiraterone acetate and an amount of bicalutamide. For instance, the method involves administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/day to about 300 mg/day of bicalutamide.

In yet another embodiment, the method for the treatment of a cancer in a mammal comprises administering an amount of

abiraterone acetate and an amount of flutamide. For example, the method comprises administering an amount of about 0.01 mg/kg/day to about 100 mg/kg/day of abiraterone acetate and an amount of about 1 mg/day to about 2000 mg/day of flutamide.

Moreover, the method for the treatment of a cancer in a mammal can comprise administering an amount of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor such as abiraterone acetate and an amount of a glucocorticoid including, but not limited to, hydrocortisone, prednisone or dexamethasone. For example, the method can comprise administering an amount of about 50 mg/day to about 2000 mg/day of abiraterone acetate and an amount of about 0.01 mg/day to about 500 mg/day of hydrocortisone. In other instances, the method can comprise administering an amount of about 500 mg/day to about 1500 mg/day of abiraterone acetate and an amount of about 10 mg/day to about 250 mg/day of hydrocortisone.

The method for the treatment of a cancer can also comprise administering an amount of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, such as abiraterone acetate, and an amount of a glucocorticoid, such as prednisone. For example, the method can comprise administering an amount of about 50 mg/day to about 2000 mg/day of abiraterone acetate and an amount of about 0.01 mg/day to about 500 mg/day of prednisone. Also, the method can comprise administering an amount of about 500 mg/day to about 1500 mg/day of abiraterone acetate and an amount of about 10 mg/day to about 250 mg/day of prednisone.

In addition, the method for the treatment of a cancer can also comprise administering an amount of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, such as abiraterone acetate, and an amount of a glucocorticoid, such as dexamethasone. For example, the method can comprise administering an amount of about 50 mg/day to about 2000 mg/day of abiraterone acetate and an amount of about 0.01 mg/day to about 500 mg/day of dexamethasone. Also, the method can comprise administering an amount of about 500 mg/day to about 1500 mg/day of abiraterone acetate and an amount of about 0.5 mg/day to about 25 mg/day of dexamethasone.

Compositions Containing a 17α-Hydroxylase/$C_{17,20}$-Lyase Inhibitor and an Additional Therapeutic Agent

In certain embodiments, the compositions can contain a combination of a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, preferably abiraterone acetate, and any of the therapeutic agents recited above. Whether the 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and the additional therapeutic agent are administered in separate compositions or as a single composition, the compositions can take various forms. For example, the compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders or sustained-release formulations, depending on the intended route of administration.

For topical or transdermal administration, the compositions can be formulated as solutions, gels, ointments, creams, suspensions or salves.

For oral administration, the compositions may be formulated as tablets, pills, dragees, troches, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

The composition may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas that contain conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the composition may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Additionally, the composition may be delivered using a sustained-release system, such as semi-permeable matrices of solid polymers containing the composition. Various forms of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature can release the composition over a period of hours, days, weeks, months. For example a sustained release capsule can release the compositions over a period of 100 days or longer. Depending on the chemical nature and the biological stability of the composition, additional strategies for stabilization may be employed.

The compositions can further comprise a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered.

For parenteral administrations, the composition can comprise one or more of the following carriers: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium hi sulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

For oral solid formulations suitable carriers include fillers such as sugars, e.g., lactose, sucrose, mannitol and sorbitol; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, fats and oils; granulating agents; and binding agents such as microcrystalline cellulose, gum tragacanth or gelatin; disintegrating agents, such as cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate, Primogel, or corn starch; lubricants, such as magnesium stearate or Sterotes; glidants, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; or flavoring agents, such as peppermint, methyl salicylate, or orange flavoring. If desired, solid dosage forms may be sugar-coated or enteric-coated using standard techniques.

For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy injectability with a syringe. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents,

for example, sugars; polyalcohols such as mannitol, sorbitol; sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Also for intravenous administration, the compositions may be formulated in solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. In a preferred embodiment, the compositions are formulated in sterile solutions.

For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories.

For administration by inhalation, the compositions may be formulated as an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the composition and a suitable powder base such as lactose or starch.

The pharmaceutical compositions may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

One example of a composition comprising a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor and an additional therapeutic agent is an oral composition or composition suitable for oral administration comprising abiraterone acetate in combination with a steroid. For example, the oral composition can be a solid dosage form such as a pill, a tablet or a capsule. The oral composition can comprise about 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, or 1000 mg of abiraterone acetate. The oral composition can comprises about 0.25 mg, 0.5 mg, 0.75 mg, 1.0 mg, 1.25 mg, 1.5 mg, 1.75 mg, 2.0 mg, 2.25 mg, 2.5 mg, 2.75 mg, 3.0 mg, 3.25 mg, 3.5 mg, 3.75 mg, 4.0 mg, 4.25 mg, 4.5 mg, 4.75 mg, 5.0 mg, 7.5 mg, 10 mg, 20 mg, 30 mg, 40 mg or 50 mg of a steroid, such as a glucocorticoid.

In one embodiment, the oral composition can comprise about 50 mg to about 500 mg of abiraterone acetate and an amount of about 0.25 mg to about 3.5 mg of the steroid, such as hydrocortisone, prednisone or dexamethasone. In other instances, the composition can comprise about 50 mg to about 300 mg of abiraterone acetate and an amount of about 1.0 mg to about 2.5 mg of the steroid, such as hydrocortisone, prednisone or dexamethasone. In another embodiment the composition can comprise about 50 mg to about 300 mg of abiraterone acetate and about 0.5 mg to about 3.0 mg of a steroid. For example, the oral composition can be a tablet containing 250 mg of abiraterone acetate; 1.25 mg or 2.0 mg of a steroid, such as hydrocortisone, prednisone or dexamethasone; and one or more carriers, excipients, diluents or additional ingredients. Additionally, the oral composition can be a capsule containing 250 mg of abiraterone acetate; 1.25 mg or 2.0 mg of a steroid, such as hydrocortisone, prednisone or dexamethasone; and one or more carriers, excipients, diluents or additional ingredients.

The description contained herein is for purposes of illustration and not for purposes of limitation. The methods and compositions described herein can comprise any feature described herein either alone or in combination with any other feature(s) described herein. Changes and modifications may be made to the embodiments of the description. Furthermore, obvious changes, modifications or variations will occur to those skilled in the art. Also, all references cited above are incorporated herein, in their entirety, for all purposes related to this disclosure.

What is claimed is:

1. A method for the treatment of a prostate cancer in a human comprising administering to said human a therapeutically effective amount of abiraterone acetate or a pharmaceutically acceptable salt thereof and a therapeutically effective amount of prednisone.

2. The method of claim **1**, wherein the therapeutically effective amount of the abiraterone acetate or pharmaceutically acceptable salt thereof is from about 50 mg/day to about 2000 mg/day.

3. The method of claim **2**, wherein the therapeutically effective amount of the abiraterone acetate or pharmaceutically acceptable salt thereof is from about 500 mg/day to about 1500 mg/day.

4. The method of claim **3**, wherein the therapeutically effective amount of the abiraterone acetate or pharmaceutically acceptable salt thereof is about 1000 mg/day.

5. The method of claim **1**, wherein the therapeutically effective amount of the abiraterone acetate or a pharmaceutically acceptable salt thereof is administered in at least one dosage form comprising about 250 mg of abiraterone acetate or a pharmaceutically acceptable salt thereof.

6. The method of claim **1**, wherein the therapeutically effective amount of the prednisone is from about 0.01 mg/day to about 500 mg/day.

7. The method of claim **6**, wherein the therapeutically effective amount of the prednisone is from about 10 mg/day to about 250 mg/day.

8. The method of claim **7**, wherein the therapeutically effective amount of the prednisone is about 10 mg/day.

9. The method of claim **1**, wherein the therapeutically effective amount of the prednisone is administered in at least one dosage form comprising about 5 mg of prednisone.

10. The method of claim **1**, comprising administering to said human about 500 mg/day to about 1500 mg/day of abiraterone acetate or a pharmaceutically acceptable salt thereof and about 0.01 mg/day to about 500 mg/day of prednisone.

11. The method of claim **10**, comprising administering to said human about 1000 mg/day of abiraterone acetate or a pharmaceutically acceptable salt thereof and about 10 mg/day of prednisone.

12. The method of claim **1**, wherein said prostate cancer is refractory prostate cancer.

13. The method of claim **12**, wherein the refractory prostate cancer is not responding to at least one anti-cancer agent.

14. The method of claim **13**, wherein the at least one anti-cancer agent comprises a hormonal ablation agent, an anti-androgen agent, or an anti-neoplastic agent.

15. The method of claim **14**, wherein the hormonal ablation agent comprises deslorelin, leuprolide, goserelin, or triptorelin.

16. The method of claim **14**, wherein the anti-androgen agent comprises bicalutamide, flutamide, or nilutamide.

17. The method of claim 14, wherein the anti-neoplastic agent comprises docetaxel.

18. The method of claim 12, comprising administering to said human about 500 mg/day to about 1500 mg/day of abiraterone acetate or a pharmaceutically acceptable salt thereof and about 0.01 mg/day to about 500 mg/day of prednisone.

19. The method of claim 18, comprising administering to said human about 1000 mg/day of abiraterone acetate or a pharmaceutically acceptable salt thereof and about 10 mg/day of prednisone.

20. The method of claim 17, comprising administering to said human about 1000 mg/day of abiraterone acetate or a pharmaceutically acceptable salt thereof and about 10 mg/day of prednisone.

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[45] **Date of Patent: Feb. 18, 1997**

[54] **17-SUBSTITUTED STEROIDS USEFUL IN CANCER TREATMENT**

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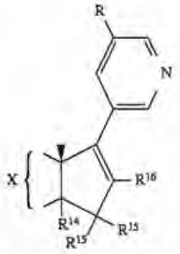
(List continued on next page.)

Primary Examiner—Mukund J. Shah
Assistant Examiner—Anthony Bottino
Attorney, Agent, or Firm—Nixon & Vanderhye

[57] **ABSTRACT**

Compounds of the general formula (1)



(I)

wherein X represents the residue of the A, B and C rings of a steroid, R represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, R^{14} represents a hydrogen atom and R^{15} represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, or a hydroxy or alkylcarbonyloxy group of 2 to 5 carbon atoms or R^{14} and R^{15} together represent a double bond, and R^{16} represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts, are useful for treatment of androgen-dependent disorders, especially prostatic cancer, and also oestrogen-dependent disorders such as breast cancer.

22 Claims, No Drawings

OTHER PUBLICATIONS

D. H. R. Barton et al., "Studies on the oxidation of Hydrazones with iodide . . . ", Tetrahedron 44, 147–162 (1988).

J. Wicha et al. 'Cardiotonic Steroids. Part 8. Synthesis of 17.beta. (3'pyridyl)–14.beta.–Androst–4–ene–3.beta.14–diol from 17–Oxoandrostane Derivatives' Bulletin of the Polish Academy of Sciences. Chemistry. vol. 33, No. 1–2, 1985, pp. 19–27.

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17-SUBSTITUTED STEROIDS USEFUL IN CANCER TREATMENT

This specification is a continuation-in-part of PCT Application PCT/GB93/00531, filed Mar. 15, 1993 and which designated the United States of America.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to 17-substituted steroids and their use in the treatment of androgen-dependent and oestrogen-dependent disorders, especially prostatic cancer and breast cancer respectively.

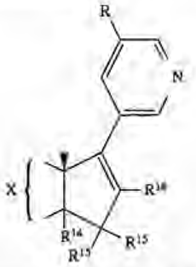
2. Description of the Related Art

The 17α-hydroxylase/C_{17-20} lyase enzyme complex (hereinafter "hydroxylase/lyase") is known to be essential for the biosynthesis of androgens and oestrogens. In the treatment of androgen-dependent disorders, especially prostatic cancer, there is a need for strong inhibitors of hydroxylase/lyase. Certain anti-androgenic steroids are well known, for example Cyproterone acetate (17α-acetoxy-6-chloro-1α, 2α-methylene-4,6-pregnadiene-3,20-dione). Many other steroids have been tested as hydroxylase/lyase inhibitors. See, for example, PCT Specification WO 92/00992 (Schering AG) which describes anti-androgenic steroids having a pyrazole or triazole ring fused to the A ring at the 2,3-position, or European Specifications EP-A 288053 and EP-A 413270 (Merrell Dow) which propose 17β-cyclopropylamino androst-5-en-3β-ol or -4-en-3-one and their derivatives.

SUMMARY OF THE INVENTION

It has now surprisingly been found that steroids lacking a C_{20} side chain and having a 17-(3-pyridyl) ring in its place, together with a 16,17-double bond, are powerful hydroxylase/lyase inhibitors, useful for the above-stated purposes.

According to the invention, there are provided compounds of the general formula



(I)

wherein X represents the residue of the A, B and C rings of asteroid, R represents a hydrogen atom or an alkyl group of 1–4 carbon atoms, R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms and each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1–4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, and R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts.

The term "steroid" herein includes any compound having the steroidal B and C rings, but in which all or part of the A ring is missing e.g. ring not closed (lacking the 2- or 3-position C-atom or both) or takes the form of a cyclopentane ring. It also includes azasteroids having a ring nitrogen atom in place of a ring carbon atom, especially in the A-ring such as in 4-azasteroids.

In general, the compounds of formula (1) are new and such compounds per se are included in the invention. However, certain of them have been disclosed as intermediates in the synthesis of certain steroids having a 3-pyridyl or 3-pyridonyl group in the 17β-position, see J. Wicha and M. Masnyk, Bulletin of the Polish Academy of Sciences: Chemistry 33 (1–2), 19–27 and 29–37 (1985). The first of these papers says that a 17β-side chain of the form —C=C—C=O or —C=C—C=N favours cardiotonic properties and describes the synthesis of 17β-(3-pyridyl)-14β-androst-4-ene-3β,14-diol, while the second uses this compound to prepare 17β-[3-pyrid-2(1H)onyl]-14β-androst-4-ene-3β,14-diol. Those final compounds differ from those of the present invention by having a saturated D-ring and the paper contains no test results. Insofar as certain compounds within formula (1) are known as intermediates in these syntheses, the invention extends to the compounds only for use in therapy. These are 3β-acetoxy-17-(3-pyridyl)androsta-5,14,16-triene and 3β,15α- and 3β,15β-diacetoxy-17-(3-pyridyl)androsta-5,16-diene. See also J. Wicha et. al., Heterocycles 20, 231–234 (1983) which is a preliminary communication of the first of the above two papers.

J. Wicha et. al., Bulletin of the Polish Academy of Sciences, Chemistry 32 (1–2), 75–83 (1984) have also described the preparation of 3β-methoxy-17β-(3-pyridyl)androstane and pyridone analogues thereof via the intermediate 3β-methoxy-17-(3-pyridyl)-5α-androst-16-ene. Accordingly, the invention extends to the latter compound only for use in therapy. A preliminary communication of this paper, by J. Wicha and M. Masynk, appeared in Heterocycles 16, 521–524 (1981).

The invention also includes pharmaceutical compositions comprising a compound of formula (1) in association with a pharmaceutically acceptable diluent or carrier. The terminology "pharmaceutical compositions" implies that injectible formulations are sterile and pyrogen-free and thereby excludes any compositions comprising the compound of formula (1) and a non-sterile organic solvent, such as may be encountered in the context of the final stages of preparing these above-mentioned compounds of formula (1) which have been described in the literature but without any therapeutic use being mentioned.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the compounds of the invention the essential structural features comprise all of:

a 3-pyridyl ring in the 17-position

a ring double bond in the 16,17-position of the D-ring

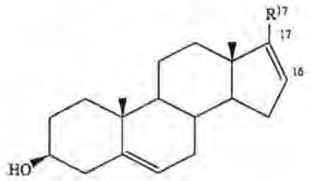
the 18-position methyl group

It is critical that the pyridine nitrogen atom be in the 3-position, not the 2- or 4-position. It is also critical that the pyridine ring be joined directly to the 17-carbon atom. This criticality is demonstrated by tests of inhibiting activity against hydroxylase and lyase (Table 1). The concentration of test compound required to achieve 50% inhibition of the

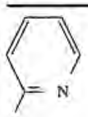
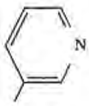
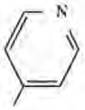
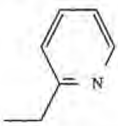
enzyme is far greater for the 2-pyridyl, 4-pyridyl and 2-pyridylmethyl compounds tested than for the 3-pyridyl. The methods of determination were as described in the Examples hereinafter.

TABLE 1

Effect of variations in the 17-substituent on inhibition of hydroxylase and lyase, demonstrating the criticality of the 17-substituent in this invention.



(2)

R^{17}	Type	IC_{50} (μM) Lyase	Hydroylase
	2-Pyridyl (for comparison)	0.13	0.32
	3-pyridyl (present invention)	0.003	0.004
	4-pyridyl (for comparison)	2.0	5.0
	2-picolyl (for comparison)	>10	>10

Note:
all the compounds of formula (2) tested were poor inhibitors of aromatase: IC_{50} >20 μM.

Our modelling of the geometry of the putative transition state of the lyase component of the hydroxylase-lyase enzyme complex, in the putative mechanism of action of the lyase component, suggests that the 16,17-double bond is essential to allow the 3-pyridine ring to adopt the orientation required for co-ordination to the haem group of the hydroxylase-lyase complex.

Elsewhere, the D-ring can have any other simple substituent. Certain simple substituents are defined in connection with the preferred general formula (1), but it will be appreciated that others could be substituted for those of formula (1). In the compounds of formula (1), R^{15} is preferably hydrogen or alkyl of 1 to 3 carbon atoms, R^{16} hydrogen, alkyl of 1 to 3 carbon atoms, fluorine, chlorine, bromine or iodine, and R hydrogen or methyl, in the 5-position of the pyridine ring.

The remainder of the molecule, designated "X" in formula (1), can be of any kind conventional in steroid chemistry or have any other feature known in steroids having anti-androgenic activity, for example the pyrazole or triazole ring, fused to the A ring at the 2- and 3- positions, disclosed in the above-cited Specification WO 92/00992, or oxazole ring fused in the same positions.

By way of example, X can represent the residue of

androstan-3α- or 3β-ol,
androst-5-en-3α- or 3β-ol,
androst-4-en-3-one,
androst-2-ene,
androst-4-ene,
androst-5-ene,
androsta-5,7-dien-3α or 3β-ol,
androsta-1,4-dien-3-one,
androsta-3,5-diene,
estra-1,3,5[10]-triene,
estra-1,3,5[10]-trien-3-ol,
5α-androstan-3-one,
androst-4-ene-3,11-dione,
6-fluoroandrost-4-ene-3-one or
androstan-4-ene-3,6-dione

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

to form 3-esters, especially 3-alkanoates and -benzoates,
to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7-7,8-, 9,11- and 11,12-positions
as 3-oximes
as 3-methylenes
as 3-carboxylates
as 3-nitriles
as 3-nitros
as 3-desoxy derivatives
to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring
to be 19-nor.

Preferred C_{1-4}-alkyl and alkoxy groups are methyl and ethoxy.

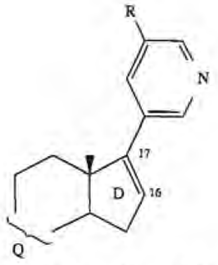
Preferred C_{1-4}-alkanoyloxy groups are acetoxy and propanoyloxy.

Preferred halo groups are fluoro, bromo and chloro and the preferred substitution position is the 6-position.

The substituents include, for instance, 2-fluoro, 4-fluoro, 6-fluoro, 9-fluoro, 3-trifluoromethyl, 6-methyl, 7-methyl, 6-oxo, 7-oxo, 11-oxo, 6-methylene, 11-methylene, 4-hydroxy, 7-hydroxy, 11-hydroxy or 12-hydroxy, each in any appropriate epimeric form, and, subject to structural compatibility (well known in general steroid chemistry), in any combination of two or more such groups.

Compounds which are likely to be unstable are considered excluded from consideration. Such compounds will be evident to steroid chemists. Compounds having esoteric substituents likely to interfere with the stereochemical alignment of the steroid molecule with the enzymes to be inhibited, by virtue of steric or electronic distribution effects, are to be avoided. For example, a 2,3,5,6-tetrafluoro-4-trifluoromethylphenoxy substituent in the 3-position is not recommended. Androst-5-en-3β-ol having such an ether substituent in place of the 3β-hydroxy group has been shown to be a very poor inhibitor for lyase and hydroxylase.

The currently preferred compounds of formula (1) include those which are saturated and unsubstituted at the 11- and 12-positions and which therefore are of the general formula (3):



(3)

wherein Q represents the residue of A, B and C rings of asteroid, and R is a hydrogen atom or an alkyl group of 1–4 carbon atoms.

However, 11- and/or 12-substituted compounds are also active. Particularly preferred are 11-oxo and 11β-hydroxy derivatives of compounds of formula (3).

Specifically preferred compounds of the invention comprise

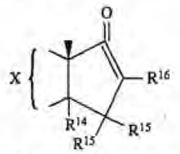
17-(3-pyridyl)androsta-5,16-dien-3β-ol,
17-(3-pyridyl)androsta-3,5,16-triene,
17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
17-(3-pyridyl)-5α-androst-16-en-3α-ol
and their acid addition salts and 3-esters.

Other notable compounds of the invention comprise

17-(3-pyridyl)-5α-androst-16-en-3-one,
17-(3-pyridyl)-androsta-4,16-diene-3,11-dione,
17-(3-pyridyl)-androsta-3,5,16-trien-3-ol,
6α- and 6β-fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one
17-(3-pyridyl)androsta-4,16-dien-3,6-dione,
17-[3-(5-methyl pyridyl)]androsta-5,16 dien-3β-ol
3α-trifluoromethyl-17-(3-pyridyl)androsta-16-en-3β-ol
and their acid addition salts and 3-esters.

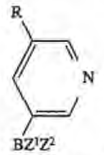
Insofar as certain compounds within formula (1) are known per se and these are compounds which are less easy to prepare than many of the others, a preferred class of compounds of formula (1) is those which do not have a 3β-alkoxy group, a 14,15-double bond or a 15-ester group.

The compounds of formula (1) can be prepared by a method which is in itself novel and inventive. Starting from a 17-oxo compound of general formula (4):



(4)

wherein X, R^{14}, R^{15} and R^{16} are as defined above and any other oxo groups and hydroxy groups in the molecule are first appropriately protected, the method comprises replacing the 17-hydroxy group of compound (4) in its enol form by a leaving group (L) which is capable of being replaced by a 3-pyridyl group in a palladium complex-catalysed cross-coupling reaction with a pyridyl ring-substituted boron compound of formula (5):

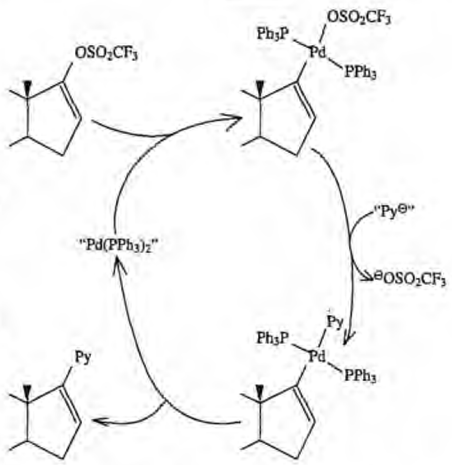


(5)

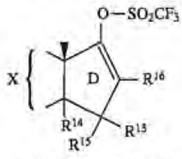
wherein Z^1 and Z^2 independently represent hydroxy or alkoxy or alkyl of 1–4 carbon atoms each, preferably 1–3 carbon atoms, most preferably ethyl or methoxy, or Z^1 and Z^2 together represent an alkylenedioxy group of 2 or 3 carbon atoms and R is as defined above and carrying out said cross-coupling reaction.

The palladium complex-catalysed cross-coupling reaction of the 17-substituted steroid with the boron compound is believed to involve the steps indicated in the following illustrative reaction scheme 1 (Py=3-pyridyl). The pyridyl anionic species is provided by the boron compound.

Scheme 1

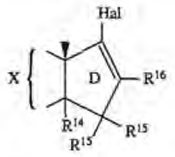


The replacement of the 17-enol group can be, for example, to form a 16,17-ene trifluoromethanesulphonate ("triflate") of formula (6):



(6)

or a 17-iodo or bromo-16,[17]-ene (a "vinyl halide") of formula (7):



(7)

(Hal=I or Br)

Compounds of formula (6) can be prepared by reacting the 17-oxo compound of formula (4) with an enol ester-forming trifluoromethanesulphonic acid derivative such as the anhydride, see S. Cacchi, E. Morera and G. Ortar, Tetrahedron Letters, 25, 4821 (1984). The 17-oxo compound can be considered notionally to exist in the enol form, the reaction being one of esterification of the enol.

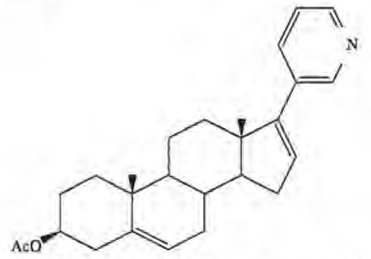
For the preparation of the 17-position derivatives of formula (6) or (7) any necessary protection of other groups in the molecule may be first carried out. For example in the triflate route hydroxyl groups are conveniently protected as their acetates, whilst in the vinyl halide route the 3-oxo group of steroids can be selectively protected as their perfluorotolyl enol ethers, see M. Jarman and R. McCague, J.Chem. Soc. Perkin Trans. 1, 1129 (1987).

Compounds of formula (7) can be prepared by first hydrazinating the 17-oxo compounds of formula (4) by a standard method to form the 17-hydrazone, which is then reacted with bromine or iodine in the presence of an amine or guanidine base, see D. Barton, G. Bashiardes and J. Fourmy, Tetrahedron Letters, 24, 1605 (1983).

The 17-position derivative (whether triflate or vinyl halide) is then reacted with the boron compound of formula (5) using as catalyst a palladium(0) phosphine complex, for example tetrakis(triphenylphosphine)palladium(0), or a palladium (II) phosphine complex which is reducible in situ to a palladium(0) phosphine species, especially bis(triphenylphosphine)palladium (II) chloride.

SUMMARY OF THE INVENTION

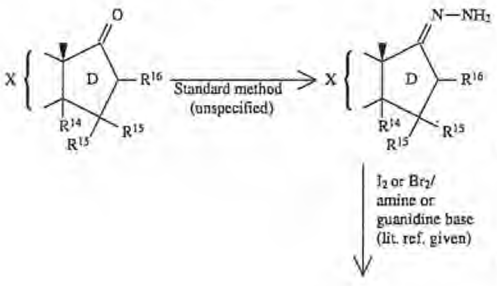
The vinyl halide route, via a compound of formula (7), is particularly well suited to the preparation of 3β-acyloxy-16,17-ene-17-(3-pyridyl) steroids, especially the preferred compound, 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene, of formula (8):



(8)

but using the unprotected 3β-hydroxy compound as starting material. By-products can be reduced either (a) by keeping the proportion of organoboron compound (borane) used in the cross-coupling reaction within the range 1.0 to 1.2 equivalents per equivalent of steroid or (b) by crystallising the reaction product of the cross-coupling reaction from a mixture of acetonitrile and methanol. This route via the vinyl iodide intermediate is therefore amenable to large scale synthesis, and is shown in Scheme 2 below.

Scheme 2



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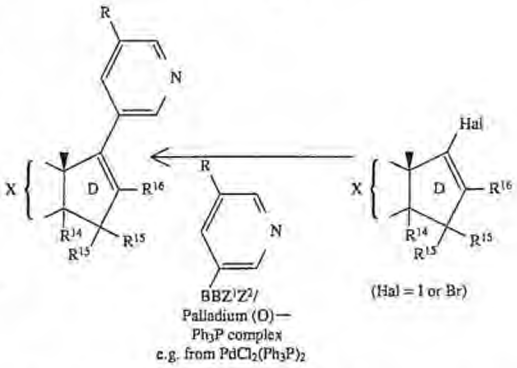
Scheme 2



The principle of this aspect of the invention may be expressed as a method of preparing a 3β-hydroxy- or 3β-(lower acyloxy)-16,17-ene-17-(3-pyridyl)-substituted steroid, wherein the 3β-(lower acyloxy) group of the steroid has from 2 to 4 carbon atoms, which comprises subjecting a 3β-hydroxy-16,17-ene-17-iodo or-bromo steroid to a palladium complex-catalysed cross-coupling reaction with a (3-pyridyl)-substituted borane, in which the pyridine ring is substituted at the 5-position by an alkyl group of 1 to 4 carbon atoms or is unsubstituted thereat, especially with a said borane of formula (5), wherein R is a hydrogen atom or an alkyl group of 1–4 carbon atoms and Z^1 and Z^2 independently represent hydroxy or alkoxy or alkyl or 1–3 carbon atoms each or Z^1 and Z^2 together represent an alkylenedioxy group of 2 or 3 carbon atoms, in a proportion of at least 1.0 equivalent of boron compound per equivalent of steroid, in an organic liquid, which is a solvent for the 3β-hydroxy steroidal reaction product, and optionally esterifying the 3β-hydroxy reaction product to the 3β-acyloxy ester, which method comprises feature (a) or (b) above.

Preferably the vinyl iodide or bromide is unsubstituted in the 14, 15 and 16-positions, in which case it can be prepared from dehydroepiandrosterone (DHEA). In the hydrazination it is preferable to use hydrazine hydrate together with a catalytic amount of a proton provider which is most preferably hydrazine sulfate.

The hydrazone is preferably iodinated with iodine or brominated with bromine in the presence of a strong base such as a tetraalkylguanidine, especially tetramethylguandine which is cheaply and readily available.

In the cross-coupling reaction, the boron compound is preferably a diethylborane or a dimethoxyborane (Z^1=Z^2=Et or OMe). Other boranes include those in which the boron atom is part of a cyclic ether ring e.g. as in Z^1, Z^2=1,2-ethylenedioxy or 1,3-propylenedioxy. In embodiment (a) of this aspect of the invention the proportion of borane added is at least 1.0, but no more than 1.2 equivalents of boron per equivalent of steroid, preferably about 1.1, but in the embodiment (b) a higher proportion is preferred, e.g. from 1.2:1 to 1.5:1 equivalents of boron compound to steroid. The higher proportion will give the better yield of product but also more of the contaminating boron, phosphine and/or palladium compounds. According to embodiment (b), however, these are removed with the acetonitrile solvent. In either embodiment, the palladium compound is a palladium (0) phosphine complex such as tetrakis(triphenylphosphine) palladium (0) or a compound reducible to a palladium (0)

phosphine species, especially bis(triphenylphosphine) palladium (II) chloride. The reaction vessel is preferably purged with an inert gas, especially argon or nitrogen, to minimise the possibility of oxidation with a corresponding redox reduction of palladium to the metallic state.

The cross-coupling reaction is preferably carried out in two phases, one aqueous, one organic. The organic phase comprises an organic solvent for the 3β-hydroxy steroidal reaction product, especially tetrahydrofuran (THF). Other cyclic ethers such as dioxane could be used in place of THF. Preferably, a nucleophilic activator, such as sodium carbonate, is used, in which case it is normally present in the aqueous phase.

After the reaction, inorganic salts can be removed by first adding another organic solvent, preferably diethyl ether, which is a solvent for the organoboron contaminants produced in the reaction product, and miscible with the first-mentioned organic solvent (e.g. THF), but immiscible with water, whereafter the organic, e.g. THF-diethyl ether, phase and water (aqueous phase) can be separated. After this separation, various work-up procedures are operable. In one procedure, particularly suited to embodiment (a), the THF and diethyl ether are removed, e.g. evaporated as a mixture, and the remaining reaction product is washed with a third organic solvent, which can be diethyl ether, preferably cooled to below room temperature, most especially to 10° C. or lower. The third organic solvent is one in which the 3β-hydroxy steroid reaction product has a low solubility and which, importantly, removes the organoboron compound/s (and also the contaminating phosphine and palladium compound/s). Diethyl ether is preferred.

A different work-up procedure, used in embodiment (b), comprises crystallisation from acetonitrile/methanol. Acetonitrile is a preferred crystallisation solvent to keep boron compound as well as palladium compound in solution and is therefore used in an excess over methanol e.g. an excess of at least 5:1 and preferably about 8:1 by volume.

To prepare the 3β-acyloxy (alkylcarbonyloxy) compounds, of which the acetoxy compound is preferred, standard acylating (acyl-esterification) agents such as acetyl, propionyl or butyryl chloride or anhydride can be used. The final esterification product may be crystallised direct from hexane, rather than from ethanol/water followed by hexane. Preferably, the work-up procedure comprises reverse phase chromatography, i.e. using a relatively lipophilic solid phase. In this procedure, the chief by-product, a bis-steroidal compound, is preferentially retained on the solid phase and can be eluted with a good separation from the desired product.

Further compounds of the invention can be prepared by standard steroid to steroid inter-conversion chemistry, so long as the D-ring chemical structure is not affected thereby. If the D-ring structure is likely to be affected, it would usually be necessary to prepare the desired compound de novo, i.e. by choosing the appropriate starting compound of formula (4), protected if necessary, and carrying out the reactions of 17-substitution of the enol and cross-coupling with the boron compound as described above.

By way of example, the 3-esters of asteroid 3-ol with an alkanoic acid of 1 to 6 carbon atoms, or a cycloalkanoic acid or aralkanoic acid such as phenylacetic or phenylpropionic acid, an aroic acid such as benzoic acid, or other simple organic acid such as methanesulphonic acid, can be converted into the 3-ol or the 3-ol to the 3-ester. Other examples of simple conversions which would not affect the D-ring structure are

i) Oppenauer oxidation using cyclohexanone and aluminium isopropoxide to convert 3-hydroxy to 3-oxo steroids and notably $\Delta^{5,6}$-3-hydroxy to $\Delta^{4,5}$-3-oxo steroids;

ii) Wittig olefination to convert oxo groups to methylene groups [D. D. Evans et al., J. Chem. Soc., 4312–4317, (1963)];

iii) Oxidation of Δ^5-3β-hydroxy to Δ^4-3,6-dione steroids using N-methylmorpholine N-oxide and tetra-n-propylammonium perruthenate catalyst [M. Moreno et al., Tetrahedron Letters, 32, 3201–3204, (1991)];

iv) 6-Methylenation of Δ^4-3-oxo steroids using formaldehyde dimethylacetal [K. Annen et al., Synthesis, 34–40 (1982)];

v) Conversion of Δ^4-3-oxo to 4,4-dimethyl-Δ^5-3-oxo, $\Delta^{1,4}$-3-oxo, $\Delta^{1,4,6}$-3-oxo, 7α-methyl-Δ^4-3-oxo, $\Delta^{4,6}$-3-oxo, 6-chloro-$\Delta^{4,6}$-3-oxo, $\Delta^{2,4}$-2,3-isoxazole, 6α-methyl-Δ^4-3-oxo and Δ^4-3-desoxy; Δ^5-3β-ol to 5α-fluoro-6-oxo, 5α,6,6-trifluoro, 6,6-difluoro and 6α-fluoro-Δ^4-3-oxo; and 11-oxo to 11-hydroxy and $\Delta^{9,11}$ steroids [D. Lednicer and L. A. Mitscher, The Organic Chemistry of Drug Synthesis, ls. 2 and 3, Wiley (1980 and 1984)] or

vi) Electrophilic fluorination of steroids using N-fluoropyridinium reagents [T. Umenoto et al., Organic Synthesis 69, 129–143 (1990)].

The compounds of formula (1) may be prepared as salts, e.g. the hydrochloride and converted to the free base form and thereafter to such other conventional pharmaceutically acceptable salts as acetates, citrates and lactates, as may seem appropriate.

The present invention also provides a pharmaceutical composition which comprises a therapeutically effective amount of a compound of the invention, in association with a therapeutically acceptable carrier or diluent. The composition of the invention can, for example, be in a form suitable for parenteral (e.g. intravenous, intramuscular or intracavital), oral, topical or rectal administration. Particular forms of the composition may be, for example, solutions, suspensions, emulsions, creams, tablets, capsules, lipsomes or micro-reservoirs, especially compositions in orally ingestible or sterile injectable form. The preferred form of composition contemplated is the dry solid form, which includes capsules, granules, tablets, pills, boluses and powders. The solid carrier may comprise one or more excipients, e.g. lactose, fillers, disintegrating agents, binders, e.g. cellulose, carboxymethylcellulose or starch or anti-stick agents, e.g. magnesium stearate, to prevent tablets from adhering to tabletting equipment. Tablets, pills and boluses may be formed so as to disintegrate rapidly or to provide slow release of the active ingredient.

The present invention also includes a method of treating androgen- and oestrogen-dependent disorders, especially tumours, and most especially pro static tumours, in the mammalian body, which comprises administering a compound of the invention to a mammalian patient in a therapeutically effective dose, e.g. in the range 0.001–0.04 mmole/kg body weight, preferably 0.001–0.01 mmole/kg, administered daily or twice daily during the course of treatment. This works out (for humans) at 20–800 mg/patient per day. The preferred use is in treating prostatic cancer. Another use is in treating breast cancer.

The following Examples illustrate the invention.

EXAMPLE 1

(a) 3β-Acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate

To a stirred solution of dehydroepiandrosterone-3-acetate (24.8 g, 75 mmol) in dry dichloromethane (500 ml) containing 2,6-di-t-butyl-4-methylpyridine (18.5 g, 90 mmol) was added trifluoromethanesulphonic anhydride (12.6 ml,

75 mmol). After 12 h the mixture was filtered and washed with water (50 ml), dried ($MgSO_4$), and the solvent evaporated. Chromatography, on elution with light petroleum-dichloromethane (6:1), gave firstly androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (3.02 g, 10%) as an oil. ^{1}H-NMR($CDCl_3$) inter alia δ0.99 (3H,s,18-CH_3), 1.02(3H, s,19-CH_3), 5.39(1H,m,6-H), 5.59(1H,m,16-H), 5.62(1H,m, 3-H), 5.93(1H,dm,J 9.4 Hz,4-H); MS m/z 402(M^+). Further elution with light petroleum-dichloromethane (3:1) afforded the title compound (20.1 g, 58%) which crystallised from hexane, m.p. 75°–76° C. ^{1}H-NMR($CDCl_3$) inter alia δ1.00(3H,s, 18-CH_3), 1.06(3H, s,19-CH_3), 2.04(3H,s,CH_3CO_2), 4.59(1H,m,3α-H), 5.39(1H,dm,J 4.9 Hz,6-H), 5.58(1H,m,16-H). Anal. Calcd: C,57.13; H,6.32; S,6.93. Found: C,57.29; H,6.31; S,6.96%.

(b) 3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene

Diethyl(3-pyridyl)borane (3.38 g, 23 mmol) from Aldrich Chemical Co. Ltd. was added to a stirred solution of 3β-acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate (6.94 g, 15 mmol) in THF (75 ml) containing bis(triphenylphosphine)palladium(II) chloride (0.105 g, 0.15 mmol). An aqueous solution of sodium carbonate (2M, 30 ml) was then added and the mixture heated, with stirring, by an oil bath at 80° C. for 1 h, and allowed to cool. The mixture was partitioned between diethyl ether and water, the ether phase was dried (Na_2CO_3), filtered through a short plug of silica, and concentrated. Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound (4.95 g, 84%) which crystallised from hexane, m.p. 144°–145° C., ^{1}H-NMR($CDCl_3$) inter alia δ1.05(3H,s, 19-CH_3), 1.08(3H,s,18-CH_3), 2.04(3H,s,CH_3CO_2), 4.60(1H,m,3α-H), 5.42(1H,dm, J 4.7 Hz,6-H), 5.99(1H,m, 16-H), 7.23(1H,m,Py 5-H) 7.65(1H,m,Py 4-H), 8.46(1H,m, Py 6-H), 8.62(1H,m,Py 2-H). Anal. Calcd: C, 79.75; H, 8.50; N, 3.58. Found: C, 79.78; H, 8.52; N, 3.54%.

EXAMPLE 2

17-(3-Pyridyl)androsta-5,16-dien-3β-ol

To a solution of 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene (4.90 g, 12.5 mmol) in methanol (50 ml) was added an aqueous solution of sodium hydroxide (10% w/v, 10 ml) and the mixture heated, with stirring, on an oil bath at 80° C. for 5 min., then allowed to cool. The mixture was poured into water, neutralised with hydrochloric acid (1M), rebasified with saturated sodium bicarbonate solution, and extracted with hot toluene (3×100 ml). The toluene extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with toluene-diethyl ether (2:1) afforded the title compound (3.45 g, 79%) which crystallised from toluene, mp 228°–229° C.; ^{1}H-NMR ($CDCl_3$ inter alia δ1.05(3H,s,19-CH_3), 1.07(3H,s,18-CH_3), 3.54(1H,m,3α-H), (5.40H,dm,J 5.0 Hz, 6-H), 5.99(1H,m,16-H), 7.22(1H, m,Py5-H), 7.65(1H,m,Py 4-H), 8.46(1H,m,Py 6-H), 8.62(1H,m,Py 2-H). Anal. Calcd: C, 82.47; H, 8.94; N, 4.01. Found: C, 82.40; H, 8.91; N, 3.97%.

EXAMPLE 3

17-(3-Pyridyl)androsta-3,5,16-triene

The method followed that described in Example 1, using in step (b) diethyl(3-pyridyl)borane (0.88 g, 6.0 mmol), androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (2.01 g, 5.0 mmol), prepared in step (a), THF (25 ml), bis(triphenylphosphine)palladium(II) chloride (35 mg, 0.05 mmol), and aqueous sodium carbonate (2M, 10 ml). Chromatography, on elution with dichloromethane, afforded the title compound (1.39 g, 84%) which crystallised from hexane, m.p. 110°–112° C. ^{1}H-NMR ($CDCl_3$) inter alia δ1.02(3H,s,19-CH_3), 1.07(3H,s,18-CH_3), 5.44(1H,m,6-H), 5.61(1H,m,3-H), 5.95(1H,dm, J 9.8 Hz, 4-H), 6.01(1H,m, 16-H), 7.23(1H,m,Py 5-H), 7.66(1H,m,Py 4-H), 8.46(1H,m, Py 6-H), 8.63(1H,m,Py 2-H); MS m/z 331 (M^+).

EXAMPLE 4

(a) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a) but using 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5-dien-17-one (5.03 g, 10 mmol), prepared as described in M. Jarman and R. McCague, J. Chem. Soc., Perkin Trans. 1, 1129 (1987), dichloromethane (80 ml), 2,6-di-t-butyl-4-methylpyridine (2.87 g, 14 mmol), and trifluoromethanesulphonic anhydride (1.85 ml, 11 mmol). Chromatography, on elution with light petroleum-dichloromethane (10:1), afforded the title compound (1.93 g, 30%) which crystallised from ethanol, m.p. 106°–107° C. ^{1}H-NMR ($CDCl_3$) inter alia δ1.02(6H,s,18 and 19-CH_3), 5.16(1H,s,4-H), 5.28(1H,m,6-H), 5.59(1H,m,16-H); MS m/z 634 (M^+).

(b) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene

The method essentially followed that of Example 1(b) but using diethyl(3-pyridyl)borane (0.44 g, 3.0 mmol), 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (1.27 g, 2.0 mmol), THF (10 ml), bis(triphenylphosphine)palladium(II) chloride (70 mg, 0.1 mmol), and aqueous sodium carbonate (2M, 5 ml). Chromatography, on elution with light petroleum-diethyl ether (3:1), afforded the title compound (0.82 g, 73%) which crystallised from hexane, m.p. 166.0°–166.5° C. ^{1}H-NMR ($CDCl_3$) inter alia δ1.05(3H,s,19-CH_3), 1.07(3H,s,18-CH_3), 5.18(1H,s,4-H), 5.32(1H,m,6-H), 6.01(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.66(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.63(1H,m,Py 2-H). Anal. Calcd: C, 66.07; H, 5.01; N, 2.49; F, 23.60. Found: C, 65.97; H, 5.02; N, 2.47; F, 23.41%.

(c) 17-(3-Pyridyl)androsta-4,16-dien-3-one

To solution of 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene (0.423 g, 0.75 mmol) in THF (5 ml) was added ethanol (5 ml) followed by aqueous hydrochloric acid (1M, 5 ml) and the mixture heated, with stirring, by an oil bath at 65° C. for 48h and allowed to cool. The mixture was poured into water (20 ml), neutralised with aqueous sodium hydroxide (1M), and extracted with diethyl ether (3×30 ml). The ether extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether, afforded the title compound (185 mg, 71%) which crystallised from diethyl ether, m.p. 148°–150° C. IR νmax 1674 cm^{-1}; ^{1}H-NMR($CDCl_3$) inter alia δ1.07(3H,s,18-CH_3), 1.24(3H,s,19-CH_3), 5.76(1H,s,4-H), 5.99(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.64(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.62(1H,m,Py 2-H); MS m/z 347 (M^+).

EXAMPLE 5

(a) 3-Acetoxyestra-1,3,5[10],16-tetraen-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a), but using oestrone-3-acetate (4.69 g, 15 mmol), dichloromethane (120 ml), 2,6-di-t-butyl-4-methylpyridine (4.00 g, 19.5 mmol), and trifluoromethanesulphonic anhydride (2.8 ml, 16.5 mmol). Chromatography, on elution with light petroleum-dichloromethane (3:1), afforded the title com-

pound (5.21 g, 78%). ^{1}H-NMR($CDCl_3$) inter alia δ1.00(3H, s,18-C$\underline{H}_3$), 2.29(3H,s,C$\underline{H}_3CO_2$), 5.62(1H,m, 16-$\underline{H}$), 6.81(1H,m,ArH), 6.85(1H,m,ArH), 7.26(1H,m,ArH). Anal. Calcd. for $C_{21}H_{23}O_5F_3S_1.½H_2O$: C, 55.62; H, 5.34. Found: C, 55.58: H, 5.14%.

(b) 3-Acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene

The method followed that described in Example 1(b), but using diethyl(3-pyridyl)borane (1.65 g, 11.2 mmol), 3-acetoxyestra-1,3,5[10],16-tetraen-17-yl trifluoromethanesulphonate (3.56 g, 8.0 mmol), THF (40 ml), bis(triphenylphosphine)palladium(II) chloride (56 mg, 0.08 mmol), and aqueous sodium carbonate (2M, 15 ml).

Chromatography, on elution with light petroleum-diethyl-ether (2:1) afforded the title compound (2.40 g, 80%). ^{1}H-NMR($CDCl_3$) inter alia δ1.04(3H, s,18-C$\underline{H}$), 2.29(3H, s, C$\underline{H}_3CO_2$), 6.03(1H,m,16-$\underline{H}$), 6.82(1H,m,ArH), 6.85(1H,m, ArH), 7.24(1H,m,Py 5-$\underline{H}$), 7.29(1H,m,ArH), 7.69(1H,m,Py 4-$\underline{H}$), 8.48(1H,m,Py 6-$\underline{H}$), 8.65(1H,m,Py 2-$\underline{H}$); MS m/z 373. (M^+).

EXAMPLE 6

17-(3-Pyridyl)estra-1,3,5[10],16-tetraen-3-ol

The method followed that described in Example 2, but using 3-acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene (2.36 g, 6.3 mmol), methanol (40 ml), aqueous sodium hydroxide (10% w/v, 5 ml), and the mixture was heated at 80° C. for 10 min. Chromatography, on elution with toluene-methanol (8:1), afforded the title compound (1.40 g, 67%) which crystallised from toluene, m.p. 256°–258° C.: ^{1}H-NMR(DMSO) inter alia δ1.01(3H,s,18-C$\underline{H}_3$), 6.15(1H, m,16-$\underline{H}$), 6.47(1H,m,ArH), 6.52(1H,m,ArH), 7.04(1H,m, ArH), 7.35(1H,m,Py 5-$\underline{H}$), 7.79(1H,m,Py 4-$\underline{H}$), 8.45(1H,m, Py 6-$\underline{H}$), 8.62(1H,m,Py 2-$\underline{H}$). Anal. Calcd: C, 83.34; H, 7.60; N, 4.23. Found: C, 83.39; H, 7.78; N, 4.06%.

EXAMPLE 7

3α-Acetoxy-17-(3-pyridyl)-5α-androst-16-ene

The method followed that described in Example 1, using in step (b) diethyl(3-pyridyl)borane (1.41 g, 9.6 mmol), 3α-acetoxy-5α-androst-16-en-17-yl trifluoromethanesulphonate (3.44 g, 7.4 mmol), prepared from the 3α-acetoxy-5α-androstan-17-one by the method of step (a), THF (40 ml), bis(triphenylphosphine)palladium(II) chloride (52 mg, 0.07 mmol), and aqueous sodium carbonate (2M, 15 mmol). Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound (2.39 g, 82%), ^{1}H-NMR ($CDCl_3$) inter alia δ0.85(3H,s, 19-C$\underline{H}_3$), 1.01(3H,s,18-C$\underline{H}_3$), 2.06(3H,s,C$\underline{H}_3CO_2$), 5.02(1H,m,3β-H), 6.00(1H,m,16-$\underline{H}$), 7.24(1H,m,Py 5-$\underline{H}$), 7.68(1H,m,Py 4-$\underline{H}$), 8.47(1H,m,Py 6-$\underline{H}$), 8.63(1H,m,Py 2-$\underline{H}$); MS m/z 393 (M^+).

EXAMPLE 8

17-(3-Pyridyl)-5α-androst-16-en-3α-ol

The method followed that described in Example 2, but using 3α-acetoxy-17-(3 -pyridyl)-5α-androst-16-ene (2.33 g, 5.9 mmol), methanol (40 ml), aqueous sodium hydroxide (10% w/v, 8 ml), and the mixture was heated at 80° C. for 20 min. Chromatography, on elution with toluene-methanol (40:1), afforded the title compound (1.62 g, 78%) which crystallised from toluene, m.p. 198°–199° C.: ^{1}H-NMR($CDCl_3$) inter alia. δ0.84(3H,s,19-C$\underline{H}_3$), 1.00(3H,s,18-C$\underline{H}_3$), 4.06(1H,m,3β-$\underline{H}$), 5.97(1H,m,16-$\underline{H}$), 7.21(1H,m,Py 5-$\underline{H}$), 7.64(1H,m,Py 4-$\underline{H}$), 8.45(1H,m,Py 6-$\underline{H}$), 8.61(1H,m,Py 2-$\underline{H}$). Anal. Calcd: C, 82.00; H,9.46; N,3.99. Found: C,81.78; H,9.47; N,3.96%.

EXAMPLE 9

17-(3-Pyridyl)-5α-androst-16-en-3-one

From a solution of 17-(3-Pyridyl)-5α-androst-16-en-3α-ol (1.05 g, 3.0 mmol) in dry toluene (60 ml) and cyclohexanone (10 ml) was distilled off part of the solvent (20 ml) to eliminate moisture. After allowing to cool to 90° C., aluminium isopropoxide (1.02 g, 5.0 mmol) was added and the mixture heated under reflux for 90 min. then allowed to cool. The mixture was diluted with diethyl ether (250 ml), washed with aqueous trisodium citrate (15% w/v; 2×30 ml), dried (Na_2CO_3), and concentrated. Chromatography, on elution with toluene-methanol (40:1), afforded the title compound (0.90 g, 86%) which crystallised from toluene, m.p. 190°–192° C. IR νmax 1713 cm^{-1}; ^{1}H-NMR ($CDCl_3$) inter alia δ1.04 (3H,s,19-C$\underline{H}_3$), 1.07 (3H,s,18-C$\underline{H}_3$), 5.98 (1H,M, 16-$\underline{H}$), 7.22 (1H,m,Py 5-$\underline{H}$), 7.64 (1H,m,Py 4-$\underline{H}$), 8.46 (1H,m,Py 6-$\underline{H}$), 8.61 (1H,m,Py 2-$\underline{H}$); MS m/z 349 (M+). Anal. Calcd: C,82.47; H,8.94; N,4.01. Found: C,82.00; H,8.94; N,3.84[{]jf44a

EXAMPLE 10

a) 3-(tert-Butyldimethylsiloxy)androsta-3,5-diene-11,17-dione

To a solution of adrenosterone (6.0 g, 20 mmol) in dry dichloromethane (120 ml) was added triethylamine (8.4 ml, 60 mmol) followed by tert-butyldimethylsilyl trifluoromethanesulfonate (5.0 ml, 22 mmol) and the mixture stirred at room temperature for 3 h. The dichloromethane was evaporated and the residue redissolved in diethyl ether (100 ml), then allowed to stand for 30 min, after which time an oil separated. The ether phase was decanted off the oil and the solvent evaporated to give the title compound which was used directly in step (b). IR νmax 1705, 1747 cm^{-1}; ^{1}H-NMR($CDCl_3$) inter alia δ0.12 (6H,s,Me_2Si), 0.85 (3H,s,18-C$\underline{H}$3), 0.92 (9H,s,^{t}BuSi) 1.17(3H,s,19-C$\underline{H}_3$), 4.73 (1H,dm, J 6.9 Hz, 6-$\underline{H}$), 5.36 (1H,m,4-$\underline{H}$).

b) 13-(tert-Butyldimethylsiloxy)-11-oxo-androsta-3,5,16-trien-17-yl trifluoromethanesulfonate

To a solution of the product from step (a) in dry THF (100 ml), cooled to −78° C., was added a freshly prepared solution of lithium diisopropylamide [prepared by adding n-butyllithium (1.6M; 13.8 ml, 22 mmol) in hexane to a solution of diisopropylamine (3.1 ml, 22 mmol) in dry THF (25 ml) at −18° C.] and the resultant yellow solution stirred at −78° C. for 30 min. A solution of N-phenyltrifluoromethanesulfonimide (7.15 g, 20 mmol) in dry THF (20 ml) was then added and after an additional 1 h. at −78° C. was allowed to reach ambient temperature. The reaction mixture was poured into water (200 ml) and extracted with diethyl ether (2×200 ml), the combined ether extracts were washed with water (20 ml), dried Na_2CO_3), and concentrated to give the title compound which was used directly in step (c). IR νmax 1710 cm^{-1}, ^{1}H-NMR ($CDCl_3$) inter alia δ0.13 (6H, S,Me_2Si), 0.92 (9H,s,^{t}Bu Si), 1.35 (6H,2s,18-C$\underline{H}_3$ and 19-C$\underline{H}_3$), 4.75 (1H,m,6-$\underline{H}$) 5.38 (1H,s,4-$\underline{H}$), 5.68 (1H,m,16-H).

c) 3-(tert-Butyldimethylsiloxy)-17-(3-pyridyl)androsta-3,5,16-trien-11-one

The method essentially followed that described in Example 1(b), but using the 13-(tert-butyldimethylsiloxy)-11-oxo-androsta-3,5,16-trien-17-yltrifluoromethanesulfonate from step (b), diethyl (3-pyridyl)borane (3.53 g, 24 mmol), THF (100 ml), bis(triphenylphosphine)palladium (II) chloride (280 mg, 0.4 mmol), and aqueous sodium carbonate (2M;50 ml). Following work-up as described in

Example 1(b) the title compound was obtained, which was used directly in step (d). IR vmax 1705 cm^{-1}, ^{1}H-NMR ($CDCl_3$) inter alia δ0.13 (3H,s,Me_2Si), 0.93 (9H,s,^{t}BuSi), 0.99 (3H,s,18-CH_3), 1.18 (3H,s,19-CH_3), 4.75 (1H,m,6-H) 5.37 (1H,m,4-H), 6.09 (1H,m,16-H), 7.26 (1H,m,Py 5-H), 7.62 (1H,m,Py 4-H), 8.50 (1H,m,Py 6-H), 8.60 (1H,m,Py 2-H). MS m/z 475 (M+).

d) 17-(3-Pyridyl)androsta-4,16-diene-3,11-dione

To a solution of the product from step (c) in wet THF (60 ml) was added a solution of tetrabutylammonium fluoride (1.0M; 10 ml, 10 mmol) in THF, and the mixture stirred at room temperature for 12 h. The mixture was partitioned between diethyl ether and water basified with saturated aqueuous sodium bicarbonate, the ether phase isolated, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether, afforded the title compound (4.30 g, 60% overall yield from adrenosterone) which crystallised from diethyl ether, m.p. 181°–183° C.

IR vmax 1669, 1703 cm^{-1}; ^{1}H-NMR($CDCl_3$) inter alia δ1.02 (3H,s, 18-CH_3), 1.45 (3H,s,19-CH_3), 5.76 (1 H,s,Py 4-H), 6.08 (1H,m, 16-H) 7.24 (1H,m,Py 5-H), 7.59 (1 H,m,Py 4-H), 8.50 (1H,m,Py 6-H), 8.59 (1H,m,Py 2-H). MS m/z 361 (M+). Anal Calcd: C, 79.74; H,7.53: N,3.88. Found: C,79.58; H,7.57; N,3.89%.

EXAMPLE 11

3-Acetoxy-17-(3-pyridyl)androsta-3,5,16-triene

17-(3-pyridyl)androsta-4,16-dien-3-one (174 mg, 0.50 mmol) was dissolved in isopropenyl acetate (2 ml). p-Toluenesulfonic acid (130 mg, 0.68 mmol) was then added and the mixture heated at 80° C. for 12 h. After allowing to cool the mixture was poured into diethyl ether, washed with saturated aqueous sodium bicarbonate, dried (Na_2CO_3) and concentrated. Chromatography on elution with light petroleum-diethyl ether (1:1), afforded the title Compound (86 mg, 44%), IR vmax 1755 cm^{-1}, ^{1}H-NMR ($CDCl_3$) inter alia δ1.05 (6H,s,18-CH_3 and 19-CH_3), 2.15 (3H,s,$COCH_3$) 5.44 (1H,m,6-H), 5.72(1H,m,4-H), 6.00 (1H,m,16-H), 7.25 (1H, m,Py 5-H), 7.66 (1H,m,Py 4-H), 8.47 (1H,M,Py 6-H), 8.63 (1H,m,Py 2-H). MS m/z 389 (M+).

EXAMPLE 12

6β-Fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one and

EXAMPLE 13

6α-Fluoro-17-(3-pyridyl)androsta-4,16-dien-13-one

To a solution of 3-acetoxy-17-(3-pyridyl)androsta-3,5,16-triene (80 mg, 0.21 mmol) in dry dichloromethane (2 ml) was added N-fluoropyridinium trifluoromethanesulfonate (180 mg, 0.73 mmol) and the mixture heated under reflux for 12 h. The mixture was diluted with diethyl ether (30 ml), washed with dilute aqueous sodium hydroxide (0.5M; 2×5 ml), dried Na_2CO_3), and concentrated. ^{1}H and ^{19}F-NMR at this stage showed the 6-fluorinated products were formed as a 3:2 mixture of the β and α-epimers. Chromatography, on elution with light petroleum-diethyl ether (1:2), gave firstly:-i) the title 6β-epimer (13 mg), 17%) as white crystals, m.p. 167°–169° C. IR vmax 1684 cm^{-1}; ^{1}H-NMR($CDCl_3$) inter alia δ1.11 (3H,s,18-CH_3), 1.37 (3H,s,19-CH_3), 5.06 (1H,dd, $J_{H\text{-}H}$ 2.4 Hz, $J_{H\text{-}F}$ 49 Hz, 6α-H), 5.92 (1H,m,4-H), 6.01 (1H,m,16-H), 7.24 (1H,m,Py 5-H), 7.65 (1H,m,Py 4-H), 8.48 (1H,m,Py 6-H), 8.63 (1H,m,Py 2-H). ^{19}F-NMR δ-165.9 (dt, $J_{H\text{-}F}$ 49 Hz, 6β-F). MS m/z 365 (M+).

Further elution afforded:

ii) The title 6α-epimer (8 mg, 11%) as white crystals, m.p. 167°–169° C., IR vmax 1681 cm^{-1}; ^{1}H-NMR ($CDCl_3$) inter alia δ1.07 (3H,s,18-CH_3), 1.24 (3H,s,19-CH_3), 5.18 (1H, dm,$J_{H\text{-}F}$ 48 Hz, 6β-H), 5.98 (ZH,m,4-H and 16-H), 7.26 (1H,m,Py 5-H), 7.64 (1H,m,Py 4-H), 8.40 (1H, m,Py6-H), 8.63 (1H,m,Py 2-H). ^{19}F-NMR ($CDCl_3$) δ-183.9 (d,$J_{H\text{-}F}$ 48 Hz, 6α-F). MS m/z 365 (M+).

EXAMPLE 14

17-(3-pyridyl)androsta-4,16-dien-3-one (via Oppenauer Oxidation)

This Example illustrates a better method of preparing the compound already prepared in Example 4. The method followed that described in Example 9, but using 17-(3-pyridyl)androsta-5,16-dien-3β-ol (1.05 g, 3.0 mmol). Chromatography, on elution with toluene-methanol (20:1), afforded the title compound (0.85 g, 82%), which crystallised from diethyl ether, m.p. 148°–150° C. Spectroscopic data was identical to that given in Example 4(c). Anal. Calcd: C,82.95; H,8.41; N,4.03 Found: C,83.00; H, 8.50; N,3.99%

EXAMPLE 15

17-(3-pyridyl)androsta-4,16-dien-3-one oxime

To a suspension of 17-(3-pyridyl)androsta-4,16-dien-3-one (125 mg, 0.36 mmol) in ethanol (2 ml) was added hydroxylimine hydrochloride (50 mg, 0.72 mmol), followed by pyridine (0.2 ml), and the mixture heated under reflux for 1 h. then allowed to cool. The solvent was evaporated and the crystalline product triturated under water, collected on a sinter, washed with cold water, and dried in vacuo to give the title oxime as a 1:1 mixture of syn and anti geometric isomers. ^{1}H-NMR ($CDCl_3$) inter alia δ1.06 (3H,s,18-CH_3), 1.13 (3H,s,19-CH_3), 5.75 and 5.80 (1H,2m, isomeric 4-H), 6.01 (1H,m, 16-H), 7.26 (1H,m,Py 5H), 7.68 and 7.88 (1H, 2m, isomeric Py 4-H), 8.48 and 8.53 (1H, 2m, isomeric Py 6-H), 8.63 (1H,m,Py 2-H). MS m/z 362 (M+).

EXAMPLE 16

17-(3-pyridyl)androsta-4,16-diene-3,6-dione

To a solution of 17-(3-pyridyl)androsta-5,16-dien-3β-ol (350 mg, 1.0 mmol) in dry dichloromethane (10 ml) was added N-methylmorphine N-oxide (351 mg, 3.0 mmol) followed by 400 mg of freshly dried and powdered 4 Å molecular sieves and the mixture stirred for 10 min. Tetrapropylammonium perruthenate catalyst (35 mg), 0.1 mmol) was then added, the reaction flask placed in an ultrasonic bath, and the mixture irradiated whilst maintaining the temperature between 20°–30° C. for 2 h. The mixture was then filtered, diluted with diethyl ether, washed with water, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether-ether acetate (5:1), afforded the title compound (26 mg, 7%) as white crystals m.p. 210°–212° C. IR vmax 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) inter alia δ1.10 (3H,s,18-CH_3), 1.44 (3H,s,19-CH_3), 4.42 (1H,m, enolic 2-H), 5.84 (1H,s,4-H), 6.01 (1H,m,16-H), 7.24 (1H, m,Py 5-H), 7.65 (1H,m,Py 4-H), 8.45 (1H,m,Py 4-H), 8.45 (1H,m,Py 6-H), 8.60 (1H,m,Py 2-H). FAB-MS MS m/z 362 (M+1).

EXAMPLE 17

3α-(Trifluoromethyl)-17-(3-pyridyl)androst-16-en-3β-ol

To a solution of 17-(3-pyridyl)androst-16-en-3-one (100 mg, 0.29 mmol) in THF (2 ml) cooled to 0° C. was added trifluoromethyltrimethylsilane (200 μl, 1.3 mmol) followed

by tetrabutylammonium fluoride trihydrate (10 mg, 0.03 mmol). After 30 min., dilute aqueous hydrochloric acid (1M; 1 ml) was added and the mixture stirred at room temperature for 12 h. The mixture was then basified with saturated aqueous sodium bicarbonate and extracted with diethyl ether. The three extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with light petroleum-diethyl ether (1:1), afforded the title compound (87 mg, 73%) which crystallised from toluene, m.p. 192°–193° C. ^{1}H-NMR ($CDCl_3$) inter alia δ0.92 (3H,s,19-CH_3), 1.01 (3H,s,18-CH_3), 5.98 (1H,m,16-H), 7.22 (1H,m, Py 5-H), 7.64 (1H,m,Py 4-H),8.45 (1H,m,Py 6-H), 8.60 (1H,m,Py 2-H); ^{19}F-NMR ($CDCl_3$) δ-79.1 (s,3α-CF_3). MS m/z 419 (M+). Anal. Calcd: C,71.57; H,7.69; N,3.34; F,13.59 Found: C,71.67; H,7.71; N,3.25; F,13.30%.

EXAMPLE 18

(a) Diethyl[3-(5-methylpyridyl)]borane

3-Bromo-5-methylpyridine, which can be prepared as described in the literature, e.g. L. van der Does and H. J. van Hertog, Rec. Trav. Chem. Pays Bas 84, 957–960 (1985) or R. A. Abramovitch and M. Saha, Can. J. Chem. 44, 1765–1771 (1966), is reacted with n-butyllithium, according to the method of M. Terashima et al., Chem. Pharm. Bull. 31, 4573–4577, (1983). The product is treated with triethylborane and then iodine.

(b) 17-[3-(5-Methylpyridyl)]androsta-5,16-dien-3β-ol

Diethyl [3-(5-methylpyridyl)]borane is reacted with 3β-acetoxyandrosta-5,16-dien-17-yl trifluoromethane sulphonate analogously to Example 1(b) and the resulting 3β-acetate is hydrolysed with sodium hydroxide, analogously to Example 2, to yield the title compound.

The following Examples illustrate preparation of compounds of the invention by the vinyl halide route. In Example 19, the 3β-hydroxy product is produced without chromatography, by embodiment (a). In Example 20, the 3β-hydroxy product is not isolated, but in step (d) an impurity has been identified as a 16,17'-bis(steroidal) by-product. This can be removed by reverse phase chromatography, but now that the by-product has been identified, those skilled in the art will be able more easily to identify procedures which will remove it, without the need for chromatography. Further, it is believed that with the higher organoboron:steroid ratios suggested above, the side-reaction leading to this impurity will be reduced.

EXAMPLE 19

(a) Dehydroepiandrosterone-17-hydrazone

To a stirred solution of dehydroepiandrosterone (28.8 g, 0.1 mol) in ethanol (500 ml) was added hydrazine hydrate (19.5 ml, 0.4 mol), followed by a solution of hydrazine sulfate (65 mg, 0.5 mmol) in water (2 ml). After stirring for 3 days the mixture was poured into water (3 liters) to precipitate the product as a white crystalline solid. The product was collected by filtration on a sinter, washed with cold water (2×50 ml), then with Et_2O (50 ml). The product was then dried in vacuo, firstly over silica gel, and finally over P_2O_5, to give the title compound as a white cyrstalline solid (29.6 g, 98%).

Notes

1) The method of Schweder et al., p.202, compound No. 2 therein (using triethylamine) gave a very fine crystalline product which was difficult to filter.

2) The method of Schweder et al. p. 203, compound No. 4 therein (using sodium acetate buffer) gave a slightly lower yield (96%) in trial experiments, whereas the modified procedure used above gave a product amenable for filtration, and in excellent yield (98%).

(b) 17-Iodo-androsta-5,16-dien-3β-ol

To a solution of iodine (53.3 g, 0.21 mol) in THF (2 L), cooled by an ice/water bath to 0° C., was added 1,1,3,3-tetramethylguanidine (63 ml, 57.6 g, 0.50 mol).

A solution of dehydroepiandrosterone-17-hydrazone (30.25 g, 0.10 mol) in THF (750 ml) was then added slowly to the above iodine solution via a transfer needle over about 2 h, whilst maintaining the reaction temperature at 0° C. After all the hydrazone solution was added, the mixture was filtered, and the filtrate concentrated. The remaining oil was then heated on an oil bath for 4 h, allowed to cool, and dissolved in Et20. The Et_2O solution was washed with 1M HCl until the aqueous phase was acidic, washed with 0.5M NaOH, then 1M $Na_2S_2O_3$, and finally with water. The Et_2O phase was separated, dried ($MgSO_4$), and concentrated to give the crude product. Recrystallisation from Et_2O/hexane (3:2) afforded the title compound as off-white crystals (35.8 g, 90%).

(c) 17-(3-Pyridyl)androsta-5,16-dien-3β-ol

Diethyl(3-pyridyl)borane (3.23 g, 22 mmol) from Aldrich Chemical Co. Ltd. was added to a stirred solution of 17-iodo-androsta-5,16-dien-3β-ol (7.96 g, 20 mmol) in THF (120 ml) containing bis(triphenylphosphine)palladium (II) chloride (140 mg, 0.2 mmol). An aqueous solution of sodium carbonate (2M, 50 ml) was then added and the mixture heated, with stirring, by an oil bath at 80° C. for 48 h, and allowed to cool.

The mixture was partitioned between Et_2O and water the organic phase was separated, dried (Na_2CO_3) and twice concentrated from Et_2O by evaporation to remove THF (with Et_2O). The residual solid was then washed with Et_2O (100 ml), the Et_2O solution decanted off, and the remaining white solid recrystallised from toluene (3.94 g, 56%).

Notes

1) The time required for completion needs to be made longer than when using the vinyl triflate (48 h vs 1 h) since it has been found that the vinyl iodide reacts much more slowly.

2) It has been found that a smaller excess of borane than described in the earlier applications (for the vinyl triflate) aids in isolation of product.

3) The work-up procedure enables the product to be isolated without chromatography, thereby enabling scaling up.

(d) 3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene

To a stirred suspension of finely powdered 17-(3-pyridyl)androsta-5,16-dien-3β-ol (3.50 g, 10 mmol) in dry diethyl ether (150 ml) containing triethylamine (2.3 ml, 16 mmol) and dimethylaminopyridine (0.012 g, 0.1 mmol) was added acetyl chloride (1.0 ml, 14 mmol). The mixture was then stirred at ambient temperature for 12 h, over which time a thick white precipitate of triethylammonium chloride had formed. The mixture was then filtered and the filtrate concentrated to afford the crude product which was recrystallised firstly from ethanol/water (1:1), then finally from hexane to afford the title compound (3.30 g, 84%).

EXAMPLE 20

(a) Dehydroepiandrosterone-17-hydrazone

Into a 10 L round-bottomed flask, fitted with a magnetic stirrer bar, was placed dehydroepiandrosterone (288 g, 1.0 mol) and ethanol (5.0 L). To the resultant stirred solution was added hydrazine hydrate (195 ml, 4.0 mol), followed by a solution of hydrazine sulfate (0.65 g, 0.005 mol) in water

(20 ml) [note: the hydrazine sulfate dissolved in this volume of water at about 40° C.]. After stirring at room temperature for 5 days, water (4.5 L) was added, the mixture poured into water (10 L), and the white crystalline precipitate allowed to settle. The product was collected by filtration on a sinter, washed with cold water (2×500 ml), then with Et_2O (2×500 ml). The product was then dried in vacuo, firstly over silica gel, and finally over P_2O_5, to give the title compound as a whim crystalline solid, mp 204°–206° C. (284.8 g, 94%).

(b) 17-Iodo-androsta-5,16dien-3β-ol

A 10 L round-bottomed flask, fitted with a magnetic stirrer bar, was charged with iodine (156.1 g, 0.615 mol), THF (4.0 L; GPR grade), and Et_2O (2.0 L; BDH specially dried grade). The resultant stirred solution was cooled by an ice/water bath to 0° C. and 1,1,3,3-tetramethylguanidine (188 ml, 173 g, 1.50 mol) was added. A solution of dehydroepiandrosterone-17-hydrazone from step (a) (90.74 g, 0.30 mol) in THF (2.25 L) was then added slowly to the above iodine solution via a canula over about 2 h, whilst maintaining the reaction temperature at 0° C. [note: N_2 is evolved as the hydrazone is added to the iodine solution]. After all the hydrazone solution was added, the mixture was stirred for an additional hour and the precipitate allowed to settle [note: a precipitate of tetramethylguanidium iodide forms during the reaction]. The mixture was then filtered, and the filtrate concentrated to an oil on a rotary evaporator.

This reaction was carried out a total of three times, thus using in total 272.22 g (0.90 mol) of dehydroepiandrosterone-17-hydrazone from step (a). The concentrated residues from the three separate reactions were combined and heated on an oil bath for 4 h, then allowed to cool [note: this converts any 17,17-diiodo by-product into the 17-vinyl iodide product]. This oil was then dissolved in Et_2O (5 L), filtered, and further diluted with additional Et_2O (4 L).

The Et_2O solution was washed with aqueous HCl (1M; 3×500 ml) until the aqueous phase was acidic [note: the ether solution changes colour from brown to yellow when the aqueous phase remains acidified] then finally with water (500 ml). The Et_2O phase was separated, dried ($MgSO_4$), and concentrated to a volume of 3 L, then left to allow the product to crystallise. The yellow crystals were collected by filtration on a sinter, washed with hexane (3×500 ml) and dried under vacuum (335.4 g, 94%). Recrystallisation from ethanol-water (5:1) afforded the product as white crystals (297.3 g, 83%) mp 175°–176° C., lit. mp 173°–174 ° C.

(c) 17-(3-Pyridyl)androsta-5,16-dien-3β-ol

In a 2 L round-bottomed flask, fitted with a magnetic stirrer bar, was placed the steroidal 17-iodo product from step (b) (98.0 g, 0.246 mol) and this was dissolved in THF (1.1 L). The flask was purged with argon and bis(triphenylphosphine)palladium (II) chloride catalyst (1.73 g, 0.0025 mol) was added, followed by diethyl(3-pyridyl)borane (43.35 g, 0.295 mol). To the resultant orange THF solution was added an aqueous solution of sodium carbonate (2M; 500 ml). The flask was fitted with a reflux condenser, and the apparatus purged again with argon. The mixture was then heated under reflux (at about 80° C.) with stirring on a stirrer/heating mantle (Electrothermal MA) for 4 days [note: upon completion of the reaction the organic phase darkens in colour from orange to dark orange/brown], then allowed to cool. This reaction was carried out a total of three times, thus using a total of 294.0 g (0.74 mol) of the steroidal 17-iodo product from step (b).

The reaction mixtures were combined and Et_2O (5 L) added. The organic phase was separated, washed with water (2 L), and left to give a first crop of crystals which were collected by filtration on a sinter. The filtrate was concentrated and the residue redissolved in Et_2O to afford a second crop of crystals. The aqueous phase and washings from the above work-up were extracted with hot toluene (2 L) on a steam bath and concentration of the toluene extracts afforded further product. The combined crude product from the above procedures was then dissolved in the minimum volume of hot methanol, filtered through a plug of "Celite" (Registered Trade Mark) and an equal volume of acetonitrile added to the methanol solution. The acetonitrile/methanol solution was then concentrated to half its original volume on a rotary evaporator and the solution left to crystallise. The resultant white crystals were collected by filtration on a sinter, washed with acetonitrile and dried in vacuo to constant weight (191.1 g, 74%), mp 202°–212° C. A second recrystallisation from toluene-methanol (50:1) afforded the product as white crystals (146.8 g, 57%) mp 214°–218° C., lit. mp 228°–229° C.

(d) 3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene

The following reaction was carried out in a 500 ml round-bottomed flask, fitted with a magnetic stirrer bar. To a suspension of the steroidal product from step (c) (26.5 g, 0.104 mol) in dry pyridine (200 ml), was added acetic anydride (75 ml) and the mixture stirred at room temperature for 24 h. The pyridine and excess acetic anydride were removed on a rotary evaporator, initially with the water bath at 70° C., and finally at 800° C. for 30 min. The resulting oil was dissolved in Et_2O (500 ml), washed with saturated aqueous $NaHCO_3$ (2×200 ml), dried (Na_2CO_3), and concentrated to an oil which crystallised on standing. ^{1}H-NMR spectroscopy at this stage showed the product contained about 5% of a 16,17'-bi(steroidal) contaminant, 3β-acetoxy-16-(3'-β-acetoxyandrosta-5',16'-dien-17'-yl)-17-(3-pyridyl)androsta-5,16-diene, which originated as a by-product from the coupling reaction of step (c).

The product was therefore further purified by preparative flash chromatography using a 9 cm diameter column, with silica stationary phase (Merck 15111), eluting with dicholoromethane. The by-product eluted first followed by the desired product, although the separation was incomplete. Fractions containing a significant amount of by-product were combined and subjected to further chromatographic purification.

The foregoing reaction and purification procedure was carried out a total of four times, thus using a total of 146 g (0.418 mol) of the steroidal product from step (c).

The product-containing dichloromethane fractions from the chromatographic purification were concentrated and recrystallised from hexane to afford white crystals which were dried in vacuo to constant weight. The total amount of product obtained was 136.0 g (83%).

The dichloromethane fractions containing the least by-product were combined, and following recrystallisation from hexane, afforded the title compound as white crystals with mp 142°–144° C. Analysis showed this material ("A") contained 6.8% w/w of the bis(steroidal) by-product.

A second crop of white crystals ("B") of the product, containing 21.8% w/w of bis(steroidal) by-product (25 g), was obtained.

The two products were purified using reverse phase chromatography. The column was packed with "LiChroprep" (Registered Trade Mark) RP-8 reverse-phase C_8 packing, Art. No. 9324, supplied by E. Merck, Darmstadt, Germany. The course of the chromatography was followed by UV detection at 253 nm, with purity checks by HPLC.

Product "A" (10.17 g) was dissolved in 200 ml. hot acetonitrile and 40 ml. hot methanol, and, after being allowed to cool, the filtrate was applied to a 10 cm. diameter

column containing about 500 g. of the packing. The eluant was 5% 0.05M aqueous ammonium acetate/95% v/v acetonitrile. 7.51 g. of product was recovered in fractions 4–10. Fractions (500 ml) 4–11 contained the product with some impurities, but not the bis-steroidal byproduct. The eluant was changed to 2.5% acetic acid/95.5% v/v acetonitrile and then to 5% acetic acid/95% v/v acetonitrile. A pink colour seen in fractions 16 and 17 evidenced the bis-steroidal by-product. Fraction 18 was colourless. The column can be washed with 100% acetonitrile, for re-use.

Product "B" (1 g) was separated by a similar method except that the product was dissolved initially in 100% acetonitrile and the filtrate applied to a 2 cm. column packed with 100 g. of the solid phase. Excellent separation of the product was achieved with the aqueous ammonium acetate/acetonitrile eluant.

Although, in this Example, the reverse phase column was used in addition to a conventional column, it is clear that the conventional column achieved little separation of the bis-steroidal by-product and it is intended to omit the conventional column in future preparations.

TEST RESULTS

(a) Preparation of testicular material

Human testes were obtained from previously untreated patients undergoing orchidectomy for prostatic cancer. The testes were decapsulated and stored in liquid nitrogen until use. A microsomal preparation was prepared essentially as described by S. E. Barrie et al., J. Steroid Biochem. 6, 1191–5, (1989). The material was then thawed, finely chopped, and homogenised in 0.25M sucrose (5 ml/g wet weight) using a Potter homogeniser. The homogenate was centrifuged at 12000 g for 30 min, and then the microsomes were pelleted by spinning the supernatant at 100,000 g for 1 hr. The pellet was washed by being resuspended in 0.25M sucrose and repelleted. The microsomal pellet was then resuspended in 50 mM sodium phosphate pH 7.4/glycerol (3/1 v/v) and stored in aliquots in liquid nitrogen.

(b) Determination of 17α-hydroxylase

The basic assay mixture was EDTA (0.2 mM), dithiothreitol (DTT; 0.1 mM), NADPH (0.25 mM), glucose 6-phosphate dehydrogenase (G6PDH; 6.25 μg/ml), $MgCl_2$ (1 mM), glucose 6-phosphate (G6P; 10 mM) and the substrate, 3H-progesterone (3 μM) in sodium phosphate (50 mm), pH 7.4. The compounds under test were dissolved in 50% DMSO and the final concentrations of ethanol and DMSO were 1% each. The assay reaction was carded out for 1 hour and was terminated by the addition of 2 vols. of methanol-acetonitrile (2:1) containing approx. 100 μM unlabelled progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone, and 16α-hydroxyprogesterone. The last-mentioned steroid was added as it appeared that the human enzyme was capable of 16α-hydroxylation as well as 17α-hydroxylation.

The separation of the steroids by HPLC was carried out using an "Uptight" guard column packed with 40–63 μm Nucleosil C18 and a 10 cm main column packed with 5 μm Nucleosil C18 and 60% methanol as eluant. The radioactivity in the peaks of interest was monitored on-line by mixing the HPLC effluent 1:1 with Ecoscint A (National Diagnostics) scintillation fluid, containing 25% acetonitrile, and passing the mixture through a Berthold LB506C radiochemical monitor. The hydroxylase activity was measured as the production of 17α-hydroxyprogesterone, androstenedione and testosterone.

(c) Determination of C_{17}–C_{20} lyase

The mixture was the same as described above for the 17α-hydroxylase except that the substrate was ^{3}H-17α-hydroxy- progesterone. The reaction was carried out for 1–2 h. and was stopped by the addition of 2 vols. of methanol/acetonitrile (2/1 containing approx. 100 μM 17α-hydroxyprogesterone, androstenedione and testosterone.

The HPLC separation used for the lyase involved a mini-re-column "Uptight Guard Column" packed with PELL-ODS (pellicular octadecyl silica) and a 10 cm. main column "Apex C18" column packed with 5μ APEX-CAT silica.

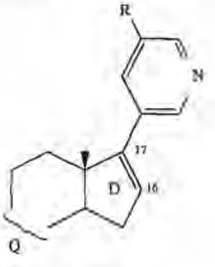
The eluant was 38:12:50 methanol:acetonitrile:water flowing at 1 ml/min. The effluent was mixed 1:1 with Ecoscint A containing 5% methanol and 5% acetonitrile and the radioactivity was measured directly by a Berthold LB506C radiochemical detector. The lyase activity was measured as the production of androstenedione and testosterone.

(d) Calculation of IC_{50}

The enzyme activity was measured in the presence of at least 4 concentrations of each compound. The data were for the 4-pyridyl and 2-picolyl compounds of Table 1 fitted by linear regression to the Dixon equation (M. Dixon, E.C. Webb, Enzymes, 2nd ed., Academic Press, New York, 1964). Data for all the other compounds were fitted by non-linear regression to the median effect equation of Chou, J. Theoret. Biol. 39, 253–276 (1976). The correlation coefficients were greater than 0.95 except for the compound of Example 1, where it was 0.91. All the assays were carried out with approx. 4 nM enzyme (as calculated from kinetic measurements) except those for Ketoconazole and the 2- and 4-pyridyl and 2-picolyl compounds of Table 1, in which 25 nM lyase and 10 nM hydroxylase were used. The IC_{50} values are dependent on enzyme concentration when the inhibitor binds tightly (all the compounds tested except the 4-pyridyl and 2-picolyl). Results are shown in Table 2 below.

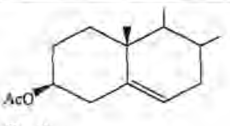
TABLE 2

(a) Confirmation that variations in the A and B rings of compounds of the invention have little effect on inhibition of hydroxylase and lyase.



(3)

Compounds tested are of formula (3) wherein R = H:

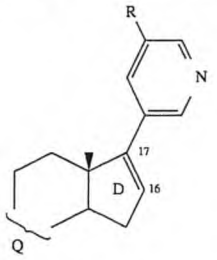
Q	IC_{50} (μM) Lyase	Hydroxylase
	0.0097	0.0130

(Ex. 1)

TABLE 2-continued

(a) Confirmation that variations in the A and B rings of compounds of the invention have little effect on inhibition of hydroxylase and lyase.

(3)



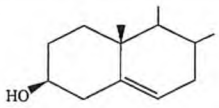
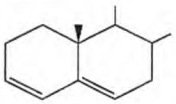
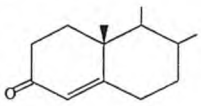
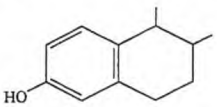
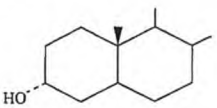
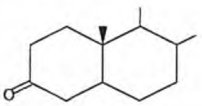
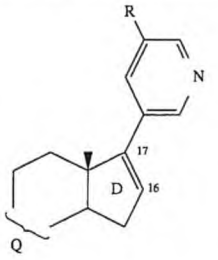
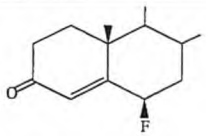
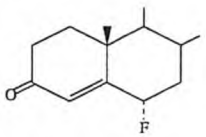
Compounds tested are of formula (3) wherein R = H: Q	IC_{50} (μM) Lyase	Hydroxylase
 (Ex. 2)	0.0029	0.0040
 (Ex. 3)	0.0056	0.0125
 (Ex. 4)	0.0021	0.0028
 (Ex. 6)	0.0018	0.0026
 (Ex. 8)	0.0025	0.0043
 (Ex. 9)	0.0030	0.0047

TABLE 2-continued

(a) Confirmation that variations in the A and B rings of compounds of the invention have little effect on inhibition of hydroxylase and lyase.

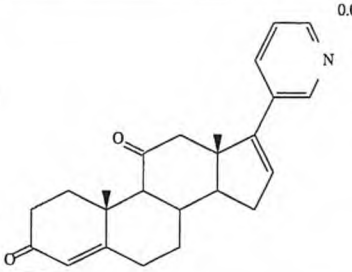
(3)



Compounds tested are of formula (3) wherein R = H: Q	IC_{50} (μM) Lyase	Hydroxylase
 (Ex. 12)	0.0022	0.0033
 (Ex. 13)	0.0032	0.0053

(b) Confirmation that variation in the C ring of compounds of the invention has little effect on the inhibition of hydroxylase and lyase.

Compound Tested

	IC_{50} (μM) Lyase	Hydroxylase
 (Ex. 10)	0.0025	0.0091

The comparative IC_{50} figures for Ketoconazole are 0.026 against lyase and 0.065 against hydroxylase.

Assay of aromatase activity

Aromatase activity was determined by the method of A. B. Foster et al., J. Med. Chem. 26, 50–54 (1983), using human placental microsomes. For the microsomes used, the Michaelis constant K_m for [1β-^{3}H] androstenedione was 0.039 μM.

The compounds having a pregnenolone-like skeleton in the A and B rings, i.e. 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene and its 3-alcohol of Examples 1 and 2, had IC_{50}>20 μM. The compound having a progesterone-like skeleton in the A and B rings, i.e. 17-(3-pyridyl)-androsta-4,16-dien-3-one of Example 4 exhibited also aromatase inhibitory activity with IC_{50}=1 μM.

In vivo organ weight and endocrine test in mice

Male HWT mice, 12 weeks old, were treated daily for 2 weeks, with 5 animals per treatment group. The test compounds were the compound of Examples 1 and 4 (as representative of compounds of the invention having the pregenolone-like and progesterone-like skeletons respectively). Ketoconazole was also tested at three different doses. The test compounds were made up in 5% benzyl alcohol, 95% safflower oil, and were given i.p. In addition to an untreated control group of animals, there was also a solvent control group which received the same volume of liquid as the test group (5 ml/kg) but no test compound. All animals were sacrificed 24 hours after the last injection. Blood was collected by cardiac puncture into heparinized tubes, and the plasma used for RIA (radio immunoassay) of testosterone and luteinising hormone. The following organs were removed and weighed: adrenals, prostate, seminal vesicles, testes, kidneys. There was no significant body weight loss in any group of mice during the experiments.

Post mortem examination of the mice revealed oil/white deposits i.p. in those treated with compound of Ex. 1 and white deposits throughout the abdomen in those treated with compound of Ex. 4. In all these mice, all organs looked normal. In Ketoconazole-treated animals, adhesions were found in 2/5,2/5,4/5 of the low/middle/top dose groups. The gut and peritoneal wall seemed to be stuck to the seminal vesicles. The livers were brown in the middle/top dose groups.

The weights of organs found in the animals post mortem are shown in Table 3 below. The reductions in weight of all of the prostate, seminal vesicles, testes and kidneys were much greater for the test compounds of the invention than for Ketoconazole. Ketoconazole caused an increase in adrenal weight at the two highest doses, whereas the compounds of the invention had no significant effect, suggesting that they did not inhibit corticosterone biosynthesis.

TABLE 3

Dose	Mean weight (mg.) ± standard error: Adrenals	Prostate	Seminal Vesicles	Testes	Kidneys
		Compound of Ex 1.			
Controls	4.5 ± 0.1	10.1 ± 0.7	189 ± 9	146 ± 3	709 ± 17
Solvent controls	4.5 ± 0.4	10.2 ± 1.3	171 ± 6	122 ± 7	615 ± 28
0.02 mmol/ /kg/day	4.3 ± 0.2	8.0 ± 0.6	136 ± 4	134 ± 4	604 ± 24
0.1 mmol /kg/day	4.0 ± 0.2	5.3 ± 0.3	51 ± 6	95 ± 3	500 ± 8
0.5 mmol /kg/day	4.7 ± 0.2	5.6 ± 0.6	25 ± 2	56 ± 2	449 ± 12

TABLE 3-continued

Dose	Mean weight (mg.) ± standard error: Adrenals	Prostate	Seminal Vesicles	Testes	Kidneys
		Compound of Ex. 4			
Controls	4.3 ± 0.4	8.4 ± 0.2	165 ± 18	142 ± 8	652 ± 45
Solvent controls	4.4 ± 0.0	9.2 ± 0.9	152 ± 9	122 ± 8	589 ± 24
0.02 mmol/ /kg/day	4.7 ± 0.2	5.9 ± 0.8	108 ± 4	117 ± 9	599 ± 29
0.1 mmol /kg/day	4.6 ± 0.4	6.4 ± 0.5	61 ± 9	105 ± 5	549 ± 28
0.5 mmol /kg/day	4.9 ± 0.1	4.1 ± 0.5	25 ± 1	59 ± 2	468 ± 15
		Ketoconazole			
Controls Solvent controls	4.2 ± 0.2	8.9 ± 0.8	193 ± 8	145 ± 4	670 ± 12
0.01 mmol/ /kg/day	4.7 ± 0.4	9.3 ± 1.2	198 ± 18	146 ± 3	615 ± 25
0.225 mmol /kg/day	4.8 ± 0.2	9.1 ± 0.8	235 ± 18	141 ± 5	637 ± 22
0.5 mmol /kg/day	6.1 ± 0.3	10.8 ± 1.4	171 ± 5	127 ± 7	574 ± 23
	6.9 ± 0.3	9.3 ± 0.9	179 ± 20	133 ± 6	710 ± 30

The results indicate the inhibition by the components of the invention of androgen and particularly testosterone synthesis. They are confirmed by endocrinological results shown in Table 4.

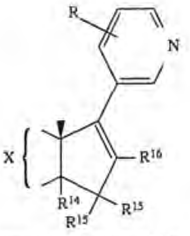
Although the solvent itself produced marked depression of testosterone levels, probably due to stress on the animals, the further decrease resulting from the administration of test compounds was much more marked for the compounds of the invention than for ketoconazole. The rise in LH levels is ascribed to a feedback mechanism associated with depletion of testosterone.

TABLE 4

	Endocrinological Results (Mean ± standard error): Testosterone nM	LH ng/ml
	Compound of Ex. 1	
Controls	9.8 ± 5.6	0.63 ± 0.16
Solvent Controls	2.5 ± 1.2	0.80 ± 0.09
0.02 Mmol/Kg/Day	2.7 ± 0.5	3.4 ± 0.5
0.1 Mmol/Kg/Day	0.2 ± 0.1	2.55 ± 0.45
0.5 Mmol/Kg/Day	0.1 ± 0.0	2.25 ± 0.67
	Compound of Ex. 4	
Control	27.8 ± 11.4	Not determined
Solvent Control	11.0 ± 5.6	Not determined
0.02 Mmol/Kg/Day	4.5 ± 0.3	Not determined
0.1 Mmol/Kg/Day	3.5 ± 1.0	Not determined
0.5 Mmol/Kg/Day	0.4 ± 0.1	Not determined
	Ketoconazole	
Controls	17.3 ± 7.1	0.66 ± 0.05
Solvent Controls	1.3 ± 0.4	0.25 ± 0.13
0.1 Mmol/Kg/Day	0.9 ± 0.2	0.39 ± 0.14
0.225 Mmol/Kg/Day	0.7 ± 0.1	0.75 ± 0.02
0.5 Mmol/Kg/Day	0.4 ± 0.1	0.76 ± 0.03

We claim:

1. A compound of the formula (I)



(I)

wherein X represents the residue of the A, B and C rings of a steroid selected from the group consisting of
androstan-3α- or 3β-ol,
androst-5-en-3α- or 3β-ol,
androst-4-en-3-one,
androst-2-ene,
androst-4-ene,
androst-5-ene,
androsta-5,7-dien-3α or 3β-ol,
androsta-1,4-dien-3-one,
estra-1,3,5[10]-trien-3-ol,
5α-androstan-3-one,
androst-4-ene-3,11-dione,
6-fluoroandrost-4-ene-3-one,
androstan-4-ene-3,6-dione,
each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

to form 3-esters

to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions

as 3-oximes

as 3-methylenes

as 3-carboxylates

as 3-nitriles

as 3-nitros

as 3-desoxy derivatives

to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B, or C-ring

to be 19-nor;

R represents a hydrogen atom or an alkyl group of 1–4 carbon atoms;

androsta-3,5-diene,

androsta-3,5-diene-3-ol,

estra-1,3,5[10]-triene and

estra-1,3,5[10]-trien-3-ol,

5α-androstan-3-one:

androst-4-ene-3,11-dione,

6-fluoroandrost-4-ene-3-one,

androstan-4-ene-3,6-dione,

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

to form 3-esters

to have one or more carbon or carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions

as 3-oximes

as 3-methylenes

as 3-carboxylates

as 3-nitriles

as 3-nitros

as 3-desoxy derivatives

to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B, or C-ring

to be 19-nor;

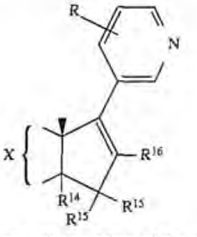
R represents a hydrogen atom or an alkyl group of 1–4 carbon atoms;

R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms;

each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1–4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms; and

R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts, but excluding 3β-acetoxy-17-(3-pyridyl)androsta-5,14,16-triene, 3β,15α- and 3β,15β-diacetoxy-17-(3-pyridyl)androsta-5,16-diene and 3β-methoxy-17-(3-pyridyl-5α-androst-16-ene.

2. A method of treating an androgen-dependent or estrogen-dependent disorder which comprises administering to a patient in a therapeutically effective dose a compound of the formula (1):



(I)

wherein X represents the residue of the A, B and C rings of asteroid selected from the group consisting of
androstan-3α- or 3β-ol,
androst-5-en-3α- or 3β-ol,
androst-4-en-3-one,
androst-2-ene,
androst-4-ene,
androst-5-ene,
androsta-5,7-dien-3α or 3β-ol,
androsta-1,4-dien-3-one,
androsta-3,5-diene,
androsta-3,5-dien-3-ol,
estra-1,3,5[10]-triene and

R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms;

each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1–4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms; and

R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts.

3. A compound according to claim **1**, which is saturated and unsubstituted at the 11- and 12-positions.

4.
17-(3-Pyridyl)androsta-5,16-dien-3β-ol,
17-(3-pyridyl)androsta-3,5,16-triene,
17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
17-(3-pyridyl)-5α-androst-16-en-3α-ol
and their acid addition salts and 3-esters.

5. A compound according to claim **1** wherein R represents a hydrogen atom.

6.
17-(3-Pyridyl)-5α-androst-16-en-3-one,
17-(3-pyridyl)-androsta-4,16-diene-3,11-dione,
17-(3-pyridyl)-androsta-3,5,16-trien-3-ol,
6α- and 6β-fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)androsta-4,16-dien-3,6-dione,
3α-trifluoromethyl-17-(3-pyridyl)androst-16-en-3β-ol
and their acid addition salts and 3-esters.

7. 3β-Alkanoyloxy-17-(3-pyridyl)androsta-5,16-dienes in which the alkanoyloxy group has from 2 to 4 carbon atoms.

8. 3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene.

9. A pharmaceutical composition comprising a compound of claim **1** in association with a pharmaceutically acceptable carrier or diluent.

10. A pharmaceutical composition comprising a compound of claim **3** in association with a pharmaceutically acceptable carrier or diluent.

11. A pharmaceutical composition comprising a compound of claim **1** wherein R represent a hydrogen atom in association with a pharmaceutically acceptable carrier or diluent.

12. A pharmaceutical composition comprising a compound of claim **4** in association with a pharmaceutically acceptable carrier or diluent.

13. A pharmaceutical composition comprising a compound of claim **6** in association with a pharmaceutically acceptable carrier or diluent.

14. A pharmaceutical composition comprising a compound of claim **7** in association with a pharmaceutically acceptable carrier or diluent.

15. A pharmaceutical composition comprising a compound of claim **8** in association with a pharmaceutically acceptable carrier or diluent.

16. A method according to claim **2** wherein the patient has prostatic cancer.

17. A method according to claim **2** wherein the patient has breast cancer.

18. A method according to claim **2** wherein the compound defined in claim **2** is saturated and unsubstituted at the 11- and 12-positions.

19. A method according to claim **2** wherein the compound is selected from the group consisting of:
17-(3-pyridyl)androsta-5,16-dien-3β-ol,
17-(3-pyridyl)androsta-3,5,16-triene,
17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
17-(3-pyridyl)-5α-androst-16-en-3α-ol
and their acid addition salts and 3-esters.

20. A method according to claim **2** wherein the compound is a 3β-alkanoyloxy-17-(3-pyridyl)androsta-5,16-diene wherein the alkanoyloxy group has 2 to 4 carbon atoms.

21. A method according to claim **2** wherein the compound is 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene.

22. An orally ingestible solid composition or a sterile injectable liquid composition comprising respectively a solid or liquid pharmaceutically acceptable carrier or diluent and a compound as defined by general formula (1) of claim **2**.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,604,213
DATED : February 18, 1997
INVENTOR(S) : Barrie, et. al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 27, lines 22-46, delete "estra-1,3,5[10]-trien-3-ol ... R represents a hydrogen atom or an alkyl group of 1-4 carbon atoms;"

Column 28, line 52, insert --,-- after "estra-1,3,5[10]-triene"

Column 28, line 52, insert the following after "estra-1,3,5[10]-triene,"

--estra-1,3,5[10]-trien-3-ol,

5α-androstan-3-one,

androst-4-ene-3,11-dione,

6-fluoroandrost-4-ene-3-one,

androstan-4-ene-3,6-dione,

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

to form 3-esters

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It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions

as 3-oximes

as 3-methylenes

as 3-carboxylates

as 3-nitriles

as 3-nitros

as 3-desoxy derivatives

to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring

to be 19-nor;

R represents a hydrogen atom or an alkyl group of 1-4 carbon atoms;

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office

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LOW DOSE KETOCONAZOLE WITH REPLACEMENT DOSES OF HYDROCORTISONE IN PATIENTS WITH PROGRESSIVE ANDROGEN INDEPENDENT PROSTATE CANCER

KATHERINE A. HARRIS, VIVIAN WEINBERG, ROBERT A. BOK, MIKA KAKEFUDA AND ERIC J. SMALL

From the University of California, San Francisco, UCSF Comprehensive Cancer Center, San Francisco, California

ABSTRACT

Purpose: High-dose (400 mg.) oral ketoconazole 3 times daily with replacement doses of hydrocortisone has become a standard treatment option for patients with advanced prostate cancer which progresses after androgen deprivation. However, toxicity can hinder the ability to deliver treatment and the cost of the regimen can be substantial. Therefore, a prospective phase II study was conducted to assess the efficacy and safety of a regimen of low dose (200 mg.) oral ketoconazole 3 times daily with replacement doses of hydrocortisone in men with androgen independent prostate cancer.

Materials and Methods: The study included 28 patients with progressive prostate cancer despite anorchid levels of testosterone and ongoing testicular androgen suppression. Treatment consisted of low dose ketoconazole and replacement doses of oral hydrocortisone (20 mg. every morning and 10 mg. at bedtime). At the time of disease progression patients were treated with high dose ketoconazole and continued on the same dose of hydrocortisone. Adrenal androgen levels were measured, and baseline and followup levels correlated with clinical outcome.

Results: Overall, 13 (46%) of 28 patients had a prostate specific antigen decrease of more than 50% (95% confidence interval 27.5% to 66.1%). Median duration of prostate specific antigen decrease for all responders was 30+ weeks and 5 patients continue to exhibit a response, ranging from 36+ to 53+ weeks. In general, therapy was well tolerated. There were no grade 4 toxicities. Grade 3 toxicities included hepatotoxicity in 1 patient and depression in 2. The most common toxicities were nausea (29% grades 1 and 2), dry skin (18% grade 1) and fatigue (14% grade 1). Four (14%) patients discontinued low dose ketoconazole due to toxicities. Of the 16 patients who received high dose ketoconazole after disease progression with low dose ketoconazole, 3 were removed from treatment due to toxicity and no patient responded to high dose ketoconazole. There was no difference in the distribution of pretreatment endocrine values between responders and nonresponders, and the magnitude of change in adrenal androgen levels was not associated with response to therapy, although a potential association could easily have been missed due to small sample size.

Conclusions: The regimen of low dose ketoconazole with replacement doses of hydrocortisone is well tolerated and has moderate activity in patients with progressive androgen independent prostate cancer.

KEY WORDS: prostatic neoplasms, ketoconazole, hydrocortisone

It has been reported that in some patients with progressive androgen independent prostate cancer some cells retain some degree of hormonal sensitivity and can be stimulated by adrenal androgens.[1] In this context it is of note that ketoconazole, a systemic antifungal agent, is a clinically useful antagonist of adrenal steroidogenesis. Ketoconazole inhibits cytochrome P-450 and effectively suppresses testicular and adrenal androgen production.[2,3] Interestingly, it may also have direct cytotoxic effects on prostate tumor cells.[4] Originally used as a therapy for advanced prostate cancer before the advent of prostate specific antigen (PSA) as a tumor marker, high dose (400 mg.) oral ketoconazole 3 times daily was reported to produce objective clinical responses in approximately 10% of patients with androgen independent prostate cancer and stable disease in another 35%.[5,6] More recently, we have reported that ketoconazole has moderate activity in patients with androgen independent prostate cancer with durable PSA decrease of greater than 50% observed in 63% of patients who had previously undergone antiandrogen withdrawal[7] and in 55% of those undergoing simultaneous antiandrogen withdrawal.[8] While toxicity is generally mild to moderate, up to 20% of patients experience grades 1 and 2 nausea and emesis,[7,8] requiring dose reduction or cessation of the drug. In addition, 20% of patients will have minor skin toxicity, 10% grade 1 or 2 fatigue and 10% reversible grade 1 or 2 hepatotoxicity.[7,8] Patients with unacceptable toxicity are frequently treated empirically with a dose reduction of oral ketoconazole by half to 200 mg. 3 times daily.

High dose ketoconazole with replacement doses of hydrocortisone has become a commonly used treatment option for patients with disease progression after androgen deprivation due to its efficacy and relative ease of administration.[9] While the mechanism of action of high dose ketoconazole is not fully understood, this dose of ketoconazole has been shown to suppress adrenal androgen production.[5] However, response to treatment with ketoconazole has to date not been shown to correlate with suppression of adrenal androgen levels. LDK

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may be an attractive alternative to high dose ketoconazole. Toxicity with high dose ketoconazole can limit the ability to deliver treatment. Anecdotal evidence suggests that low dose ketoconazole is better tolerated, although this question has not been addressed in a rigorous fashion. In addition, the cost of the high dose regimen is substantial, and a low dose regimen would bring down costs. While clinical responses to low dose ketoconazole have been reported,[10] the low dose has not been systematically studied.

Given anecdotal evidence suggesting that low dose ketoconazole has reduced toxicity while maintaining efficacy, a prospective phase II study of this regimen in patients with androgen independent prostate cancer was conducted. Replacement doses of oral hydrocortisone (20 mg. every morning, 10 mg. at bedtime) were also administered, since hydrocortisone as a single agent is known to have activity in advanced prostate cancer and we wished to compare the efficacy of low dose with historical high dose ketoconazole data. In addition, there were no compelling data available to suggest whether adrenal insufficiency occurred at this dose of ketoconazole,[11] so that concerns regarding patient safety mandated the use of replacement hydrocortisone.

PATIENTS AND METHODS

Eligible patients had histologically confirmed adenocarcinoma of the prostate with progressive disease, as defined by the Prostate Specific Antigen Working Group Consensus Criteria.[12] All patients had previously undergone antiandrogen withdrawal for a minimum of 8 weeks. Any other hormonal therapies, including any dose of megestrol acetate, finasteride or systemic corticosteroids, had to have been discontinued at least 4 weeks before study enrollment. Required laboratory parameters included total bilirubin and aspartate transaminase less than 2 times the upper limit of normal. All patients had a Karnofsky performance status of 60% or greater and a life expectancy of at least 3 months. Prior therapy with 1 nonchemotherapeutic, nonhormonal investigational agent was permitted, provided it had been discontinued at least 30 days before study enrollment. Exclusion criteria were prior treatment with chemotherapy, aminoglutethimide, ketoconazole or herbal products such as saw palmetto or PC-SPES. Given drug interactions with ketoconazole, the concurrent use of terfenadine, astemizole or cisapride was not allowed. Radiation therapy within 28 days or radiopharmaceutical therapy within 60 days was not permitted.

Eligible patients were evaluated with a history and physical examination at study entry and monthly thereafter. In addition to a complete history and physical examination at each visit, patients were evaluated for adverse events. Formal quality of life or pain assessment was not performed. Complete blood count, PSA, total bilirubin, alkaline phosphatase, aspartate transaminase, creatinine and glucose were measured at baseline and then monthly. An endocrine panel including androstendione, dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA) and testosterone was obtained at baseline and after 2 months of treatment. A bone scan and, if clinically indicated, computerized tomography of the abdomen and pelvis were obtained at baseline. If imaging studies were positive at baseline they were repeated every 3 months.

Patients received 200 mg. ketoconazole orally 3 times daily on an empty stomach along with replacement doses of oral hydrocortisone (20 mg. every morning with food and 10 mg. at bedtime with food). At the time progressive disease was documented as defined by the consensus criteria,[12] the dose of ketoconazole was increased to 400 mg. orally 3 times daily and continued until disease progression. Replacement doses of hydrocortisone were continued as long as the patient was receiving ketoconazole. When the patient was no longer receiving ketoconazole, hydrocortisone was tapered by 5 mg. every 3 days until completely discontinued. Antacids, H-2 blockers and proton pump inhibitors were avoided.

At each visit toxicity was graded according to the National Cancer Institute common toxicity criteria (version 2.0) and recorded. In the event of grade 3 or higher hepatotoxicity or symptomatic peptic ulcer or gastritis, patients were removed from protocol treatment. If nausea was reported, the patient was instructed to take ketoconazole with meals. Antiemetics other than corticosteroids were permitted. If grade 2 or 3 nausea persisted despite these measures, the patient was removed from therapy. Patients with other grade 3 or higher toxicity had treatment withheld until toxicity resolved to grade 1 or better. Any patient with grade 4 toxicity or grade 3 toxicity persisting for more than 4 weeks (except as outlined previously) was removed from protocol treatment.

The primary end points of this prospective phase II study were to determine the response proportion and duration of response to low dose ketoconazole and replacement doses of hydrocortisone in patients with progressive prostate cancer despite androgen deprivation. PSA responses were defined according to the consensus criteria.[12] The secondary objectives of this trial were to determine the toxicity profile of this regimen, to assess the use of increasing ketoconazole to high dose in patients with disease progression on low dose and to correlate response to treatment with suppression of adrenal androgen levels.

The study included 28 patients. This sample size was sufficient to detect a response frequency of at least 40% compared to the null hypothesis of 20% with power of approximately 80% (0.79). Descriptive statistics were used to characterize the patient and disease features as well as toxicity. Nonparametric Spearman correlation was calculated to evaluate the association between adrenal androgens. The change in endocrine measurements from before treatment to 2 months after low dose ketoconazole was analyzed using the nonparametric Wilcoxon test for paired data. The distributions of adrenal androgens for responders and nonresponders were compared using the Mann-Whitney test.

RESULTS

Patient characteristics. A total of 28 patients with androgen independent prostate cancer was treated and all were on study at least 2 months and were evaluable for toxicity and response. Pretreatment patient characteristics are listed in table 1. Median patient age was 76 years (range 49 to 91). Of the patients 17 had bone only disease, 3 had bone and soft tissue disease, and 8 had PSA elevation as the only manifestation of progressive disease. At the start of therapy median PSA was 48.9 ng./ml. (range, 6.3 to 557.8), alkaline phosphatase 97 units/l. (range, 61 to 489), hemoglobin 13.1 gm./dl.

TABLE 1. *Pretreatment patient characteristics*

Age:	
Median	76
Range	49–91
No. extent of disease:	
PSA only	8
Bone only	17
Bone and soft tissue	3
PSA (ng./ml.):	
Median	48.9
Range	6.3–557.8
Alkaline phosphatase (units per l.):	
Median	97
Range	61–489
Hemoglobin (gm./dl.):	
Median	13.1
Range	10.1–14.3
Performance status:	
Median	0
Range	0–1

(range 10.1 to 14.3) and Eastern Cooperative Oncology Group performance status 0 (range, 0 to 1).

Clinical outcome. Overall, 13 (46%) of 28 patients had a PSA decrease of more than 50% (95% confidence interval 27.5%–66.1%). PSA responses were seen regardless of distribution of disease, although proportionately more PSA decreases were seen in patients with PSA only disease. PSA decreases of greater than 50% were seen in 6 (35%) of 17 patients with bone only disease, 1 (33%) of 3 with bone and soft tissue disease, and 6 (75%) of 8 patients with PSA elevation only. No responses were observed on bone scans or CT. Median duration of PSA decrease for all responders was 30+ weeks and 5 patients continued to exhibit a response ranging from 36+ to 53+ weeks. In 16 patients disease was unresponsive to low dose ketoconazole and the ketoconazole dose was increased to 400 mg. orally 3 times daily. Of these 16 patients 3 were taken off high dose ketoconazole due to toxicity and the remaining 13 did not respond to high dose ketoconazole. However, 2 patients remain on high dose ketoconazole with stable disease at 16+ and 19+ weeks.

Toxicity. Toxicity following low dose ketoconazole for all 28 patients is listed in table 2. In general, therapy was well tolerated. There were no grade 4 toxicities. Grade 3 toxicities included hepatotoxicity in 1 patient and depression in 2. The most common toxicities were grades 1 and 2 nausea (29%), grade 1 dry skin (18%) and grade 1 fatigue (14%). Four patients (14%) discontinued low dose ketoconazole due to persistent grade 2 nausea (1), grade 3 depression (2) and grade 3 hepatotoxicity (1). High dose ketoconazole was discontinued due to persistent grade 2 nausea in 2 patients and grade 2 gastric ulcer with grade 1 gastrointestinal bleeding in 1.

Adrenal androgen levels. Adrenal androgen (androstenedione, DHEA, and DHEAS) levels were measured at baseline and after 2 months of treatment (table 3). The baseline DHEA value significantly correlated with androstenedione ($p = 0.02$) and DHEAS ($p = 0.002$). There was no significant difference in the baseline endocrine values between responders and nonresponders but there was a significant decrease in all 3 parameters after 2 months of therapy. Median change was −0.49 ng./ml. (range −1.98, 0.02) in androstenedione, −1.4 ng./ml. (range −5.2, −0.3) in DHEA and −327.1 ng./ml. (range −1318.9, −23.0) in DHEAS. However, the degree of adrenal androgen suppression was not associated with response nor was the degree of suppression predicted by response to low dose ketoconazole.

TABLE 2. *Toxicity to low dose ketoconazole*

Toxicity*	No. (%)		
	Grade 1	Grade 2	Grade 3
Nausea	5 (18)	3 (11)	0
Dry skin	5 (18)	0	0
Fatigue	4 (14)	0	0
Bruising	1 (4)	0	0
Liver (aspartate transaminase, alanine transaminase)	0	1 (4)	1 (4)
Stomatitis	0	1 (4)	0
Depression	0	0	2 (7)
Insomnia	0	1 (4)	0

TABLE 3. *Adrenal androgen levels*

	Before Treatment		After 2 Mos.	
	No.	Median Ng./Ml. (range)	No.	Median Ng./Ml. (range)
Androstenedione	27	0.71 (0.33–2.62)	25	0.30 (0.10–0.73)
DHEA	27	2.3 (0.9–6.0)	25	1 (0.5–1.7)
DHEAS*	27	364.8 (50–1,907)	25	50 (9–588.1)

* Two-month values which were reported as less than 50 were coded as 50 for calculations.

DISCUSSION

A PSA response proportion of 46% was demonstrated in this prospective trial of low dose ketoconazole with replacement doses of hydrocortisone. While the use of PSA as an intermediate marker of response and outcome remains controversial, an emerging body of literature supports the use of a greater than 50% decrease in PSA as an intermediate marker of survival[13–16] in patients with androgen independent prostate cancer treated with secondary hormones, cytotoxic agents or suramin. Although it is not appropriate to compare the results for this trial directly with the results of retrospective series using high dose ketoconazole with hydrocortisone replacement, the PSA response proportion observed seemed close to the historic high dose ketoconazole response rate of 50%. Furthermore, increase to high dose ketoconazole failed to "capture" any patients who had progressive disease despite low dose ketoconazole. While this observation clearly does not demonstrate equivalence between low dose ketoconazole and high dose ketoconazole, it suggests that the mechanism of action is unlikely to be significantly different.

Ketoconazole is a substituted imidazole that suppresses testicular and adrenal steroidogenesis by inhibition of the conversion of cholesterol to pregnenolone. Because ketoconazole is a potent inhibitor of all adrenal steroid synthetic pathways, replacement doses of hydrocortisone may be required. Glucocorticoids alone may have antitumor effects mediated either by direct interaction with androgen receptors or by feedback inhibition of the hypothalamic-pituitary-adrenal axis. Contemporary studies have tried to control for the beneficial effects of antiandrogen withdrawal in this setting. Kelly et al reported on 30 patients treated with 40 mg. hydrocortisone daily and found that 20% had a decrease of at least 50% in serum PSA maintained for a median of 4 months.[14] Dawson et al treated 34 patients with hormone refractory disease with 30 mg. hydrocortisone daily and found a 29% PSA response proportion.[17] In a recent randomized trial hydrocortisone was evaluated alone or in combination with mitoxantrone in patients with hormone refractory prostate cancer.[18] A greater than 50% decrease in PSA was observed in 22% of patients treated with hydrocortisone alone. Finally, in a large study comparing suramin plus hydrocortisone to placebo plus hydrocortisone in patients with metastatic hormone refractory disease and opioid analgesic requirements 38 (16%) of 230 treated with 40 mg. hydrocortisone daily plus placebo had a greater than 50% reduction in PSA.[19] Taken together, these data suggest that 16% to 29% of patients will have a greater than 50% reduction in PSA when treated with 30 to 40 mg. hydrocortisone daily. The response proportion observed with hydrocortisone plus low dose ketoconazole appears to be considerably higher than that expected with hydrocortisone alone, and supports prior experience that suggested that ketoconazole is an active agent in this group of patients. However, the true benefit of adding ketoconazole to hydrocortisone can only be adequately addressed in a randomized trial.

Baseline levels of androstenedione, DHEA and DHEAS did not differ between responders and nonresponders. While all 3 of these endocrine parameters decreased significantly after 2 months of therapy, the degree of decrease was not predicted by response. Of note, low dose ketoconazole clearly suppresses adrenal steroidogenesis. Therefore, replacement doses of hydrocortisone are recommended, even when ketoconazole is administered at low doses, despite the possibility that it may not be necessary in all patients. While high dose and low dose ketoconazole have been shown to suppress adrenal androgen production,[5, 11] no study has shown an association between response to treatment and changes in adrenal androgen levels. While our study was likely too small to detect an association with adequate power, this question is being addressed in a recently completed cancer and leukemic

group B phase III study of antiandrogen withdrawal alone or with high dose ketoconazole. It is intriguing that preliminary results from this trial revealed that the baseline level of DHEA appeared to be an independent predictor of survival.[20]

Low dose ketoconazole is attractive because of its favorable toxicity profile, ease of use and its reduced cost compared to high dose ketoconazole. All medications are given orally and patients need only monthly laboratory followup. As with high dose ketoconazole, nausea was the most frequent toxicity. However, nausea was generally mild to moderate, and only 1 patient discontinued low dose ketoconazole due to persistent grade 2 nausea. Only 3 patients (11%) experienced grade 3 toxicity (hepatotoxicity in 1 and depression in 2), and no patient experienced grade 4 toxicity. While the relative efficacy and toxicity of low dose compared with high dose ketoconazole can only be assessed in a phase III trial, these data suggest that low dose ketoconazole should be considered as a treatment option in this patient population.

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Two Prevalent *CYP17* Mutations and Genotype-Phenotype Correlations in 24 Brazilian Patients with 17-Hydroxylase Deficiency

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We performed molecular genetic analysis of 24 subjects from 19 families with 17-hydroxylase deficiency in Brazil. Of 7 novel *CYP17* mutations, 2 (W406R and R362C) account for 50% and 32% of the mutant alleles, respectively. Both mutations were completely inactive when studied in COS-7 cells and yeast microsomes; however, phenotypic features varied among subjects. Some 46,XY individuals with these genotypes had ambiguous genitalia, and other subjects had normal blood pressure and/or serum potassium. We found mutations W406R and R362C principally in families with Spanish and Portuguese ancestry, respectively, suggesting that two independent founder effects contribute to the increased prevalence of 17-hydroxylase deficiency in Brazil. Mutations Y329D and P428L retained a trace of activity, yet the two individuals with these mutations had severe hypertension and hypokalemia. The 46,XX female with mutation Y329D reached Tanner stage 5, whereas the 46,XY subject with mutation P428L remained sexually infantile. The severity of hypertension, hypokalemia, 17-deoxysteroid excess, and sex steroid deficiency varied, even among patients with completely inactive CYP17 protein(s). Spontaneous sexual development occurred only in 46,XX females with partial deficiencies. We conclude that other factors, in addition to *CYP17* genotype, contribute to the phenotype of individual patients with 17-hydroxylase deficiency. (*J Clin Endocrinol Metab* 89: 49–60, 2004)

MUTATIONS IN THE *CYP17* gene cause 17-hydroxylase deficiency (17OHD), a rare form of congenital adrenal hyperplasia (CAH) with an estimated incidence of about 1:50,000 newborns (1). Individuals with 17OHD account for roughly 1% of all cases of CAH, and most reports involve isolated cases from consanguineous families (2). Since cloning of the *CYP17* gene encoding cytochrome P450c17 (CYP17, 17α-hydroxylase/17,20-lyase) (3), nearly 40 different mutations in *CYP17* have been described (4–9), although a few more common mutations reoccur in certain ethnic groups (10–12).

The typical features of complete 17OHD were described almost 40 yr ago (13), as hypertension, hypokalemia, and sexual infantilism in phenotypic females. Subsequent reports identified 17OHD as a cause not only of incomplete male pseudohermaphroditism (14), but also sexual infantilism in 46,XY subjects (15). The lack of adrenal 17α-hydroxylase activity drives massive overproduction of the 17-deoxysteroids 11-deoxycorticosterone (DOC) and corticosterone (B), which are the mineralocorticoids that cause hypertension and hypokalemia in 17OHD (4). Concomitant lack of gonadal 17,20-lyase activity precludes sex steroid production and hence the development of the male phenotype *in utero* or of secondary sexual characteristics at puberty.

Nevertheless, there is considerable variation in the clinical and biochemical features of 17OHD (16), including the variant of isolated 17,20-lyase deficiency (17, 18). The severity of clinical disease tends to be milder with mutations that retain partial catalytic activity in assays using heterologous expression systems (4), but the age of onset of hypertension, the degree of hypokalemia, and the aldosterone production rate appear to vary, even among patients with mutations that completely inactivate the enzyme (2). However, because there have been no studies of multiple individuals bearing the same genotype who have been studied by the same investigators, it is not clear to what extent genotype alone determines phenotype in 17OHD.

Worldwide, the most common form of CAH is 21-hydroxylase deficiency (19), and the second most common form appears to be lipoid CAH in Japan and Korea (20) and 11-hydroxylase deficiency in the Middle East (21); founder effects that yield a single prevalent mutation account for the high prevalence of these two disorders in their respective populations. In contrast, 17OHD appears to be the second most common form of CAH in Brazil (16, 22). Founder effects may also contribute to the high prevalence of 17OHD in Brazil, but the population of Brazil is among the most ethnically heterogeneous in the world (23). The Portuguese settled Brazil beginning in the 1500s, and the indigenous Amerindian people, Africans derived from the extensive slave trade, and waves of immigration from Italy, Spain, Germany, Asia, and The Netherlands contribute to the genetic diversity (23–25).

Abbreviations: CAH, Congenital adrenal hyperplasia; CPR, cytochrome P450-oxidoreductase; DOC, 11-deoxycorticosterone; 17OHD, 17-hydroxylase deficiency.

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The Brazilian Congenital Adrenal Hyperplasia Multicenter Study Group has had the opportunity to evaluate the clinical features of 30 subjects with 17OHD from 24 kindreds, the largest group of 17OHD cases studied by a single group. To provide insight into the phenotypic variations in 17OHD and to define the genetics of 17OHD in Brazil, we analyzed the *CYP17* gene in these subjects. We now report the results of molecular genetic and functional analyses of the mutations.

Subjects and Methods

Subjects, clinical presentation, and hormonal evaluation

Of 30 subjects in whom the diagnosis of 17OHD was established (at Escola Paulista de Medicina by C.E.K.), DNA was analyzed in 24, constituting the cohort for the genetic analysis. These 24 subjects derived from 19 kindreds, and consanguinity was known to occur in 6 of the 19 families. The study protocol was approved by the committee on ethics in human research from Escola Paulista de Medicina (n.1703/98), and all patients provided written informed consent. Blood pressure was measured by aneroid sphygmomanometer in the seated position on at least three occasions. For diagnostic studies, blood samples were obtained before and 60 min after the iv injection of 250 μg cosyntropin [synthetic ACTH-(1–24)], and Table 1 lists the mean and ranges of basal and stimulated hormone values in these subjects. The diagnosis of 17OHD was established by the reduced circulating concentrations of cortisol and gonadal steroids, elevated gonadotropins, and high [>3 SD above normal, with or without ACTH-(1–24) stimulation] concentrations of the diagnostic steroids DOC and/or B, as well as frequently elevated concentrations of 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone (16). In our subjects, basal hormone concentrations alone established the diagnosis. The clinical features are summarized in Table 2.

DNA preparation, PCR, and sequencing

DNA was extracted from peripheral leukocytes (Pure Gene DNA Isolation Kit D-5000, Gentra Systems). The 6.4-kb *CYP17* gene was amplified into 1–4 pieces from 0.5–1 μg genomic DNA using TaKaRa Ex Taq DNA polymerase (Takara Shuzo Co., Shiga, Japan) in 100-μl reactions using buffer and deoxy-NTPs provided by the manufacturer and 3% dimethylsulfoxide. The primers are listed in Table 3, and the locations of the primers are illustrated in Fig. 1. To amplify 3- to 4-kb products, PCR parameters included 40 cycles of 3 min at 94 C, 1 min at 65 C, and 3 min at 70 C. For amplification of the entire gene, the annealing time was increased to 1.5 min, and the extension parameters were 72 C for 5.5 min. The final PCR products were precipitated with ethanol and purified on 1% agarose gels using the QIAEX II kit (Qiagen, Chatsworth, CA). Amplicons were submitted for direct sequencing of the 8 exons and flanking intronic DNA by the dye termination method on a PE Applied Biosystems instrument (McDermott Center Sequencing Facility at University of Texas Southwestern Medical Center, Dallas, TX). The mutations were identified by comparison with the GenBank sequence (accession no. M19489) for *CYP17* (3) using MacVector 6.5.3 (Accelrys Corp., San Diego, CA). Identified mutations were confirmed by sequencing the product of a second PCR amplification in the opposite direction.

Heterologous expression and enzyme assay

The cDNAs for missense CYP17 mutations were generated by sequential PCR using overlapping mutagenic oligonucleotides (Table 3) with template plasmid pLW01-c17 and Ex Taq polymerase with 1% dimethylsulfoxide as previously described (26). The final PCR product was extracted with phenol-chloroform, precipitated with ethanol, digested with *Bam*HI and *Eco*RI, gel-purified, ligated into the eukaryotic expression vector pcDNA3 (Invitrogen, Carlsbad, CA), and later subcloned into yeast expression vector V10 (27). Each cDNA insert was sequenced in its entirety to ensure that only the desired mutations were introduced.

The enzymatic activities of the four missense mutations were studied by transient transfection of COS-7 and HEK-293 cells with 1–2 μg of the pcDNA3 expression vectors using FuGENE6 (3 μl) in 100 μl serum-free medium as previously described (26). Incubations with 0.1 μM [^{3}H]progesterone, -pregnenolone, or -17α-hydroxypregnenolone (90,000 cpm; PerkinElmer Life Sciences, Norwalk, CT) for up to 16 h were repeated three times using COS-7 cells and were confirmed with an additional experiment using HEK-293 cells under similar assay conditions. In some cases, incubations were repeated with 0.01 μM steroids to increase assay sensitivity. Extraction, chromatography, and autoradiography were performed as previously described (28).

The P450 content and enzymatic activities of the mutations were also studied in *Saccharomyces cerevisiae* strain W303B. Yeast were transformed with 1 μg expression vector V10 (empty, and with wild-type or mutant *CYP17* cDNA) with or without pYcDE2-OR to provide cytochrome P450-oxidoreductase (CPR) (29), using the lithium acetate method as previously described (26). CO-reduced P450 difference spectra were performed by resuspending yeast harvested from 80 ml culture in 12 ml 0.1 mM potassium phosphate (pH 7.4) with glucose, adding 3-ml aliquots to two cuvettes, and bubbling CO gas into the sample cuvette for 1 min

TABLE 1a. Basal and ACTH-stimulated adrenal steroid values in 24 Brazilian patients with 17OHD

Steroids	Basal	Post-ACTH	Reference values[a]	
			Basal	Post-ACTH
Cortisol (μg/dl)	2.3 ± 2.9 [0.1–11.9]	6.2 ± 7.9 [0.1–19.7]	6–25	18–42
DOC (ng/dl)	291 ± 124 [120–504]	415 ± 159 [150–737]	4–12	12–61
B (μg/dl)	15.5 ± 7.7 [0.84–32.9]	23.9 ± 10.6 [2.66–44.4]	0.1–0.5	1.7–4.8
18OHDOC (ng/dl)	271 ± 163 [35–704]	478 ± 253 [41–1045]	0–10	56–158
18OHB (ng/dl)	261 ± 139 [11–456]	411 ± 232 [43–855]	10–35	84–174

Values are the mean ± SD [range]. 18OHB, 18-hydroxycorticosterone; 18OHDOC, 18-hydroxydeoxycorticosterone.

[a] Basal and ACTH-stimulated reference values from Nichols Institute and from Kater *et al.* (25a); normal ranges for adolescents and adults are similar. Conversion factors for Systeme International units are: DOC, ×0.03026 nmol/liter; B, ×28.86 nmol/liter; 18OHDOC, ×0.02886 nmol/liter; 18OHB, ×0.02759 nmol/liter; cortisol, ×27.59 nmol/liter.

TABLE 1b. Baseline gonadotropin and gonadal steroid values in 24 Brazilian patients with 17OHD

Gonadotropins/gonadal steroid	Patients		Reference values
	46,XX	46,XY	
LH (IU/liter)	51 ± 31 [12.5–79]	50 ± 28 [12.7–87]	2–15
FSH (IU/liter)	87 ± 40 [38–170]	77 ± 42 [20–164]	2–12
Testosterone (ng/dl)	30 ± 16 [12.5–65]	18 ± 16 [2–48]	<50[a]
Estradiol (ng/dl)	1.1 ± 0.7 [0.1–2.2]	1.5 ± 1.0 [0.3–3.1]	<30[a]

Values are the mean ± SD [range].

[a] Reference values for testosterone and estradiol are given for prepubertal children and are higher for Tanner stages 2–5. Conversion factors for Systeme International units are: testosterone, ×0.03467 nmol/liter; estradiol, ×35.71 pmol/liter.

TABLE 2. Clinical characteristics of 24 Brazilian subjects with 17OHD at diagnosis

Kindred and subjects	Age (yr)	Remarkable features[a]	Karyotype[b]	Tanner stage	EG[c]	Social sex	SBP (mm Hg)	DBP (mm Hg)	K^+ (mEq/liter)	Mutations
1-1	10.3	Amb/MPH	XY	B1P1	Amb	F	140	100	3.3	R362C
1-2	16.5	Classical	XX	B1P1	Fem	F	130	95	3.6	R362C
2	13.7	Normal K^+/MPH	XY	B1P1	Fem	F	140	105	3.9	R362C
3	13.8	Hip Fx/MPH	XY	B1P2	Fem	F	145	110	2.4	W406R
4	14.4	Classical/MPH	XY	B1P1	Fem	F	135	90	3.6	W406R
5-1	15.5	Classical/MPH	XY	B1P1	Fem	F	140	100	3.2	W406R
5-2	11.8	Classical	XX	B1P1	Fem	F	155	97	3.6	W406R
6	19	Arrh/MPH	XY	B1P2	Fem	F	145	100	2.6	P428L
7-1	34	Stroke/MPH	CN	B1P2	Fem	F	200	130	2.5	R362C
7-2	34	Classical	XX	B3P2[d]	Fem	F	200	130		R362C
8	15.7	Classical	XX	B1P1	Fem	F	150	120	3.4	W406R
9	16.6	Normal BP	XX	B1P1	Fem	F	115	70	1.8	W406R
10-1	17.4	Classical	XX	B1P1	Fem	F	137	102	2.0	W406R
10-2	18	↓K^+ Myop	XX	B1P2[d]	Fem	F	145	110	3.8	W406R
11	27	Tanner 5	XX	B5P5	Fem	F	170	100	2.8	Y329D/A–G
12	16	Classical/MPH	XY	B3P2[d]	Fem	F	110	70	3.6	W406R
13	19.7	Classical/MPH	XY	B1P1	Fem	F	160	100	2.9	W406R/R362C
14	15	Classical/MPH	XY/XO; CN	B1P1	Fem	F	125	80	3.1	W406R
15	14	Classical/MPH	XY	B2P1	Fem	F	180	110	2.6	W406R/R362C
16	27	Stroke/MPH	XY	B5P2[d]	Fem	F	160	120	2.3	Y329X
17	40	↓K^+ Myop/MPH	XY/XXY	B2P1[d]	Fem	F	130	90	3.2	W406R/M1T
18-1	17	GB	XX	B3P2	Fem	F	120	80	3.4	R362C
18-2	27	Classical	XX	B3P2[d]	Fem	F	130	90		R362C
19	19	Classical/MPH	XY	B1P1	Fem	F			3.1	W406R
Median	18						146	100	3.2	
Range	10.3–40						110–200	70–120	1.8–4.1	

[a] Classical, Hypertension, hypokalemia, pubertal delay; MPH, male pseudohermaphrodite; Hip Fx, hip fracture; Arrh, arrhythmia; Myop, myopathy; GB, gonadoblastoma in streak ovaries.

[b] CN, Chromatin (buccal smear inactive X) negative; case 17, 1 cell in 39 was XXY.

[c] EG, External genitalia; Amb, ambiguous genitalia; Fem, female/infantile.

[d] Gonadal steroid replacement begun before diagnosis.

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TABLE 3. Oligonucleotide primers for DNA amplification and sequencing

Primer no.	Oligonucleotide primer	Sequence	Oligo pairs and amplicons
	CYP17 gene[a]		
1	c17geneS1a[b]	5′-CTCCACCGCTGTCTATCTTGCCTGCC-3′	6.4 kb
2	c17geneAS1	5′-CTCTAAATCTGTGTTGTGGGGCCAC-3′	
3	I3S1	5′-GCTGGAGAAGCAAAATGGAAGAAGGGTGG-3′	3.0 kb (1+4, 2+3)
4	I4AS1	5′-CCTACTATGTGCCAGGTTCTCTGCTTG-3′	0.25 kb (3+4)
5	I1AS1	5′-TGGTCTGAAGACCTGAACCAATCCC-3′	0.4 kb (1+5)
6	I1S1	5′-CAAGAGTGGGGTGGATGGGTGTGAG-3′	
7	I3AS1	5′-GATTGGGGACAATGTCAGGGTCTAC-3′	0.6 kb (6+7)
8	I4S1	5′-GAGTGTCACAGATGGGGCTCCTTCC-3′	0.75 kb (8+9)
9	I6AS1	5′-TGGGCTGGCAAGCAGTGAATGCATC-3′	
10	I6S1	5′-GCATGAGGCTGAGCAAGGAAGGGAG-3′	1.0 kb (2+10)
11	I5S1	5′-CCTCTCCTGGGCTTACACACACTAG-3′	1.5 kb (11+12)
12	I7AS1	5′-AGCAGAGTCCAGGCTCGCTGTGTG-3′	
	Promotor		
13	c17PS1	5′-GAAGGGACTGCTGGAGCCATGGCAG-3′	0.9 kb (13+14)
14	c17PAS1	5′-GGAGGGGGTGTAAGAACAGGGAGAG-3′	
15	c17PS2	5′-GCCCTTTGTCCTTTCCCTCAGAAGC-3′	0.9 kb (15+16)
16	c17PAS2	5′-CAGCAAGAGAGCCACGAGCTGCCAC-3′	
17	c17PS3	5′-ACCTATCTCTCCCTTCCCTTCCACC-3′	0.9 kb (5+17)
	Mutagenesis[c]		
18	W406R-S1	5′-AATGAGAAGGAGCGGCACCAGCCGGATCAG-3′	0.5 kb (18+26)
19	W406R-AS1	5′-ATCCGGCTGGTGCCGCTCCTTCTCATTGTG-3′	1.2 kb (T7+19)
20	R362C-S1	5′-CGAGAGGTGCTTTGCCTCAGGCCCGTGGCC-3′	0.6 kb (20+26)
21	R362C-AS1	5′-ACGGGCCTGAGGCAAAGCACCTCTCGGATG-3′	1.1 kb (T7+21)
22	P428L-S1	5′-CAGCTCATCTCACTGTCAGTAAGCTATTTG-3′	0.4 kb (22+26)
23	P428L-AS1	5′-TAGCTTACTGACAGTGAGATGAGCTGGGTC-3′	1.3 kb (T7+23)
24	Y329D-S1	5′-AAGAAGAAGCTCGACGAGGAGATTGACCAG-3′	0.6 kb (24+26)
25	Y329D-AS1	5′-GTCAATCTCCTCGTCGAGCTTCTTCTTCAC-3′	1.1 kb (T7+25)
26	pLW01-AS1	5′-TCAGCAAAAAACCCCTCAAGACCCG-3′	1.7 kb (T7+26)

[a] Ix, Intron x; S and AS, sense and antisense, respectively (see Fig. 1 for primer locations).
[b] Primer c17geneS1 was modified (*underlined* C) to c17geneS1a (see *Results*).
[c] T7 (sense) and pLW01-AS1 (antisense) oligonucleotides were used as terminal primers. The mutated base pair is *underlined*.

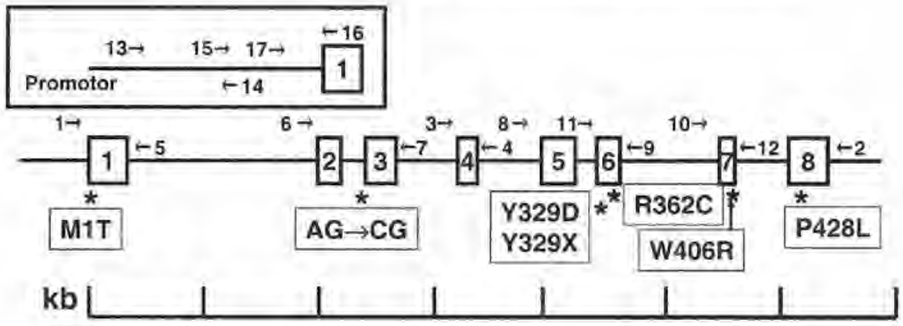

FIG. 1. Schematic representation of the human *CYP17* gene, indicating the approximate locations and sizes of exons (*numbered boxes*), oligonucleotide primers (*arrows*, numbered as explained in Table 3), and identified mutations (*labeled asterisks*).

(26). Using the same suspension of whole yeast used for CO-reduced spectra, substrate-induced difference spectra were recorded with up to 40 μM progesterone as previously described (30).

Microsomes were prepared from 1 liter yeast culture grown to an A_{600} of 1.0–1.8 in defined medium by sonication of spheroplasts as previously described (26), and protein content was determined by colorometric assay. Microsomes containing CPR and wild-type CYP17 (25 μg protein) or the mutations (250 μg) were incubated at 37 C with 0.1 μM [^{3}H]progesterone, -pregnenolone, or -17α-hydroxypregnenolone for 60 min in 200 μl 50 mM potassium phosphate, pH 7.4, with 1 mM NADPH. Extraction, chromatography, and autoradiography (26, 29) and immunoblotting were performed as previously described (28).

Results

Mutation analysis

Seven *CYP17* gene mutations were found, none of which has been described previously. We found 5 missense mutations in exons 1, 6 (two), 7, and 8; a nonsense mutation in exon 6; and an AG to CG mutation at g.2306 in the splice acceptor site of intron 2 (Table 4 and Fig. 1). Mutation W406R in exon 7 was the most common, accounting for half of the mutant alleles, including 11 homozygotes. Mutation R362C accounted for almost one third of the mutant alleles with 7 homozygotes, and 2 subjects were compound heterozygotes for W406R plus R362C. Together, mutations W406R and R362C accounted for 23 of 28 (82%) of the alleles identified in 25 (52%) and 16 (33%) of the 48 sequenced *CYP17* genes, respectively (Table 4 and Fig. 2). One subject was homozygous for P428L, and another was homozygous for Y329X. One 46,XX female who reached Tanner stage 5 (Table 2) was a compound heterozygote for Y329D and the AG to CG substitution in the splice acceptor site of intron 2, and 1 subject was heterozygous for M1T and W406R.

Only the M1T mutation altered the restriction map of the

TABLE 4. *CYP17* mutations in 24 Brazilian subjects

	Mutations[a]		Exon/Intron	Homozygotes	Heterozygotes[b]	Affected alleles[c]
	Amino acid	Nucleotide				
1	W406R	TGG/CGG	7	11	3 (2× R362C; 1× M1T)	14 (50)
2	R362C	CGC/TGC	6	7	2 (2× W406R)	9 (32)
3	P428L	CCG/CTG	8	1	0	1 (3.5)
4	Y329X	TAC/TAG	6	1	0	1 (3.5)
5	Y329D	TAC/GAC	6	0	1 (AG/CG splice)	1 (3.5)
6	M1T	ATG/ACG	1	0	1 (W406R)	1 (3.5)
7	AG/CG splice (position 2306)	AG/CG	Intron 2	0	1 (Y329D)	1 (3.5)
Total				20 subjects	4 subjects	28 (100)

[a] Base substitutions are *underlined*.
[b] The second mutation affecting the compound heterozygotes is in *parentheses*, such that each subject is listed twice.
[c] Allele frequency is calculated based on one allele per homozygote (see text). Percentages are given in *parentheses*.

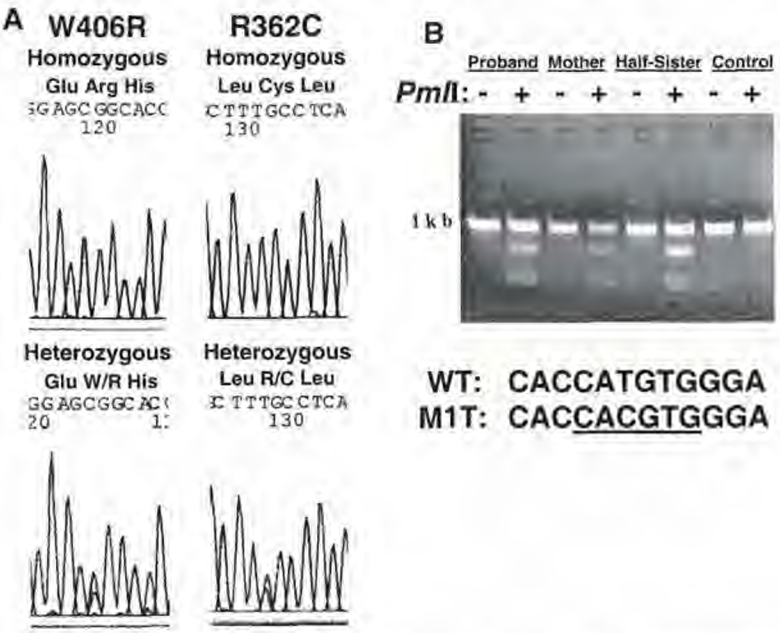

FIG. 2. Brazilian *CYP17* mutations. A, Electropherograms corresponding to homozygous and heterozygous patients for the common mutations W406R and R362C. PCR-amplified DNA was purified and submitted for direct sequencing using oligonucleotide I6S1 (for W406R) or I4S1 (for R362C) as described in the text. B, *Pml*I digest from patient and family members bearing the M1T mutation. Half of the DNA, PCR-amplified using oligonucleotides c17PS3 and I1AS1 (1 kb amplicon), is digested by *Pml*I, indicating heterozygosity for M1T in all three family members. The sequences of this region for the wild-type and mutant alleles are shown with the *Pml*I site *underlined*.

surrounding region, introducing a *Pml*I site (CACGTG). Half of the DNA that was amplified from the affected proband with this mutation, from her mother, and from her half-sister was digested by *Pml*I at the expected site, indicating that all 3 are heterozygous for this mutation (Fig. 2). For the family members of all other kindreds, zygosity was determined by sequencing amplified DNA from the region surrounding the mutation(s). DNA from all 29 available parents and from 36 of 46 available siblings (78%) contained 1 copy of the same mutation found in the affected family member.

After sequencing the exons and flanking intronic DNA from 24 patients, we consistently observed 5 differences from the *CYP17* sequence M19489 deposited in GenBank (3): 1) a polymorphism at D283 (GAT to GAC) in exon 5, 2) a third C at the CC in positions −26 to −28 at the 3′ end of intron 2, 3) a third C at the CC in positions −3 to −5 at the 3′ end of intron 3, 4) an A to T substitution in position −5 at the 3′ end of intron 7, and 5) a C in place of the T 34 bp upstream from the ATG start codon. This last difference was incorporated into primer c17geneS1a, which gave better PCR amplifications than primer c17geneS1 (Table 3). These differences, except for 2 and 5 above, have been noted previously (31), suggesting that these minor changes correspond to the correct sequence in our population.

Heterologous expression, enzyme assay, and difference spectroscopy

To determine whether the mutant enzymes retained any residual 17α-hydroxylase activity, cDNAs bearing the four missense mutations (W406R, R362C, P428L, and Y329D) were constructed and subcloned into mammalian and yeast expression vectors pcDNA3 and V10, respectively. COS-7 cells transiently transfected with pcDNA3 containing the wild-type CYP17 cDNA metabolized progesterone to the expected 4:1 mixture of 17α-hydroxyprogesterone and 16α-hydroxyprogesterone (Fig. 3) (32, 33). In contrast, COS-7 cells expressing the CYP17 mutations W406R and R362C produced only the same background metabolites as mock-transfected cells (Fig. 3). Unlike mutations W406R and R362C, mutation Y329D always exhibited a small amount (~5%) of residual activity when expressed in COS-7 cells, and muta-

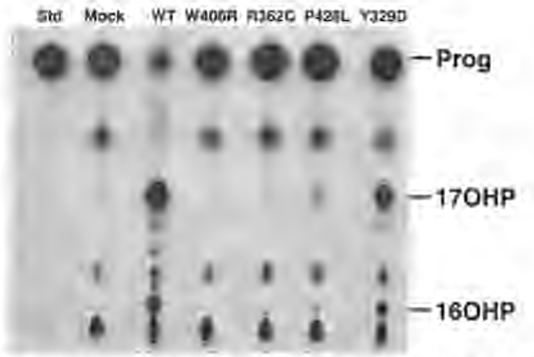

FIG. 3. Progesterone metabolism by transiently transfected COS-7 cells expressing the cDNA for CYP17, wild type or mutations W406R, R362C, P428L, and Y329D. A representative autoradiogram of chromatographed steroid products is shown, with the locations of steroid standards indicated. The film was intentionally overexposed to reveal trace 17α-hydroxylation by mutations P428L and Y329D, so that by-products of progesterone metabolism by endogenous enzymes appear more abundant than is typical. Similar results were obtained in three separate experiments with COS-7 cells and in one identical experiment using HEK-293 cells. Prog, Progesterone; 17OHP and 16OHP, 17α-hydroxy- and 16α-hydroxyprogesterone, respectively.

tion P428L yielded a trace of 17α-hydroxyprogesterone in most experiments (Fig. 3).

The activity and spectral properties of the mutations were further characterized in yeast. Yeast microsomes containing wild-type human CYP17 and human P450-oxidoreductase are an abundant source of enzyme activity (29), as demonstrated by the pattern of [^{3}H]pregnenolone metabolism in Fig. 4. As was observed when the mutations were expressed in COS-7 and HEK-293 cells, the 17α-hydroxylase and 17,20-lyase activities of microsomes containing mutations W406R and R362C did not exceed background levels (Fig. 4A). These data confirm that mutations W406R and R362C are not active under these *in vitro* conditions. In contrast, microsomes containing mutations Y329D and P428L metabolized some progesterone and pregnenolone by 17α-hydroxylation, and both mutant enzymes demonstrated little 17,20-lyase activity even in the presence of cytochrome b_5 (Fig. 4B). The 17α-hydroxylation rates for Y329D and P428L were too low to determine meaningful kinetic constants, but we estimate that mutations Y329D and P428L retain less than 5% of the wild-type activity.

Intact yeast that express wild-type human CYP17 afford CO-reduced difference spectra with a peak at 450 nm, derived from the CO adduct with the heme thiolate in CYP17 (34). This difference spectrum, characteristic of all cytochromes P450, indicates the presence of properly folded, functional CYP17 protein, and we obtain 50–100 nmol of spectroscopically active wild-type human CYP17 per liter of culture using vector V10 in strain W303B (26). Likewise, addition of progesterone to a suspension of yeast expressing wild-type human CYP17 yields a type I substrate binding spectrum (30), and these difference spectra can be used to monitor the expression of functional protein in yeast. Because mutations Y329D and P428L retain some activity, some of the protein must fold and incorporate heme properly. If these molecules form a stable CO adduct typical of active cytochromes P450, we would observe a peak at 450 nm in the CO-reduced difference spectrum. However, CO-reduced difference spectra using yeast expressing the four missense mutations did not demonstrate detectable P450 absorbances (Fig. 4C). Similarly, yeast expressing mutations Y329D and P428L did not form a type I difference spectrum in the presence of up to 40 μM progesterone (not shown). The lack of discernable spectral changes upon addition of CO or progesterone indicates that only a small fraction of the mutant CYP17 proteins is expressed in a functional form in yeast.

Immunoblots using fresh yeast microsomes containing the four missense mutations all contained immunoreactive protein that comigrated with wild-type human CYP17 at approximately 57 kDa as well as degradation products (Fig. 4D). The amount of full-length protein remained relatively constant in microsomes containing wild-type CYP17 after multiple freeze-thaw cycles. In contrast, the quantity of full-length CYP17 protein declined rapidly with freeze-thawing or warming in sodium dodecyl sulfate sample buffer for the four missense mutations, and products of lower mass increased in parallel, presumably through proteolysis (not shown). We conclude from these data that all four missense mutations impair activity primarily by destabilizing the enzyme structures, thus impairing the capacity to incorporate and/or retain heme. For mutations Y329D and P428L, a sufficient portion of the protein molecules remain properly folded to exhibit catalytic activity at least transiently, but this activity is barely detectable using sensitive radiochemical assays at low substrate concentrations. Mutations Y329D and P428L are examples of partial, combined deficiencies in both 17α-hydroxylase and 17,20-lyase activities.

Correlation of genotype and phenotype: mutations W406R and R362C

We compared the phenotypic characteristics in the 11 homozygotes for W406R and the 7 homozygotes for R362C whose CYP17 enzymes are completely inactive in heterologous assay systems. Although DOC concentrations pre- and post-ACTH-(1–24) administration were higher in W406R homozygotes than in subjects homozygous for R362C ($P < 0.01$), blood pressure and circulating concentrations of potassium or other hormones were similar in the two groups (Table 5). Aldosterone values were low in both groups, and in fact, plasma aldosterone values in untreated subjects were uniformly suppressed regardless of genotype (data not shown). Thus, despite equally inactive CYP17 enzymes, homozygotes for mutations W406R and R362C showed some trends to phenotypic differences, and clinical features varied even among subjects with these two common mutations (Tables 2 and 3). Although variations in blood pressure and potassium values may be influenced by dietary and environmental differences, the range of genital differentiation among these male pseudohermaphrodites remains unexplained.

The finding of the W406R or R362C allele in an affected or obligate heterozygous subject who recently emigrated from the Iberian peninsula would argue against these mutations arising *de novo* in Brazil. We studied the family of a compound heterozygote for W406R and R362C (Fig. 5) whose deceased father was born in Spain (DNA not available). The

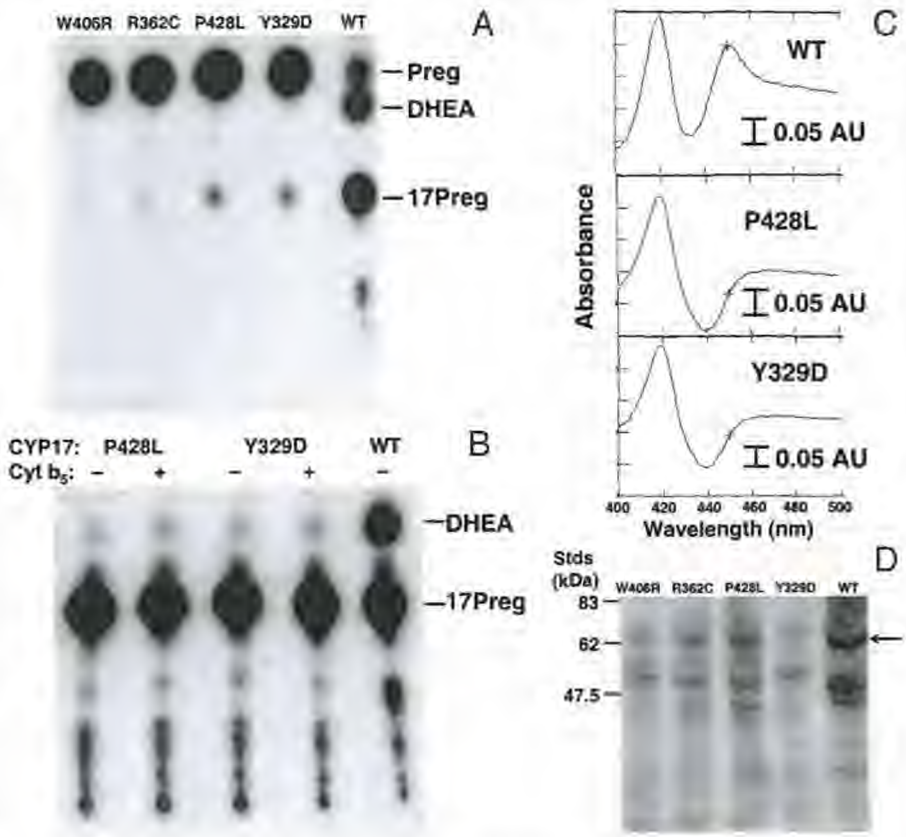

FIG. 4. Activities and spectral properties of mutant CYP17 enzymes in *S. cerevisciae*. A and B, Autoradiograms of thin layer chromatograms (overexposed as in Fig. 3) showing pregnenolone (A) or 17α-hydroxypregnenolone (B) metabolism by yeast microsomes containing human CPR and wild-type (WT) or mutant CYP17 as indicated. Preg, Pregnenolone; DHEA, dehydroepiandrosterone; 17Preg, 17α-hydroxypregnenolone. B, Purified cytochrome b_5 (5 pmol) was added where indicated. C, CO-reduced difference spectra from yeast expressing wild-type CYP17 (*top*), P428L (*middle*), or Y329D mutation (*bottom*). The *cursor* is set at 450 nm, and the *bar* represents 0.05 absorbance units (AU). D, Immunoblot of yeast microsomes containing wild-type (WT) CYP17 and for the four missense mutations (30 μg protein/lane). The *arrow* denotes bands corresponding to full-length CYP17 proteins, which migrate near the chemically modified 62-kDa protein standard (26).

father migrated to Brazil and married a Brazilian of Portuguese ancestry, and the mother was heterozygous for allele R362C. Of their children, one with 17OHD bore one copy of each mutation, one was a heterozygote for W406R, one is wild type at both alleles, and one died of an unknown cause. The genetics within this kindred suggest that the W406R mutation arose in Spain from an ancestor common to many Brazilians bearing mutation W406R.

Discussion

In this study we report the largest series of 17OHD subjects studied in a single country by 1 group of investigators. In 1994, Kater and Biglieri (16) first observed an unusual large number of Brazilian cases of 17OHD, and 17OHD remains the second most common cause of CAH in Brazil (22). The finding in the present study that 82% of the mutant alleles can be explained by 2 mutations suggests founder effects for the *CYP17* mutations, an unusual result for an autosomal recessive disease in a country with such an extensive racial admixture (24). Only 3 recurring *CYP17* mutations have been described previously: a CATC duplication following Ile^{479} found in Canadian Mennonites and Dutch Frieslanders (10), the deletion of Phe at codon 53 or 54 in Japan (11); and deletion of residues 487–489 in East Asia (35). The fact that founder effects can be manifested in a country with great ethnic heterogeneity also suggests a high coefficient of inbreeding in local areas, even though known consanguinity occurred in only 6 of our 19 families (32%). Recurring mutations have also been identified in Brazilian series of 3 other autosomal recessive disorders, namely 17β-hydroxysteroid dehydrogenase 3 deficiency (36), 5α-reductase deficiency (37), and 21-hydroxylase deficiency (38), and these observations are consistent with a high rate of inbreeding in some populations.

Analysis of 6 intronic polymorphisms found within *CYP17* itself (www.pga.swmed.edu) provided further evidence of founder effects. All homozygotes for mutations W406R and R362C were homozygous at all 6 positions, including the rare C and T variants at g.3274 (intron 2) and g.7028 (intron 7), respectively. Among 42 family members of subjects bearing mutation W406R, 4 relatives (heterozygotes or wild type at W406) were also heterozygous for C and A at the adjacent polymorphism in the 3′ end of intron 6 (g.6787), but all W406R homozygotes had only the C variant. We discovered another polymorphism in intron 6 at g.4617, C or the T found in the GenBank sequence, adjacent to the R362C mutation in exon 6. All R362C homozygotes had 2 copies of the C variant, whereas 8 of 29 wild-type and heterozygous relatives studied possessed 1 copy each with C and T. The patterns at these polymorphisms associated with both mutations R362 and W406R were identical, consistent with our suggestion that the 2 mutations arose in similar genetic backgrounds. Compound heterozygotes for R362C and W406R were also homozygous at all polymorphisms. Finally, both copies of intron 7 from the subject homozygous for P428L contained 2 additional C's near the 3′ end, and the heterozygous relatives

TABLE 5. Clinical and hormonal characteristics of the W406R and R362C homozygotes

Parameter[a]	W406R (n = 11)	R362C (n = 7)	Normal range[b]
Systolic BP (mm Hg)	140 (113–175)	159 (130–200)	90–130
Diastolic BP (mm Hg)	100 (75–125)	109 (90–130)	60–85
Serum potassium (mEq/L)	3.1 (1.9–3.6)	3.5 (2.8–3.9)	3.8–5.0
Plasma steroids (ng/dL)			
Testosterone			
46,XY	11.2 (2–20)	36 (13.7–48)	<50
46,XX	31 (12.5–65)	26 (13–35)	<50
Estradiol			
46,XY	1.8 (0.5–3.1)	0.9 (0.8–1.0)	<3
46,XX	1.0 (0.1–2.2)	1.0 (1.0)	<3
Deoxycorticosterone			
Pre	384 (216–504)[c]	240 (132–376)	4–12
Post	516 (307–737)[c]	338 (249–400)	12–61
Corticosterone[d]			
Pre	19.5 (12.1–32.9)	16.1 (8.9–24.0)	0.1–0.5
Post	25.0 (14.3–43.6)	27.9 (18.6–44.4)	1.7–4.8
18OHDOC[e]			
Pre	337 (162–561)	361 (153–704)	0–10
Post	422 (198–588)	585 (300–1045)	56–158
18OHB[f]			
Pre	331 (181–635)	303 (169–436)	10–35
Post	399 (190–616)	429 (196–855)	84–174

[a] Mean values (ranges) for blood pressures (BP) and serum potassium are averages of at least three measurements. Normal BP values in adolescents are less than 5 mm Hg lower.

[b] Reference values from Nichols Institute and from Kater *et al.* (25a); reference values for testosterone and estradiol are given for prepubertal children. Conversion factors for Systemme International units are same as in Tables 1a and 1b.

[c] $P < 0.01$ W406R *vs.* R362C.

[d] Corticosterone values in micrograms per deciliter.

[e] 18-Hydroxydeoxycorticosterone.

[f] 18-Hydroxycorticosterone.

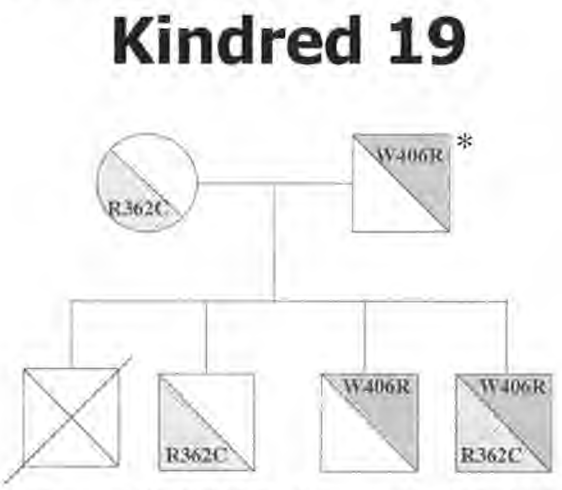

FIG. 5. Genetics of kindred 19. The father was born in Spain (*asterisk*) and carried mutation W406R (*shaded*). The mother, of Portuguese descent, was heterozygous for mutation R362C (*stippled*). One offspring was a compound heterozygote for both mutations and had complete, combined 17OHD.

exhibited 1 copy of each variant. Based on the uniform patterns of polymorphisms, we scored 1 allele for each homozygote in Table 4 (28 total), although the true number may be as high as 32.

Mutations W406R and R362C

Previously, only 2 Brazilian patients with 17OHD had been characterized by molecular genetics, and both were cases of isolated 17,20-lyase deficiency (18). In this study, 7 *CYP17* mutations not previously described were identified in 19 families; 2 of these (W406R and R362C) are responsible for 82% of the affected alleles. Patients affected by W406R and R362C mutations are distributed in south/southeast and northeast Brazil, respectively, consistent with separate founder effects in these 2 regions. The majority of subjects with W406R and R362C mutations are of Spanish or Portuguese descent, respectively. In contrast, among the nearly 40 currently reported mutations in the *CYP17* gene (4–9), none is of Spanish or Portuguese origin. This discrepancy with our data may be due to the higher prevalence and greater awareness of other conditions with similar clinical features as 17OHD (16), leading to underdiagnosis of 17OHD worldwide. Mutation W406R is now the most common genetic defect known to cause 17OHD. Given the high prevalence of mutations W406R and R362C, neither of which alters a restriction site, we now screen new Brazilian patients with 17OHD with a single 1.5-kb PCR reaction spanning exons 6 and 7 using primers I5S1 and I7AS1, sequencing one or both exons depending on the family's ethnic background.

The only phenotypic feature that was significantly different between homozygotes for mutations W406R and R362C was the higher DOC concentration found in subjects with mutation W406R, yet mean blood pressure and serum potassium concentrations did not differ in the two groups. When these two mutations were expressed in COS-7 or HEK-293 cells and in yeast, we consistently found no residual enzymatic activity for either mutation. It is therefore unlikely that any clinical divergence found in these two groups can be explained by differences in the enzymatic properties of the

CYP17 mutations alone. Regional or ethnic preferences in dietary sodium content as well as other genetic differences related to ethnic origin may influence the typical features of subjects with these common mutations.

Although most subjects homozygous for mutations W406R and R362C presented with hypertension, hypokalemia, and sexual infantilism (13), phenotypic variations were occasionally observed within each group. Among 3 46,XY homozygotes for mutation R362C, 1 presented at birth with ambiguous genitalia, a second had female external genitalia but normokalemia at diagnosis, and the third demonstrated the classical, complete 17OHD syndrome. Analogously, the appearance of the external genitalia can vary among genetically male siblings with the same mutation in the androgen receptor gene (39). None of the 4 46,XX patients manifested any degree of spontaneous sexual development, and all were hypertensive. All 11 homozygotes for mutation W406R remained sexually infantile, yet 1 subject was consistently normotensive for up to 2 yr of continuous follow-up before the diagnosis of 17OHD despite concurrent, marked hypokalemia. Testosterone concentrations varied within the prepubertal range where this RIA lacks accuracy, limiting the significance of these data. We conclude that environmental and other genetic factors may modulate the phenotypic features of patients with severe 17OHD. These genetic modifier loci might influence steroid production and action by altering the activity of transcription factors (40, 41), the CYP17 cofactor proteins CPR and cytochrome b_5 (29), or downstream mediators of mineralocorticoid, androgen, and estrogen action.

Other mutations and partial 17OHD

The homozygote for a TAG nonsense mutation at tyrosine 329 in exon 6 had refractory hypertension in childhood. A TAA stop codon at the same position has been previously reported in a compound heterozygote from Japan, and this patient also had severe clinical manifestations (42). Curiously, our subject who is a compound heterozygote for mutations W406R and M1T, like a previously reported case bearing mutation M1I (43), presented with hypokalemic myopathy and serum potassium values as low as 1.0 mEq/liter. The researchers suggested that CYP17 protein translation from mutation M1I might begin at methionine 49, which would yield an inactive protein (43), but it is not known whether a truncated protein is produced from mutation M1I or M1T, or if this process can contribute to potassium wasting.

Partial 17OHD has been reported in women with normal or abnormal menstrual cycles and breast development as well as in males with incomplete virilization (14, 44, 45). In addition, approximately 10–15% of subjects with the diagnosis of 17OHD are normotensive and/or normokalemic at diagnosis (16), although few of these individuals have been genotyped (4). Our 46,XX compound heterozygote for Y329D and the AG to CG substitution in intron 2 had spontaneous Tanner stage 5 breast development at puberty. The AG to CG mutation should alter RNA splicing and introduce a frameshift that yields a truncated, inactive enzyme (See companion paper, Ref. 45a), yet some correctly spliced transcripts may be produced from this allele as well. In addition, mutation Y329D retains approximately 5% of the catalytic activity when expressed in heterologous systems, but the protein is unstable and readily degraded. Although *in vitro* experiments do not necessarily reflect true *in vivo* conditions, as little as 5–8% of 17,20-lyase activity may be sufficient to promote secondary sexual development (2, 11), particularly in genetic females due to the potency of estradiol (46).

In contrast, the phenotype of our 46,XY homozygote for mutation P428L was complete, combined 17OHD, despite some residual activity of the mutant enzyme in heterologous expression experiments. The carboxyl terminus of CYP17 is important for both heme and substrate binding (47), and even small C-terminal alterations can destroy most (48) or all (10, 12, 49) enzyme activity. Mutations closer to the heme at C442 tend to destroy all activity (6, 26), although mutation R415C retains some activity (8). Both our homozygote for P428L and our heterozygote for Y329D developed hypertension and hypokalemia, indicating that the low residual activities of P428L and Y329D are insufficient to prevent mineralocorticoid excess. Consequently, the threshold CYP17 activity level to yield atypical phenotypic features in 17OHD appears to be lowest for pubertal breast development in 46,XX patients, which reflects sufficient C_{19} steroid production, driven by high precursor concentrations, via a defective CYP17 enzyme. In contrast, 17-deoxysteroid metabolism to cortisol is impaired by the low adrenal 17α-hydroxylase activity, such that 17-deoxysteroids with mineralocorticoid activity accumulate universally in 17OHD. Nonetheless, differences in target tissue sensitivity to these mineralocorticoids may account for the variability in hypertension and hypokalemia seen among individuals with similar genotypes, but the rare examples of partial virilization in subjects with mutant proteins that have no activity in heterologous systems remain enigmatic.

Structural basis of protein instability and phenotype variability

To understand how the four missense mutations (W406R, R362D, P428L, and Y329D) cause partial or complete loss of enzymatic activity, we located the mutated residues in a computer model of human CYP17 (47). Residue Y329 lies in the middle of the J helix and appears to form a hydrophobic packing interaction with L460 on the C-terminal end of the adjacent L helix (Fig. 6, A and B). The substitution of a charged aspartate at position 329 weakens this hydrophobic interaction and destabilizes the enzyme structure, but does not directly perturb the active site. Residues R362, W406, and P428 all lay within a contiguous three-dimensional space in a region of the protein that is critical for heme binding and proper folding. Residue R362 comprises part of the ExxR motif at the C terminus of the K helix, a motif present in all known cytochromes P450 (50). The serpiginous chain of residues that follows and leads to the heme-liganding cysteine (C442) tends to unwind the K helix, but hydrogen bonding between the adjacent E and R residues in this motif stabilizes this structure and helps to form the redox partner binding site (47, 51) (Fig. 6C). W406 abuts R362 from its position at the start of the meander region (Fig. 6D), another conserved motif that precedes the heme-binding decapeptide. Two

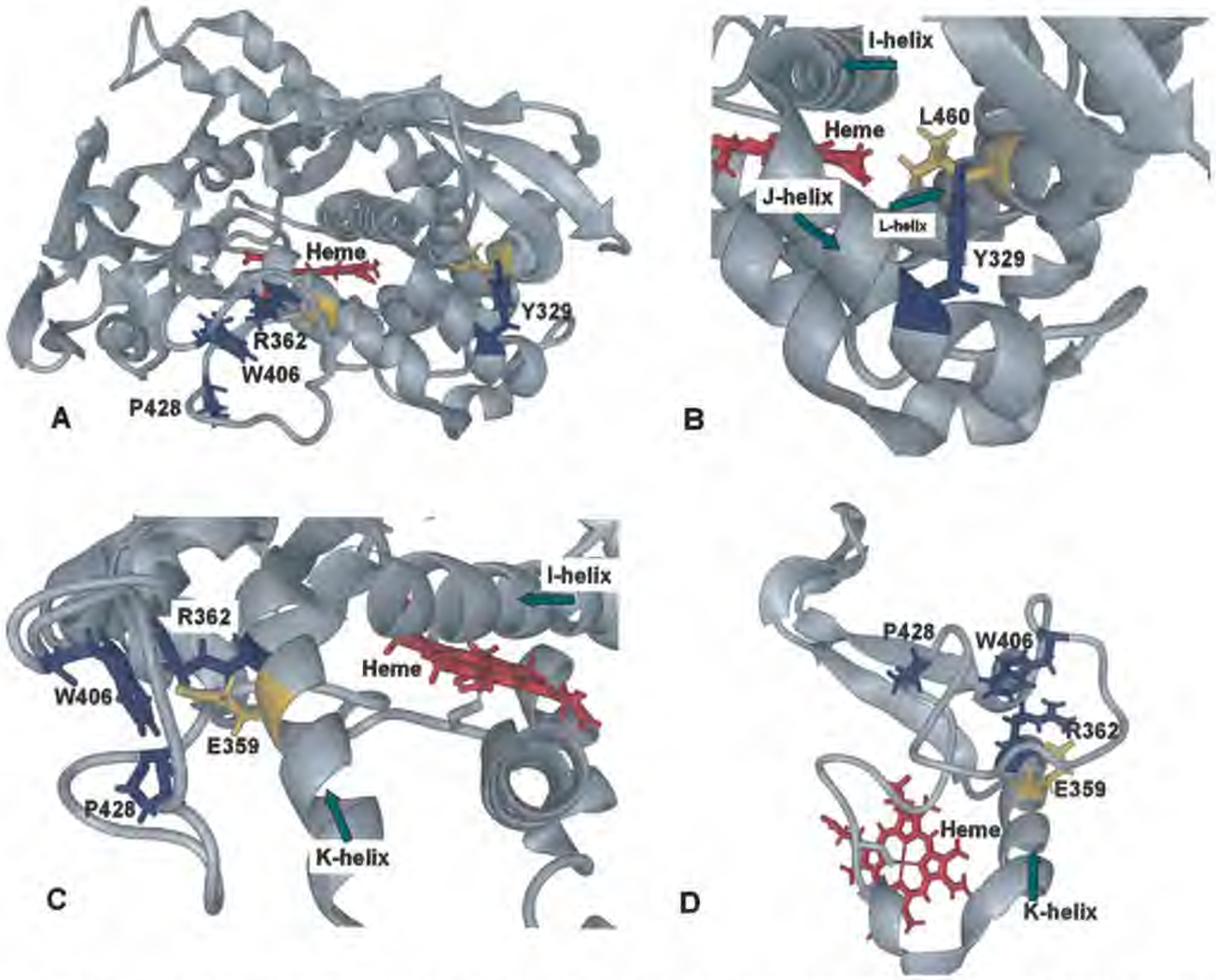

FIG. 6. Computer model of human CYP17, highlighting locations of missense mutations Y329D, R362C, W406R, and P428L. A, Ribbon diagram of protein backbone, with heme (*red*) and all atoms of mutated residues (*blue*) displayed and labeled. Also shown in *yellow* are residues E359 and L460, which interact with R362 and Y329, respectively. B, Hydrophobic interaction of Y329 (*blue*, aromatic ring viewed edge-on) in J helix with L460 (*yellow*) in L helix. *Arrows* indicate directions of I, J, and L helices. C, Proximity of mutated residues R362, W406, and P428 (*blue*). Residue E359, the hydrogen-bonding partner of R362, is shown in *yellow*, and the directions of the I and K helices are indicated. The front clipping plane has been recessed for clarity. D, Redox partner binding surface of human CYP17 viewed face-on with only backbone atoms of residues 347–447 displayed, plus all atoms of heme (*red*), E359 (*yellow*), and mutated residues R362, W406, and P428 (*blue*). The hydrogen bonding of E359 and R362 maintains the integrity of the K helix, and W406 and P428 lay adjacent to R362 in the serpiginous chain of residues connecting the K helix with the heme-binding decapeptide. Images were generated with MidasPlus software on a Silicon Graphics (Mountain View, CA) Octane workstation using CYP17 structure 2c17 (www.rcsb.org).

CYP17 mutations that change residues in or near the meander domain are F417C (52) and P409R (6), and both of these mutations completely destroy enzyme activity (26). The W406R mutation juxtaposes two positively charged arginine residues, which would weaken hydrogen bonding within the ExxR motif. Thus, it is remarkable that the two common Brazilian mutations derived from different ethnic backgrounds alter two residues that lie adjacent in the protein structure, and these two mutations appear to destabilize the enzyme by a common mechanism. Mutation P428L probably impairs heme incorporation directly, rather than by disrupting hydrogen bonding in the ExxR motif.

Our genetic and biochemical studies suggest that the missense mutations described in this study, and perhaps most mutations that cause severe 17OHD, primarily impair enzyme activity by destabilizing enzyme structure. This hypothesis is supported by the lack of spectroscopically demonstrable P450 (CO-reduced or substrate-induced difference spectra) in CYP17 mutations studied in this and previous (26, 53) reports. We postulate that intracellular factors may variably enhance the stability of mutant CYP17 proteins within the adrenals and/or gonads of 17OHD individuals, which may allow for transient maintenance of some enzyme activity in rare cases when enzyme expression

is maximally induced despite the lack of demonstrable activity in heterologous expression systems. This paradigm may also explain how high doses of gonadotropins enabled oocyte development leading to *in vitro* fertilization in one female with complete 17OHD (54). Therefore, the activities of CYP17 mutations in heterologous expression systems aid in defining the molecular basis of 17OHD, but additional undefined factors may still modify the clinical features in individual subjects.

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Urological Oncology

The use of C17,20-lyase inhibitors in prostate cancer is described by authors from Los Angeles. These agents suppress the generation of testosterone and potentially active androgenic precursors, perhaps reversing castration resistance. Abiraterone is an orally bioavailable lyase inhibitor structurally related to pregnenolone, and is currently under clinical assessment.

Another paper is presented by the same group of authors in Los Angeles addressing some of the controversies about the continual need for the traditional radical open surgical management of RCC, and evaluates the oncological principles which ensure the persistent need for this approach.

Selective blockade of androgenic steroid synthesis by novel lyase inhibitors as a therapeutic strategy for treating metastatic prostate cancer

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THE NEED FOR NOVEL AGENTS TO TREAT PROSTATE CANCER

Prostate cancer is the most common malignancy in Western societies and the second most common cause of male cancer-related death in the UK and USA, accounting for ≈12% of all male cancer-related deaths [1,2]. When confined to the prostate gland, the disease is curable with local therapy (radical prostatectomy, external beam radiotherapy, brachytherapy or cryotherapy). However, despite the use of PSA for screening, in 15–33% of men local therapy fails and they develop incurable metastatic disease [3,4]. Activation of the androgen receptor (AR) by androgenic steroids including testosterone and its more potent 5α-reduced metabolite, 5α-dihydrotestosterone (DHT), regulates the transcription of a diverse range of target genes involved in prostate cell proliferation, differentiation, and apoptosis [5]. Androgen deprivation by medical or surgical castration remains the mainstay of treatment and >90% of men with metastatic prostate cancer initially respond rapidly and often dramatically to castration, with improvements in bone pain, regression of soft tissue metastasis, and steep declines in PSA level. However, the duration of response is frequently short (12–33 months) and in almost all patients is followed by the emergence of castration-resistant prostate cancer (CRPC) that, untreated, is invariably fatal within 9–12 months [3]. Systemic chemotherapy with docetaxel for patients with CRPC confers a modest survival advantage (2–3 months) and is effective for palliating symptoms [6], but the duration and rate of response is limited. Other chemotherapy, e.g. mitoxantrone, has not been shown to improve survival but might help with symptom control [6]. There is an urgent need for new agents that provide palliation and improve survival.

Continued androgen-dependent activation of a 'hypersensitive' AR in castrated patients secondary to AR gene amplification [7], mutations in the AR gene [5], increased AR expression [8], or alterations in AR co-repressor/co-activator function [9] appear to be important mechanisms of castration resistance. These could be reversed by suppressing circulating androgens, inhibiting the binding of biologically active androgenic steroids to the ARs or disrupting AR

generation with drugs such as HSP90 inhibitors. This review discusses the rationale behind using selective lyase inhibitors (e.g. abiraterone acetate, also known as CB7630), to suppress circulating androgens.

TARGETING THE AR

The benefit of androgen deprivation therapy (ADT) was first reported by Huggins and Hodges in 1941 [10] when surgical castration was found to give symptomatic benefit and in some cases, complete responses in metastatic prostate cancer. The role of ADT by castration has since expanded to the neoadjuvant and, more commonly, the adjuvant settings, and to patients with a high PSA level but no clinical or radiological evidence of metastatic disease [3]. Medical castration with LHRH agonists (e.g. goserelin and leuprorelin) is often a more popular alternative to orchidectomy. These act by continuous stimulation of the anterior pituitary gland, resulting in inhibition of LH secretion and suppression of testicular androgen synthesis. Antiandrogens (either steroidal, e.g. cyproterone acetate, or nonsteroidal, e.g. bicalutamide or flutamide) prevent androgen binding to the AR. These can be used in combination with LHRH analogues to inhibit binding of residual androgens to the AR to attempt 'maximum androgen blockade' [3]. However, this combined strategy has not significantly prolonged the survival of patients with advanced prostate cancer [3]. In addition, up to 30% of patients show a decrease in PSA level after stopping AR antagonists [11]. This can partly be explained by the development of AR gene mutations or increased AR expression that might cause AR antagonists to behave as weak agonists [7,8]. For many years, oestrogens, including diethylstilbestrol, were the primary medical treatment for metastatic prostate cancer, until they were superseded by LHRH agonists [3]. Now they have a role in treating castrated patients in whom castration and antiandrogen therapy have failed, but although PSA response rates in a series of phase II trials were 21–80%, the median response duration is <6 months and the concomitant use of prophylactic warfarin has not eliminated the risk of thromboembolic events [12].

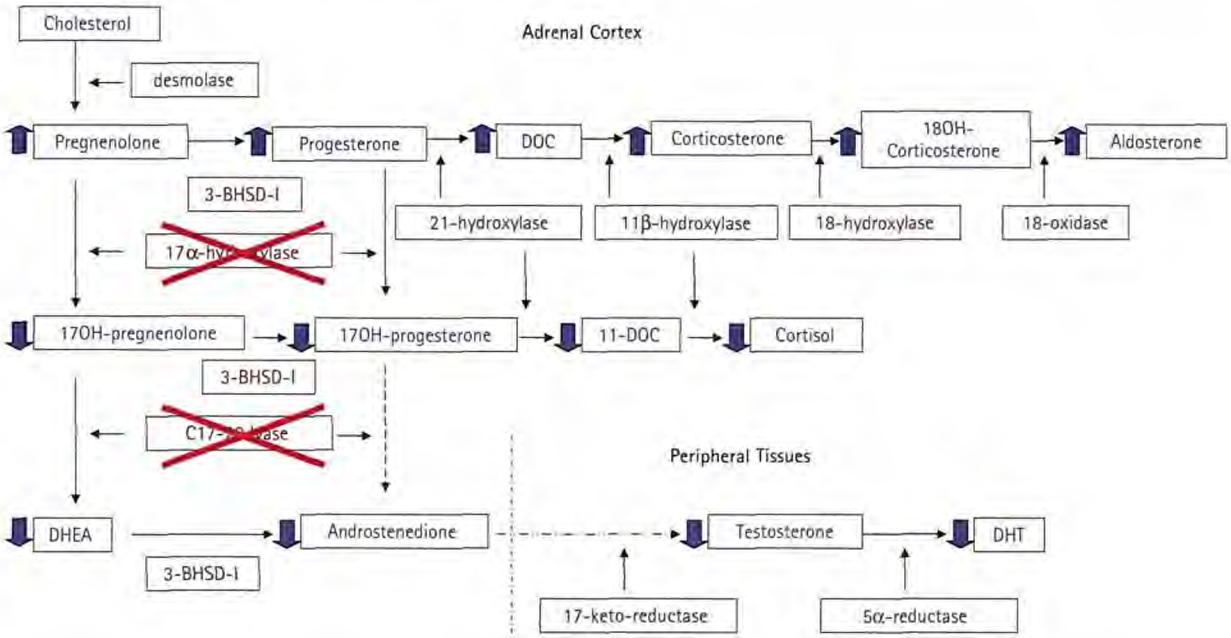
The presence of AR protein expression and significant AR mRNA levels in tumour samples from patients with CRPC strongly suggest that prostate tumours evolve mechanisms to reactivate AR signalling and AR-responsive pathways after ADT [7,13]. This is supported by preclinical models, which have suggested that, as prostate cancer cells become castration resistant (otherwise, but less appropriately known as hormone- or androgen-resistant), they acquire the ability to grow in the presence of low levels of androgens (equivalent to castrate), by the up-regulation of AR expression [8]. It was also reported that, despite castration, intraprostatic levels of testosterone and DHT in CRPC might remain sufficient to maintain tumour growth [14]. The source of these androgens is unclear, but altered regulation of enzymes involved in the synthesis and inactivation of androgens might be one cause of their accumulation. In support of this, increased expression of enzymes involved in androgen synthesis were reported in prostate cancer cells acquired from biopsies of CRPC [13,15]. These data suggest the possibility of endogenous production of steroids by CRPC. Overall, these reports indicate that the currently used ADTs fail to reverse AR signalling in CRPC and support the use of novel drugs that target the AR directly or indirectly by suppressing the generation of ligands.

Plasma testosterone is not completely suppressed by castration, in part because of peripheral conversion of adrenal androgenic steroids to testosterone by 17-ketoreductase [3]. Adrenal androgen synthesis can be inhibited by targeting the hypothalamo-pituitary-adrenal axis or by inhibiting key enzymes involved in adrenal steroid biosynthesis. Suppression of the hypothalamo-pituitary-adrenal axis and consequently the generation of adrenal androgens by low-dose steroids has not been unequivocally shown to occur in patients with CRPC, but it could be one explanation for their anti-neoplastic activity [16,17]. However, ketoconazole, an imidazole antifungal agent that weakly and non-selectively inhibits several cytochrome P450 enzymes involved in adrenal steroid synthesis, induces a transient PSA response in 20–30% of patients with CRPC [11]. Interestingly, patients who respond to ketoconazole and subsequently progress show a significant decrease, associated with an increase with disease progression of adrenal androgens (dehydroepiandrosterone (DHEA) and androstenedione) suggesting that ketoconazole resistance is caused by adrenal androgens [11]. These clinical results lend credence to the inhibition of the adrenal steroid synthesis pathway as a therapeutic strategy.

THE ROLE OF CYTOCHROME P450c17 IN ANDROGEN BIOSYNTHESIS

The two sites thought to produce most of the androgenic steroids in humans are the testis and the adrenal cortex. The principal enzymatic reaction in steroid biosynthesis involves cleavage of a six-carbon group from cholesterol by CYP450ssc (desmolase) [18]. A series of subsequent reactions catalysed by members of the cytochrome P450 family then produce the glucocorticoid and mineralocorticoid 21-carbon steroid hormones vital to human survival (Fig. 1). CYP450c17 is a single microsomal enzyme, encoded for by the single human CYP17 gene, that catalyses the two independently-regulated steroids 17α-hydroxylase and $C_{17,20}$-lyase activities needed to produce the 19-carbon precursors of the sex steroids in both the adrenal cortex and the testis [18]. The 17α-hydroxylase activity typically converts pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone, and the $C_{17,20}$-lyase activity converts 17α-hydroxypregnenolone to DHEA and 17α-hydroxyprogesterone to androstenedione (Fig. 1). The $C_{17,20}$-lyase activity is roughly 50 times more efficient for converting 17α-hydroxypregnenolone to DHEA than for converting 17α-hydroxyprogesterone to androstenedione [18,19]. The products of steroid biosynthesis, androstenedione and DHEA, are weak as androgens but in the testis can be converted to testosterone by the enzyme 17α-hydroxysteroid dehydrogenase [20]. Castration blocks testosterone from this source but does not prevent the synthesis of adrenal androgens. These androgens might be a clinically important source of androgenic steroids for activating the AR on prostate cancer cells. Steroidogenesis in the human adrenal is divided into three morphologically and functionally distinct zones. The zona glomerulosa, located just below the adrenal capsule, does not express CYP450c17 and produces the 17-hydroxy 21-carbon steroid aldosterone, the principal mineralocorticoid, under the regulation of angiotensin II [21]. The middle layer, the zona fasciculata, expresses CYP450c17 and has abundant 17α-hydroxylase but very little $C_{17,20}$-lyase activity, and produces the 17-hydroxy 21-carbon steroid cortisol, the principal glucocorticoid,

FIG. 1. The impact of abiraterone on the adrenal steroid synthesis pathway. Abiraterone inhibits CYP450c17 17α-hydroxylase and $C_{17,20}$-lyase activity (crossed out in red) and suppresses androstenedione, DHEA and their androgenic precursors (blue arrows). Suppression of cortisol and its precursors causing a compensatory rise in ACTH and excess synthesis of aldosterone and its precursors is predicted (blue arrows).



under the regulation of adrenocorticotropic hormone (ACTH) corticotrophin [21]. The inner zona reticularis, which does not become morphologically identifiable until the onset of adrenarche, expresses CYP450c17 and has both 17α-hydroxylase and $C_{17,20}$-lyase activities, and thus produces 17-hydroxy 19-carbon precursors of sex steroids [18,21]. The $C_{17,20}$-lyase activity of human CYP450c17 is enhanced by serine phosphorylation of CYP450c17 and by the presence of cytochrome b5. The expression of cytochrome b5 increases in the adrenal zona reticularis at the onset of adrenarche [18].

Congenital deficiencies in CYP450c17 are a rare form of congenital adrenal hyperplasia in which not only adrenal but also gonadal steroidogenesis is impaired [19]. This results in abrogation of gonadal sex steroid production and adrenal biosynthesis of cortisol and androgens. However, corticosterone synthesis is not impaired, and as corticosterone has glucocorticoid properties, patients rarely manifest symptoms of adrenal insufficiency. In fact, rodents lack CYP450c17 and use corticosterone as their principal glucocorticoid [19]. However, because corticosterone is a weaker glucocorticoid than cortisol, abnormally high corticosterone production is necessary before feedback inhibition of pituitary ACTH secretion occurs, establishing a new steady state. To produce enough corticosterone to compensate for the absence of cortisol, more intermediate steroids might be generated. These include progesterone, 11-deoxycorticosterone (DOC), 18-hydroxycorticosterone and 19-nor-DOC. This ACTH-driven overproduction of mineralocorticoids often leads to hypertension, a characteristic presenting feature of this disease, usually in early adulthood [19]. At diagnosis, sexual infantilism in 46 XX females and ambiguous genitalia in 46 XY males is usually manifest and laboratory investigations will often find hypokalaemia [19]. An extremely rare disorder is isolated $C_{17,20}$-lyase deficiency caused by mutations that destroy most $C_{17,20}$-lyase activity but preserve most 17α-hydroxylase activity. Patients do not have mineralocorticoid excess but show the consequences of absent sex steroids [19], so CYP450c17 is a logical target for the development of new drugs to treat CRPC. The most potent and selective inhibitor of CYP450c17 currently in clinical studies is abiraterone acetate.

PRECLINICAL DEVELOPMENT OF ABIRATERONE ACETATE

The serendipitous discovery that some esters of 4-pyridylacetic acid are effective inhibitors of the hydroxylase-lyase enzyme in rat testis [22] led to the study of a variety of esters of 3- and 4-pyridylacetic acid and their α-alkylated derivatives on human testicular 17α-hydroxylase/$C_{17,20}$-lyase [23,24]. The most potent inhibitors of human testicular 17α-hydroxylase/$C_{17,20}$-lyase had, as a common structural feature, the 17-(-3-pyridyl) substituent that contains nitrogen capable of forming a co-ordinate bond with the haem iron of the enzyme (Fig. 2) [23,24]. The 3-pyridyl substituent results in a several orders more potent inhibition of CYP450c17 than the 2-pyridyl and 4-pyridyl substituents [23]. The double bond in the 16,17-position of the steroidal skeleton is essential for the irreversible inhibition of CYP450c17 [25]. Two compounds, CB7598 (abiraterone), which is closely related structurally to the natural substrate pregnenolone, and CB7627, were identified as the most potent, and were selected for further development at the Institute of Cancer Research in London. With a Ki_{app} of <1 nM, both compounds were

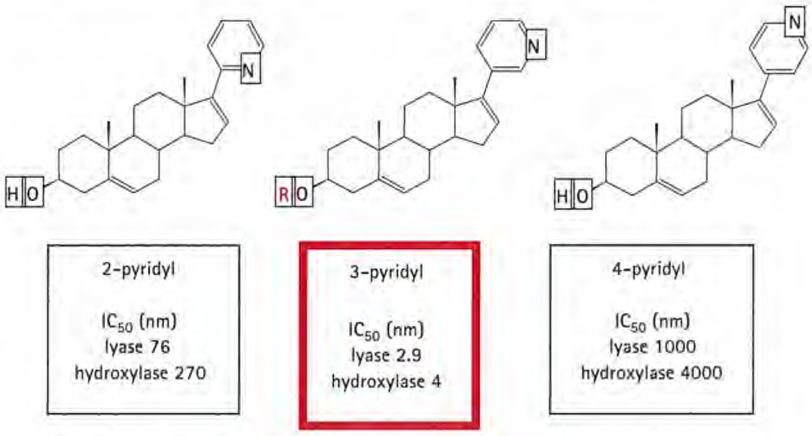
TABLE 1 Summary of phase I evaluation of abiraterone acetate

Variable	Phase I study patient population		
	Single dose in castrate men	Single dose in intact men	Continuous dosing (12 days) in intact men
Number of patients	16	4	6
Dose levels, mg	10, 30, 100, 200, 500	200, 500	500, 800
Testosterone level at inclusion, nmol/L	<2	>9	>9
Effect on testosterone (T)	Target suppression at 500 mg	Suppression with nadir on second day, recovery 6–9 days.	Suppression with nadir on first to third day and sustained for up to 9 days
Effect on androstenedione	Same as for T	Same as for T	Same as for T
Effect on LH	Suppressed at inclusion	Maximal rise on third day, recovery by 10th day	Maximal rise on third day

several times more potent inhibitors of rat hydroxylase/lyase activity than ketoconazole [23,24]. Unlike CB7627, abiraterone was a highly selective inhibitor of CYP450c17 and so was chosen as the main candidate for further development [24]. However, due to poor bioavailability and a susceptibility to hydrolysis by esterases, a prodrug for abiraterone was sought [26,27]. The amide was a several orders less potent inhibitor of hydroxylase/lyase [24] but the 3β-*O*-acetate form (abiraterone acetate, CB7630) (Fig. 2) was resistant to esterases and *in vivo* was rapidly deacetylated to abiraterone resulting in potent inhibition of CYP450c17 [26,27].

When adult mice given abiraterone acetate or ketoconazole i.p. daily for 14 days were compared to a control group dosed with vehicle alone, abiraterone significantly reduced plasma testosterone concentrations for at least 24 h despite a four-fold increase in LH concentrations [27]. This was associated with reduced weights of the ventral prostate (48%) and seminal vesicles (87%), compared to the controls. The kidneys and testis were also reduced in weight by 37% and 62%, respectively [27]. There was no weight loss in the androgen-dependent organs of the group given ketoconazole [27]. There was a marked increase in the weight of the adrenal glands in the group administered ketoconazole, but no change in the control group or in the animals dosed with abiraterone, indicating no inhibition of corticosterone production by abiraterone. Animal toxicology studies found no effects on haematological, biochemical or renal function variables and only mild toxicities. Abiraterone acetate was therefore considered safe enough to be studied in humans [27].

FIG. 2. The structure of abiraterone and abiraterone acetate (centre). The effect variations in the orientation of the pyridyl substituent have on the potency of 17α-hydroxylase/$C_{17,20}$-lyase inhibition.



R = H: Abiraterone (CB7598); 17-(3-pyridyl)androsta-5,16-dien-3β-ol

R = Ac: Abiraterone acetate (CB7630)

CLINICAL EVALUATION OF ABIRATERONE ACETATE

A series of three phase I studies were performed in men with histologically confirmed prostate cancer who had shown a biochemical (PSA) relapse after two lines of hormone treatment but for whom, at the time of participation, no alternative treatment for symptomatic or progressive disease was considered necessary (Table 1). The first in-human study investigated the effect of a single dose of abiraterone acetate in castrate patients (testosterone confirmed <2 nmol/L, 58 ng/mL) with prostate cancer in whom treatment with antiandrogens had failed.

Nine patients were treated in groups of three at doses of 10, 30 and 100 mg. At these doses, the plasma level of abiraterone was below the level of detection and there was no consistent effect on testosterone [28]. One patient was then given 200 mg and six of them 500 mg. There were detectable plasma levels of abiraterone at doses of >200 mg [28]. Abiraterone at 500 mg suppressed testosterone concentrations, to <0.14 nmol/L (4 ng/mL), or by 75% when baseline testosterone levels were >0.6 nmol/L (20 ng/mL), and androstenedione concentrations. Suppression was sustained from the second to the fifth day after abiraterone acetate. There was no corresponding suppression of

17α-hydroxyprogesterone. This first study was followed by a second investigating the effect of a single dose of abiraterone acetate in intact men with prostate cancer who had previously been treated with an antiandrogen and an LHRH agonist. One patient was given 200 mg, but the testosterone level was not suppressed. Three patients were then given 500 mg, and in all three there was a reduction in testosterone level of more than half from baseline. The testosterone nadir was on the second day after therapy, with recovery to pretreatment levels 6–9 days later. There was a corresponding compensatory surge in LH levels, maximal on the third day, with recovery to pretreatment levels by the 10th day. There was no change in cortisol levels in either study [28]. The third and last in this series of phase I evaluations investigated 12-day continuous dosing in intact men with prostate cancer who had previously received antiandrogens and LHRH agonists. The duration of drug treatment was limited by a lack of availability of the drug. Three patients were given 500 mg daily and another three were given 800 mg daily. The testosterone level was suppressed after the first day of dosing and was sustained for 3 days, and then reversed by a rise in LH. The patients given 800 mg appeared to show greater suppression of testosterone but as in the 500 mg cohort, the effect of abiraterone was insufficient to offset the rise in LH. All six patients had a reduced cortisol response to ACTH stimulation on the 11th day after dosing, suggesting reduced adrenocortical reserve. There was a mild reduction in evening cortisol levels also in the three patients given 800 mg but there were no clinical manifestations of adrenocortical insufficiency [28]. Pharmacokinetic studies suggested good oral bioavailability at doses of >200 mg. The mean (SD) T_{max} for abiraterone was 2.70 (2.71) h with an elimination half-life of 27.6 (20.17) h, supporting once-daily oral dosing. There was an inter-patient 10-fold variation in the area under the curve for a given dose, making analysis of dose-dependent pharmacokinetic relationships difficult. Several factors could have caused this wide level of variation. As with all oral drugs, absorption might have been altered by residual food in the stomach despite a 2-h fast, by intrinsic inter-individual differences in upper gastrointestinal pH, by concomitant medication(s) effecting gastric pH, or by variable first-pass hepatic metabolism. Abiraterone acetate was well tolerated, and no serious adverse events or haematological or biochemical changes were reported. Mild mood variations, flushing attacks, testicular discomfort, and headaches were described in individual patients [28].

FUTURE DEVELOPMENT OF NOVEL LYASE INHIBITORS

Several other derivatives of naturally occurring steroidal substrates, including pregnane and androstane, are potent inhibitors of 17α-hydroxylase/$C_{17,20}$-lyase [29,30]. Sa40 (17-(5-pyrimidyl)androsta-5,16-diene-3β-ol) and its 3-acetyl derivative, Sa41, are three times more potent for *in vitro* inhibition of human CYP450c17 than abiraterone and abiraterone acetate, respectively, but have a poorer pharmacokinetic profile in *in vivo* rodent experiments [29]. Clinical assessment in patients is required. L-2 (20-hydroximinio-4,16-pregnadien-3-one), L-36 (17-(3′-pyrazolyl)androsta-4,16-dien-3-one) and L-39 (17-(5′-isoxazolyl)androsta-4,16-dien-3-one) are steroidal compounds that in addition to inhibiting 17α-hydroxylase/$C_{17,20}$-lyase, also inhibit 5α-reductase with a potency similar to finasteride [30]. They also interact with wild-type AR and the mutated AR on cells from the LNCaP cell line [30]. L-36 shows an agonistic interaction whereas L-39 is antagonistic and inhibits *in vitro* LNCaP cell growth [30]. L-39 has therefore been proposed as a candidate for further development. However, the benefit of 5α-reductase inhibitors for treating metastatic prostate cancer is unclear [3]. Although conversion of testosterone to its more active metabolite, DHT, is inhibited, testosterone consequently accumulates and can activate the AR. Also, interaction with the AR might not be desirable as changes in the AR can lead to antagonists behaving agonistically [7,8]. Other nonsteroidal compounds, including TX-977 and its two diastereoisomers, reported to be more potent *in vivo* inhibitors of testosterone synthesis, have been associated with an increase in weight of the adrenal glands in rodent models, suggesting lower specificity of lyase inhibition vs glucocorticoid synthesis [31]. Abiraterone acetate's selectivity for CYP450c17 and its failure to interact with the AR might be preferable for treating patients with CRPC. However, at present, while the available data are encouraging with respect to side-effects and endocrine effectiveness with short-term dosing, there is no evidence of clinical efficacy. A safety and efficacy evaluation of abiraterone acetate administered daily and continuously to castrate men with advanced prostate cancer progressing despite hormone treatment is planned. Concomitant castration is expected to prevent a compensatory LH rise, and sustained, profound suppression of serum testosterone and androgenic precursor levels is predicted. Patients will be closely monitored for the development of glucocorticoid insufficiency or hypertension. Synthesis of corticosterone, a precursor of aldosterone and the primary glucocorticoid in rodents, will not be inhibited by abiraterone and its continued synthesis might prevent clinical manifestations of glucocorticoid insufficiency. In fact, hypertension due to increased ACTH and not glucocorticoid insufficiency is described in congenital CYP45017c deficiency [19]. The results of these studies are keenly awaited.

CONFLICT OF INTEREST

A. Belldegrun: Vice Chairman, Board of Directors and Chairman, Scientific Advisory Board, Cougar Biotechnology.

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Abbreviations: **AR**, androgen receptor; **DHT**, 5α-dihydrotestosterone; **CRPC**, castration-resistant prostate cancer; **ADT**, androgen deprivation therapy; **DHEA**, dehydroepiandrosterone; **DOC**, deoxycorticosterone; **ACTH**, adrenocorticotropic hormone.

THE GENETICS, PATHOPHYSIOLOGY, AND MANAGEMENT OF HUMAN DEFICIENCIES OF P450c17

Richard J. Auchus, MD, PhD

P450c17 commands a central role in human steroidogenesis as the qualitative regulator of steroid hormone flux (Fig. 1). Analysis of P450c17 deficiencies in humans illustrates many aspects of the physiology of steroid biosynthesis and demonstrates poignant features of the genetics and biochemistry of P450c17. 17-Hydroxylase deficiency was first described in patients with sexual infantilism and hypertension.[10] It is now recognized to occur in partial and selective forms with variable phenotypes. This article reviews the genetics and biochemistry of P450c17 as a prelude for understanding the pathophysiology of such deficiencies and approaches to their diagnosis and management.

P450c17 AND *CYP17*

Patients who carry the diagnosis of 17-hydroxylase deficiency harbor alterations in the *CYP17* gene that encodes the P450c17 enzyme. P450c17 actually performs multiple chemical transformations. Human P450c17 17α-hydroxylates Δ^5-pregnenolone and Δ^4-progesterone with roughly equal catalytic efficiency,[3, 35] whereas all other reactions show prominent differences between Δ^5 and Δ^4 substrates. The 17,20-lyase activity is roughly 50 times more efficient for the 17α-hydroxypregneno-

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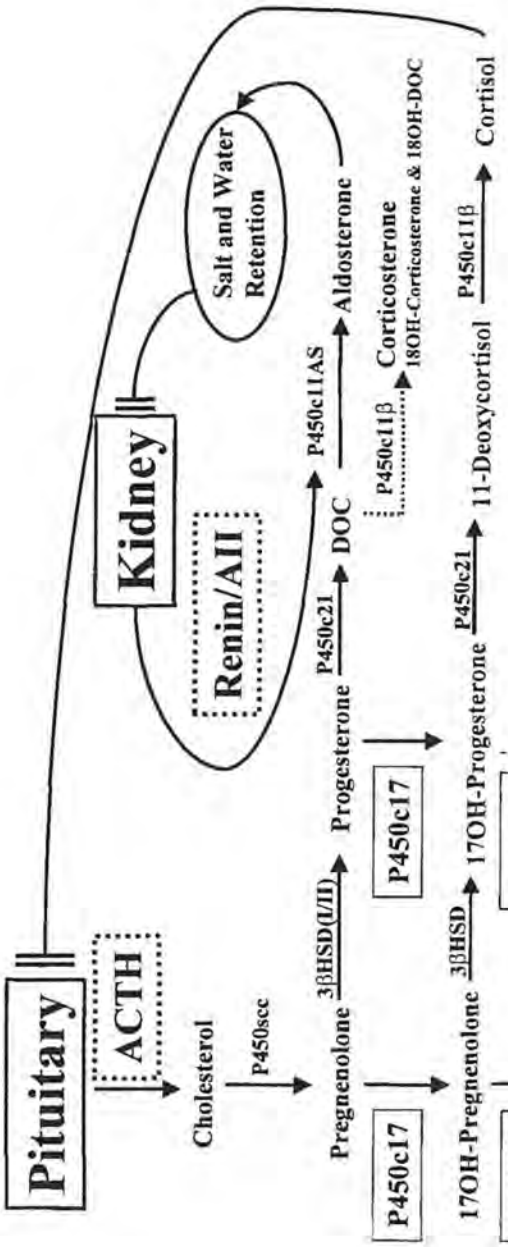

Figure 1. *See legend on opposite page.*

lone-to-dehydroepiandrosterone (DHEA) reaction than for the 17α-hydroxyprogesterone-to-androstenedione reaction.[3, 35] Although the rate of the lyase reaction can be increased more than 10-fold by the addition of cytochrome b_5,[3, 31, 35] the Δ^5 preference persists, and the lyase rate never quite achieves the rate of the hydroxylase reactions. In addition, human P450c17 16α-hydroxylates progesterone but not pregnenolone.[3, 37, 62] In the presence of cytochrome b_5, P450c17 converts approximately 10% of pregnenolone substrate to a Δ^{16} andiene product,[35] which is also formed by porcine P450c17 and acts as a pheromone precursor in pigs.[48] Although experiments to study the chemistry of P450c17 often require certain conditions, such as detergent solubilization that could be considered nonphysiologic, the remarkable consistency of substrate preferences and kinetic constants observed for the modified solubilized P450c17 expressed in *Escherichia coli*,[31, 35] the native P450c17 expressed in yeast microsomes[3] or intact COS-1 cells,[37, 38] and that obtained from human tissues and cells[3, 62] strengthens these conclusions.

One consequence of this Δ^5 preference of human P450c17 for the 17,20-lyase reaction is that the vast majority of sex steroids in humans derive from DHEA as an intermediate. This Δ^5 preference also allows the phenomenon of adrenarche to occur in humans, an event that is characterized by a dramatic rise in adrenal DHEA production that occurs at about age 8 to 10 years,[12, 60] whereas cortisol production remains relatively constant. Adrenarche is an exemplary manifestation of the biochemistry of P450c17, in which the 17α-hydroxylase and 17,20-lyase activities are differentially regulated. In fact, this dichotomy between adrenal 17α-hydroxylase activity, reflected by relatively constant cortisol production, and 17,20-lyase activity, reflected by drastically age-dependent changes in DHEA production, previously suggested that distinct enzymes performed the two transformations; however, later copurification of the 17α-hydroxylase and 17,20-lyase activities of neonatal pig testes suggests otherwise.[47] This controversy was settled when the cDNA for bovine P450c17 was expressed in COS-1 cells, conferring 17α-hydroxylase and 17,20-lyase activities to these nonsteroidogenic cells[77] and proving genetically that the 17α-hydroxylase and 17,20-lyase enzymes

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Figure 1. Major steroidogenesis pathways in humans and feedback loops controlling glucocorticoid and mineralocorticoid production. Ordinarily, cortisol is the major glucocorticoid produced by the adrenal zona fasciculata/reticularis, and cortisol exerts negative feedback inhibition *(double vertical bars)* to regulate pituitary adrenocorticotropic hormone (ACTH) production. Aldosterone is the principal mineralocorticoid of the adrenal zona glomerulosa, and aldosterone synthase (P450c11AS) expression is stimulated by volume depletion, which activates the renin-angiotensin (AII) system, and to a lesser extent, by ACTH. Aldosterone acts to stimulate kaluresis and salt and water retention, which feeds back on the kidney to suppress renin production. The production of corticosterone, a weak glucocorticoid, and of 11-deoxycorticosterone (DOC), a potent mineralocorticoid, is relatively low and unimportant in healthy individuals with intact feedback systems. Note that P450c11β in the zona fasciculata also 18-hydroxylates (18OH) DOC and corticosterone as minor products.

were, in fact, both embodied in a single enzyme, P450c17. Differential regulation of the two principal activities of P450c17 is possible because the abundance of P450-oxidoreductase[37] and the addition[31, 35, 50] or coexpression[3] of cytochrome b_5 preferentially augments the 17,20-lyase activity, and phosphorylation[7, 76] also selectively enhances 17,20-lyase activity. Recent data showing high expression of b_5 in the zona reticularis of monkeys[40] and humans[71] suggest that the developmentally regulated expression of b_5 might be a key event in the genesis of adrenarche in higher primates.

PATHOPHYSIOLOGY

P450c17 deficiencies are a form of congenital adrenal hyperplasia in which not only adrenal but also gonadal steroidogenesis is impaired. In humans, one gene for P450c17 is expressed in the adrenals and gonads[11] instead of two tissue-specific isozymes. A single 2.1-kb mRNA species yields a 57-kd protein in these tissues, and mutations in this gene produce a spectrum of deficiencies in 17-hydroxysteroids and C_{19} steroids. Loss of P450c17 in the adrenal gland impairs cortisol and DHEA production, whereas gonadal deficiency of P450c17 abrogates sex steroid production. The initial description of 17-hydroxylase deficiency was a case in which both 17α-hydroxylase and 17,20-lyase products were absent.[10] When the gene for human P450c17 was cloned,[54] patients with 17-hydroxylase deficiency were found to harbor mutations in the *CYP17* gene,[4, 67] but molecular techniques and subsequent clinical evaluations failed to implicate *CYP17* mutations as the cause of isolated 17,20-lyase deficiency.[73] Recently, three cases of isolated 17,20-lyase deficiency have been confirmed by molecular genetics,[8, 20] demonstrating that amino acid substitution mutations in P450c17 can cause an isolated loss of 17,20-lyase activity.

Combined 17α-Hydroxylase/17, 20-Lyase Deficiency

Loss of P450c17 in the human adrenal gland prohibits the biosynthesis of cortisol and C_{19} steroids. Curiously, the adrenal glands of patients with 17-hydroxylase deficiency are similar to those of rodents, which do not express P450c17,[63] such that rodents rely on corticosterone as their principal glucocorticoid, and their adrenal glands cannot make C_{19} steroids. Patients with 17-hydroxylase deficiency rarely[26] manifest symptoms of adrenal insufficiency owing to sustained corticosterone production. Because corticosterone is a weaker glucocorticoid than cortisol, abnormally high corticosterone production is necessary before feedback inhibition on pituitary corticotropin (ACTH) secretion occurs,[45] establishing a new steady state (Fig. 2). To produce sufficient corticosterone to make up for the absence of cortisol, dramatically elevated quantities of intermediate steroids, such as progesterone and 11-deoxycorticosterone

(DOC), must accumulate, as well as unusual metabolites, such as 18-hydroxycorticosterone[33] and 19-nor-deoxycorticosterone.[23] This ACTH-driven overproduction of mineralocorticoids leads to hypertension, a characteristic presenting feature of this disease. The hypertension usually develops in early adulthood[9] but can present in infancy[15] and can be severe.[46] As is true in other hypertensive disorders caused by mineralocorticoid excess,[39] the hypertension can become fixed if the disease is not treated for many years.[52]

Although the general description given herein is true for most patients with this disorder, considerable variation in phenotype and laboratory findings has been described. These variables include the degree of genital virilization in 46,XY subjects and the capacity for menstruation in 46,XX subjects; the severity of the hypertension and hypokalemia; the aldosterone secretion rate; the type and amount of adrenocortical hyperplasia; the gonadal morphology and histology; and the coexistence of additional disorders, such as 21-hydroxylase deficiency[53] or maternal androgen excess.[14] This heterogeneity has not been completely explained, but many factors, including the severity of the P450c17 deficiency, variations in genes regulating hormone responsiveness, diet (sodium consumption), and environment, undoubtedly contribute. The reader is referred to a detailed discussion of case reports,[72] which is beyond the scope of this article.

Isolated 17, 20-Lyase Deficiency

This disoder is extremely rare because mutations that cause this phenotype must not only destroy most 17,20-lyase activity but preserve most 17α-hydroxylase activity. Patients who are 46,XY present with ambiguous genitalia at birth or with inguinal hernias with or without pubertal delay as adolescents[72] (Table 1). Patients do not show the consequences of mineralocorticoid excess because preserved cortisol production prevents excessive DOC and corticosterone accumulation. Clinical laboratory findings vary considerably owing to the age of diagnosis, the severity of the disease, and the discrepancy between the 17α-hydroxylase and 17,20-lyase activities in a given individual. Nonetheless, C_{19} steroid production is severely, although not completely, impaired, whereas 17-hydroxylated steroid production is nearly or completely normal.

DIAGNOSIS

Unlike forms of congenital adrenal hyperplasia, such as the lipoid type and 21-hydroxylase deficiency, in which glucocorticoid and mineralocorticoid production are impaired, patients with 17-hydroxylase deficiency do not have an adrenal crisis in the postnatal period. Consequently, the diagnosis is often not entertained until hypertension, hypokalemia, or pubertal delay is evaluated during adolescence or early

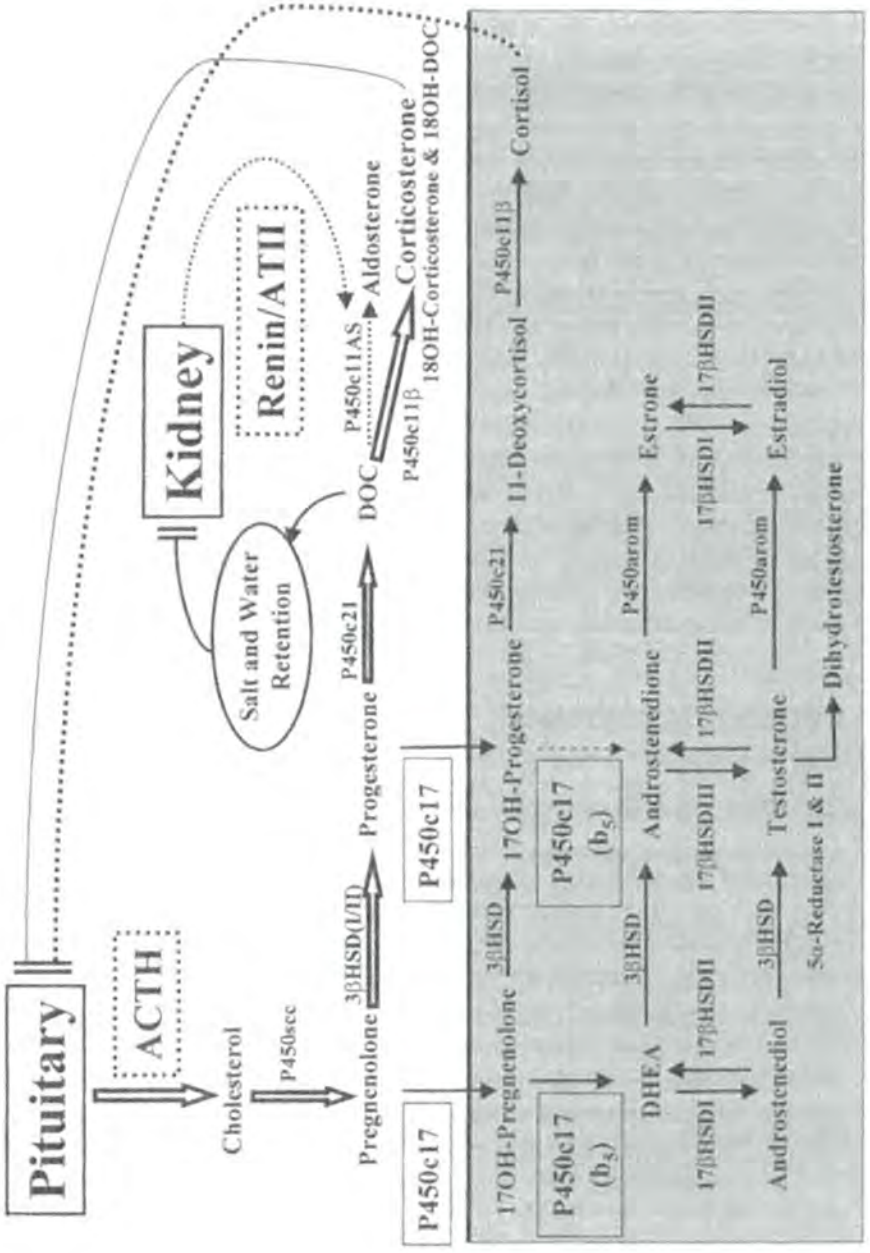

Figure 2. *See legend on opposite page.*

adulthood. Patients with a 46,XY karyotype and incomplete deficiency may be misdiagnosed with androgen insensitivity or defects in later steps of dihydrotestosterone biosynthesis. As is true for all steroidogenic enzyme deficiencies, the diagnosis is most convincingly established by measuring precursor-to-product ratios during ACTH stimulation testing. In particular, circulating concentrations of the 17-deoxysteroids progesterone, corticosterone, and DOC rise to 5 to 10 times normal after ACTH administration.[15] In addition, 17-hydroxylase deficiency, in distinct contrast to 11-hydroxylase and 21-hydroxylase deficiencies, is characterized by elevated production of 18-hydroxycorticosterone and 18-hydroxy-DOC (Table 2).[33] The ratio of corticosterone to DOC (or of their 18-hydroxy-derivatives) distinguishes 17- from 11-hydroxylase deficiency. Table 3 compares the clinical, laboratory, and genetic characteristics of the various mineralocorticoid excess states that may arise in children and young adults.

Although production of the precursors corticosterone and DOC is markedly elevated in 17-hydroxylase deficiency, DOC production can be much greater in 11-hydroxylase deficiency, whereas plasma 18-hydroxy-DOC concentrations are not elevated.[33] The reason for this apparent discrepancy is that P450c11β (the product of the *CYP11B1* gene) is not exclusively an 11β-hydroxylase but exhibits weak 18-hydroxylase activity[49, 64] (Fig. 2) and trace amounts of aldosterone synthase activity.[75] The low 18-hydroxy-DOC production in 11-hydroxylase deficiency, despite enormous DOC concentrations, is compelling genetic evidence that P450c11β is responsible for elevated 18-hydroxy-DOC and 18-hydroxy-corticosterone production in 17-hydroxylase deficiency. Analogously, in glucocorticoid-remediable aldosteronism, abundant 18-oxygenase activities in the zona fasciculata owing to the presence of a chimeric *CYP11B2/11B1* gene[36] lead to excessive 18-oxygenated steroid production.[16] Patients with 17-hydroxylase deficiency with paradoxically measurable, if

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Figure 2. Physiologic disturbances in glucocorticoid, mineralocorticoid, and sex steroid homeostasis in complete 17-hydroxylase deficiency. The inability to 17α-hydroxylate C_{21} steroids in the adrenal gland eliminates all steroids within shaded region and shunts pregnenolone flux to progesterone, 11-deoxycorticosterone (DOC), corticosterone, and possibly aldosterone *(large open arrows)*. Absence of negative feedback by cortisol *(dashed line)* causes overproduction of adrenocorticotropic hormone (ACTH) *(top-most large open arrow)*, and the resultant abundance of the weak glucocorticoid corticosterone provides adequate systemic glucocorticoid action and feedback on ACTH secretion *(solid line)*. The hypothalamic-pituitary-adrenal axis then reaches a steady state at a higher set-point; however, the drive to overproduce corticosterone allows the accumulation of intermediates such as the potent mineralocorticoid DOC, and high DOC production stimulates salt and water retention, which suppresses renin secretion *(dashed arrow)*. Thus, aldosterone production is low *(dashed arrow)*, but hypertension and hypokalemia develop because of DOC excess. In addition, the unusually high concentrations of DOC and corticosterone in the presence of robust P450c11β expression leads to excessive production of ordinarily minor metabolites 18-hydroxy (18OH)-DOC and 18-OH-corticosterone. Because 17-hydroxy (17OH)-pregnenolone and dehydroepiandrosterone (DHEA) synthesis is nil *(shaded region)* in the fetus and at puberty, no androgen or estrogen synthesis is possible, and sexual infantilism results.

Table 1. COMPARISON OF COMBINED 17α-HYDROXYLASE/17,20-LYASE DEFICIENCY AND ISOLATED 17,20-LYASE DEFICIENCY

Deficiency	Plasma Steroids	Urinary Steroids	Clinical Presentation
Combined 17α-hydroxylase and 17,20-lyase	↓ 17-OH-steroids, DHEA, androgens, estrogens ↑ Progesterone, DOC, DOC metabolites, corticosterone	↓ 17-OHCS, 17-KS pregnanetriolone ↑ Tetrahydro-DOC	Hypertension/hypokalemia, sexual infantilism
Isolated 17,20-lyase	Normal 17-OH-steroids ↓ DHEA, androgens ↑ 17-OHP/AD (>10 after hCG)	↓ 17-KS	Ambiguous genitalia in 46,XY

17-OHP = 17-hydroxyprogesterone; AD = androstenedione; hCG = human chorionic gonadotropin; DHEA = dehydroepiandrosterone; 17-OHCS = 17-hydroxycorticosteroids; 17-KS = 17-ketosteroids; DOC = 11-deoxycorticosterone.

Table 2. COMPARISON OF STEROID PROFILES IN ADULTS WITH 17-, 11-, AND 21-HYDROXYLASE DEFICIENCES

Type of Deficiency	DOC ng/dL	18-OH-DOC ng/dL	Corticosterone ng/dL	18-OH-Corticosterone ng/dL	Aldosterone ng/dL
17-OH	25–500	100–600	4000–40,000	60–1000	<10
11-OH	50 to >1000	<10	<200	<10	<3
21-OH	10–100	3–20	100–500	10–200	10–60
Normal	2–20	1–20	100–500	10–40	10–30

DOC = 11-deoxycorticosterone; 18-OH-DOC = 18-hydroxy-DOC; OH = hydroxylase.
Data adapted from Kater CE, Biglieri EG: Disorders of steroid 17 alpha-hydroxylase deficiency. Endocrinol Metab Clin North Am 23:341–357, 1994.

not elevated, aldosterone production have been described. It is possible that, in these instances, artifacts owing to laboratory methods or intercurrent glucocorticoid therapy confound the data.[32] It is equally likely that other genetic and environmental modifiers contribute to these variations in disease manifestations, such as polymorphisms that alter the aldosterone synthase activity of P450c11β. The latter hypothesis is consistent with the finding that most 17-hydroxylase deficiency cases with measurable aldosterone production are from Japan.[32, 72] Until a large series of patients with 17-hydroxylase deficiency is compiled with uniform evaluation, these conundrums will persist.

Although heterozygous family members of patients with 17-hydroxylase deficiency without other endocrine abnormalities usually have clinically normal adrenal and gonadal physiology, it is sometimes possible to detect heterozygosity using biochemical testing. Elevated corticosterone and 18-hydroxycorticosterone concentrations, as well as the 18-hydroxycorticosterone-to-aldosterone ratio, after ACTH stimulation are perhaps the most readily available means to detect heterozygotes if an index case has been identified.[65] More precisely, the ratio of total urinary metabolites of corticosterone to those of cortisol is elevated (reflecting low 17α-hydroxylation), and the ratio of total urinary metabolites of C_{19} steroids to those of C_{21} steroids is low (reflecting low 17,20-lyase activity).[13] If a compelling reason for ascertainment of an individual's zygosity exists, molecular genetics provides a highly sensitive, although tedious, method that must be performed in a research laboratory.[20]

MOLECULAR GENETICS

Deletions, Premature Truncations, Frameshifts, and Splicing Errors

Among the genetic abnormalities described in the *CYP17* gene, the largest deletion reported involves the substitution of 518 bp (most of exon 2 and part of exon 3) with 469 bp of unknown DNA, disrupting the protein near its beginning and causing complete 17α-hydroxylase

Table 3. COMPARISON OF MINERALOCORTICOID EXCESS STATES WITH SUPPRESSED PLASMA RENIN ACTIVITY

Disease	Laboratory Findings	Key Features	Molecular Basis
*17-Hydroxylase deficiency	↑ DOC, corticosterone, and 18-OH derivatives ↓ 17-OH-steroids, C_{19} steroids ↓ Aldosterone	Sexual infantilism/ambiguity	*CYP17* mutations
Primary aldosteronism	↑ Aldosterone, often ↑ 18-OH-corticosterone	Normal cortisol axis	Unknown
DOC-oma	↑ DOC, normal aldosterone, variable other steroids	Normal cortisol axis	Unknown
*Syndrome of apparent mineralocorticoid excess	↓ Cortisone metabolites ↓ Aldosterone	Dexamethasone suppression of hypertension, kaluresis	*HSD11B2* mutations
*Glucocorticoid-remediable aldosteronism	↑ 18-OH- and 18-oxocortisol, variable aldosterone	Dexamethasone suppression of hypertension, kaluresis	*CYP11B2/11B1* chimeric gene
*Cushing syndrome	↑ Cortisol production, variable other steroids	Symptoms of cortisol excess, variable mineralocorticoid excess	Unknown
*Glucocorticoid resistance	↑ Cortisol production ↑ C_{19} steroids	Symptoms of cortisol insufficiency, androgen excess	GR mutations, other

*ACTH-dependent mineralocorticoid excess.
DOC = 11-deoxycorticosterone; OH = hydroxy; GR = glucocorticoid receptor.

deficiency.[6] A 4-bp duplication of the sequence CATC following Ile479[30] was originally observed in Canadian Mennonites[29] and has been subsequently found in at least six Dutch Frieslander families.[28] This duplication leaves 95% of the protein unaffected and creates a mutant P450c17 that has an altered sequence in its last 25 residues, that is truncated three residues prematurely, and that is wholly devoid of enzymatic activity. The crucial nature of the carboxy terminus of P450c17 is also shown by the complete absence of activity in the 9-bp, in-frame deletion of residues Asp487, Ser488, and Phe489[19] and in a Gln461→stop mutation.[73] Although these mutants retain the heme-binding region, these ostensibly minor alterations in the extreme carboxy terminus are catastrophic for enzymatic activity.

A computer model of human P450c17 suggests why the enzyme is so sensitive to alterations in its carboxy terminus.[5] The last 48 residues of P450c17 are involved in an extended β-sheet structure that folds down from the protein surface to form the "roof" of the active site, which is critical for proper substrate binding and subsequent catalysis. The CATC duplication after Ile479,[30] the deletion of residues 487 to 489,[19] and the mutant Gin461→stop,[73] which all retain the heme-binding site, disrupt or lack this critical stretch of residues required for activity.

The mutation delTG300,301 shifts the reading frame and alters the codon use beginning within exon 5.[43] Mutation 7bp dup 120 changes the reading frame from exon 2 onward.[70] The premature truncation Trp 17→stop has been found in a homozygous[68] and a compound heterozygous[61] patient, and mutations Glu194→stop and Arg239→stop each comprise separate alleles in a single patient with complete 17α-hydroxylase deficiency.[56] These three early truncations are not informative for structure/function studies because they delete the heme-binding region as well as residues important for substrate and redox partner binding.

Two deleterious intronic mutations have been described, a G to T substitution at nucleotide +5 in intron 2[61] and an analogous G to A substitution at position +5 of intron 7.[66] These splice junction mutations delete exons 2 or 7, respectively, during RNA processing ("exon skipping"). The excision of these exons introduces early premature stop codons well before the heme-binding region. The deletion of a G within codon 438 has been found in a homozygous patient.[51] This mutant gene encodes a protein in which the Gly-Pro-Arg-Ser-Cys-Ile motif at residues 438–443 (the underlined Cys ordinarily donates the axial sulfhydryl to the heme iron) is converted to Asp-Leu-Ala-Pro-Val-Stop, which destroys all enzymatic activity. An ATG→ATC substitution in the intiating methionine codon has been described in a patient with complete 17α-hydroxylase deficiency and hypokalemic myopathy.[58]

Amino Acid Substitutions—Combined 17α-Hydroxylase/17,20-Lyase Deficiency

Careful biochemical and computational analyses of mutant enzymes from patients with unusual phenotypes can provide insight into the

functional roles of specific amino acids in P450c17. For example, the mutation His373Leu, when expressed in *E. coli*, lacks the classical P450 difference spectrum,[44] strong evidence that this protein does not bind heme properly. Modeling studies[5] predict that His373 lies distant from the heme moiety, suggesting that structural changes elsewhere in the His373Leu mutant secondarily abolish heme binding. In contrast, the mutation Arg440His[18] lies two residues away from the heme-liganding Cys442, and the reason for loss of activity in this mutant is more straightforward. In most P450 enzymes, an analogous arginine residue in this position is critical for neutralization of a negative charge on a heme proprionate and stabilization of heme incorporation[25]; hence, this mutation also interferes with heme binding.

The mutation Ser106Pro, found in two apparently unrelated Guamanian patients,[38] introduces a helix-breaking proline into what is predicted to be the B′-helix, near residues that form a lateral boundary of the substrate-binding pocket. P450c17 is sensitive to perturbations in this region, such that even the conservative replacement of Ser106 with Thr (the corresponding residue found in rainbow trout P450c17[57]) abolishes most enzymatic activity.[37] Specifically, Ile112 is predicted to interact directly with substrate, suggesting why mutation insIle112 is devoid of measurable activity.[27] Nearby, mutations Gly90Asp[67] and Arg96Trp[34] are predicted to reposition the second strand of β-sheet 1, containing the key residue Gly95. Computer simulations predict that 3β-hydroxyl and 3-keto groups of Δ^5 and Δ^4 substrates, respectively, form hydrogen bonds to the carbonyl group or the amide hydrogen of Gly95.[5, 41] The four mutants insIle112, Ser106Pro, Arg96Trp, and Gly90Asp may all primarily impair substrate binding.

Three mutations that retain partial enzymatic activity have also been described. Mutations Tyr64Ser[27] and Pro342Thr[1] retain approximately 15% and 20% of wild-type activity, respectively. The loss of one of two contiguous Phe residues in the ΔPhe53/54 mutation[69] destroys all but a trace of enzymatic activity,[72] and this mutation has been found in other cases of 17-hydroxylase deficiency in Japan,[42] suggesting a founder effect. The structural alterations responsible for the loss of activity in these mutants are not entirely clear, but these regions of the protein must be somewhat more tolerant of such structural changes than, for example, the active site and the heme-binding region.

Mutations Causing Isolated 17,20-Lyase Deficiency

The first patient with isolated 17,20-lyase deficiency in whom the *CYP17* gene was sequenced proved to be a compound heterozygote for the Gln461→stop and Arg496Cys mutations.[73] When studied in transfected cells, the Gln461→stop mutant was inactive, but the Arg496Cys mutant retained a small amount of 17α-hydroxylase and 17,20-lyase activities.[73] When restudied as an adult,[74] the patient's steroid hormone profile reflected nearly complete deficiencies of 17α-hydroxylase and 17,20-lyase activities, consistent with the molecular genetics and bio-

chemistry of the mutant proteins. This case illustrates many of the pitfalls in the diagnosis of isolated 17,20-lyase deficiency and emphasizes that the clinical features, the molecular genetics, and the biochemistry of the mutant P450c17 protein(s) must all be congruent to ensure an accurate diagnosis.

Recently, two 46,XY Brazilian patients presented with convincing clinical evidence of isolated 17,20-lyase deficiency, that is, genital ambiguity and diminished C_{19} steroid production yet normal 17-hydroxycorticosteroid production. One patient was homozygous for mutation Arg347His and the other for Arg358Gln, whereas each parent was heterozygous for the respective mutant allele.[20] When expressed in COS-1 cells, the mutants hydroxylated progesterone and pregnenolone,[20] but only a trace of 17,20-lyase activity could be reconstituted by coexpressing an excess of oxidoreductase and b_5.[21] Although 17α-hydroxypregnenolone is a poor substrate for the mutant enzymes, competition experiments unequivocally show that the affinity of the mutant proteins for 17α-hydroxypregnenolone is equivalent to that of the wild-type enzyme,[20, 21] suggesting that arginines 347 and 358 do not lie in or near the active site.

Computer modeling studies demonstrate that R347H and R358Q neutralize positive charges in the redox partner binding site.[5, 20] Biochemical studies confirm that mutations R347H and R358Q impair interactions of P450c17 with its electron donor P450-oxidoreductase and with cytochrome b_5[21]; therefore, isolated 17,20-lyase deficiency is not caused by an inability of the mutant enzymes to bind the intermediate 17α-hydroxypregnenolone but rather by subtle disturbances in interactions with redox partners.[5, 20, 21] Another patient subsequently shown to have isolated 17,20-lyase deficiency was found to harbor mutation F417C,[8] which is predicted to lie on the edge of this redox partner binding surface.[5] The biochemistry of the F417C mutant has not been studied in detail, so it is not known if the same mechanisms as for the R347H and R358Q mutants apply to F417C.

A male pseudohermaphrodite with congenital methemoglobinemia owing to a mutation in the gene for cytochrome b_5 has been described.[22] It is possible that this patient was incompletely virilized because of low (but not absent) testicular 17,20-lyase activity and testosterone deficiency in utero owing not to a P450c17 mutation but rather to the loss of b_5, the cofactor protein that stimulates 17,20-lyase activity. Neither circulating steroid hormone concentrations nor a genetic analysis of the *CYP17* gene were reported for this subject. If this patient has isolated 17,20-lyase deficiency owing to the loss of b_5, the physiologic importance of b_5 in P450c17 chemistry would be proved.

MANAGEMENT

The child with 17-hydroxylase deficiency is chronically exposed to elevated circulating mineralocorticoid (DOC) concentrations but roughly normal amounts of glucocorticoids (as corticosterone). Mineralocorticoid

excess in the neonatal period is of no consequence because mineralocorticoid (aldosterone) production is normally high in infants[17]; however, as the child ages and begins to consume solid foods, sodium intake rises, and mineralocorticoid excess can lead to sodium retention, hypertension, and hypokalemia. The hypertension can become fixed if not treated for many years[39]; hence, some control of DOC production is desirable. Moderation of dietary sodium content is prudent as an adjunct to pharmacologic therapy, which consists of glucocorticoid supplementation to reduce aberrant DOC production. Special considerations in the child with 17-hydroxylase deficiency include the avoidance of highly potent fluorinated glucocorticoids, such as dexamethasone, that have disproportionately large detrimental effects on linear growth and bone mineral accrual. Hydrocortisone administered in two or three divided doses will generally suffice, although direct comparison of steroid regimens in this uncommon disease are lacking. The glucocorticoid dose should be titrated to normalization of blood pressure and plasma potassium concentrations, as well as restoring plasma renin activity to the measurable range as endpoints. The frank normalization of plasma DOC and corticosterone concentrations may require overtreatment with glucocorticoids.[52] It is preferable to err on the side of undertreatment because the dire consequences of glucocorticoid excess throughout childhood are less desirable than modest mineralocorticoid excess.

As is true for patients with Turner's syndrome, gonodal dysgenesis, androgen insensitivity, or some other steroid biosynthetic defects, patients with 17-hydroxylase deficiency fail to exhibit pubertal development, and fetal testosterone deficiency causes all but the most mildly affected patients to present phenotypically as prepubertal females. In addition, the testosterone surge that occurs during the first year of life in 46,XY children is absent in 17-hydroxylase deficiency, which could theoretically impair responsiveness to testosterone later in life for mildly affected individuals. In most cases, estrogen replacement therapy is initiated at the time of expected puberty or on diagnosis if that time has already passed. Estrogen replacement not only allows the development of female secondary sexual characteristics but stimulates the increase in bone mass that normally occurs during puberty.[24] In a few cases, testosterone supplementation has been given to mildly affected 46,XY patients to stimulate penile development[15]; however, as is true for patients with partial androgen insensitivity, the rearing of these individuals as males and the choice of appropriate therapy are complex decisions that unfortunately may yield less than satisfactory results.

The treatment of 17-hydroxylase deficiency in the adult patient strives to achieve four goals: (1) reduction of the production or action of mineralocorticoids; (2) avoidance of the untoward effects of glucocorticoid excess; (3) replacement of sex steroids; and (4) prevention of the long-term consequences of the abnormal physiology. Although the caveats and special considerations are somewhat different in the two age groups, the cornerstone of the therapeutic plan remains sodium restriction plus glucocorticoid supplementation, traditionally consisting of a

daily dose of dexamethasone. Patients with 17-hydroxylase deficiency given dexamethasone demonstrate a prompt reduction in DOC and corticosterone production, with naturesis and resolution of kaluresis.[33] The hypertension usually resolves with glucocorticoid therapy,[52] but if the diagnosis has been delayed for many years, the hypertension can become fixed.[39] The goal of glucocorticoid therapy is to restore the blood pressure and plasma potassium concentration to normal using the minimal amount of drug possible, usually 0.25 to 1 mg/d of dexamethasone or 2 mg/d to 5 mg/d of prednisone. Circulating concentrations of DOC and corticosterone may not completely normalize on this regimen,[52] but a rise in renin and aldosterone during glucocorticoid administration indicates that the therapeutic goal of eliminating ACTH-dependent mineralocorticoid excess has been achieved.[33] As is true in other states of ACTH-driven mineralocorticoid excess, such as glucocorticoid-remediable aldosteronism and apparent mineralocorticoid excess, care must be taken not to suppress the hypothalamic-pituitary-adrenal axis overzealously, which can lead to complications of glucocorticoid excess.[16] Instead, small doses of mineralocorticoid antagonists, such as spironolactone or potassium canrenoate, can be added to the regimen, allowing modest glucocorticoid doses during long-term therapy in the adult as well.[39] Particularly with the development of newer mineralocorticoid antagonists lacking the side effects of spironolactone, such as eplerenone, blockade of mineralocorticoid action is likely to assume a larger role in the management of ACTH-dependent mineralocorticoid excess states. If hypertension persists despite adequate blockade of mineralocorticoid production or action, the addition of a calcium channel blocker to the regimen is usually sufficient.[32, 39]

Patients who have 17-hydroxylase deficiency also fail to produce DHEA, and beneficial effects of DHEA supplementation in women (but not in men) with adrenal insufficiency have been demonstrated.[2] Because it is not clear whether the benefits of DHEA in these women are caused by the direct action of DHEA or the conversion of DHEA to active androgens, definitive recommendations cannot be made, and any regimen would require a preparation and dosing that accommodates a narrow therapeutic window to avoid the undesirable consequences of androgen excess. Nonetheless, some form of C_{19} steroid supplementation could be beneficial in 17-hydroxylase deficiency. Some 46,XX females with partial 17-hydroxylase deficiency have been reported to have spontaneous menses,[59] but, as a general rule, 46,XX patients require cyclical or combined estrogen-progestin replacement therapy to prevent endometrial hyperplasia from unopposed estrogen stimulation. In contrast, 46,XY females lack Müllerian structures and can be treated with an estrogen replacement regimen without the progestin. One astonishing report describes the successful in vitro fertilization of a 46,XX patient with 17-hydroxylase deficiency after stimulating ovarian development, despite low intrafollicular estradiol concentrations.[55]

Affected 46,XY individuals require gonadectomy to prevent malignant degeneration in their intra-abdominal testes. The need for genetic

and psychologic counseling of these individuals, particularly if some intersex features exist, should not be neglected. Most of these patients are diagnosed at an age at which their gender identity and role have been firmly established but before an age when they can fully grasp the complexity of their condition.

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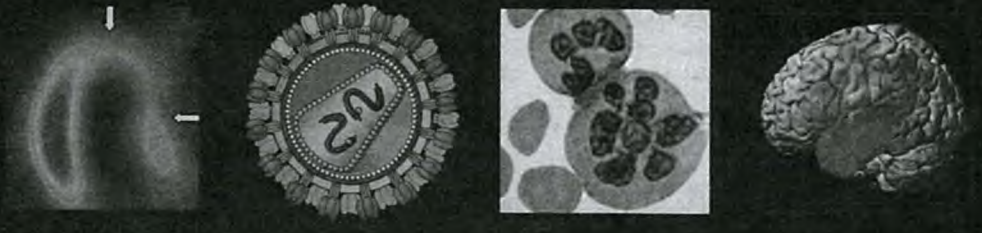

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survival of 80 to 90% for low-grade lesions. More invasive or histologically poorly differentiated tumors are more likely to recur locally and metastasize. Metastatic disease is treated with the chemotherapy used in bladder cancer, and the outcome is similar to that for metastatic transitional cell cancer of bladder origin.

READING

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81 HYPERPLASTIC AND MALIGNANT DISEASES OF THE PROSTATE

Howard I. Scher

The frequency of both benign and malignant changes in the prostate increases with age. Autopsies of men in the eighth decade of life show hyperplastic changes in >90% and malignant changes in >70% of individuals. The high prevalence of these diseases in an elderly population with competing causes of morbidity and mortality mandates a risk-adapted approach to diagnosis and treatment. This can be achieved by considering these diseases as a series of states. Each state represents a different clinical milestone for which intervention(s) may be recommended based on the presence or risk of developing symptoms or death from disease in a given time frame (Fig. 81-1). For benign proliferative disorders, symptoms of urinary frequency, infection, and potential for obstruction are weighed against the side effects and complications of medical or surgical therapy. For prostate malignancies, the risk of developing the disease, symptoms, or death from cancer are balanced against the morbidities of interventions recommended and preexisting medical conditions.

In 2004, around 230,000 prostate cancer cases were diagnosed, of which 29,900 succumbed. The absolute number of prostate cancer deaths has decreased in the past 5 years; this has been attributed by some to the widespread use of detection strategies based on monitoring prostate-specific antigen (PSA). However, screening has not been shown to improve survival in prospective randomized trials. The paradox of management is that although the disease remains the second leading cause of cancer deaths in men, the almost 8:1 ratio in incidence to prostate cancer–specific mortality shows that the majority of men with it die of their disease.

ANATOMY AND PATHOLOGY

The prostate is located in the pelvis and is surrounded by the rectum, the bladder, the periprostatic and dorsal vein complexes that are responsible for erectile function, and the urinary sphincter that is responsible for passive urinary control. The prostate is composed of branching tubuloalveolar glands arranged in lobules and surrounded by a stroma. The acinal unit includes an epithelial compartment made up of epithelial, basal, and neuroendocrine cells and a stromal compartment that includes fibroblasts and smooth-muscle cells. The compartments are separated by a basement membrane. PSA and acid phosphatase (ACP) are produced in the epithelial cells. Both cell types express androgen receptors and depend on androgens for growth. Testosterone, the major circulating androgen, is converted by the enzyme 5-α reductase to dihydrotestosterone in the gland. Changes in prostate size occur during puberty and after the age of 55 in the periurethral portion of the gland. Most cancers develop in the peripheral zone, which can often be palpated by a digital rectal examination (DRE). Nonmalignant growth occurs predominantly in the transition zone around the urethra.

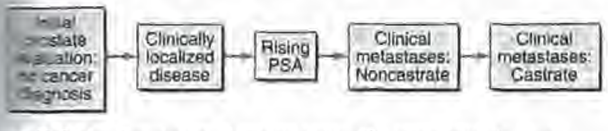

FIGURE 81-1 Clinical states of prostate cancer. PSA, prostate-specific antigen.

EPIDEMIOLOGY

The development of a prostate cancer involves a multistep process. Hypermethylation of the GSTP1 gene promoter, leading to a loss of function of a gene that detoxifies carcinogens, is one early change. Epidemiologic studies show that the risk of being diagnosed with prostate cancer increases by a factor of 2 if one first-degree relative is affected and by 4 if two or more are affected. Current estimates are that 40% of early-onset and 5 to 10% of all cancers are hereditary and follow a Mendelian inheritance pattern. Prostate cancer affects ethnic groups differently. Matched for age, the prostates of African-American males have both a greater number of precursor prostatic intraepithelial neoplasia (PIN) lesions and larger tumors than white males, possibly related to the higher levels of testosterone seen in African-American males. These lesions are highly unstable, and typically multifocal. Polymorphic variants of the androgen receptor gene, the cytochrome P450 C17 gene, and the steroid 5α-reductase type II (*SRD5A2*) gene have also been implicated in the variations in incidence. The incidence of autopsy-detected cancers is similar around the world, while the incidence of the clinical disease varies. Thus, environmental factors may play a role. High consumption of dietary fats, such as α-linoleic acid, or polycyclic aromatic hydrocarbons that form when red meats are cooked is believed to increase risk. Similar to breast cancer in Asian women, the risk of prostate cancer in Asian males increases when they move to western environments. Protective factors include the isoflavinoid genistein (which inhibits 5α-reductase), cruciferous vegetables that contain isothiocyanate sulfuraphane, retinoids such as lycopene (in pizza and tomatoes), and inhibitors of cholesterol biosynthesis. The antioxidant α-tocopherol (vitamin E) and selenium may also reduce risk.

DIAGNOSIS AND TREATMENT BY CLINICAL STATE

The clinical states framework considers the risk of morbidity from an enlarging but nonmalignant gland; the probability that a clinically significant cancer is present in an individual with or without urinary symptoms; or, for those with a prostate cancer diagnosis; the probability of developing symptoms or dying from disease. At any point in time, a patient resides in one state and remains there until the disease has progressed to the next state. Applying this paradigm, a patient with localized prostate cancer who has had all cancer removed surgically remains in the state of localized disease as long as the PSA remains undetectable. The time within a state becomes a measure of the impact of an intervention on the natural history of disease, be it benign or malignant in etiology, recognizing that the impact may not be assessable for years. It also allows a distinction between *cure*—the elimination of all cancer cells, the primary therapeutic objective when treating most cancers—and *cancer control*, in which the tempo of the illness is modulated and symptoms controlled until the patient dies of other causes. It is the concept of cancer control that makes the man-

agement of prostate cancer unique. Even when a recurrence is documented, immediate therapy is not always necessary. Rather, as at the time of diagnosis, the need for intervention is based on the tempo of the illness as it unfolds in the individual, relative to the risk:reward ratio of the therapy being considered.

NO CANCER DIAGNOSIS ■ Symptoms The need to pursue a diagnosis of prostate cancer is based on symptoms, an abnormal DRE, or an elevated serum PSA. The urologic history should focus on symptoms of outlet obstruction, continence, potency, or a change in ejaculatory pattern. Benign proliferative disease may produce hesitancy, intermittent voiding, a diminished stream, incomplete emptying, and postvoid leakage. The severity of these symptoms can be quantitated with the self-administered American Urological Association (AUA) *Symptom Index* (Table 81-1) recognizing that the degree of symptoms does not always relate to gland size. Resistance to urine flow reduces bladder compliance, leading to nocturia, urgency, and, ultimately, to retention. Infection, tranquilizing drugs, antihistamines, or alcohol can precipitate urinary retention. Prostatitis often produces pain or induration. Symptoms of metastatic disease include pain secondary to osseous metastases, although many are asymptomatic despite extensive spread. Less common are symptoms related to marrow compromise (myelophthisis), a coagulopathy, or spinal cord compression.

Physical Examination The DRE focuses on the size, consistency, and abnormalities within or beyond the gland. Many cancers occur in the peripheral zone and can be palpated on DRE. Carcinomas are characteristically hard, nodular, and irregular, while induration may be due to benign prostatic hypertrophy (BPH) or to calculi or tumor. Overall, 20 to 25% of men with an abnormal DRE have cancer.

PROSTATE-SPECIFIC ANTIGEN

PSA is a kallikrein-like serine protease that causes liquefaction of seminal coagulum. It is produced by both nonmalignant and malignant epithelial cells. PSA is prostate specific, not prostate cancer specific, and increases may occur from prostatitis, nonmalignant enlargement of the gland (BPH), prostate cancer, and prostate biopsies. The level is not affected by the performance of a DRE. It circulates in the blood as an inactive complex with the protease inhibitors α_1-antichymotrypsin and β_1-macroglobulin and has an estimated half-life in the serum of 2 to 3 days. Levels should be undetectable if the prostate has been removed. PSA immunostaining is used to establish a prostate cancer diagnosis.

PSA testing was approved for early detection in 1994. It is recommend on an annual basis along with a DRE for men over age 50 (with an anticipated survival of >10 years; this includes men up to age 76 years). For African Americans and men with a family history, testing is advised to begin at age 40. The normal range of PSA is 0 to 4 ng/mL. For values >4, the sensitivity for prostate cancer detection is 57 to 79%, the specificity is 59 to 68%, and the positive predictive value is 40 to 49%.

The PSA-based criteria used to recommend a diagnostic prostate biopsy have evolved over time. PSA values may fluctuate for no apparent reason; thus, an isolated abnormal value should be confirmed before proceeding with further testing. These evolving criteria aim to increase the sensitivity of the test for younger men more likely to die of the disease and to reduce the frequency of detecting cancers of low malignant potential in elderly men more likely to die of other causes. Age-specific reference ranges apply a lower "upper" limit of normal for younger males and higher "upper" limit for older individuals. Different thresholds alter sensitivity and specificity of detection. The threshold for performance of a biopsy is now 2.6 ng/mL for men under age 60. Prostate-specific antigen density (PSAD) measurements were developed to correct for the contribution of BPH to the total PSA level. PSAD is calculated by dividing the serum PSA by the estimated prostate weight calculated by transrectal ultrasound (TRUS). Values <0.10 are consistent with BPH, while those >0.15 suggest cancer. *PSA velocity* is the rate of change in PSA levels over time. It is particularly useful for men with values that are rising in the seemingly "normal" range. Rates of rise >0.75 ng/mL per year suggest cancer. As an example, an increase from 2.5 to 3.9 in a 1-year period would warrant further testing. Free and complexed PSA measurements are used when levels are between 4 and 10 ng/mL to decide who needs a biopsy. In cancer, the level of free PSA is lower. The ratios of free to total, complexed to total, and free to complexed PSA have also been used. In one series, specificity improved by 20% using a normal range of free/total >0.15; complexed/total <0.70; and free/complexed >0.25. A diagnostic algorithm based on the DRE and PSA findings is illustrated in Fig. 81-2. In general, a biopsy is recommended if the DRE or PSA are abnormal.

Prostate Biopsy A diagnosis of cancer is established by a TRUS-guided needle biopsy. Direct visualization assures that all areas of gland are sampled. A minimum of six separate cores, three from the right and three from the left, are advised, as is a separate biopsy of the transition zone, if clinically indicated. Performance of a biopsy is not advised in a patient with prostatitis until a course of antibiotics has been administered. The positive predictive value of an abnormal DRE is 21%, while 25% of men with a PSA > 4 ng/mL and an abnormal DRE, and 17% of men with a PSA of 2.5 to 4.0 ng/mL and normal DRE, have cancer. Those with an abnormal PSA and negative biopsy are advised to undergo a repeat biopsy.

TABLE 81-1 AUA System Index

Questions to Be Answered	AUA Symptom Score (Circle 1 Number on Each Line)					
	Not at All	Less than 1 Time in 5	Less than Half the Time	About Half the Time	More than Half the time	Almost Always
Over the past month, how often you have had a sensation of not emptying your bladder completely after you finished urinating?	0	1	2	3	4	5
Over the past month, how often have you had to urinate again less than 2 h after you finished urinating?	0	1	2	3	4	5
Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5
Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5
Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5
Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5
Over the past month, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	(None)	(1 time)	(2 times)	(3 times)	(4 times)	(5 times)
Sum of 7 circled numbers (AUA Symptom Score): ———						

Note: AUA, American Urological Association.

Source: Barry MJ et al: J Urol 148:1549, 1992. Used with permission.

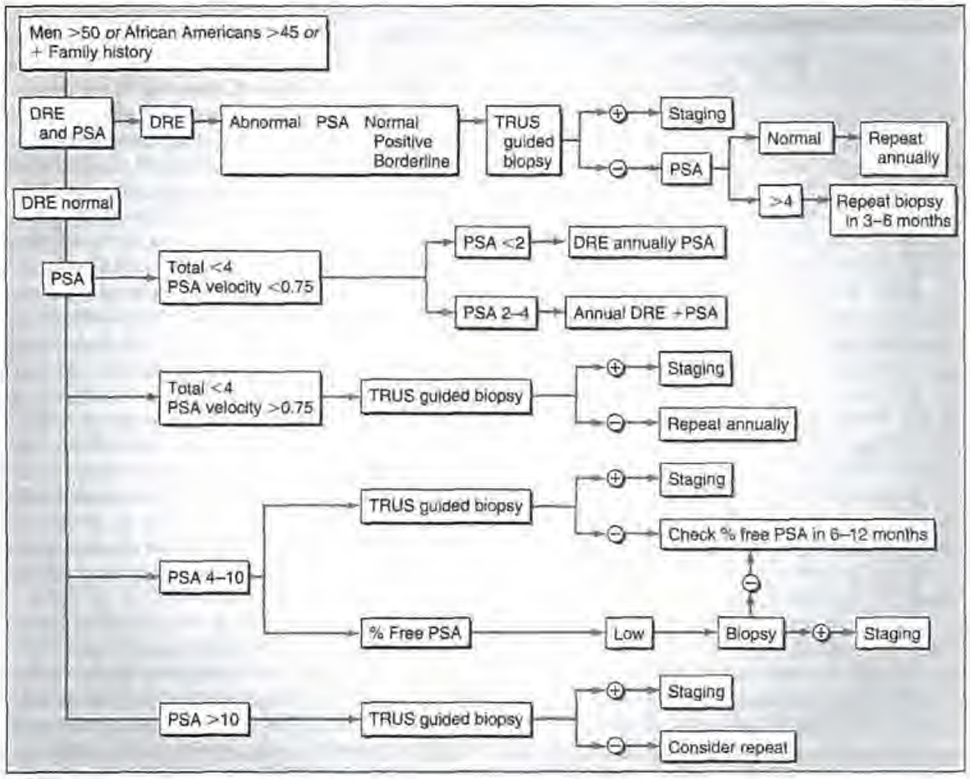

FIGURE 81-2 Algorithm for diagnostic evaluation of men based on digital rectal examination and prostate-specific antigen levels.

Pathology The noninvasive proliferation of epithelial cells within ducts is termed *prostatic intraepithelial neoplasia*. PIN is a precursor of cancer, but not all PIN lesions develop into invasive cancers. Of the cancers identified, >95% are adenocarcinomas; the remaining are squamous, transitional cell tumors, and rarely, carcinosarcomas. Metastases to the prostate are rare, but in some cases, transitional cell tumors of the bladder or colon cancers invade the gland by direct extension. Each core of the biopsy is examined for the presence of cancer, and the amount of cancer present is quantified. A measure of histologic aggressiveness is also assigned using the *Gleason grading system*, in which the dominant and secondary glandular histologic patterns are scored from 1 (well differentiated) to 5 (undifferentiated) and summed to give a total score of 2 to 10 for each tumor. The most poorly differentiated area of tumor (i.e., the area with the highest histologic grade) often determines biologic behavior. The presence or absence of perineural invasion and extracapsular spread are also recorded.

PSA-based detection strategies have changed the clinical spectrum of the disease. Now, 95 to 99% of newly diagnosed cancers are clinically localized, 40% are not palpable, and, of these, 70% are pathologically organ-confined. The downside of widespread use is the detection and treatment of cancers with such a low malignant potential that they would not have shortened survival or produced symptoms during the patient's lifetime. The side effects of treatment, including impotence, incontinence, and bowel dysfunction, are unacceptable for these cases. Formal clinical trials to assess the value of screening on prostate cancer morbidity and mortality are ongoing. Until the results of these studies are available, men are advised to make an informed decision about whether to undergo testing.

Prevention It is difficult to identify individuals who are at risk for developing a cancer that is clinically significant. The Prostate Cancer Prevention Trial is a double-blinded, randomized multicenter trial designed to investigate the ability of finasteride, a 5α-reductase inhibitor, to prevent the development of prostate cancer in men age ≥55 years. The prostate cancer detection rate was 18.4% (803 of 4364) for finasteride and 24.4% (1147 of 4692) for placebo-treated men. However, more of the cancers detected in the finasteride group were high-grade [37% (280 of 757) vs. 22% (237 of 1068 cancers) for the placebo]. No effect on survival was detected. Vitamin E and selenium (the SELECT study) are also being tested as preventive agents.

Treatment of Benign Disease Asymptomatic patients do not require treatment regardless of the size of the gland, while those with an inability to urinate, gross hematuria, recurrent infection, or bladder stones may require surgery. Typically, obstruction does not occur and the symptoms remain stable over time. In these cases, uroflowmetry can identify patients with normal flow rates who are unlikely to benefit from treatment and those with high postvoid residuals who may need other interventions. Pressure-flow studies detect primary bladder dysfunction. Cystoscopy is recommended if hematuria is documented and to assess the urinary outflow tract before surgery. Imaging of the upper tracts is advised for patients with hematuria, a history of calculi, or prior urinary tract problems. Therapies such as finasteride, which blocks the conversion of testosterone to dihydrotestosterone, have been shown to decrease prostate size, increase urine flow rates, and improve symptoms. They will also lower baseline PSA levels by 50%, an important consideration when using PSA to guide biopsy recommendations. α-Adrenergic blockers such as terazosin act by relaxing the smooth muscle of the bladder neck and increasing peak urinary flow rates. No data show that these agents influence the progression of the disease. Surgical approaches include a transurethral resection of the prostate (TURP), transurethral incision, or removal of the gland via a retropubic, suprapubic or perineal approach. TULIP (transurethral ultrasound-guided laser-induced prostatectomy), coils, stents, and hyperthermia are also utilized.

PROSTATE CANCER STAGING The TNM staging system includes categories for cancers that are palpable on DRE, those identified solely on the basis of an abnormal PSA (T1c), those that are palpable but clin-

ically confined to the gland (T2), and those that have extended outside of the gland (T3 and T4) (Table 81-2). The assessment of disease extent based on DRE alone is inaccurate with respect to the extent of the disease within the gland, the presence or absence of capsular invasion, involvement of seminal vesicles, and extension of disease to lymph nodes. This led to a modification of the staging system to include the results of imaging studies on the assignment of T stage. Unfortunately, no single test has proven to predict the pathologic stage accurately, be it the presence of organ-confined disease, seminal vesical involvement, or lymph node spread.

TRUS is most frequently used to assess the primary tumor, but no consistent finding predicts cancer with certainty. TRUS is used primarily to direct prostate biopsies. Computed tomography (CT) scans lack sensitivity and specificity to detect extraprostatic extension and are inferior to magnetic resonance imaging (MRI) in visualization of lymph nodes. MRI specificity is improved with an endorectal coil and aids in planning radiation therapy. T1-weighted images demonstrate the periprostatic fat, periprostatic venous plexus, perivesicular tissues, lymph nodes, and bone marrow. T2-weighted images demonstrate the internal architecture of the prostate and seminal vesicles. Most cancers have a low signal, while the normal peripheral zone has a high signal, although the technique lacks sensitivity and specificity.

Radionuclide bone scans are used to evaluate spread to osseous sites. This test is sensitive but relatively nonspecific because areas of increased uptake are not always related to metastatic disease. Healing fractures, arthritis, Paget's disease, and other conditions will also show abnormal uptake. True-positive bone scan results are rare if the PSA <8 ng/mL and uncommon when the PSA < 10 ng/mL unless the tumor is high grade. When the PSA <10 ng/mL, a positive bone scan is usually falsely positive, which in turn, leads to additional low-yield testing.

CLINICALLY LOCALIZED DISEASE Localized prostate cancers are clinically confined to the prostate. Patients with localized disease are managed by radical surgery, radiation therapy, or watchful waiting. Data from the literature do not provide clear evidence for the superiority of any one treatment. Choice of therapy needs consideration of several factors: the presence of symptoms, the probability that the untreated tumor will adversely affect the patient during his lifetime and thus require treatment, and whether the tumor can be cured by single-modality therapy directed at the prostate or requires both local and systemic therapy to achieve cure. As most of the tumors detected are deemed clinically significant, most men undergo treatment.

Comparing the outcomes of various forms of therapy is limited by the lack of prospective trials, referral bias, and differences in the outcomes used. The primary outcomes are cancer control and treatment-related morbidities. These benchmarks of success or failure vary by modality. Often, PSA relapse–free survival is used because an effect on metastatic progression or survival may not be apparent for years. Based on a half-life in the blood of 3 days, PSA should be undetectable in the blood 4 weeks after all prostate tissue has been removed by radical surgery. If PSA remains detectable, the patient is considered to have persistent disease. In contrast, the PSA does not become undetectable after radiation therapy because the remaining nonmalignant elements of the gland continue to produce PSA even if all cancer cells have been eliminated. Similarly, there is no adequate cancer control definition for a patient treated with watchful waiting because PSA levels will continue to rise in the absence of therapy. Other outcomes are the time to objective progression (local or systemic) and cancer-specific and overall survival; however, these outcomes may take years to define.

The more advanced the disease, the lower the probability of local control and the higher the probability of systemic relapse. More important is that within the categories of T1, T2, and T3 disease are tumors with a range of prognoses. Some T3 tumors are curable with therapy directed solely at the prostate, and some T1 lesions have a high probability of systemic relapse that requires the integration of local and systemic therapy to achieve cure. T1c tumors particularly require the use of other factors to predict outcomes and select treatment. Many groups have developed prognostic models based on a combination of the initial T stage, Gleason score, and baseline PSA. Some are based on discrete cut points (PSA <10 or ≥ 10; Gleason score of ≤6, 7, or ≥8). Others are nomograms that use PSA and Gleason score as continuous variables. These algorithms are used to predict disease extent: organ confined vs. nonorgan confined, node negative or positive, and the probability of success using a PSA-based definition of failure specific to the local therapy under consideration. Exactly what cut-off value would lead a patient to accept one form of therapy vs. another is an area of active debate. One nomogram to predict PSA relapse–free survival following radical surgery is illustrated in Fig. 81-2. Specific nomograms have been developed for radical prostatectomy, external-beam radiation therapy, and brachytherapy (seed implantation). These are being refined continually to incorporate other clinical parameters and biologic determinants. Surgical technique, radiation therapy delivery, and criteria for watchful waiting continue to be refined and improved; the year treatment was given affects outcomes independent of other factors. The improvements make treatment decisions a dynamic process.

The frequency of adverse events for the different modalities is highly variable. Of greatest concern to patients are the effects on continence, sexual potency, and bowel function. Part of the variability relates to the definition used for a specific complication and whether the patient or physician is reporting the event. Incontinence figures range from 2% to 47% and impotence rates range from 25% to 89% following radical prostatectomy. The time of the assessment is also important. After surgery, impotence is immediate but may reverse over time, while with radiation therapy, impotence is not immediate but may develop over time.

TABLE 81-2 *Comparison of Clinical Stage by the TNM Classification System and the Whitmore-Jewett Staging System*

TNM Stage	Description	Whitmore-Jewett Stage	Description
T1a	Nonpalpable, with 5% or less of resected tissue with cancer	A1	Well differentiated tumor on few chips from 1 lobe
T1b	Nonpalpable, with >5% of resected tissue with cancer	A2	Involvement more diffuse
T1c	Nonpalpable, detected due to elevated serum PSA		
T2a	Palpable, half of one lobe or less	BIN	Palpable, < one lobe, surrounded by normal tissue
T2b	Palpable, > half of one lobe but not both lobes	B1	Palpable, < one lobe
T2c	Palpable, involves both lobes	B2	Palpable, one entire lobe or both lobes
T3a	Palpable, unilateral extracapsular extension	C1	Palpable, outside capsule, not into seminal vesicles
T3b	Palpable, bilateral extracapsular extension		
T3c	Tumor invades seminal vesicle(s)	C2	Palpable, seminal vesicle involved
M1	Distant metastases	D	Metastatic disease

Source: Adapted from PF Schröder et al: TNM classification of prostate cancer. Prostate (Suppl) 4:129, 1992; and American Joint Committee on Cancer, 1992.

Radical Retropubic Prostatectomy (RRP) The goal of radical prostatectomy is to excise the cancer completely with a clear margin, to maintain continence by preserving the external sphincter, and to preserve potency by sparing the autonomic nerves in the neurovascular bundle. RRP is advised for patients with a life expectancy of >10 years and is performed using a retropubic, perineal, or laparoscopic approach.

Outcomes can be predicted using postoperative nomograms that consider pretreatment factors and the pathologic findings at surgery. PSA failure is defined as a detectable value of 0.2 or 0.4 ng/mL, although the exact definition varies among series. The techniques continue to improve as the ability to localize the tumor within or beyond the prostate are refined with different biopsy algorithms and with imaging. The result is better case selection and surgical planning, which in turn have led to more rapid recovery and higher rates of continence and potency. Factors associated with incontinence include older age, shorter urethra length, surgical technique, preservation of neurovascular bundles, and development of an anastomotic stricture. Surgical experience is also a factor. In one series, 6% of patients had mild stress urinary incontinence (SUI) (requiring 1 pad/day), 2% moderate SUI (>1 pad/day), and 0.3% severe SUI (requiring an artificial urinary sphincter). At 1 year, 92% were completely continent. In contrast, the results in a Medicare population treated at multiple centers showed that at 3, 12, and 24 months following surgery, 58, 35, and 42% wore pads in their underwear, and 24, 11, and 15% reported "a lot" of urine leakage. Factors associated with recovery of erectile function include younger age, quality erections before surgery, and the absence of damage to the neurovascular bundles. Erectile function returns in a median of 4 to 6 months if both bundles are preserved. Potency is reduced by half if at least one nerve bundle is sacrificed. In cases where cancer control requires the removal of both bundles, sural nerve grafts are being explored. Overall, with the availability of drugs such as sildenafil, intraurethral inserts of alprostadil, and intracavernosal injections of vasodilators, many patients recover satisfactory sexual function.

High-risk patients are those with a predicted high probability of failure with surgery alone based on pretreatment factors. In these situations, nomograms and predictive models can only go so far. Exactly what probability of success or failure would lead a physician to recommend and a patient to seek alternative approaches is controversial. For example, it may be appropriate to recommend radical surgery for a younger patient with a low probability of cure. To improve the outcomes of surgery for high-risk patients, neoadjuvant hormonal therapy has been explored. The results of several large trials testing 3 or 8 months of androgen ablation before surgery showed that serum PSA levels decreased by 96%, prostate volumes reduced by 34%, and margin positivity rates declined from 41 to 17%. Unfortunately, hormones did not produce an improvement in PSA relapse–free survival. Thus, neoadjuvant hormonal therapy is not recommended.

Radiation Therapy Radiation therapy is given by external beam, the implantation of radioactive sources into the gland, or a combination of both. Contemporary external beam radiation techniques now use three-dimensional conformal treatment plans to maximize the administered dose to the tumor and to minimize the exposure of the surrounding normal structures. The addition of intensity modulation (IMRT) has allowed further shaping of the isodose curves and the delivery of higher doses to the tumor and a further reduction in normal tissue exposure. These advances have allowed the safe administration of doses >80 Gy, higher local control rates, and fewer side effects. Overall, radiation therapy is associated with a higher frequency of bowel complications (mainly diarrhea) than surgery. Measures of cancer control include the proportion of patients who show a decline in PSA to <0.5 or 1 ng/mL, the proportion with "nonrising" PSA values, or the proportion with a negative biopsy of the prostate 2 years after completion of treatment. PSA relapse is defined as three consecutive rising PSA values from the nadir value, with the time to failure as the midpoint between the nadir and first rising value.

Radiation dose is important. A PSA nadir of <1.0 ng/mL was observed in 90% of patients receiving 75.6 or 81.0 Gy vs. 76 and 56% for those receiving 70.2 Gy and 64.8 Gy, respectively. The positive biopsy rates at 2.5 years were 4% for those treated with 81 Gy, vs. 36 and 27% for those receiving 70.2 or 75.6 Gy. The frequency of rectal complications relates directly to the volume of the anterior rectal wall receiving full-dose treatment. Grade 3 rectal or urinary toxicities were seen in 2.1% of cases at a median dose of 75.6 Gy. Grade 3 urethral strictures requiring dilatation developed in 1% of cases, all of whom had undergone a TURP. Pooled data show that the frequency of grade 3 to 4 toxicities is 6.9 and 3.5%, respectively, for patients who received >70 Gy. The frequency of erectile dysfunction is related to the quality of erections pretreatment, the dose administered, and the time of assessment. The etiology is related to a disruption of the vascular supply and not the nerve fibers.

Neoadjuvant hormone therapy has also been studied in combination with radiation therapy to increase local control rates, decrease the size of the prostate so that the exposure of normal tissues to full-dose radiation is reduced, and decrease the rate of systemic failure. Short-term hormone exposures can reduce toxicities and improve local control rates, but long-term (2 to 3 years) treatment is needed to prolong the time to PSA failure and the development of metastatic disease. The impact on survival has been less clear.

Brachytherapy involves the direct implantation of the prostate with radioactive sources. It is based on the principle that the deposition of radiation energy in tissues decreases exponentially as a function of the square of the distance from the source. The goal is to deliver intensive irradiation to the prostate, minimizing the exposure of the surrounding tissues. Techniques have evolved from intraoperative manual insertion methods to the current standard, in which customized templates based on CT and ultrasonographic assessment of the tumor are used for seed placement based on computer-optimized dosimetry to achieve more homogeneous dose distributions. The implants themselves are now performed transperineally, without an open procedure, with real-time imaging. The result is a marked reduction in local failure rates with fewer complications. In a series of 197 patients followed for a median of 3 years, 5-year actuarial PSA relapse–free survival for patients with pretherapy PSA levels of 0 to 4, 4 to 10, and >10 μg/mL were 98, 90 and 89%, respectively. In a separate report of 201 patients who underwent posttreatment biopsies, 80% were negative, 17% indeterminate, and 3% were positive. The results did not change with longer follow-up. Nevertheless, many physicians feel that implantation is best reserved for patients with good or intermediate prognostic features. The procedure is well tolerated, although most patients experience urinary frequency and urgency that can persist for several months. Incontinence has been seen in 2 to 4% of cases. Higher complication rates are observed in patients who have undergone a prior TURP or who have obstructive symptoms at baseline. Proctitis has been reported in <2% of patients.

Watchful waiting, or deferred therapy, is a policy of no therapeutic intervention(s) until the tumor progresses. Progression can be based on PSA changes, local tumor growth, the development of symptoms, or metastatic disease. The practice evolved from studies of predominantly elderly men with well-differentiated tumors in whom clinically significant progression could not be demonstrated for protracted periods, during which a significant proportion died of intercurrent disease. In a structured literature review of patients treated by radical surgery, a deferred approach, or external beam radiation, the 10-year mean survivals were 93% for radical prostatectomy, 84% for deferred treatment, and 74% for external beam radiation. Risk of progression was related to grade. Men with grade 1 or 2 tumors had a 13% risk of death and 19% risk of metastases at 10 years; those with grade 3 tumors had 63 and 74% risks, respectively.

Case selection is critical, and the criteria to select those to whom watchful waiting can be applied safely are under intense study. In a recent prostatectomy series, it was estimated that 10 to 15% of patients had "insignificant" cancers. Given the multifocality of the disease, a concern is the limited ability to predict pathologic findings on the basis of a needle biopsy, even when multiple cores are obtained. Arguing against this approach is the result of a randomized trial of radical prostatectomy vs. watchful waiting from Sweden. With a median follow-up of 6.2 years, men treated by radical surgery had a lower risk of prostate cancer death relative to watchful waiting patients (4.6 vs. 8.9%) and a lower risk of metastatic progression, hazard ratio .63.

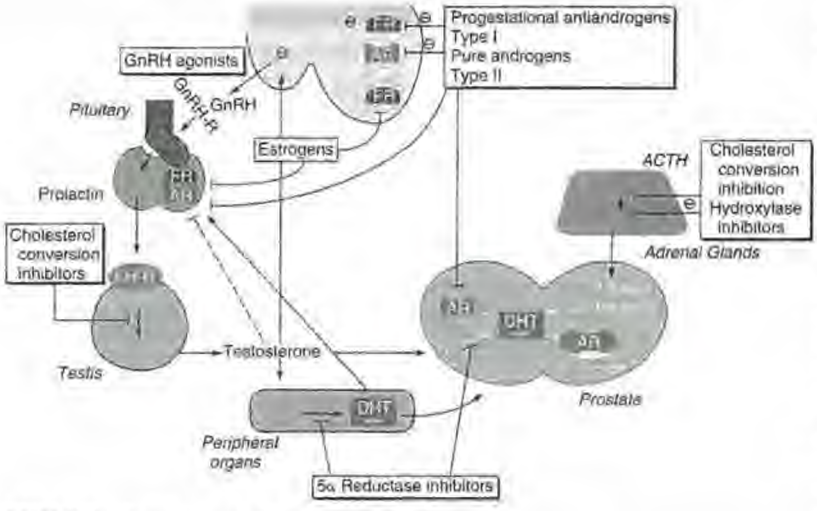

FIGURE 81-3 Sites of action of different hormone therapies.

Nevertheless, it can be anticipated that more patients may be candidates for a deferred approach as PSA testing is applied more widely and earlier.

RISING PSA This state includes patients in whom the sole manifestation of disease is a rising PSA after surgery and/or radiation therapy. By definition, no evidence of disease is found on scan. For these patients the central issue is whether the rise in PSA is the result of persistent disease in the primary site, a systemic recurrence, or both. In theory, disease that persists or has recurred in the primary site may be curable by additional local treatment. For patients who had undergone surgery, the question is whether external beam radiation therapy to the prostate bed can eliminate the disease and lead to an undetectable PSA. For radiation therapy–treated patients, the question is whether a prostatectomy would achieve cure.

The decision to recommend radiation therapy is often made on clinical grounds, as imaging studies such as CT and bone scan are typically uninformative. Some recommend a Prostascint scan: imaging with a radiolabeled antibody to prostate-specific membrane antigen (PSMA), which is highly expressed on prostate epithelial cells. Antibody localization to the prostatic fossa suggests local recurrence; localization to extrapelvic sites predicts failure of radiation therapy. Others recommend that a biopsy of the urethrovesical anastamosis be obtained before considering radiation. Factors that predict for response to salvage radiation are a positive surgical margin, a lower Gleason grade, a long interval from surgery to PSA failure, a slow PSA doubling time, and a low (<0.5 to 1.0 ng/mL) PSA value at the time of treatment. Radiation is generally not recommended if the PSA was persistently elevated after surgery (indicating that disease-free status was not achieved).

For patients with a rising PSA after radiation therapy, a salvage prostatectomy can be considered if the disease was "curable" at the onset, persistent disease has been documented by a biopsy of the prostate, and no metastatic disease is seen on imaging studies. Unfortunately, case selection is poorly defined in most series, and morbidities are significant. As currently performed, virtually all patients are impotent, and ~45% have either total urinary incontinence or stress incontinence. Major bleeding, bladder neck contractures, and rectal injury are not uncommon.

In the majority of cases, the rise in PSA indicates systemic disease. In these cases, the need for treatment should consider the probability of developing clinically detectable disease on scan and in what time frame. That immediate therapy is not required was shown in a series where patients did not receive systemic therapy until metastatic disease was documented. Overall, the median time to metastatic progression was 8 years and 63% of the patients with rising PSA values remained free of metastases at 5 years. Factors associated with progression include Gleason grade, time to recurrence, and PSA doubling times. For those with Gleason grade ≥8 tumors, the probability of metastatic progression was 37, 51, and 71% at 3, 5, and 7 years, respectively. If the time to recurrence was <2 years and PSA doubling time was long (> 10 months), the proportion with metastatic disease was 23, 32, and 53% vs. 47, 69, and 79% if the doubling time was short (< 10 months) during the same time intervals. These models continue to be refined. A difficulty making these predictions is that most patients with a rising PSA receive some form of therapy before the development of metastases.

METASTATIC DISEASE: NONCASTRATE *Metastatic disease noncastrate* refers to patients with tumors visible on an imaging study and noncastrate levels of testosterone. The patient may be newly diagnosed or have recurrent disease after treatment for localized disease. Standard treatment is to block androgen action or decrease androgen production by medical or surgical means. Over 90% of male hormones originate in the testes; <10% are synthesized in the adrenal gland. Surgical orchiectomy is the "gold standard" approach but is least acceptable by patients. Medical therapies can be divided into those that lower testosterone levels, e.g., gonadotropin-releasing hormone (GnRH) agonists and antagonists, estrogens and progestational agents, and the antiandrogens that bind to the androgen receptor but do not signal (Fig. 81-3). Ketoconazole inhibits adrenal androgen synthesis and is used after first-line castration is no longer effective. In this setting, the adrenal glands may contribute up to 40% of the active androgens in the prostate.

GnRH analogues (leuprolide acetate and goserelin acetate) initially produce a rise in luteinizing hormone and follicle-stimulating hormone (FSH), followed by a downregulation of receptors in the pituitary gland, which effects a chemical castration. They were approved on the basis of randomized comparisons showing an improved safety profile (specifically, reduced cardiovascular toxicities) relative to diethylstilbestrol (DES), with equivalent potency. The initial rise in testosterone may result in a clinical flare of the disease. As such, these agents are contraindicated in men with significant obstructive symptoms, cancer-related pain, or spinal cord compromise. Estrogens such as DES also lower testosterone levels but have fallen out of favor due to the risk of vascular complications such as fluid retention, phlebitis, emboli, and stroke.

In contrast, nonsteroidal antiandrogens such as flutamide, bicalutamide, or nilutamide block the binding of androgens to the receptor. Given alone, testosterone levels remain the same or increase. These agents were approved initially to block the flare associated with the initial rise in testosterone that results following GnRH administration. They have also been studied as part of a combined androgen blockade (CAB) or maximal androgen blockade (MAB) and as monotherapy. The concept of CAB was developed to inhibit testicular and adrenal androgens at the outset, and it preoccupied the field for many years. It is achieved clinically by combining an antiandrogen with a GnRH agonist or surgical orchiectomy. Cumulative results of randomized comparisons involving thousands of patients showed no advantage for combining an antiandrogen with surgical orchiectomy, while separate analyses of trials combining an antiandrogen with a GnRH analogue have shown a modest (<10%) survival advantage. Meta-analysis of all combined androgen blockade trials concluded that there was no benefit to the approach. In practice, most patients treated with GnRH analogue therapy receive an antiandrogen for the first 2 to 4 weeks of treatment.

The anti–prostate cancer effects of agents that lower serum testos-

some levels are similar, and the clinical course is predictable: an initial response, a period of stability in which the cells are dormant and not proliferating, followed by regrowth after a variable period of time as a hormone-independent tumor. Androgen ablation is not curative. Cells that survive castration are present when the disease is first diagnosed. Considered by disease manifestation, PSA levels return to normal in 60 to 70% of cases and measurable disease regression occurs in 30%; while improvements in bone scan occur in 25% of cases, the majority remain stable. Survival is inversely proportional to disease extent. Agents that lower testosterone are associated with an androgen-deprivation syndrome that includes hot flushes, weakness, fatigue, impotence, loss of muscle mass, changes in personality, anemia, depression, and a reduction in bone density. The bone changes can be prevented by treatment with bisphosphonates along with vitamin D and calcium supplementation.

A question often asked is whether antiandrogens, which are associated with fewer hot flashes, less of an effect on libido, less muscle wasting, fewer personality changes, and less bone loss, can be used alone without compromising outcomes. Gynecomastia remains a significant problem but can be alleviated in part with the addition of tamoxifen. Most reported randomized trials suggest that the cancer-specific outcomes are inferior. Even a comparison of bicalutamide, 150 mg (three times the recommended dose of 50 mg), versus surgical castration showed a shorter time to progression and inferior survival for patients with established metastatic disease. Nevertheless, some men may accept the trade-off of a potentially inferior cancer outcome for an improved quality of life.

Another question is whether hormones should be given early, in the adjuvant setting or at the time recurrence is first documented, or later, when metastatic disease or symptoms are manifest. Trials in support of early therapy have often been underpowered relative to the "net benefit" reported or have been criticized on methodologic grounds. In one, although a survival benefit was shown for patients treated with radiation therapy and 3 years of androgen ablation relative to radiation alone, the trial was criticized for the poor outcomes for the control group. Another showing a survival benefit for patients with positive nodes randomized to medical or surgical castration compared to observation ($p = .02$) was criticized because the confidence intervals around the 5- and 8-year survival distributions overlapped between the two groups. A large randomized study comparing early to late hormone treatment (orchiectomy or GnRH analogue) in patients with locally advanced or asymptomatic metastatic disease showed that patients treated early were less likely to progress from M0 to M1 disease, develop pain, and die of prostate cancer. This trial was criticized because therapy was delayed "too long" in the late-treatment group. When patients treated by radical surgery, radiation therapy, or watchful waiting were randomly assigned to receive bicalutamide, 150 mg, or placebo, hormone treatment produced a significant reduction in the proportion of patients who developed osseous metastases at 2 years (9% for bicalutamide; 13.8% for placebo). This result has not gained acceptance in part because too many "good-risk" patients were treated and because no effect on survival was demonstrated. These criticisms are valid; however, the net influence on survival from early hormone intervention is similar to that observed in patients with breast cancer where adjuvant hormonal therapy is routinely given.

Another way to reduce the side effects of androgen ablation is to administer hormones on an intermittent basis. This was proposed as a way to prevent the emergence of castration-resistant cells by "forcing" the cells that survive androgen ablation into a normal differentiation pathway by repleting testosterone. Theoretically, surviving cells that are allowed to proliferate in the presence of androgen will retain sensitivity to androgen ablation. The duration of treatment varies from 2 to 8 months beyond the point of maximal response. Once therapy is stopped, endogenous testosterone levels increase, and the symptoms associated with androgen ablation abate. PSA levels also begin to rise, and, at some level, androgen ablation is restarted. Using this approach, multiple cycles of regression and proliferation have been documented in individual patients. It is unknown whether the intermittent approach increases, decreases, or does not change the overall duration of sensitivity to androgen ablation. A trial to address this question is ongoing.

METASTATIC DISEASE: CASTRATE Castration-resistant disease can be manifest in many ways. For some it is a rise in PSA with no change in radiographs and no new symptoms. In others, it is a rising PSA and progression in bone, with or without symptoms of disease. Still others will show soft tissue disease with or without osseous metastases, and others have a pattern of visceral spread. The prognosis, highly variable, can also be predicted using nomograms designed for this cohort. The important distinction is that despite the failure of first-line hormone treatment, the majority of these tumors remain sensitive to second- and third-line hormonal treatments. Castration resistance does not indicate hormonal resistance. The rising PSA is an indication of continued signaling through the androgen receptor axis.

The manifestations of disease in this patient group hinder the development of drugs and treatment standards because the traditional measures of outcome such as tumor regression do not apply. No PSA-based outcomes are true surrogates for a survival benefit, and assessing changes in osseous disease using bone scans is notoriously inaccurate. It is essential to define therapeutic objectives before initiating treatment, as standards of care have changed on the basis of randomized comparisons that provide clinical benefits without prolonging life. These endpoints include the relief of symptoms and delaying metastases or the time to the development of new symptoms of disease.

The management of these patients requires first that the castrate status be documented. Patients receiving an antiandrogen alone who have elevated levels of serum testosterone should be treated first with a GnRH analogue or orchiectomy and observed for response. Patients on an anti-androgen in combination with a GnRH analogue should have the antiandrogen discontinued, as ~30% will respond to the withdrawal of the antiandrogen. Any response occurs within weeks of stopping flutamide, but may take 8 to 12 weeks with nilutamide and bicalutamide (they have a long terminal half-life). At the time of progression, a different antiandrogen can be given as these agents are not cross-resistant. Other hormones that may be active include estrogens, progestins, ketoconazole, and glucocorticoids. Those who respond to estrogens or progestins should also be evaluated for a withdrawal response at the time of progression. Cytotoxic agents are considered when hormone responses stop.

No chemotherapy regimen has been proven to prolong life in these patients. However, responses to chemotherapy that improve symptom control are not uncommon. Drugs directed at the tumor cell cytoskeleton such estramustine (Emcyt) and a taxane such as paclitaxel or docetaxel can induce responses in ≥50% using measurable disease regression as the endpoint. Seventy percent will show a >50% decline in PSA from baseline. Studies evaluating survival effects are nearly done.

Management of pain is a critical part of therapy. Optimal palliation requires assessing whether the symptoms and metastases are focal or diffuse and whether disease threatens the spinal cord, the cauda equina, or the base of the skull. Neurologic symptoms require emergent evaluation because loss of function may be permanent if not addressed in a timely manner. Single sites of pain or areas of neurologic involvement are best treated with external beam radiation. As the disease is often diffuse, palliation at one site often leads to the emergence of symptoms at another. An important principle of management was established in two randomized trials of mitoxantrone and prednisone vs. prednisone alone. In both studies, mitoxantrone-treated patients had a greater reduction in pain, used fewer narcotics, were more mobile, and had less fatigue. No survival benefit was shown.

Given the bone-dominant nature of prostate cancer spread, bone-directed therapies may be useful in patients with diffuse disease. Two bone-seeking radioisotopes, ^{89}Sr (metastron) and ^{153}Sm-EDTMP (quadramet), are approved for palliation of pain although they have no effect on PSA or on survival. Fewer patients treated with an isotope

developed new areas of pain or required additional radiation therapy compared to patients receiving external beam radiation therapy alone. Addition of zoledronate to "standard therapy" in patients with castration-resistant disease resulted in fewer skeletal events relative to placebo-treated patients. The bone events included development of new pain, need for radiation therapy, and microfractures. Finally, patients randomly assigned to a combination of ^{89}Sr and doxorubicin after induction chemotherapy had fewer skeletal events and longer survival than patients treated with doxorubicin alone. Confirmatory studies are ongoing.

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82 TESTICULAR CANCER

Robert J. Motzer, George J. Bosl

Primary germ cell tumors (GCTs) of the testis, arising by the malignant transformation of primordial germ cells, constitute 95% of all testicular neoplasms. Infrequently, GCTs arise from an extragonadal site, including the mediastinum, retroperitoneum, and, very rarely, the pineal gland. This disease is notable for the young age of the afflicted patients, the totipotent capacity for differentiation of the tumor cells, and its curability; about 95% of all newly diagnosed patients will be cured. Experience in the management of GCTs leads to improved outcome.

INCIDENCE AND EPIDEMIOLOGY Nearly 9000 new cases of testicular GCT were diagnosed in the United States in 2004; the incidence of this malignancy has increased slowly over the past 40 years. The tumor occurs most frequently in men between the ages of 20 and 40. A testicular mass in a man ≥50 years should be regarded as a lymphoma until proved otherwise. GCT is at least four to five times more common in white than in African-American males, and a higher incidence has been observed in Scandinavia and New Zealand than in the United States.

ETIOLOGY AND GENETICS Cryptorchidism is associated with a severalfold higher risk of GCT. Abdominal cryptorchid testes are at a higher risk than inguinal cryptorchid testes. Orchiopexy should be performed before puberty, if possible. Early orchiopexy reduces the risk of GCT and improves the ability to save the testis. An abdominal cryptorchid testis that cannot be brought into the scrotum should be removed. About 2% of men with GCTs of one testis will develop a primary tumor in the other testis. Testicular feminization syndromes increase the risk of testicular GCT, and Klinefelter's syndrome is associated with mediastinal GCT.

An isochromosome of the short arm of chromosome 12 [i(12p)] is pathognomonic for GCT of all histologic types. Excess 12p copy number either in the form of i(12p) or as increased 12p on aberrantly banded marker chromosomes occurs in nearly all GCTs, but the gene(s) on 12p involved in the pathogenesis are not yet defined.

CLINICAL PRESENTATION A painless testicular mass is pathognomonic for a testicular malignancy. More commonly, patients present with testicular discomfort or swelling suggestive of epididymitis and/or orchitis. In this circumstance, a trial of antibiotics is reasonable. However, if symptoms persist or a residual abnormality remains, then testicular ultrasound examination is indicated.

Ultrasound of the testis is indicated whenever a testicular malignancy is considered and for persistent or painful testicular swelling. If a testicular mass is detected, a radical inguinal orchiectomy should be performed. Because the testis develops from the gonadal ridge, its blood supply and lymphatic drainage originate in the abdomen and descend with the testis into the scrotum. An inguinal approach is taken to avoid breaching anatomic barriers and permitting additional pathways of spread.

Back pain from retroperitoneal metastases is common and must be distinguished from musculoskeletal pain. Dyspnea from pulmonary metastases occurs infrequently. Patients with increased serum levels of human chorionic gonadotropin (hCG) may present with gynecomastia. A delay in diagnosis is associated with a more advanced stage and possibly worse survival.

The staging evaluation for GCT includes a determination of serum levels of α fetoprotein (AFP), hCG, and lactate dehydrogenase (LDH). After orchiectomy, a chest radiograph and a computed tomography (CT) scan of the abdomen and pelvis should be performed. A chest CT scan is required if pulmonary nodules or mediastinal or hilar disease is suspected. Stage I disease is limited to the testis, epididymis, or spermatic cord. Stage II disease is limited to retroperitoneal (regional) lymph nodes. Stage III disease is disease outside the retroperitoneum, involving supradiaphragmatic nodal sites or viscera. The staging may be "clinical"—defined solely by physical examination, blood marker evaluation, and radiographs—or "pathologic"—defined by an operative procedure.

The regional draining lymph nodes for the testis are in the retroperitoneum, and the vascular supply originates from the great vessels (for the right testis) or the renal vessels (for the left testis). As a result, the lymph nodes that are involved first by a right testicular tumor are the interaortocaval lymph nodes just below the renal vessels. For a left testicular tumor, the first involved lymph nodes are lateral to the aorta (para-aortic) and below the left renal vessels. In both cases, further nodal spread is inferior and contralateral and, less commonly, above the renal hilum. Lymphatic involvement can extend cephalad to the retrocrural, posterior mediastinal, and supraclavicular lymph nodes. Treatment is determined by tumor histology (seminoma versus nonseminoma) and clinical stage (Table 82-1).

PATHOLOGY GCTs are divided into nonseminoma and seminoma subtypes. Nonseminomatous GCTs are most frequent in the third decade of life and can display the full spectrum of embryonic and adult cellular differentiation. This entity comprises four histologies: embryonal carcinoma, teratoma, choriocarcinoma, and endodermal sinus (yolk sac) tumor. Choriocarcinoma, consisting of both cytotrophoblasts and syncytiophoblasts, represents malignant trophoblastic differentiation and is invariably associated with secretion of hCG. Endodermal sinus tumor is the malignant counterpart of the fetal yolk sac and is associated with secretion of AFP. Pure embryonal carcinoma may secrete AFP or hCG, or both; this pattern is biochemical evidence of differentiation. Teratoma is composed of somatic cell types derived from two or more germ layers (ectoderm, mesoderm, or endoderm). Each of these histologies may be present alone or in combination with others. Nonseminomatous GCTs tend to metastasize early to sites such as the retroperitoneal lymph nodes and lung parenchyma. One-third of patients present with disease limited to the testis (stage I), one-third with retroperitoneal metastases (stage II), and one-third with more extensive supradiaphragmatic nodal or visceral metastases (stage III).

Seminoma represents about 50% of all GCTs, has a median age in the fourth decade, and generally follows a more indolent clinical course. Most patients (70%) present with stage I disease, about 20% with stage II disease, and 10% with stage III disease; lung or other visceral metastases are rare. Radiation therapy is the treatment of

nancy, and 20% nondiagnostic or yielding insufficient material for diagnosis. Characteristic features of malignancy mandate surgery. A diagnosis of follicular neoplasm also warrants surgery, as benign and malignant lesions cannot be distinguished based on cytopathology or frozen section. The management of patients with benign lesions is more variable. Many authorities advocate TSH suppression, whereas others monitor nodule size without suppression. With either approach, thyroid nodule size should be monitored, either by palpation or ultrasound. Repeat FNA is indicated if a nodule enlarges, and a second biopsy should be performed within 2 to 5 years to confirm the benign status of the nodule.

Nondiagnostic biopsies occur for many reasons, including a fibrotic reaction with relatively few cells available for aspiration, a cystic lesion in which cellular components reside along the cyst margin, or a nodule that may be too small for accurate aspiration. For these reasons, ultrasound-guided FNA is useful when the FNA is repeated. Ultrasound is also increasingly used for initial biopsies in an effort to enhance nodule localization and the accuracy of sampling.

The evaluation of a thyroid nodule is stressful for most patients. They are concerned about the possibility of thyroid cancer, whether verbalized or not. It is constructive, therefore, to review the diagnostic approach and to reassure patients when malignancy is not found. When a suspicious lesion or thyroid cancer is identified, an explanation of the generally favorable prognosis and available treatment options should be provided.

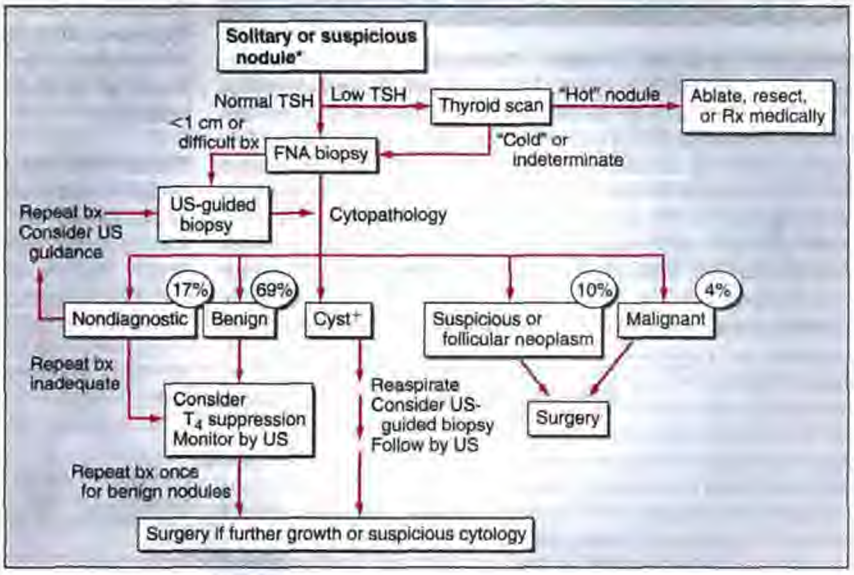

FIGURE 320-13 Approach to the patient with a thyroid nodule. *There are many exceptions to the suggested options. See text and references for details. †About one-third of nodules are cystic or mixed solid-cystic. US, ultrasound; TSH, thyroid-stimulating hormone; FNA, fine-needle aspiration.

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321 DISORDERS OF THE ADRENAL CORTEX

Gordon H. Williams, Robert G. Dluhy

BIOCHEMISTRY AND PHYSIOLOGY

The adrenal cortex produces three major classes of steroids: (1) glucocorticoids, (2) mineralocorticoids, and (3) adrenal androgens. Consequently, normal adrenal function is important for modulating intermediary metabolism and immune responses through glucocorticoids; blood pressure, vascular volume, and electrolytes through mineralocorticoids; and secondary sexual characteristics (in females) through androgens. The adrenal axis plays an important role in the stress response by rapidly increasing cortisol levels. Adrenal disorders include hyperfunction (Cushing's syndrome) and hypofunction (adrenal insufficiency), as well as a variety of genetic abnormalities of steroidogenesis.

STEROID NOMENCLATURE The basic structure of steroids is built upon a five-ring nucleus (Fig. 321-1). The carbon atoms are numbered in a sequence beginning with ring A. Adrenal steroids contain either 19 or 21 carbon atoms. The C_{19} steroids have methyl groups at C-18 and C-19. C_{19} steroids with a ketone group at C-17 are termed *17-ketosteroids*; C_{19} steroids have predominantly androgenic activity. The C_{21} steroids have a 2-carbon side chain (C-20 and C-21) attached at position 17 and methyl groups at C-18 and C-19; C_{21} steroids with a hydroxyl group at position 17 are termed *17-hydroxycorticosteroids*. The C_{21} steroids have either glucocorticoid or mineralocorticoid properties.

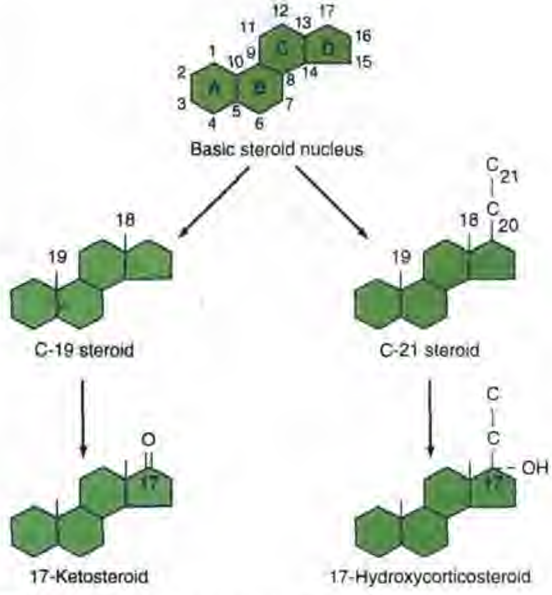

FIGURE 321-1 Basic steroid structure and nomenclature.

BIOSYNTHESIS OF ADRENAL STEROIDS Cholesterol, derived from the diet and from endogenous synthesis, is the substrate for steroidogenesis. Uptake of cholesterol by the adrenal cortex is mediated by the low-density lipoprotein (LDL) receptor. With long-term stimulation of the adrenal cortex by adrenocorticotropic hormone (ACTH), the number of LDL receptors increases. The three major adrenal biosynthetic pathways lead to the production of glucocorticoids (cortisol), mineralocorticoids (aldosterone), and adrenal androgens (dehydroepiandrosterone). Separate zones of the adrenal cortex synthesize specific hormones (Fig. 321-2). This zonation is accompanied by the selective expression of the genes encoding the enzymes unique to the formation of each type of steroid: aldosterone synthase is normally expressed only in the outer (glomerulosa) cell layer, whereas 21- and 17-hydroxylase are expressed in the (inner) faciculata-reticularis cell layers, which are the sites of cortisol and androgen biosynthesis, respectively.

STEROID TRANSPORT Cortisol circulates in the plasma as free cortisol, protein-bound cortisol, and cortisol metabolites. *Free cortisol* is the physiologically active hormone that is not protein-bound and therefore can act directly on tissue sites. Normally, <5% of circulating cortisol is free. Only the unbound cortisol and its metabolites are filterable at the glomerulus. Increased quantities of free steroid are excreted in the urine in states characterized by hypersecretion of cortisol, because the

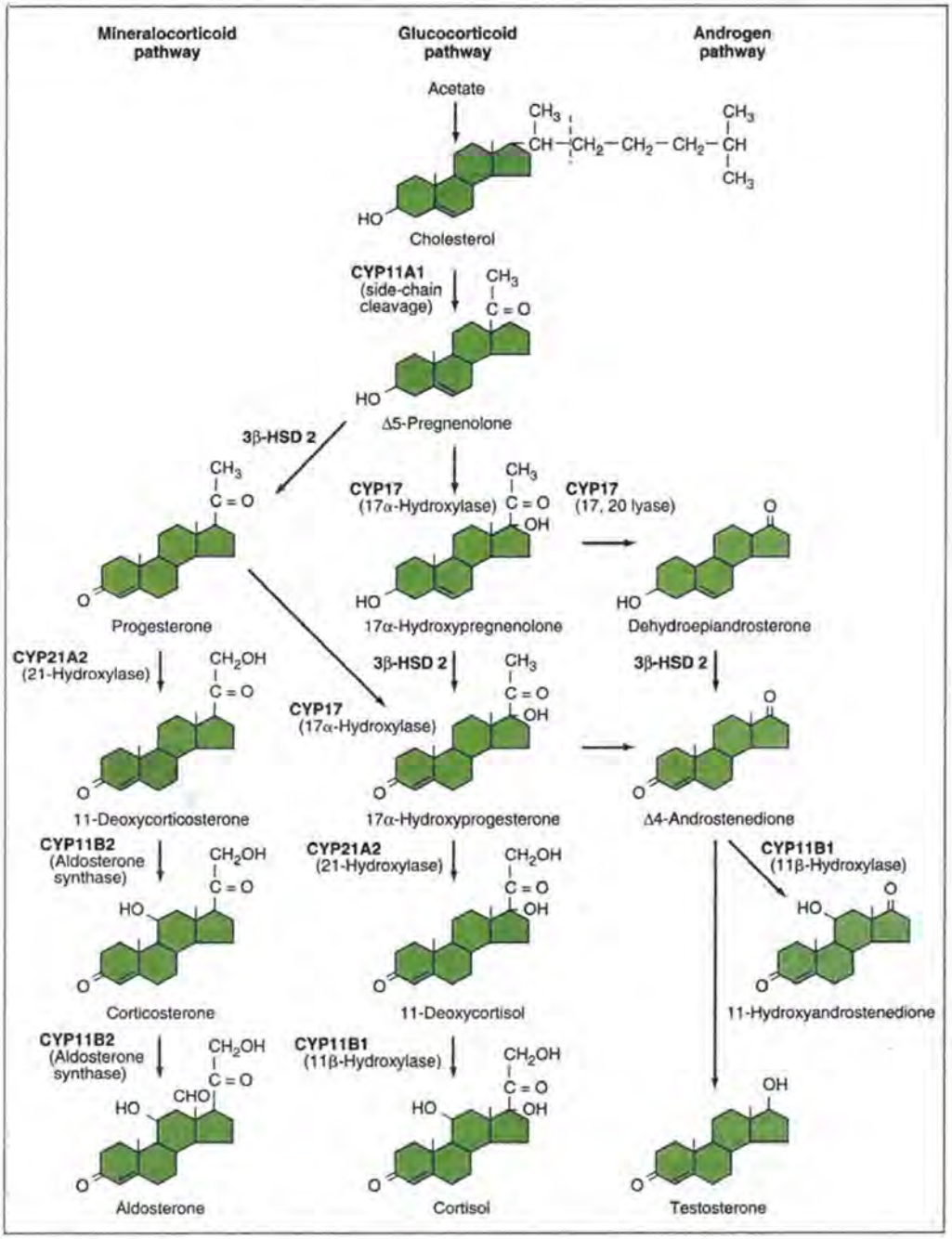

FIGURE 321-2 Biosynthetic pathways for adrenal steroid production; major pathways to mineralocorticoids, glucocorticoids, and androgens. 3β-HSD, 3β-hydroxysteroid dehydrogenase.

unbound fraction of plasma cortisol rises. Plasma has two cortisol-binding systems. One is a high-affinity, low-capacity α_2-globulin termed *transcortin* or *cortisol-binding globulin* (CBG), and the other is a low-affinity, high-capacity protein, *albumin*. Cortisol binding to CBG is reduced in areas of inflammation, thus increasing the local concentration of free cortisol. When the concentration of cortisol is >700 nmol/L (25 μg/dL), part of the excess binds to albumin, and a greater proportion than usual circulates unbound. CBG is increased in high-estrogen states (e.g., pregnancy, oral contraceptive administration). The rise in CBG is accompanied by a parallel rise in *protein-bound cortisol*, with the result that the total plasma cortisol concentration is elevated. However, the free cortisol level probably remains normal, and manifestations of glucocorticoid excess are absent. Most synthetic glucocorticoid analogues bind less efficiently to CBG (~70% binding). This may explain the propensity of some synthetic analogues to produce cushingoid effects at low doses. *Cortisol metabolites* are biologically inactive and bind only weakly to circulating plasma proteins.

Aldosterone is bound to proteins to a smaller extent than cortisol, and an ultrafiltrate of plasma contains as much as 50% of circulating aldosterone.

STEROID METABOLISM AND EXCRETION ■ Glucocorticoids The daily secretion of cortisol ranges between 40 and 80 μmol (15 and 30 mg; 8–10 mg/m²), with a pronounced circadian cycle. The plasma concentration of cortisol is determined by the rate of secretion, the rate of inactivation, and the rate of excretion of free cortisol. The liver is the major organ responsible for steroid inactivation. A major enzyme regulating cortisol metabolism is 11β-hydroxysteroid dehydrogenase (11β-HSD). There are two isoforms: 11β-HSD I is primarily expressed in the liver and acts as a reductase, converting the inactive cortisone to the active glucocorticoid, cortisol; the 11β-HSD II isoform is expressed in a number of tissues and converts cortisol to the inactive metabolite, cortisone. Mutations in the *11BHSD1* gene are associated with rapid cortisol turnover, leading to activation of the hypothalamic-pituitary-adrenal (HPA) axis and excessive adrenal androgen production in women. In animal models, excess omental expression of 11β-HSD I increases local glucocorticoid production and is associated with central obesity and insulin resistance. The oxidative reaction of 11β-HSD I is increased in hyperthyroidism. Mutations in the *11BHSD2* gene cause the syndrome of *apparent mineralocorticoid excess*, reflecting insufficient inactivation of cortisol in the kidney, allowing inappropriate cortisol activation of the mineralocorticoid receptor (see below).

Mineralocorticoids In individuals with normal salt intake, the average daily secretion of aldosterone ranges between 0.1 and 0.7 μmol (50 and 250 μg). During a single passage through the liver, >75% of circulating aldosterone is normally inactivated by conjugation with glucuronic acid. However, under certain conditions, such as congestive failure, this rate of inactivation is reduced.

Adrenal Androgens The major androgen secreted by the adrenal is dehydroepiandrosterone (DHEA) and its sulfuric acid ester (DHEAS). Approximately 15 to 30 mg of these compounds is secreted daily. Smaller amounts of androstenedione, 11β-hydroxyandrostenedione, and testosterone are secreted. DHEA is the major precursor of the urinary 17-ketosteroids. Two-thirds of the urine 17-ketosteroids in the male are derived from adrenal metabolites, and the remaining one-third comes from testicular androgens. In the female, almost all urine 17-ketosteroids are derived from the adrenal.

Steroids diffuse passively through the cell membrane and bind to intracellular receptors (Chap. 317). Glucocorticoids and mineralocorticoids bind with nearly equal affinity to the mineralocorticoid receptor (MR). However, only glucocorticoids bind to the glucocorticoid receptor (GR). After the steroid binds to the receptor, the steroid-receptor complex is transported to the nucleus, where it binds to specific sites on steroid-regulated genes, altering levels of transcription. Some actions of glucocorticoids (e.g., anti-inflammatory effects) are mediated by GR-mediated inhibition of other transcription factors, such as activating protein-1 (AP-1) or nuclear factor kappa B (NFκB), which normally stimulate the activity of various cytokine genes. Because cortisol binds to the MR with the same affinity as aldosterone, mineralocorticoid specificity is achieved by local metabolism of cortisol to the inactive compound cortisone by 11β-HSD II. The glucocorticoid effects of other steroids, such as high-dose progesterone, correlate with their relative binding affinities for the GR. Inherited defects in the GR cause glucocorticoid resistance states. Individuals with GR defects have high levels of cortisol but do not have manifestations of hypercortisolism.

ACTH PHYSIOLOGY ACTH and a number of other peptides (lipotropins, endorphins, and melanocyte-stimulating hormones) are processed from a larger precursor molecule of 31,000 mol wt—proopiomelanocortin (POMC) (Chap. 318). POMC is made in a variety of tissues, including brain, anterior and posterior pituitary, and lymphocytes. The constellation of POMC-derived peptides secreted depends on the tissue. ACTH, a 39-amino-acid peptide, is synthesized and stored in basophilic cells of the anterior pituitary. The *N*-terminal 18-amino-acid fragment of ACTH has full biologic potency, and shorter *N*-terminal fragments have partial biologic activity. Release of ACTH and related peptides from the anterior pituitary gland is stimulated by corticotropin-releasing hormone (CRH), a 41-amino-acid peptide produced in the median eminence of the hypothalamus (Fig. 321-3). Urocortin, a neuropeptide related to CRH, mimics many of the central effects of CRH (e.g., appetite suppression, anxiety), but its role in ACTH reg-

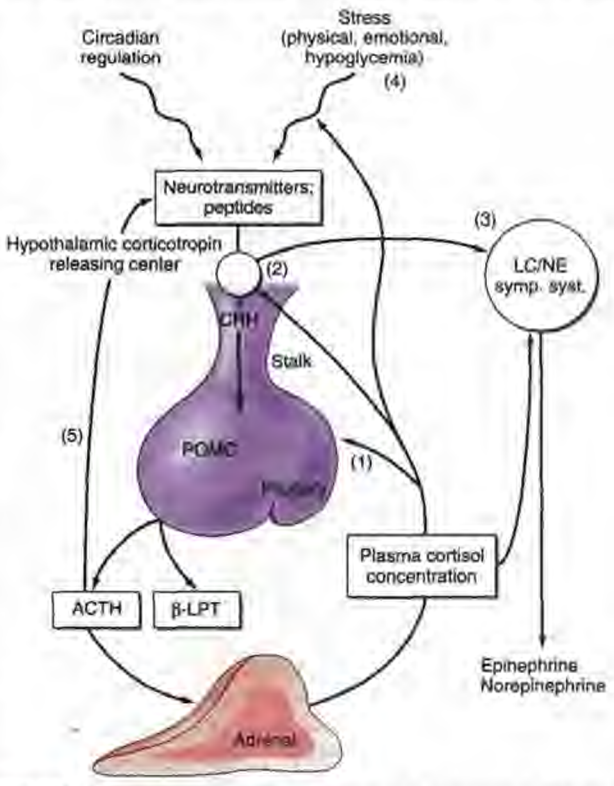

FIGURE 321-3 The hypothalamic-pituitary-adrenal axis. The main sites for feedback control by plasma cortisol are the pituitary gland (1) and the hypothalamic corticotropin-releasing center (2). Feedback control by plasma cortisol also occurs at the locus coeruleus/sympathetic system (3) and may involve higher nerve centers (4) as well. There may also be a short feedback loop involving inhibition of corticotropin-releasing hormone (CRH) by adrenocorticotropic hormone (ACTH) (5). Hypothalamic neurotransmitters influence CRH release; serotoninergic and cholinergic systems stimulate the secretion of CRH and ACTH; α-adrenergic agonists and γ-aminobutyric acid (GABA) probably inhibit CRH release. The opioid peptides β-endorphin and enkephalin inhibit, and vasopressin and angiotensin II augment, the secretion of CRH and ACTH. β-LPT, β-lipotropin; POMC, pro-opiomelanocortin; LC, locus coeruleus; NE, norepinephrine.

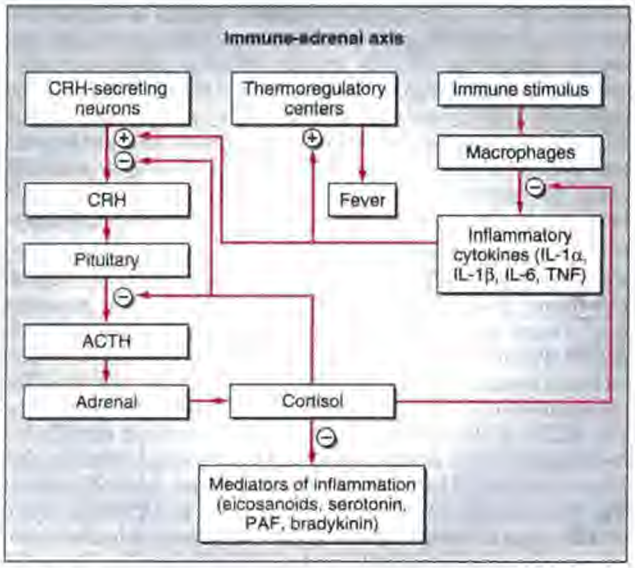

FIGURE 321-4 The immune-adrenal axis. Cortisol has anti-inflammatory properties that include effects on the microvasculature, cellular actions, and the suppression of inflammatory cytokines (the so-called immune-adrenal axis). A stress such as sepsis increases adrenal secretion, and cortisol in turn suppresses the immune response via this system. −, suppression; +, stimulation; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; IL, interleukin; TNF, tumor necrosis factor; PAF, platelet activating factor.

ulation is unclear. Some related peptides such as β-lipotropin (β-LPT) are released in equimolar concentrations with ACTH, suggesting that they are cleaved enzymatically from the parent POMC before or during the secretory process. However, β-endorphin levels may or may not correlate with circulating levels of ACTH, depending on the nature of the stimulus.

The major factors controlling ACTH release include CRH, the free cortisol concentration in plasma, stress, and the sleep-wake cycle (Fig. 321-3). Plasma ACTH varies during the day as a result of its pulsatile secretion, and follows a circadian pattern with a peak just prior to waking and a nadir before sleeping. If a new sleep-wake cycle is adopted, the pattern changes over several days to conform to it. ACTH and cortisol levels also increase in response to eating. Stress (e.g., pyrogens, surgery, hypoglycemia, exercise, and severe emotional trauma) causes the release of CRH and arginine vasopressin (AVP) and activation of the sympathetic nervous system. These changes in turn enhance ACTH release, acting individually or in concert. For example, AVP release acts synergistically with CRH to amplify ACTH secretion; CRH also stimulates the locus coeruleus/sympathetic system. Stress-related secretion of ACTH abolishes the circadian periodicity of ACTH levels but is, in turn, suppressed by prior high-dose glucocorticoid administration. The normal pulsatile, circadian pattern of ACTH release is regulated by CRH; this mechanism is the so-called open feedback loop. CRH secretion, in turn, is influenced by hypothalamic neurotransmitters including the serotoninergic and cholinergic pathways. The immune system also influences the HPA axis (Fig. 321-4). For example, inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL) 1α, IL-1β, and IL-6] produced by monocytes increase ACTH release by stimulating secretion of CRH and/or AVP. Finally, ACTH release is regulated by the level of free cortisol in plasma. Cortisol decreases the responsiveness of pituitary corticotropic cells to CRH; the response of the POMC mRNA to CRH is also inhibited by glucocorticoids. In addition, glucocorticoids inhibit the locus coeruleus/sympathetic system and CRH release. The latter servomechanism establishes the primacy of cortisol in the control of ACTH secretion. The suppression of ACTH secretion that results in adrenal atrophy following *prolonged* glucocorticoid therapy is caused primarily by suppression of hypothalamic CRH release, as exogenous CRH administration in this circumstance produces a rise in plasma ACTH. Cortisol also exerts feedback effects on higher brain centers (hippocampus, reticular system, and septum) and perhaps on the adrenal cortex (Fig. 321-4).

The biologic half-life of ACTH in the circulation is <10 min. The action of ACTH is also rapid: within minutes of its release, the concentration of steroids in the adrenal venous blood increases. ACTH stimulates steroidogenesis via activation of adenyl cyclase. Adenosine 3′,5′-monophosphate (cyclic AMP), in turn, stimulates the synthesis of protein kinase enzymes, thereby resulting in the phosphorylation of proteins that activate steroid biosynthesis.

RENIN-ANGIOTENSIN PHYSIOLOGY Renin is a proteolytic enzyme that is produced and stored in the granules of the juxtaglomerular cells surrounding the afferent arterioles of glomeruli in the kidney. Renin acts on the basic substrate angiotensinogen (a circulating α_2-globulin made in the liver) to form the decapeptide angiotensin I (Fig. 321-5). Angiotensin I is then enzymatically transformed by angiotensin-converting enzyme (ACE), which is present in many tissues (particularly the pulmonary vascular endothelium), to the octapeptide angiotensin II by the removal of the two C-terminal amino acids. Angiotensin II is a potent pressor agent and exerts its action by a direct effect on arteriolar smooth muscle. In addition, angiotensin II stimulates production of aldosterone by the zona glomerulosa of the adrenal cortex; the heptapeptide angiotensin III may also stimulate aldosterone production. The two major classes of angiotensin receptors are termed *AT1* and *AT2*; AT1 may exist as two subtypes α and β. Most of the effects of angiotensins II and III are mediated by the AT1 receptor. Angiotensinases rapidly destroy angiotensin II (half-life, ~1 min), while the half-life of renin is more prolonged (10 to 20 min). In addition to circulating renin-angiotensin, many tissues have a local renin-angiotensin system and the ability to produce angiotensin II. These tissues include the uterus, placenta, vascular tissue, heart, brain, and, particularly, the adrenal cortex and kidney. Although the role of locally generated angiotensin II is not established, it may modulate the growth and function of the adrenal cortex and vascular smooth muscle.

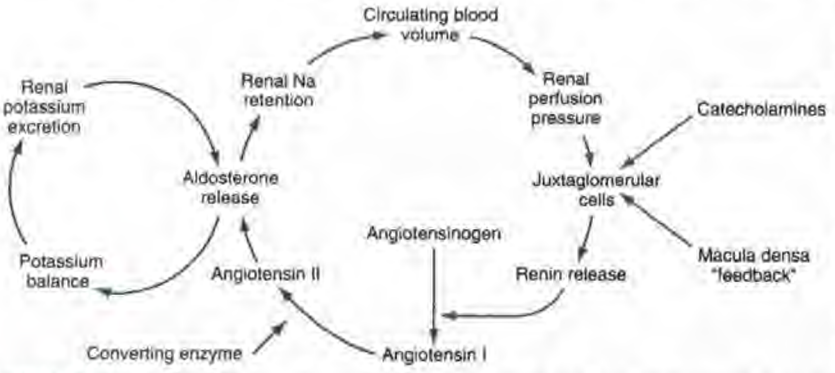

FIGURE 321-5 The interrelationship of the volume and potassium feedback loops on aldosterone secretion. Integration of signals from each loop determines the level of aldosterone secretion.

The amount of renin released reflects the combined effects of four interdependent factors. The *juxtaglomerular cells*, which are specialized myoepithelial cells that cuff the afferent arterioles, act as miniature pressure transducers, sensing renal perfusion pressure and corresponding changes in afferent arteriolar perfusion pressures. For example, a reduction in circulating blood volume leads to a corresponding reduction in renal perfusion pressure and

afferent arteriolar pressure (Fig. 321-5). This change is perceived by the juxtaglomerular cells as a decreased stretch exerted on the afferent arteriolar walls, and the juxtaglomerular cells release more renin into the renal circulation. This results in the formation of angiotensin I, which is converted in the kidney and peripherally to angiotensin II by ACE. Angiotensin II influences sodium homeostasis via two major mechanisms: it changes renal blood flow so as to maintain a constant glomerular filtration rate, thereby changing the filtration fraction of sodium, and it stimulates the adrenal cortex to release aldosterone. Increasing plasma levels of aldosterone enhance renal sodium retention and thus result in expansion of the extracellular fluid volume, which, in turn, dampens the stimulus for renin release. In this context, the renin-angiotensin-aldosterone system regulates volume by modifying renal hemodynamics and tubular sodium transport.

A second control mechanism for renin release is centered in the *macula densa cells*, a group of distal convoluted tubular epithelial cells directly opposed to the juxtaglomerular cells. They may function as chemoreceptors, monitoring the sodium (or chloride) load presented to the distal tubule. Under conditions of increased delivery of filtered sodium to the macula densa, a signal is conveyed to decrease juxtaglomerular cell release of renin, thereby modulating the glomerular filtration rate and the filtered load of sodium.

The *sympathetic nervous system* regulates the release of renin in response to assumption of the upright posture. The mechanism is either a direct effect on the juxtaglomerular cell to increase adenyl cyclase activity or an indirect effect on either the juxtaglomerular or the macula densa cells via vasoconstriction of the afferent arteriole.

Finally, circulating factors influence renin release. Increased dietary intake of potassium decreases renin release, whereas decreased potassium intake increases it. The significance of these effects is unclear. *Angiotensin II* exerts negative feedback control on renin release that is independent of alterations in renal blood flow, blood pressure, or aldosterone secretion. *Atrial natriuretic peptides* also inhibit renin release. Thus, the control of renin release involves both *intrarenal* (pressure receptor and macula densa) and *extrarenal* (sympathetic nervous system, potassium, angiotensin, etc.) mechanisms. Steady-state renin levels reflect all these factors, with the intrarenal mechanism predominating.

GLUCOCORTICOID PHYSIOLOGY The division of adrenal steroids into glucocorticoids and mineralocorticoids is arbitrary in that most glucocorticoids have some mineralocorticoid-like properties. The descriptive term *glucocorticoid* is used for adrenal steroids whose predominant action is on intermediary metabolism. Their overall actions are directed at enhancing the production of the high-energy fuel, glucose, and reducing all other metabolic activity not directly involved in that process. Sustained activation, however, results in a pathophysiologic state, e.g., Cushing's syndrome. The principal glucocorticoid is cortisol (hydrocortisone). The effect of glucocorticoids on intermediary metabolism is mediated by the GR. Physiologic effects of glucocorticoids include the regulation of protein, carbohydrate, lipid, and nucleic acid metabolism. Glucocorticoids raise the blood glucose level by antagonizing the secretion and actions of insulin, thereby inhibiting peripheral glucose uptake, which promotes hepatic glucose synthesis (gluconeogenesis) and hepatic glycogen content. The actions on protein metabolism are mainly catabolic, resulting in an increase in protein breakdown and nitrogen excretion. In large part, these actions reflect a mobilization of glycogenic amino acid precursors from peripheral supporting structures, such as bone, skin, muscle, and connective tissue, due to protein breakdown and inhibition of protein synthesis and amino acid uptake. Hyperaminoacidemia also facilitates gluconeogenesis by stimulating glucagon secretion. Glucocorticoids act directly on the liver to stimulate the synthesis of certain enzymes, such as tyrosine aminotransferase and tryptophan pyrrolase. Glucocorticoids regulate fatty acid mobilization by enhancing the activation of cellular lipase by lipid-mobilizing hormones (e.g., catecholamines and pituitary peptides).

The actions of cortisol on protein and adipose tissue vary in different parts of the body. For example, pharmacologic doses of cortisol can deplete the protein matrix of the vertebral column (trabecular bone), whereas long bones (which are primarily compact bone) are affected only minimally; similarly, peripheral adipose tissue mass decreases, whereas abdominal and interscapular fat expand.

Glucocorticoids have anti-inflammatory properties, which are probably related to effects on the microvasculature and to suppression of inflammatory cytokines. In this sense, glucocorticoids modulate the immune response via the so-called immune-adrenal axis (Fig. 321-4). This "loop" is one mechanism by which a stress, such as sepsis, increases adrenal hormone secretion, and the elevated cortisol level in turn suppresses the immune response. For example, cortisol maintains vascular responsiveness to circulating vasoconstrictors and opposes the increase in capillary permeability during acute inflammation. Glucocorticoids cause a leukocytosis that reflects release from the bone marrow of mature cells as well as inhibition of their egress through the capillary wall. Glucocorticoids produce a depletion of circulating eosinophils and lymphoid tissue, specifically T cells, by causing a redistribution from the circulation into other compartments. Thus, cortisol impairs cell-mediated immunity. Glucocorticoids also inhibit the production and action of the mediators of inflammation, such as the lymphokines and prostaglandins. Glucocorticoids inhibit the production and action of interferon by T lymphocytes and the production of IL-1 and IL-6 by macrophages. The antipyretic action of glucocorticoids may be explained by an effect on IL-1, which appears to be an endogenous pyrogen (Chap. 16). Glucocorticoids also inhibit the production of T cell growth factor (IL-2) by T lymphocytes. Glucocorticoids reverse macrophage activation and antagonize the action of migration-inhibiting factor (MIF), leading to reduced adherence of macrophages to vascular endothelium. Glucocorticoids reduce prostaglandin and leukotriene production by inhibiting the activity of phospholipase A_2, thus blocking release of arachidonic acid from phospholipids. Finally, glucocorticoids inhibit the production and inflammatory effects of bradykinin, platelet-activating factor, and serotonin. It is probably only at pharmacologic dosages that antibody production is reduced and lysosomal membranes are stabilized, the latter effect suppressing the release of acid hydrolases.

Cortisol levels respond within minutes to stress, whether physical (trauma, surgery, exercise), psychological (anxiety, depression), or physiologic (hypoglycemia, fever). The reasons why elevated glucocorticoid levels protect the organism under stress are not understood, but in conditions of glucocorticoid deficiency, such stresses may cause hypotension, shock, and death. Consequently, in individuals with adrenal insufficiency, glucocorticoid administration should be increased during stress.

Cortisol has major effects on body water. It helps regulate the extracellular fluid volume by retarding the migration of water into cells and by promoting renal water excretion, the latter effect mediated by suppression of vasopressin secretion, by an increase in the rate of glomerular filtration, and by a direct action on the renal tubule. The consequence is to prevent water intoxication by increasing solute-free water clearance. Glucocorticoids also have weak mineralocorticoid-like properties, and high doses promote renal tubular sodium reabsorption and increased urine potassium excretion. Glucocorticoids can also influence behavior; emotional disorders may occur with either an excess or a deficit of cortisol. Finally, cortisol suppresses the secretion of pituitary POMC and its derivative peptides (ACTH, β-endorphin, and β-LPT) and the secretion of hypothalamic CRH and vasopressin.

MINERALOCORTICOID PHYSIOLOGY Mineralocorticoids modify function in two classes of cells—epithelial and nonepithelial.

Effects on Epithelia Classically, mineralocorticoids are considered major regulators of extracellular fluid volume and are the major determinants of potassium metabolism. These effects are mediated by the binding of aldosterone to the MR in epithelial cells, primarily the principal cells in the renal cortical collecting duct. Because of its electro-

chemical gradient, sodium passively enters these cells from the urine via epithelial sodium channels located on the luminal membrane and is actively extruded from the cell via the Na/K-activated ATPase ("sodium pump") located on the basolateral membrane. The sodium pump also provides the driving force of potassium loss into the urine through potassium-selective luminal channels, again assisted by the electrochemical gradient for potassium in these cells. Aldosterone stimulates all three of these processes by increasing gene expression directly (for the sodium pump and the potassium channels) or via a complex process (for epithelial sodium channels) to increase both the number and activity of the sodium channels. Water passively follows the transported sodium, thus expanding intra- and extravascular volume.

Because the concentration of hydrogen ion is greater in the lumen than in the cell, hydrogen ion is also actively secreted. Mineralocorticoids also act on the epithelium of the salivary ducts, sweat glands, and gastrointestinal tract to cause reabsorption of sodium in exchange for potassium.

When normal individuals are given aldosterone, an initial period of sodium retention is followed by natriuresis, and sodium balance is reestablished after 3 to 5 days. As a result, edema does not develop. This process is referred to as the *escape phenomenon*, signifying an "escape" by the renal tubules from the sodium-retaining action of aldosterone. While renal hemodynamic factors may play a role in the escape, the level of atrial natriuretic peptide also increases. However, it is important to realize that there is no escape from the potassium-losing effects of mineralocorticoids.

Effect on Nonepithelial Cells The MR has been identified in a number of nonepithelial cells, e.g., neurons in the brain, myocytes, endothelial cells, and vascular smooth-muscle cells. In these cells, the actions of aldosterone differ from those in epithelial cells in several ways:

1. They do not modify sodium-potassium homeostasis.
2. The groups of regulated genes differ, although only a few are known; for example, in nonepithelial cells, aldosterone modifies the expression of several collagen genes and/or genes controlling tissue growth factors, e.g., transforming growth factor (TGF) β and plasminogen activator inhibitor, type 1 (PAI-1).
3. In some of these tissues (e.g., myocardium and brain), the MR is not protected by the 11β-HSD II enzyme. Thus, cortisol rather than aldosterone may be activating the MR. In other tissues (e.g., the vasculature), 11β-HSD II is expressed in a manner similar to that of the kidney. Therefore, aldosterone is activating the MR.
4. Some effects on nonepithelial cells may be via nongenomic mechanisms. These actions are too rapid—occurring within 1 to 2 min and peaking within 5 to 10 min—to be considered genomic, suggesting that they are secondary to activation of a cell-surface receptor. However, no cell-surface MR has been identified, raising the possibility that the same MR is mediating both genomic and nongenomic effects. Rapid, nongenomic effects have also been described for other steroids including estradiol, progesterone, thyroxine, and vitamin D.
5. Some of these tissues—the myocardium and vasculature—may also produce aldosterone, although this theory is controversial.

Regulation of Aldosterone Secretion Three primary mechanisms control adrenal aldosterone secretion: the renin-angiotensin system, potassium, and ACTH (Table 321-1). Whether these are also the primary regulatory mechanisms modifying nonadrenal production is uncertain. The renin-angiotensin system controls extracellular fluid volume via regulation of aldosterone secretion (Fig. 321-5). In effect, the renin-angiotensin system maintains the circulating blood volume constant by causing aldosterone-induced sodium retention during volume deficiency and by decreasing aldosterone-dependent sodium retention when volume is ample. There is an increasing body of evidence indicating that some tissues, in addition to the kidney, produce angiotensin II and may participate in the regulation of aldosterone secretion either from the adrenal or extraadrenal sources. Intriguingly, the adrenal itself is capable of synthesizing angiotensin II. What role(s) the extrarenal production of angiotensin II plays in normal physiology is still largely unknown. However, the tissue renin-angiotensin system is activated in utero in response to growth and development and/or later in life in response to injury.

TABLE 321-1 Factors Regulating Aldosterone Biosynthesis

Factor	Effect
Renin-angiotensin system	Stimulation
Sodium ion	Inhibition (?physiologic)
Potassium ion	Stimulation
Neurotransmitters	
Dopamine	Inhibition
Serotonin	Stimulation
Pituitary hormones	
ACTH	Stimulation
Non-ACTH pituitary hormones (e.g., growth hormone)	Permissive (for optimal response to sodium restriction)
β-Endorphin	Stimulation
γ-Melanocyte-stimulating hormone	Permissive
Atrial natriuretic peptide	Inhibition
Ouabain-like factors	Inhibition
Endothelin	Stimulation

Note: ACTH, adrenocorticotropic hormone.

Potassium ion directly stimulates aldosterone secretion, independent of the circulating renin-angiotensin system, which it suppresses (Fig. 321-5). In addition to a direct effect, potassium also modifies aldosterone secretion indirectly by activating the local renin-angiotensin system in the zona glomerulosa. This effect can be blocked by the administration of ACE inhibitors that reduce the local production of angiotensin II and thereby reduce the acute aldosterone response to potassium. An increase in serum potassium of as little as 0.1 mmol/L increases plasma aldosterone levels under certain circumstances. Oral potassium loading therefore increases aldosterone secretion, plasma levels, and excretion.

Physiologic amounts of ACTH stimulate aldosterone secretion acutely, but this action is not sustained unless ACTH is administered in a pulsatile fashion. Most studies relegate ACTH to a minor role in the control of aldosterone. For example, subjects receiving high-dose glucocorticoid therapy, and with presumed complete suppression of ACTH, have normal aldosterone secretion in response to sodium restriction.

Prior dietary intake of both potassium and sodium can alter the magnitude of the aldosterone response to acute stimulation. This effect results from a change in the expression and activity of aldosterone synthase. Increasing potassium intake or decreasing sodium intake sensitizes the response of the glomerulosa cells to acute stimulation by ACTH, angiotensin II, and/or potassium.

Neurotransmitters (dopamine and serotonin) and some peptides such as atrial natriuretic peptide, γ-melanocyte-stimulating hormone (γ-MSH), and β-endorphin, also participate in the regulation of aldosterone secretion (Table 321-1). Thus, the control of aldosterone secretion involves both stimulatory and inhibitory factors.

ANDROGEN PHYSIOLOGY Androgens regulate male secondary sexual characteristics and can cause virilizing symptoms in women (Chap. 44). Adrenal androgens have a minimal effect in males whose sexual characteristics are predominately determined by gonadal steroids (testosterone). In females, however, several androgen-like effects, e.g., sexual hair, are largely mediated by adrenal androgens. The principal adrenal androgens are DHEA, androstenedione, and 11-hydroxyandrostenedione. DHEA and androstenedione are weak androgens and exert their effects via conversion to the potent androgen testosterone in extraglandular tissues. DHEA also has poorly understood effects on the immune and cardiovascular systems. Adrenal androgen formation is regulated by ACTH, not by gonadotropins. Adrenal androgens are suppressed by exogenous glucocorticoid administration.

LABORATORY EVALUATION OF ADRENOCORTICAL FUNCTION

A basic assumption is that measurements of the plasma or urinary level of a given steroid reflect the rate of adrenal *secretion* of that steroid. However, urine *excretion* values may not truly reflect the secretion rate because of improper collection or altered metabolism. Plasma levels reflect the level of secretion only at the time of measurement. The plasma level (*PL*) depends on two factors: the secretion rate (*SR*) of the hormone and the rate at which it is metabolized, i.e., its metabolic clearance rate (*MCR*). These three factors can be related as follows:

$$PL = \frac{SR}{MCR} \quad \text{or} \quad SR = MCR \times PL$$

BLOOD LEVELS ■ Peptides The plasma levels of ACTH and angiotensin II can be measured by immunoassay techniques. Basal ACTH secretion shows a circadian rhythm, with lower levels in the early evening than in the morning. However, ACTH is secreted in a pulsatile manner, leading to rapid fluctuations superimposed on this circadian rhythm. Angiotensin II levels also vary diurnally and are influenced by dietary sodium and potassium intakes and posture. Both upright posture and sodium restriction elevate angiotensin II levels.

Most clinical determinations of the renin-angiotensin system, however, involve measurements of peripheral *plasma renin activity* (PRA) in which the renin activity is gauged by the generation of angiotensin I during a standardized incubation period. This method depends on the presence of sufficient angiotensinogen in the plasma as substrate. The generated angiotensin I is measured by radioimmunoassay. The PRA depends on the dietary sodium intake and on whether the patient is ambulatory. In normal humans, the PRA shows a diurnal rhythm characterized by peak values in the morning and a nadir in the afternoon. An alternative approach is to measure plasma active renin, which is easier and not dependent on endogenous substrate concentration. PRA and active renin correlate very well on low-sodium diets but less well on high-sodium diets.

Steroids Cortisol and aldosterone are both secreted episodically, and levels vary during the day, with peak values in the morning and low levels in the evening. In addition, the plasma level of aldosterone, but not of cortisol, is increased by dietary potassium loading, by sodium restriction, or by assumption of the upright posture. Measurement of the sulfate conjugate of DHEA may be a useful index of adrenal androgen secretion, as little DHEA sulfate is formed in the gonads and because the half-life of DHEA sulfate is 7 to 9 h. However, DHEA sulfate levels reflect both DHEA production and sulfatase activity.

URINE LEVELS For the assessment of glucocorticoid secretion, the urine 17-hydroxycorticosteroid assay has been replaced by measurement of urinary free cortisol. Elevated levels of urinary free cortisol correlate with states of hypercortisolism, reflecting changes in the levels of unbound, physiologically active circulating cortisol. Normally, the rate of excretion is higher in the daytime (7 A.M. to 7 P.M.) than at night (7 P.M. to 7 A.M.).

Urinary 17-ketosteroids originate in either the adrenal gland or the gonad. In normal women, 90% of urinary 17-ketosteroids is derived from the adrenal, and in men 60 to 70% is of adrenal origin. Urine 17-ketosteroid values are highest in young adults and decline with age.

A carefully timed urine collection is a prerequisite for all excretory determinations. Urinary creatinine should be measured simultaneously to determine the accuracy and adequacy of the collection procedure.

STIMULATION TESTS Stimulation tests are useful in the diagnosis of hormone deficiency states.

Tests of Glucocorticoid Reserve Within minutes after administration of ACTH, cortisol levels increase. This responsiveness can be used as an index of the functional reserve of the adrenal gland for production of cortisol. Under maximal ACTH stimulation, cortisol secretion increases tenfold, to 800 μmol/d (300 mg/d), but maximal stimulation can be achieved only with prolonged ACTH infusions.

A screening test (the so-called rapid ACTH stimulation test) involves the administration of 25 units (0.25 mg) of cosyntropin intravenously or intramuscularly and measurement of plasma cortisol levels before administration and 30 and 60 min after administration. The test can be performed at any time of the day. The most clear-cut criterion for a normal response is a stimulated cortisol level of >500 nmol/L (>18 μg/dL), and the minimal stimulated normal increment of cortisol is >200 nmol/L (>7 μg/dL) above baseline. Severely ill patients with elevated basal cortisol levels may show no further increases following acute ACTH administration.

Tests of Mineralocorticoid Reserve and Stimulation of the Renin-Angiotensin System Stimulation tests use protocols designed to create a programmed volume depletion, such as sodium restriction, diuretic administration, or upright posture. A simple, potent test consists of severe sodium restriction and upright posture. After 3 to 5 days of a 10-mmol/d sodium intake, rates of aldosterone secretion or excretion should increase two- to threefold over the control values. Supine morning plasma aldosterone levels are usually increased three- to sixfold, and they increase a further two- to fourfold in response to 2 to 3 h of upright posture.

When the dietary sodium intake is normal, stimulation testing requires the administration of a potent diuretic, such as 40 to 80 mg furosemide, followed by 2 to 3 h of upright posture. The normal response is a two- to fourfold rise in plasma aldosterone levels.

SUPPRESSION TESTS Suppression tests to document hypersecretion of adrenal hormones involve measurement of the target hormone response after standardized suppression of its tropic hormone.

Tests of Pituitary-Adrenal Suppressibility The ACTH release mechanism is sensitive to the circulating glucocorticoid level. When blood levels of glucocorticoid are increased in normal individuals, less ACTH is released from the anterior pituitary and less steroid is produced by the adrenal gland. The integrity of this feedback mechanism can be tested clinically by giving a glucocorticoid and judging the suppression of ACTH secretion by analysis of urine steroid levels and/or plasma cortisol and ACTH levels. A potent glucocorticoid such as dexamethasone is used, so that the agent can be given in an amount small enough not to contribute significantly to the pool of steroids to be analyzed.

The best *screening* procedure is the overnight dexamethasone suppression test. This involves the measurement of plasma cortisol levels at 8 A.M. following the oral administration of 1 mg dexamethasone the previous midnight. The 8 A.M. value for plasma cortisol in normal individuals should be <140 nmol/L (5 μg/dL).

The definitive test of adrenal suppressibility involves administering 0.5 mg dexamethasone every 6 h for two successive days while collecting urine over a 24-h period for determination of creatinine and free cortisol and/or measuring plasma cortisol levels. In a patient with a normal hypothalamic-pituitary ACTH release mechanism, a fall in the urine free cortisol to <80 nmol/d (30 μg/d) or of plasma cortisol to <140 nmol/L (5 μg/dL) is seen on the second day of administration.

A normal response to either suppression test implies that the glucocorticoid regulation of ACTH and its control of the adrenal glands is physiologically normal. However, an isolated abnormal result, particularly to the overnight suppression test, does not in itself demonstrate pituitary and/or adrenal disease.

Tests of Mineralocorticoid Suppressibility These tests rely on an expansion of extracellular fluid volume, which should decrease circulating plasma renin activity and decrease the secretion and/or excretion of aldosterone. Various tests differ in the rate at which extracellular fluid volume is expanded. One convenient suppression test involves the intravenous infusion of 500 mL/h of normal saline solution for 4 h, which normally suppresses plasma aldosterone levels to <220 pmol/L (<8 ng/dL) from a sodium-restricted diet or to <140 pmol/L (<5 ng/dL) from a normal sodium intake. Alternatively, a high-sodium diet can be administered for 3 days with 0.2 mg fludrocortisone twice daily. Aldosterone excretion is measured on the third day and should be <28 nmol/d (10 μg/d). These tests should not be performed in potassium-

depleted individuals since they carry a risk of precipitating hypokalemia.

TESTS OF PITUITARY-ADRENAL RESPONSIVENESS Stimuli such as insulin-induced hypoglycemia, AVP, and pyrogens induce the release of ACTH from the pituitary by an action on higher neural centers or on the pituitary itself. Insulin-induced hypoglycemia is particularly useful, because it stimulates the release of both growth hormone and ACTH. In this test, regular insulin (0.05 to 0.1 U/kg body weight) is given intravenously as a bolus to reduce the fasting glucose level to at least 50% below basal. The normal cortisol response is a rise to >500 nmol/L (18 μg/dL). Glucose levels must be monitored during insulin-induced hypoglycemia, and it should be terminated by feeding or intravenous glucose, if subjects develop symptoms of hypoglycemia. This test is contraindicated in individuals with coronary artery disease or a seizure disorder.

Metyrapone inhibits 11β-hydroxylase in the adrenal. As a result, the conversion of 11-deoxycortisol (compound S) to cortisol is impaired, causing 11-deoxycortisol to accumulate in the blood and the blood level of cortisol to decrease (Fig. 321-2). The hypothalamic-pituitary axis responds to the declining cortisol blood levels by releasing more ACTH. Note that assessment of the response depends on both an intact hypothalamic-pituitary axis and an intact adrenal gland.

Although modifications of the original metyrapone test have been described, a commonly used protocol involves administering 750 mg of the drug by mouth every 4 h over a 24-h period and comparing the control and postmetyrapone plasma levels of 11-deoxycortisol, cortisol, and ACTH. In normal individuals, plasma 11-deoxycortisol levels should exceed 210 nmol/L (7 μg/dL) and ACTH levels should exceed 17 pmol/L (75 pg/mL) following metyrapone administration. The metyrapone test does not accurately reflect ACTH reserve if subjects are ingesting exogenous glucocorticoids or drugs that accelerate the metabolism of metyrapone (e.g., phenytoin).

A direct and selective test of the pituitary corticotrophs can be achieved with CRH. The bolus injection of ovine CRH (corticorelin ovine triflutate; 1 μg/kg body weight) stimulates secretion of ACTH and β-LPT in normal human subjects within 15 to 60 min. In normal individuals, the mean increment in ACTH is 9 pmol/L (40 pg/mL). However, the magnitude of the ACTH response is less than that produced by insulin-induced hypoglycemia, implying that additional factors (such as vasopressin) augment stress-induced increases in ACTH secretion.

The rapid ACTH test can often distinguish between primary and secondary adrenal insufficiency, because aldosterone secretion is preserved in secondary adrenal failure by the renin-angiotensin system and potassium. Cosyntropin (25 units) is given intravenously or intramuscularly, and plasma cortisol and aldosterone levels are measured before and at 30 and 60 min after administration. The cortisol response is abnormal in both groups, but patients with secondary insufficiency show an increase in aldosterone levels of at least 140 pmol/L (5 ng/dL). No aldosterone response is seen in patients in whom the adrenal cortex is destroyed. Alternatively, ACTH at a physiologic dose (1 μg), the so-called low-dose ACTH test, may be used to detect secondary adrenal insufficiency. An abnormal response is similar to that in the rapid ACTH test. However, levels need to be measured at 30 min, and the ACTH needs to be directly injected intravenously because it can be absorbed by plastic tubing. Because the use of a bolus of exogenous ACTH does not invariably exclude a diagnosis of secondary adrenocortical insufficiency, direct tests of pituitary ACTH reserve (metyrapone test, insulin-induced hypoglycemia) may be required in the appropriate clinical setting.

HYPERFUNCTION OF THE ADRENAL CORTEX

Excess cortisol is associated with Cushing's syndrome; excess aldosterone causes aldosteronism; and excess adrenal androgens cause adrenal virilism. These syndromes do not always occur in the "pure" form but may have overlapping features.

CUSHING'S SYNDROME ■ Etiology Cushing described a syndrome characterized by truncal obesity, hypertension, fatigability and weakness, amenorrhea, hirsutism, purplish abdominal striae, edema, glucosuria, osteoporosis, and a basophilic tumor of the pituitary. As awareness of this syndrome has increased, the diagnosis of Cushing's syndrome has been broadened into the classification shown in Table 321-2. Regardless of etiology, all cases of endogenous Cushing's syndrome are due to increased production of cortisol by the adrenal. In most cases the cause is *bilateral adrenal hyperplasia* due to hypersecretion of pituitary ACTH or ectopic production of ACTH by a nonpituitary source. The incidence of pituitary-dependent adrenal hyperplasia is three times greater in women than in men, and the most frequent age of onset is the third or fourth decade. Most evidence indicates that the primary defect is the de novo development of a pituitary adenoma, as tumors are found in >90% of patients with pituitary-dependent adrenal hyperplasia. Alternatively, the defect may occasionally reside in the hypothalamus or in higher neural centers, leading to release of CRH inappropriate to the level of circulating cortisol. This primary defect leads to hyperstimulation of the pituitary, resulting in hyperplasia or tumor formation. In surgical series, most individuals with hypersecretion of pituitary ACTH are found to have a microadenoma (<10 mm in diameter; 50% are ≤5 mm in diameter), but a pituitary macroadenoma (>10 mm) or diffuse hyperplasia of the corticotrope cells may be found. Traditionally, only an individual who has an ACTH-producing pituitary tumor is defined as having *Cushing's disease*, whereas Cushing's syndrome refers to all causes of excess cortisol: exogenous ACTH tumor, adrenal tumor, pituitary ACTH-secreting tumor, or excessive glucocorticoid treatment.

The *ectopic ACTH syndrome* is caused by nonpituitary tumors that secrete either ACTH and/or CRH and cause bilateral adrenal hyperplasia (Chap. 86). The ectopic production of CRH results in clinical, biochemical, and radiologic features indistinguishable from those caused by hypersecretion of pituitary ACTH. The typical signs and symptoms of Cushing's syndrome may be absent or minimal with ectopic ACTH production, and hypokalemic alkalosis is a prominent manifestation. Most of these cases are associated with the primitive small cell (oat cell) type of bronchogenic carcinoma or with carcinoid tumors of the thymus, pancreas, or ovary; medullary carcinoma of the thyroid; or bronchial adenomas. The onset of Cushing's syndrome may be sudden, particularly in patients with carcinoma of the lung, and this feature accounts in part for the failure of these patients to exhibit the classic manifestations. On the other hand, patients with carcinoid tumors or pheochromocytomas have longer clinical courses and usually exhibit the typical cushingoid features. The ectopic secretion of ACTH is also accompanied by the accumulation of ACTH fragments in plasma and by elevated plasma levels of ACTH precursor molecules.

TABLE 321-2 Causes of Cushing's Syndrome

Adrenal hyperplasia
Secondary to pituitary ACTH overproduction
Pituitary-hypothalamic dysfunction
Pituitary ACTH-producing micro- or macroadenoma
Secondary to ACTH or CRH-producing nonendocrine tumors (bronchogenic carcinoma, carcinoid of the thymus, pancreatic carcinoma, bronchial adenoma)
Adrenal macronodular hyperplasia (including ectopic expression of GIP receptors in the adrenal cortex)
Adrenal micronodular dysplasia
Sporadic
Familial (Carney's syndrome)
Adrenal neoplasia
Adenoma
Carcinoma
Exogenous, iatrogenic causes
Prolonged use of glucocorticoids
Prolonged use of ACTH

Note: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; GIP, gastric inhibitory peptide.

Because such tumors may produce large amounts of ACTH, baseline steroid values are usually very high and increased skin pigmentation may be present.

Approximately 20 to 25% of patients with Cushing's syndrome have an adrenal neoplasm. These tumors are usually unilateral, and about half are malignant. Occasionally, patients have biochemical features both of pituitary ACTH excess and of an adrenal adenoma. These individuals may have *nodular hyperplasia* of both adrenal glands, often the result of prolonged ACTH stimulation in the absence of a pituitary adenoma. Two additional entities cause nodular hyperplasia: a familial disorder in children or young adults (so-called pigmented micronodular dysplasia; see below) and an abnormal cortisol response to gastric inhibitory polypeptide or luteinizing hormone, secondary to ectopic expression of receptors for these hormones in the adrenal cortex.

The most common cause of Cushing's syndrome is *iatrogenic* administration of steroids for a variety of reasons. Although the clinical features bear some resemblance to those seen with adrenal tumors, these patients are usually distinguishable on the basis of history and laboratory studies.

Clinical Signs, Symptoms, and Laboratory Findings Many of the signs and symptoms of Cushing's syndrome follow logically from the known action of glucocorticoids (Table 321-3). Catabolic responses in peripheral supportive tissue causes muscle weakness and fatigability, osteoporosis, broad violaceous cutaneous striae, and easy bruisability. The latter signs are secondary to weakening and rupture of collagen fibers in the dermis. Osteoporosis may cause collapse of vertebral bodies and pathologic fractures of other bones. Decreased bone mineralization is particularly pronounced in children. Increased hepatic gluconeogenesis and insulin resistance can cause impaired glucose tolerance. Overt diabetes mellitus occurs in <20% of patients, who probably are individuals with a predisposition to this disorder. Hypercortisolism promotes the deposition of adipose tissue in characteristic sites, notably the upper face (producing the typical "moon" facies), the interscapular area (producing the "buffalo hump"), supraclavicular fat pads, and the mesenteric bed (producing "truncal" obesity) (Fig. 321-6). Rarely, episternal fatty tumors and mediastinal widening secondary to fat accumulation occur. The reason for this peculiar distribution of adipose tissue is not known, but it is associated with insulin resistance and/or elevated insulin levels. The face appears plethoric, even in the absence of any increase in red blood cell concentration. Hypertension is common, and emotional changes may be profound, ranging from irritability and emotional lability to severe depression, confusion, or even frank psychosis. In women, increased levels of adrenal androgens can cause acne, hirsutism, and oligomenorrhea or amenorrhea. Some signs and symptoms in patients with hypercortisolism—i.e., obesity, hypertension, osteoporosis, and diabetes—are nonspecific and therefore are less helpful in diagnosing the condition. On the other hand, easy bruising, typical striae, myopathy, and virilizing signs (although less frequent) are, if present, more suggestive of Cushing's syndrome (Table 321-3).

Except in iatrogenic Cushing's syndrome, plasma and urine cortisol levels are elevated. Occasionally, hypokalemia, hypochloremia, and metabolic alkalosis are present, particularly with ectopic production of ACTH.

Diagnosis The diagnosis of Cushing's syndrome depends on the demonstration of increased cortisol production and failure to suppress cortisol secretion normally when dexamethasone is administered (Chap. 318). Once the diagnosis is established, further testing is designed to determine the etiology (Fig. 321-7 and Table 321-4).

For initial screening, the overnight dexamethasone suppression test is recommended (see above). In difficult cases (e.g., in obese or depressed patients), measurement of a 24-h urine free cortisol can also be used as a screening test. A level >140 nmol/d (50 μg/d) is suggestive of Cushing's syndrome. The definitive diagnosis is then established by failure of urinary cortisol to fall to less than <25 nmol/d (10 μg/d) or of plasma cortisol to fall to <140 nmol/L (5 μg/dL) after a standard low-dose dexamethasone suppression test (0.5 mg every 6 h for 48 h). Owing to circadian variability, plasma cortisol and, to a certain extent, ACTH determinations are not meaningful when performed in isolation, but the absence of the normal fall of plasma cortisol at midnight is consistent with Cushing's syndrome because there is loss of the diurnal cortisol rhythm.

TABLE 321-3 *Frequency of Signs and Symptoms in Cushing's Syndrome*

Sign or Symptom	Percent of Patients
Typical habitus (centripetal obesity)[a]	97
Increased body weight	94
Fatigability and weakness	87
Hypertension (blood pressure >150/90)	82
Hirsutism[a]	80
Amenorrhea	77
Broad violaceous cutaneous striae[a]	67
Personality changes	66
Ecchymoses[a]	65
Proximal myopathy[a]	62
Edema	62
Polyuria, polydipsia	23
Hypertrophy of clitoris	19

[a] Features more specific for Cushing's syndrome.

The task of determining the etiology of Cushing's syndrome is complicated by the fact that all the available tests lack specificity and by the fact that the tumors producing this syndrome are prone to spontaneous and often dramatic changes in hormone secretion (periodic hormonogenesis). No test has a specificity >95%, and it may be necessary to use a combination of tests to arrive at the correct diagnosis.

Plasma ACTH levels can be useful in distinguishing the various causes of Cushing's syndrome, particularly in separating ACTH-dependent from ACTH-independent causes. In general, measurement of plasma ACTH is useful in the diagnosis of ACTH-independent etiologies of the syndrome, since most adrenal tumors cause low or undetectable ACTH levels [<2 pmol/L (10 pg/mL)]. Furthermore, ACTH-secreting pituitary macroadenomas and ACTH-producing nonendocrine tumors usually result in elevated ACTH levels. In the ectopic ACTH syndrome, ACTH levels may be elevated to >110 pmol/L (500 pg/mL), and in most patients the level is >40 pmol/L (200 pg/mL). In Cushing's syndrome as the result of a microadenoma or pituitary-hypothalamic dysfunction, ACTH levels range from 6 to 30 pmol/L (30 to 150 pg/mL) [normal, <14 pmol/L (<60 pg/mL)],

FIGURE 321-6 A woman with Cushing's syndrome due to a right adrenal cortical adenoma. *A*, One month prior to surgery, age 20. *B*, One year after surgery, age 21.

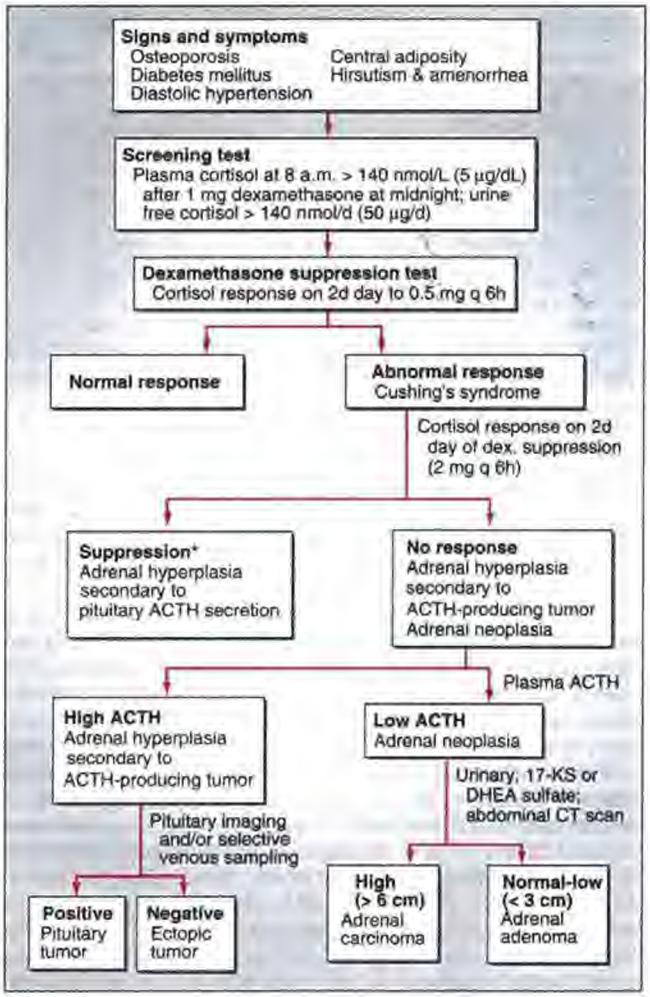

FIGURE 321-7 Diagnostic flowchart for evaluating patients suspected of having Cushing's syndrome. *This group probably includes some patients with pituitary-hypothalamic dysfunction and some with pituitary microadenomas. In some instances, a microadenoma may be visualized by pituitary magnetic resonance scanning. 17-KS, 17-ketosteroids; DHEA, dehydroepiandrosterone; ACTH, adrenocorticotropic hormone; CT, computed tomography.

with half of values falling in the normal range. However, the main problem with the use of ACTH levels in the differential diagnosis of Cushing's syndrome is that ACTH levels may be similar in individuals with hypothalamic-pituitary dysfunction, pituitary microadenomas, ectopic CRH production, and ectopic ACTH production (especially carcinoid tumors) (Table 321-4).

TABLE 321-4 Diagnostic Tests to Determine the Type of Cushing's Syndrome

Test	Pituitary Macroadenoma	Pituitary Microadenoma	Ectopic ACTH or CRH Production	Adrenal Tumor
Plasma ACTH level	↑ to ↑↑	N to ↑	↑ to ↑↑↑	↓
Percent who respond to high dose dexamethasone	<10	95	<10	<10
Percent who respond to CRH	>90	>90	<10	<10

Note: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; N, normal; ↑, elevated; ↓, decreased. See text for definition of a response.

A useful step to distinguish patients with an ACTH-secreting pituitary microadenoma or hypothalamic-pituitary dysfunction from those with other forms of Cushing's syndrome is to determine the response of cortisol output to administration of high-dose dexamethasone (2 mg every 6 h for 2 days). An alternative 8-mg, overnight high-dose dexamethasone test has been developed; however, this test has a lower sensitivity and specificity than the standard test. When the diagnosis of Cushing's syndrome is clear-cut on the basis of baseline urinary and plasma assays, the high-dose dexamethasone suppression test may be used without performing the preliminary low-dose suppression test. The high-dose suppression test provides close to 100% specificity if the criterion used is suppression of urinary free cortisol by >90%. Occasionally, in individuals with bilateral nodular hyperplasia and/or ectopic CRH production, steroid output is also suppressed. Failure of low- and high-dose dexamethasone administration to suppress cortisol production (Table 321-4) can occur in patients with adrenal hyperplasia secondary to an ACTH-secreting pituitary macroadenoma or an ACTH-producing tumor of nonendocrine origin and in those with adrenal neoplasms.

Because of these difficulties, several additional tests have been advocated, such as the metyrapone and CRH infusion tests. The rationale underlying these tests is that steroid hypersecretion by an adrenal tumor or the ectopic production of ACTH will suppress the hypothalamic-pituitary axis so that inhibition of pituitary ACTH release can be demonstrated by either test. Thus, most patients with pituitary-hypothalamic dysfunction and/or a microadenoma have an increase in steroid or ACTH secretion in response to metyrapone or CRH administration, whereas most patients with ectopic ACTH-producing tumors do not. Most pituitary macroadenomas also respond to CRH, but their response to metyrapone is variable. However, false-positive and false-negative CRH tests can occur in patients with ectopic ACTH and pituitary tumors.

The main diagnostic dilemma in Cushing's syndrome is to distinguish those instances due to microadenomas of the pituitary from those due to ectopic sources (e.g., carcinoids or pheochromocytoma) that produce CRH and/or ACTH. The clinical manifestations are similar unless the ectopic tumor produces other symptoms, such as diarrhea and flushing from a carcinoid tumor or episodic hypertension from a pheochromocytoma. Sometimes, one can distinguish between ectopic and pituitary ACTH production by using metyrapone or CRH tests as noted above. In these situations, computed tomography (CT) of the pituitary gland is usually normal. Magnetic resonance imaging (MRI) with the enhancing agent gadolinium may be better than CT for this purpose but demonstrates pituitary microadenomas in only half of patients with Cushing's disease. Because microadenomas can be detected in up to 10 to 20% of individuals without known pituitary disease, a positive imaging study does not prove that the pituitary is the source of ACTH excess. In those with negative imaging studies, selective petrosal sinus venous sampling for ACTH is now used in many referral centers. ACTH levels are measured at baseline, 2, 5, and 10 min after ovine CRH (1 μ/kg IV) injections. Peak petrosal:peripheral ACTH ratios of >3 confirm the presence of a pituitary ACTH-secreting tumor. In centers where petrosal sinus sampling is performed frequently, it has proved highly sensitive for distinguishing pituitary and nonpituitary sources of ACTH excess. However, the catheterization procedure is technically difficult, and complications have occurred.

The diagnosis of a *cortisol-producing adrenal adenoma* is suggested by low ACTH and disproportionate elevations in baseline urine free cortisol levels with only modest changes in urinary 17-ketosteroids or plasma DHEA sulfate. Adrenal androgen secretion is usually reduced in these patients owing to the cortisol-induced suppression of ACTH and subsequent involution of the androgen-producing zona reticularis.

The diagnosis of *adrenal carcinoma* is suggested by a palpable abdominal mass and by markedly elevated baseline values of both urine 17-ketosteroids and plasma DHEA sulfate. Plasma and urine cortisol levels are variably elevated. Adrenal carcinoma is usually resistant to both ACTH stimulation and dexamethasone suppression. Ex-

evated adrenal androgen secretion often leads to virilization in the female. Estrogen-producing adrenocortical carcinoma usually presents with gynecomastia in men and dysfunctional uterine bleeding in women. These adrenal tumors secrete increased amounts of androstenedione, which is converted peripherally to the estrogens estrone and estradiol. Adrenal carcinomas that produce Cushing's syndrome are often associated with elevated levels of the intermediates of steroid biosynthesis (especially 11-deoxycortisol), suggesting inefficient conversion of the intermediates to the final product. This feature also accounts for the characteristic increase in 17-ketosteroids. Approximately 20% of adrenal carcinomas are not associated with endocrine syndromes and are presumed to be nonfunctioning or to produce biologically inactive steroid precursors. In addition, the excessive production of steroids is not always clinically evident (e.g., androgens in adult men).

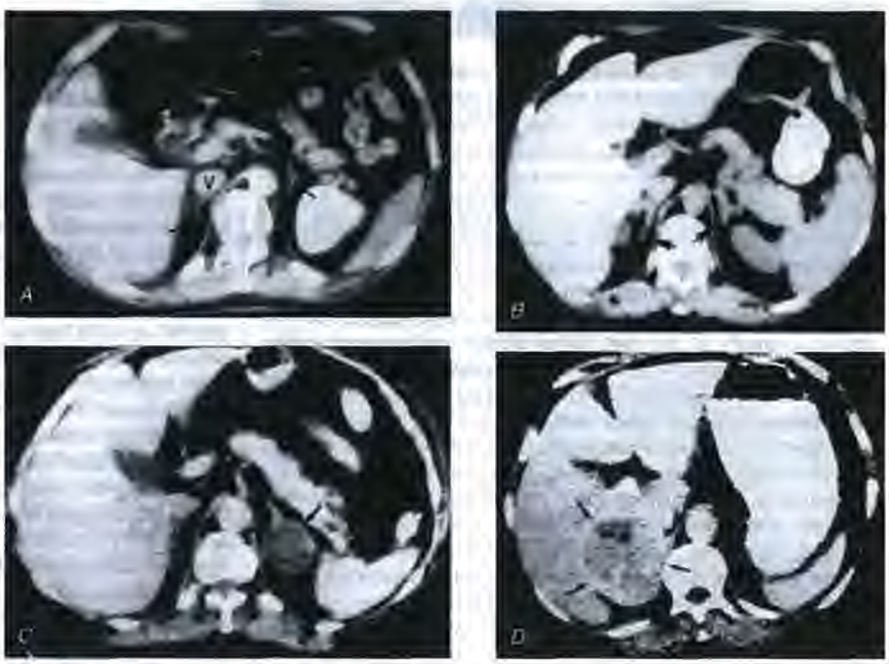

FIGURE 321-8 Computed tomography (CT) is the preferred method for visualizing the adrenal glands (*arrows*). *A*. The normal right adrenal gland is adjacent to the inferior vena cava (V) where it emerges from the liver. Approximately 90% of right adrenal glands appear as linear structures extending posteriorly from the inferior vena cava into the space between the right lobe of the liver and the crus of the diaphragm. The normal left adrenal gland is lateral to the left crus of the diaphragm and below the stomach. Most left adrenal glands are shaped like an inverted V or Y. *B*. Adrenal CT scan of a patient with ectopic ACTH production. Both adrenal glands (*arrows*) are enlarged (compare with *A*). In contrast, only 50% of patients with bilateral adrenal hyperplasia secondary to pituitary ACTH hypersecretion show enlargement of the adrenals when imaged by CT scan. *C*. CT scan of a patient with Cushing's syndrome with biochemical evidence only of cortisol overproduction. The left adrenal has been replaced by a racquet-shaped 2-cm tumor (*arrow*). Attenuation of the tumor is low because of its high lipid content. *D*. CT scan in a patient with Cushing's syndrome and biochemical evidence of an adrenal carcinoma. In contrast to the tumor in *C*, the right-sided mass in this patient is large and has a heterogeneous appearance—usual characteristics of an adrenal carcinoma.

Differential Diagnosis ■ *PSEUDO-CUSHING'S SYNDROME* Problems in diagnosis include patients with obesity, chronic alcoholism, depression, and acute illness of any type. Extreme *obesity* is uncommon in Cushing's syndrome; furthermore, with exogenous obesity, the adiposity is generalized, not truncal. On adrenocortical testing, abnormalities in patients with exogenous obesity are usually modest. Basal urine steroid excretion levels in obese patients are also either normal or slightly elevated, and the diurnal pattern in blood and urine levels is normal. Patients with *chronic alcoholism* and those with *depression* share similar abnormalities in steroid output: modestly elevated urine cortisol, blunted circadian rhythm of cortisol levels, and resistance to suppression using the overnight dexamethasone test. In contrast to alcoholic subjects, depressed patients do not have signs and symptoms of Cushing's syndrome. Following discontinuation of alcohol and/or improvement in the emotional status, results of steroid testing usually return to normal. One or more of three tests have been used to differentiate mild Cushing's syndrome and pseudo-Cushing's syndrome. The serum cortisol level following the standard 2-day low-dose dexamethasone test has very high sensitivity and specificity. Although the CRH test alone is less useful, in combination with the low-dose dexamethasone test, there is nearly complete discrimination between these two conditions. Finally, a midnight cortisol level obtained in awake patients may have similar predictive value as the low-dose dexamethasone test if a cut-off of 210 nmol/L (7.5 μg/dL) is used. Patients with *acute illness* often have abnormal results on laboratory tests and fail to exhibit pituitary-adrenal suppression in response to dexamethasone, since major stress (such as pain or fever) interrupts the normal regulation of ACTH secretion. *Iatrogenic Cushing's syndrome*, induced by the administration of glucocorticoids or other steroids such as megestrol that bind to the glucocorticoid receptor, is indistinguishable by physical findings from endogenous adrenocortical hyperfunction. The distinction can be made, however, by measuring blood or urine cortisol levels in a basal state; in the iatrogenic syndrome these levels are low secondary to suppression of the pituitary-adrenal axis. The severity of iatrogenic Cushing's syndrome is related to the total steroid dose, the biologic half-life of the steroid, and the duration of therapy. Also, individuals taking afternoon and evening doses of glucocorticoids develop Cushing's syndrome more readily and with a smaller total daily dose than do patients taking morning doses only.

Radiologic Evaluation for Cushing's Syndrome The preferred radiologic study for visualizing the adrenals is a CT scan of the abdomen (Fig. 321-8). CT is of value both for localizing adrenal tumors and for diagnosing bilateral hyperplasia. All patients believed to have hypersecretion of pituitary ACTH should have a pituitary MRI scan with gadolinium contrast. Even with this technique, small microadenomas may be undetectable; alternatively, false-positive masses due to cysts or nonsecretory lesions of the normal pituitary may be imaged. In patients with ectopic ACTH production, high-resolution chest CT is a useful first step.

Evaluation of Asymptomatic Adrenal Masses With abdominal CT scanning, many incidental adrenal masses (so-called incidentalomas) are discovered. This is not surprising, since 10 to 20% of subjects at autopsy have adrenocortical adenomas. The first step in evaluating such patients is to determine whether the tumor is functioning by means of appropriate screening tests, e.g., measurement of 24-h urine catecholamines and metabolites and serum potassium and assessment of adrenal cortical function by dexamethasone-suppression testing. However, 90% of incidentalomas are nonfunctioning. If an extra-adrenal malignancy is present, there is a 30 to 50% chance that the adrenal tumor is a metastasis. If the primary tumor is being treated and there are no other metastases, it is prudent to obtain a fine-needle aspirate of the adrenal mass to establish the diagnosis. In the absence of a known malignancy the next step is unclear. The probability of adrenal carcinoma is <0.01%, the vast majority of adrenal masses being benign adenomas. Features suggestive of malignancy include large size (a size > 4 to 6 cm suggests carcinoma); irregular margins;

and inhomogeneity, soft tissue calcifications visible on CT (Fig. 321-8), and findings characteristic of malignancy on a chemical-shift MRI image. If surgery is not performed, a repeat CT scan should be obtained in 3 to 6 months. Fine-needle aspiration is not useful to distinguish between benign and malignant primary adrenal tumors.

℞ TREATMENT

Adrenal Neoplasm When an adenoma or carcinoma is diagnosed, adrenal exploration is performed with excision of the tumor. Adenomas may be resected using laparoscopic techniques. Because of the possibility of atrophy of the contralateral adrenal, the patient is treated pre- and postoperatively as if for total adrenalectomy, even when a unilateral lesion is suspected, the routine being similar to that for an Addisonian patient undergoing elective surgery (see Table 321-8).

Despite operative intervention, most patients with adrenal carcinoma die within 3 years of diagnosis. Metastases occur most often to liver and lung. The principal drug for the treatment of adrenocortical carcinoma is mitotane (*o,p'*-DDD), an isomer of the insecticide DDT. This drug suppresses cortisol production and decreases plasma and urine steroid levels. Although its cytotoxic action is relatively selective for the glucocorticoid-secreting zone of the adrenal cortex, the zona glomerulosa may also be inhibited. Because mitotane also alters the extraadrenal metabolism of cortisol, plasma and urinary cortisol levels must be assessed to titrate the effect. The drug is usually given in divided doses three to four times a day, with the dose increased gradually to tolerability (usually <6 g daily). At higher doses, almost all patients experience side effects, which may be gastrointestinal (anorexia, diarrhea, vomiting) or neuromuscular (lethargy, somnolence, dizziness). All patients treated with mitotane should receive long-term glucocorticoid maintenance therapy, and, in some, mineralocorticoid replacement is appropriate. In approximately one-third of patients, both tumor and metastases regress, but long-term survival is not altered. In many patients, mitotane only inhibits steroidogenesis and does not cause regression of tumor metastases. Osseous metastases are usually refractory to the drug and should be treated with radiation therapy. Mitotane can also be given as adjunctive therapy after surgical resection of an adrenal carcinoma, although there is no evidence that this improves survival. Because of the absence of a long-term benefit with mitotane, alternative chemotherapeutic approaches based on platinum therapy have been used. However, there are no data presently available indicating a prolongation of life.

BILATERAL HYPERPLASIA Patients with hyperplasia usually have a relative or absolute increase in ACTH levels. Since therapy would logically be directed at reducing ACTH levels, the ideal primary treatment for ACTH- or CRH-producing tumors, whether pituitary or ectopic, is surgical removal. Occasionally (particularly with ectopic ACTH production) surgical excision is not possible because the disease is far advanced. In this situation, "medical" or surgical adrenalectomy may correct the hypercortisolism.

Controversy exists as to the proper treatment for bilateral adrenal hyperplasia when the source of the ACTH overproduction is not apparent. In some centers, these patients (especially those who suppress after the administration of a high-dose dexamethasone test) undergo surgical exploration of the pituitary via a transsphenoidal approach in the expectation that a microadenoma will be found (Chap. 318). However, in most circumstances selective petrosal sinus venous sampling is recommended, and the patient is referred to an appropriate center if the procedure is not available locally. If a microadenoma is not found at the time of exploration, total hypophysectomy may be needed. Complications of transsphenoidal surgery include cerebrospinal fluid rhinorrhea, diabetes insipidus, panhypopituitarism, and optic or cranial nerve injuries.

In other centers, total adrenalectomy is the treatment of choice. The cure rate with this procedure is close to 100%. The adverse effects include the certain need for lifelong mineralocorticoid and glucocorticoid replacement and a 10 to 20% probability of a pituitary tumor developing over the next 10 years (Nelson's syndrome; Chap. 318). It is uncertain whether these tumors arise de novo or if they were present prior to adrenalectomy but were too small to be detected. Periodic radiologic evaluation of the pituitary gland by MRI as well as serial ACTH measurements should be performed in all individuals after bilateral adrenalectomy for Cushing's disease. Such pituitary tumors may become locally invasive and impinge on the optic chiasm or extend into the cavernous or sphenoid sinuses.

Except in children, pituitary irradiation is rarely used as primary treatment, being reserved rather for postoperative tumor recurrences. In some centers, high levels of gamma radiation can be focused on the desired site with less scattering to surrounding tissues by using stereotactic techniques. Side effects of radiation include ocular motor palsy and hypopituitarism. There is a long lag time between treatment and remission, and the remission rate is usually <50%.

Finally, in occasional patients in whom a surgical approach is not feasible, "medical" adrenalectomy may be indicated (Table 321-5). Inhibition of steroidogenesis may also be indicated in severely cushingoid subjects prior to surgical intervention. Chemical adrenalectomy may be accomplished by the administration of the inhibitor of steroidogenesis ketoconazole (600 to 1200 mg/d). In addition, mitotane (2 or 3 g/d) and/or the blockers of steroid synthesis aminoglutethimide (1 g/d) and metyrapone (2 or 3 g/d) may be effective either alone or in combination. Mitotane is slow to take effect (weeks). Mifepristone, a competitive inhibitor of the binding of glucocorticoid to its receptor, may be a treatment option. Adrenal insufficiency is a risk with all these agents, and replacement steroids may be required.

ALDOSTERONISM Aldosteronism is a syndrome associated with hypersecretion of the mineralocorticoid aldosterone. In *primary* aldosteronism the cause for the excessive aldosterone production resides within the adrenal gland; in *secondary* aldosteronism the stimulus is extraadrenal.

Primary Aldosteronism In the original descriptions of excessive and inappropriate aldosterone production, the disease was the result of an *aldosterone-producing adrenal adenoma* (Conn's syndrome). Most cases involve a unilateral adenoma, which is usually small and may occur on either side. Rarely, primary aldosteronism is due to an adrenal carcinoma. Aldosteronism is twice as common in women as in men, usually occurs between the ages of 30 and 50, and is present in ~1% of unselected hypertensive patients. However, the prevalence may be as high as 5%, depending on the criteria and study population. In many patients with clinical and biochemical features of primary aldosteronism, a solitary adenoma is not found at surgery. Instead, these patients have *bilateral cortical nodular hyperplasia*. In the literature, this disease is also termed *idiopathic hyperaldosteronism*, and/or *nodular hyperplasia*. The cause is unknown.

SIGNS AND SYMPTOMS Hypersecretion of aldosterone increases the renal distal tubular exchange of intratubular sodium for secreted potassium and hydrogen ions, with progressive depletion of body potassium and development of hypokalemia. Most patients have diastolic hypertension, which may be very severe, and headaches. The hypertension is probably due to the increased sodium reabsorption and extracellular volume expansion. *Potassium depletion* is responsible for the muscle

TABLE 321-5 Treatment Modalities for Patients with Adrenal Hyperplasia Secondary to Pituitary ACTH Hypersecretion

Treatments to reduce pituitary ACTH production
Transsphenoidal resection of microadenoma
Radiation therapy
Treatments to reduce or eliminate adrenocortical cortisol secretion
Bilateral adrenalectomy
Medical adrenalectomy (metyrapone, mitotane, aminoglutethimide, ketoconazole)*

* Not curative but effective as long as chronically administered in selected patients.
Note: ACTH, adrenocorticotropic hormone.

weakness and fatigue and is due to the effect of potassium depletion on the muscle cell membrane. The polyuria results from impairment of urinary concentrating ability and is often associated with polydipsia. However, some individuals with mild disease, particularly the bilateral hyperplasia type, may have normal potassium levels and therefore have no symptoms associated with hypokalemia.

Electrocardiographic and roentgenographic signs of left ventricular enlargement are, in part, secondary to the hypertension. However, the left ventricular hypertrophy is disproportionate to the level of blood pressure when compared to individuals with essential hypertension, and regression of the hypertrophy occurs even if blood pressure is not reduced after removal of an aldosteronoma. Electrocardiographic signs of potassium depletion include prominent U waves, cardiac arrhythmias, and premature contractions. In the absence of associated congestive heart failure, renal disease, or preexisting abnormalities (such as thrombophlebitis), edema is characteristically absent. However, structural damage to the cerebral circulation, retinal vasculature, and kidney occurs more frequently than would be predicted based on the level and duration of the hypertension. Proteinuria may occur in as many as 50% of patients with primary aldosteronism, and renal failure occurs in up to 15%. Thus, it is probable that excess aldosterone production induces cardiovascular damage independent of its effect on blood pressure.

LABORATORY FINDINGS Laboratory findings depend on both the duration and the severity of potassium depletion. An overnight concentration test often reveals impaired ability to concentrate the urine, probably secondary to the hypokalemia. Urine pH is neutral to alkaline because of excessive secretion of ammonium and bicarbonate ions to compensate for the metabolic alkalosis.

Hypokalemia may be severe (<3 mmol/L) and reflects body potassium depletion, usually >300 mmol. In mild forms of primary aldosteronism, potassium levels may be normal. *Hypernatremia* is infrequent but may be caused by sodium retention, concomitant water loss from polyuria, and resetting of the osmostat. Metabolic alkalosis and elevation of serum bicarbonate are caused by hydrogen ion loss into the urine and migration into potassium-depleted cells. The alkalosis is perpetuated by potassium deficiency, which increases the capacity of the proximal convoluted tubule to reabsorb filtered bicarbonate. If hypokalemia is severe, serum magnesium levels are also reduced.

DIAGNOSIS The diagnosis is suggested by persistent hypokalemia in a nonedematous patient with a normal sodium intake who is not receiving potassium-wasting diuretics (furosemide, ethacrynic acid, thiazides). If hypokalemia occurs in a hypertensive patient taking a potassium-wasting diuretic, the diuretic should be discontinued and the patient should be given potassium supplements. After 1 to 2 weeks, the potassium level should be remeasured, and if hypokalemia persists, the patient should be evaluated for a mineralocorticoid excess syndrome (Fig. 321-9).

The criteria for the diagnosis of primary aldosteronism are (1) diastolic hypertension without edema, (2) hyposecretion of renin (as judged by low plasma renin activity levels) that fails to increase appropriately during volume depletion (upright posture, sodium depletion), and (3) hypersecretion of aldosterone that does not suppress appropriately in response to volume expansion.

Patients with primary aldosteronism characteristically *do not have edema*, since they exhibit an "escape" phenomenon from the sodium-retaining aspects of mineralocorticoids. Rarely, pretibial edema is present in patients with associated nephropathy and azotemia.

The estimation of plasma renin activity is of limited value in separating patients with primary aldosteronism from those with hypertension of other causes. Although failure of plasma renin activity to rise normally during volume-depletion maneuvers is a criterion for a diagnosis of primary aldosteronism, suppressed renin activity also occurs in ~25% of patients with essential hypertension.

Although a renin measurement alone lacks specificity, the ratio of serum aldosterone to plasma renin activity is a very useful screening test. A high ratio (>30), when aldosterone is expressed as ng/dL and plasma renin activity as ng/mL per hour, strongly suggests autonomy of aldosterone secretion. Aldosterone levels need to be >500 pmol/L (>15 ng/dL) when salt intake is not restricted. In some centers, the aldosterone/plasma renin activity ratio is used as a primary screen test in all normokalemic, difficult-to-control hypertensive patients, in addition to those with hypokalemia. Ultimately, it is necessary to demonstrate a lack of aldosterone suppression to diagnose primary aldosteronism (Fig. 321-9). The autonomy exhibited in these patients refers only to the resistance to suppression of secretion during volume expansion; aldosterone can and does respond in a normal or above-normal fashion to the stimulus of potassium loading or ACTH infusion.

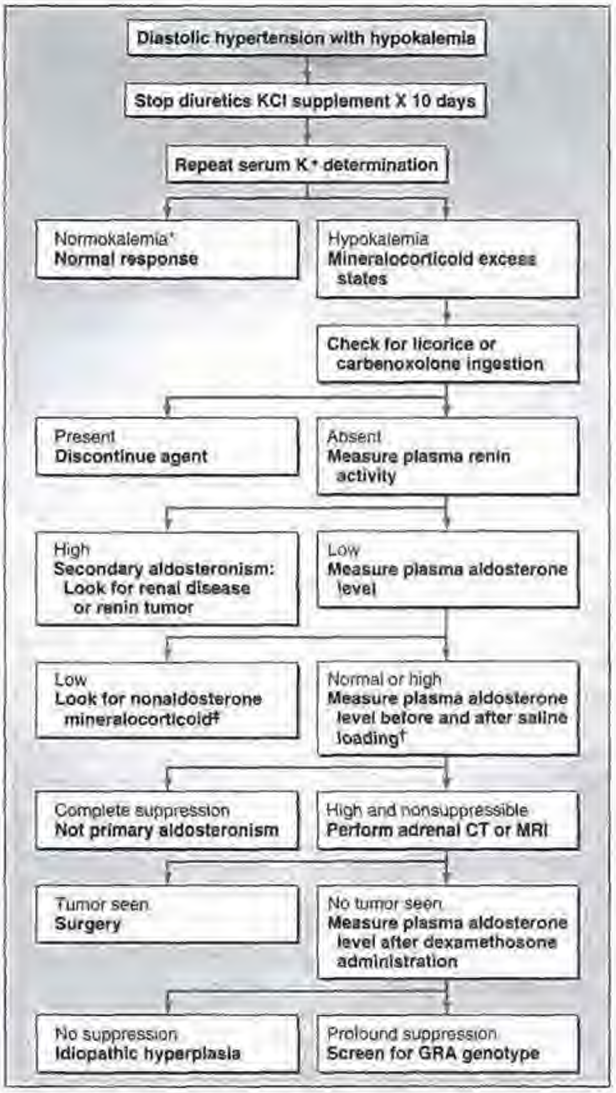

FIGURE 321-9 Diagnostic flowchart for evaluating patients with suspected primary aldosteronism. *Serum K^+ may be normal in some patients with hyperaldosteronism who are taking potassium-sparing diuretics (spironolactone, triamterene) or who have a low sodium intake and a high potassium intake. †This step should not be taken if hypertension is severe (diastolic pressure > 115 mmHg) or if cardiac failure is present. Also, serum potassium levels should be corrected before the infusion of saline solution. Alternative methods that produce comparable suppression of aldosterone secretion include oral sodium loading (200 mmol/d) and the administration of fludrocortisone, 0.2 mg bid, for 3 days. ‡For example, Liddle's syndrome, apparent mineralocorticoid excess syndrome, or a deoxycorticosterone-secreting tumor. (GRA, glucocorticoid-remediable aldosteronism; CT, computed tomography; MRI, magnetic resonance imaging.)

Once hyposecretion of renin and failure of aldosterone secretion suppression are demonstrated, aldosterone-producing adenomas should be localized by abdominal CT scan, using a high-resolution scanner as many aldosteronomas are <1 cm in size. If the CT scan is negative, percutaneous transfemoral bilateral adrenal vein catheterization with adrenal vein sampling may demonstrate a two- to threefold increase in plasma aldosterone concentration on the involved side. In cases of hyperaldosteronism secondary to cortical nodular hyperplasia, no lateralization is found. It is important for samples to be obtained simultaneously if possible and for cortisol levels to be measured to ensure that false localization does not reflect dilution or an ACTH- or stress-induced rise in aldosterone levels. In a patient with an adenoma, the aldosterone/cortisol ratio lateralizes to the side of the lesion.

DIFFERENTIAL DIAGNOSIS Patients with hypertension and hypokalemia may have either primary or secondary hyperaldosteronism (Fig. 321-10). A useful maneuver to distinguish between these conditions is the measurement of plasma renin activity. Secondary hyperaldosteronism in patients with accelerated hypertension is due to elevated plasma renin levels; in contrast, patients with primary aldosteronism have suppressed plasma renin levels. Indeed, in patients with a serum potassium concentration of <2.5 mmol/L, a high ratio of plasma aldosterone to plasma renin activity in a random sample is usually sufficient to establish the diagnosis of primary aldosteronism without additional testing. Ectopic ACTH production should also be considered in patients with hypertension and severe hypokalemia.

Primary aldosteronism must also be distinguished from other *hypermineralocorticoid states*. Nonaldosterone mineralocorticoid states will have suppressed plasma renin activity but low aldosterone levels. The most common problem is to distinguish between hyperaldosteronism due to an adenoma and that due to idiopathic bilateral nodular hyperplasia. This distinction is important because hypertension associated with idiopathic hyperplasia does not usually benefit from bilateral adrenalectomy, whereas hypertension associated with aldosterone-producing tumors is usually improved or cured by removal of the adenoma. Although patients with idiopathic bilateral nodular hyperplasia tend to have less severe hypokalemia, lower aldosterone secretion, and higher plasma renin activity than do patients with primary aldosteronism, differentiation is impossible solely on clinical and/or biochemical grounds. An anomalous postural decrease in plasma aldosterone and elevated plasma 18-hydroxycorticosterone levels are present in most patients with a unilateral lesion. However, these tests are also of limited diagnostic value in the individual patient, because some adenoma patients have an increase in plasma aldosterone with upright posture, so-called renin-responsive aldosteronoma. A definitive diagnosis is best made by radiographic studies, including bilateral adrenal vein catheterization, as noted above.

In a few instances, hypertensive patients with hypokalemic alkalosis have adenomas that secrete deoxycorticosterone. Such patients have reduced plasma renin activity levels, but aldosterone levels are either normal or reduced, suggesting the diagnosis of mineralocorticoid excess due to a hormone other than aldosterone. Several inherited disorders have clinical features similar to those of primary aldosteronism (see below).

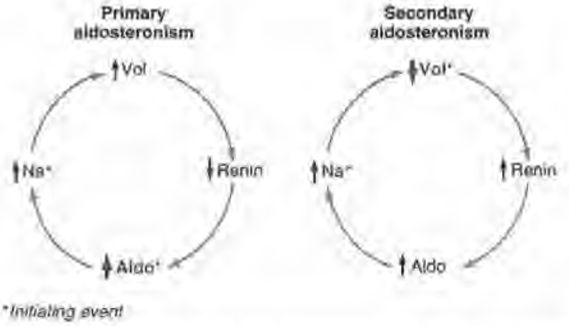

FIGURE 321-10 Responses of the renin-aldosterone volume control loop in primary versus secondary aldosteronism.

℞ TREATMENT

Primary aldosteronism due to an adenoma is usually treated by surgical excision of the adenoma. Where possible a laparoscopic approach is favored. However, dietary sodium restriction and the administration of an aldosterone antagonist—e.g., spironolactone—are effective in many cases. Hypertension and hypokalemia are usually controlled by doses of 25 to 100 mg spironolactone every 8 h. In some patients medical management has been successful for years, but chronic therapy in men is usually limited by side effects of spironolactone such as gynecomastia, decreased libido, and impotence.

When idiopathic bilateral hyperplasia is suspected, surgery is indicated only when significant, symptomatic hypokalemia cannot be controlled with medical therapy, i.e., by spironolactone, triamterene, or amiloride. Hypertension associated with idiopathic hyperplasia is usually not benefited by bilateral adrenalectomy.

Secondary Aldosteronism *Secondary aldosteronism* refers to an appropriately increased production of aldosterone in response to activation of the renin-angiotensin system (Fig. 321-10). The production rate of aldosterone is often higher in patients with secondary aldosteronism than in those with primary aldosteronism. Secondary aldosteronism usually occurs in association with the accelerated phase of hypertension or on the basis of an underlying edema disorder. Secondary aldosteronism in pregnancy is a normal physiologic response to estrogen-induced increases in circulating levels of renin substrate and plasma renin activity and to the anti-aldosterone actions of progestogens.

Secondary aldosteronism in hypertensive states is due either to a primary overproduction of renin (primary reninism) or to an overproduction of renin secondary to a decrease in renal blood flow and/or perfusion pressure (Fig. 321-10). Secondary hypersecretion of renin can be due to a narrowing of one or both of the major renal arteries by atherosclerosis or by fibromuscular hyperplasia. Overproduction of renin from both kidneys also occurs in severe arteriolar nephrosclerosis (malignant hypertension) or with profound renal vasoconstriction (the accelerated phase of hypertension). The secondary aldosteronism is characterized by hypokalemic alkalosis, moderate to severe increases in plasma renin activity, and moderate to marked increases in aldosterone levels.

Secondary aldosteronism with hypertension can also be caused by rare renin-producing tumors (primary reninism). In these patients, the biochemical characteristics are of renal vascular hypertension, but the primary defect is renin secretion by a juxtaglomerular cell tumor. The diagnosis can be made by demonstration of normal renal vasculature and/or demonstration of a space-occupying lesion in the kidney by radiographic techniques and documentation of a unilateral increase in renal vein renin activity. Rarely, these tumors arise in tissues such as the ovary.

Secondary aldosteronism is present in many forms of *edema*. The rate of aldosterone secretion is usually increased in patients with edema caused by either cirrhosis or the nephrotic syndrome. In congestive heart failure, elevated aldosterone secretion varies depending on the severity of cardiac failure. The stimulus for aldosterone release in these conditions appears to be *arterial hypovolemia* and/or hypotension. Thiazides and furosemide often exaggerate secondary aldosteronism via volume depletion; hypokalemia and, on occasion, alkalosis can then become prominent features. On occasion secondary hyperaldosteronism occurs without edema or hypertension (Bartter and Gitelman syndromes, see below).

Aldosterone and Cardiovascular Damage Although many studies have investigated the role of angiotensin II in mediating cardiovascular damage, additional evidence indicates that aldosterone has an important

role that is independent of angiotensin II. Patients with primary aldosteronism (in which angiotensin II levels are usually very low) have a higher incidence of left ventricular hypertrophy (LVH), albuminuria, and stroke than do patients with essential hypertension. Experimental animal models mimicking secondary aldosteronism (angiotensin infusion) or primary aldosteronism (aldosterone infusion) reveal a common pathophysiologic sequence. Within the first few days there is activation of proinflammatory molecules with a histologic picture of perivascular macrophage infiltrate and inflammation, followed by cellular death, fibrosis, and ventricular hypertrophy. These events are prevented if an aldosterone receptor antagonist is used or if adrenalectomy is performed initially. The same pathophysiologic sequence is seen in animals with average aldosterone levels and cardiovascular damage, i.e., diabetes mellitus, or genetic hypertensive rats. Importantly, the level of sodium intake is a critical co-factor. If salt intake is severely restricted, no damage occurs even though the aldosterone levels are markedly elevated. Thus, it is not the level of aldosterone per se that is responsible for the damage, but its level relative to the volume or sodium status of the individual.

Four clinical studies support these experimental results. In the RALES trial, patients with class II/IV heart failure were randomized to standard care or a low dose of the mineralocorticoid receptor antagonist, spironolactone. There was a 30% reduction in all-cause mortality and cardiovascular mortality and hospitalizations after 36 months. Two studies in hypertensive subjects addressed the question of the relative importance of a reduction of angiotensin II formation versus blockade of the MR in mediating cardiovascular damage. Subjects were randomized to eplerenone (an MR antagonist), enalapril (an ACE inhibitor), or both agents. In the first study the subjects had LVH, with the end point being a reduction in LVH. In the second, the subjects had diabetes mellitus and proteinuria, with the end point being a reduction in proteinuria. In both studies all three treatment arms substantially reduced the primary end point; however, the most potent effect occurred in the combination arms of the studies. In the monotherapy LVH arms, the reduction in LVH was similar, while in the proteinuria study, eplerenone produced a greater reduction than did enalapril. The final study was the EPHESUS trial, where individuals who developed congestive heart failure after an acute myocardial infarction were randomized to standard-of-care treatment with or without a small dose of eplerenone. Eplerenone administration produced a significantly greater reduction in mortality (15 to 17%) and in cardiovascular-related hospitalizations than the placebo arm. Thus, these four clinical studies provide strong support to the hypothesis that MR blockade has a significant added advantage over standard-of-care therapy in reducing cardiovascular mortality and surrogate end points. However, regulatory approval is pending.

SYNDROMES OF ADRENAL ANDROGEN EXCESS Adrenal androgen excess results from excess production of DHEA and androstenedione, which are converted to testosterone in extraglandular tissues; elevated testosterone levels account for most of the virilization. Adrenal androgen excess may be associated with the secretion of greater or smaller amounts of other adrenal hormones and may, therefore, present as "pure" syndromes of virilization or as "mixed" syndromes associated with excessive glucocorticoids and Cushing's syndrome. ***→For further discussion of hirsutism and virilization, see Chap. 44.***

HYPOFUNCTION OF THE ADRENAL CORTEX

Cases of adrenal insufficiency can be divided into two general categories: (1) those associated with primary inability of the adrenal to elaborate sufficient quantities of hormone, and (2) those associated with a secondary failure due to inadequate ACTH formation or release (Table 321-6).

PRIMARY ADRENOCORTICAL DEFICIENCY (ADDISON'S DISEASE) The original description of Addison's disease—"general languor and debility, feebleness of the heart's action, irritability of the stomach, and a peculiar change of the color of the skin"—summarizes the dominant clinical features. Advanced cases are usually easy to diagnose, but recognition of the early phases can be a real challenge.

TABLE 321-6 Classification of Adrenal Insufficiency

PRIMARY ADRENAL INSUFFICIENCY
Anatomic destruction of gland (chronic or acute)
"Idiopathic" atrophy (autoimmune, adrenoleukodystrophy)
Surgical removal
Infection (tuberculous, fungal, viral—especially in AIDS patients)
Hemorrhage
Invasion: metastatic
Metabolic failure in hormone production
Congenital adrenal hyperplasia
Enzyme inhibitors (metyrapone, ketoconazole, aminoglutethimide)
Cytotoxic agents (mitotane)
ACTH-blocking antibodies
Mutation in ACTH receptor gene
Adrenal hypoplasia congenita
SECONDARY ADRENAL INSUFFICIENCY
Hypopituitarism due to hypothalamic-pituitary disease
Suppression of hypothalamic pituitary axis
By exogenous steroid
By endogenous steroid from tumor

Note: ACTH, adrenocorticotropic hormone.

Incidence Acquired forms of primary insufficiency are relatively rare, may occur at any age, and affect both sexes equally. Because of the common therapeutic use of steroids, secondary adrenal insufficiency is relatively common.

Etiology and Pathogenesis Addison's disease results from progressive destruction of the adrenals, which must involve >90% of the glands before adrenal insufficiency appears. The adrenal is a frequent site for chronic granulomatous diseases, predominantly tuberculosis but also histoplasmosis, coccidioidomycosis, and cryptococcosis. In early series, tuberculosis was responsible for 70 to 90% of cases, but the most frequent cause now is *idiopathic* atrophy, and an autoimmune mechanism is probably responsible. Rarely, other lesions are encountered, such as adrenoleukodystrophy, bilateral hemorrhage, tumor metastases, HIV, cytomegalovirus (CMV), amyloidosis, adrenomyeloneuropathy, familial adrenal insufficiency, or sarcoidosis.

Although half of patients with idiopathic atrophy have circulating adrenal antibodies, autoimmune destruction is probably secondary to cytotoxic T lymphocytes. Specific adrenal antigens to which autoantibodies may be directed include 21-hydroxylase (CYP21A2) and side chain cleavage enzyme, but the significance of these antibodies in the pathogenesis of adrenal insufficiency is unknown. Some antibodies cause adrenal insufficiency by blocking the binding of ACTH to its receptors. Some patients also have antibodies to thyroid, parathyroid, and/or gonadal tissue (Chap. 330). There is also an increased incidence of chronic lymphocytic thyroiditis, premature ovarian failure, type 1 diabetes mellitus, and hypo- or hyperthyroidism. The presence of two or more of these autoimmune endocrine disorders in the same person defines the polyglandular autoimmune syndrome type II. Additional features include pernicious anemia, vitiligo, alopecia, nontropical sprue, and myasthenia gravis. Within families, multiple generations are affected by one or more of the above diseases. Type II polyglandular syndrome is the result of a mutant gene on chromosome 6 and is associated with the HLA alleles B8 and DR3.

The combination of parathyroid and adrenal insufficiency and chronic mucocutaneous candidiasis constitutes type I polyglandular autoimmune syndrome. Other autoimmune diseases in this disorder include pernicious anemia, chronic active hepatitis, alopecia, primary hypothyroidism, and premature gonadal failure. There is no HLA association; this syndrome is inherited as an autosomal recessive trait. It is caused by mutations in the *a*utoimmune *p*olyendocrinopathy *c*andidiasis *e*ctodermal *d*ystrophy (APECED) gene located on chromo-

some 21q22.3. The gene encodes a transcription factor thought to be involved in lymphocyte function. The type I syndrome usually presents during childhood, whereas the type II syndrome is usually manifested in adulthood.

Clinical suspicion of adrenal insufficiency should be high in patients with AIDS (Chap. 173). CMV regularly involves the adrenal glands (so-called CMV necrotizing adrenalitis), and involvement with *Mycobacterium avium-intracellulare*, *Cryptococcus*, and Kaposi's sarcoma has been reported. Adrenal insufficiency in AIDS patients may not be manifest, but tests of adrenal reserve frequently give abnormal results. When interpreting tests of adrenocortical function, it is important to remember that medications such as rifampin, phenytoin, ketoconazole, megestrol, and opiates may cause or potentiate adrenal insufficiency. Adrenal hemorrhage and infarction occur in patients on anticoagulants and in those with circulating anticoagulants and hypercoagulable states, such as the antiphospholipid syndrome.

There are several rare genetic causes of adrenal insufficiency that present primarily in infancy and childhood (see below).

Clinical Signs and Symptoms Adrenocortical insufficiency caused by gradual adrenal destruction is characterized by an insidious onset of fatigability, weakness, anorexia, nausea and vomiting, weight loss, cutaneous and mucosal pigmentation, hypotension, and occasionally hypoglycemia (Table 321-7). Depending on the duration and degree of adrenal hypofunction, the manifestations vary from mild chronic fatigue to fulminating shock associated with acute destruction of the glands, as described by Waterhouse and Friderichsen.

Asthenia is the cardinal symptom. Early it may be sporadic, usually most evident at times of stress; as adrenal function becomes more impaired, the patient is continuously fatigued, and bed rest is necessary.

Hyperpigmentation may be striking or absent. It commonly appears as a diffuse brown, tan, or bronze darkening of parts such as the elbows or creases of the hand and of areas that normally are pigmented such as the areolae about the nipples. Bluish-black patches may appear on the mucous membranes. Some patients develop dark freckles, and irregular areas of vitiligo may paradoxically be present. As an early sign, tanning following sun exposure may be persistent.

Arterial hypotension with postural accentuation is frequent, and blood pressure may be in the range of 80/50 or less.

Abnormalities of gastrointestinal function are often the presenting complaint. Symptoms vary from mild anorexia with weight loss to fulminating nausea, vomiting, diarrhea, and ill-defined abdominal pain, which may be so severe as to be confused with an acute abdomen. Patients may have personality changes, usually consisting of excessive irritability and restlessness. Enhancement of the sensory modalities of taste, olfaction, and hearing is reversible with therapy. Axillary and pubic hair may be decreased in women due to loss of adrenal androgens.

Laboratory Findings In the early phase of gradual adrenal destruction, there may be no demonstrable abnormalities in the routine laboratory parameters, but adrenal reserve is decreased—that is, while basal steroid output may be normal, a subnormal increase occurs after stress. Adrenal stimulation with ACTH uncovers abnormalities in this stage of the disease, eliciting a subnormal increase of cortisol levels or no increase at all. In more advanced stages of adrenal destruction, serum sodium, chloride, and bicarbonate levels are reduced, and the serum potassium level is elevated. The hyponatremia is due both to loss of sodium into the urine (due to aldosterone deficiency) and to movement into the intracellular compartment. This extravascular sodium loss depletes extracellular fluid volume and accentuates hypotension. Elevated plasma vasopressin and angiotensin II levels may contribute to the hyponatremia by impairing free water clearance. Hyperkalemia is due to a combination of aldosterone deficiency, impaired glomerular filtration, and acidosis. Basal levels of cortisol and aldosterone are subnormal and fail to increase following ACTH administration. Mild to moderate hypercalcemia occurs in 10 to 20% of patients for unclear reasons. The electrocardiogram may show nonspecific changes, and the electroencephalogram exhibits a generalized reduction and slowing. There may be a normocytic anemia, a relative lymphocytosis, and a moderate eosinophilia.

Diagnosis The diagnosis of adrenal insufficiency should be made only with ACTH stimulation testing to assess adrenal reserve capacity for steroid production (see above for ACTH test protocols). In brief, the best screening test is the cortisol response 60 min after 250 μg of cosyntropin given intramuscularly or intravenously. Cortisol levels should exceed 495 nmol/L (18 μg/dL). If the response is abnormal, then primary and secondary adrenal insufficiency can be distinguished by measuring aldosterone levels from the same blood samples. In secondary, but not primary, adrenal insufficiency the aldosterone increment will be normal [≥150 pmol/l (5 ng/dL)]. Furthermore, in primary adrenal insufficiency, plasma ACTH and associated peptides (β-LPT) are elevated because of loss of the usual cortisol-hypothalamic-pituitary feedback relationship, whereas in secondary adrenal insufficiency, plasma ACTH values are low or "inappropriately" normal (Fig. 321-11).

TABLE 321-7 Frequency of Symptoms and Signs in Adrenal Insufficiency

Sign or Symptom	Percent of Patients
Weakness	99
Pigmentation of skin	98
Weight loss	97
Anorexia, nausea, and vomiting	90
Hypotension (< 110/70)	87
Pigmentation of mucous membranes	82
Abdominal pain	34
Salt craving	22
Diarrhea	20
Constipation	19
Syncope	16
Vitiligo	9

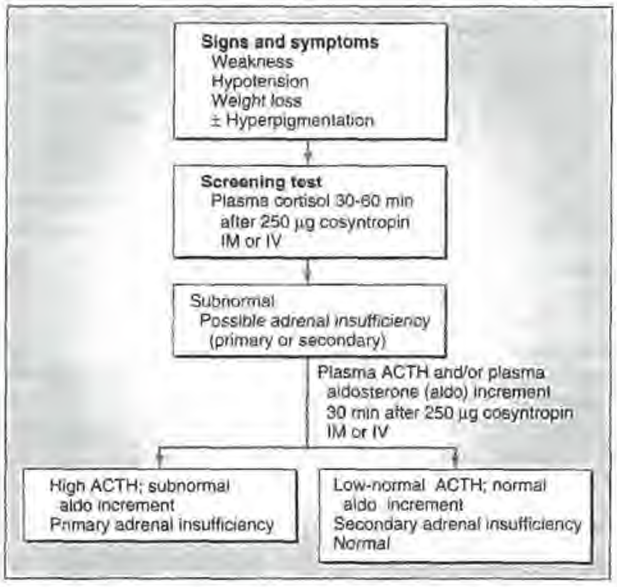

FIGURE 321-11 Diagnostic flowchart for evaluating patients with suspected adrenal insufficiency. Plasma adrenocorticotropic hormone (ACTH) levels are low in secondary adrenal insufficiency. In adrenal insufficiency secondary to pituitary tumors or idiopathic panhypopituitarism, other pituitary hormone deficiencies are present. On the other hand, ACTH deficiency may be isolated, as seen following prolonged use of exogenous glucocorticoids. Because the isolated blood levels obtained in these screening tests may not be definitive, the diagnosis may need to be confirmed by a continuous 24-h ACTH infusion. Normal subjects and patients with secondary adrenal insufficiency may be distinguished by insulin tolerance or metyrapone testing.

Differential Diagnosis Because weakness and fatigue are common, diagnosis of early adrenocortical insufficiency may be difficult. However, the combination of mild gastrointestinal distress, weight loss, anorexia, and a suggestion of increased pigmentation makes it mandatory to perform ACTH stimulation testing to rule out adrenal insufficiency, particularly before steroid treatment is begun. Weight loss is useful in evaluating the significance of weakness and malaise. Racial pigmentation may be a problem, but a *recent* and progressive *increase* in pigmentation is usually reported by the patient with gradual adrenal destruction. Hyperpigmentation is usually absent when adrenal destruction is rapid, as in bilateral adrenal hemorrhage. The fact that hyperpigmentation occurs with other diseases may also present a problem, but the appearance and distribution of pigment in adrenal insufficiency are usually characteristic. When doubt exists, measurement of ACTH levels and testing of adrenal reserve with the infusion of ACTH provide clear-cut differentiation.

TABLE 321-8 Steroid Therapy Schedule for a Patient with Adrenal Insufficiency Undergoing Surgery[a]

	Hydrocortisone Infusion, Continuous, mg/h		Hydrocortisone (Orally)		Fludrocortisone (Orally), 8 A.M.
			8 A.M.	4 P.M.	
Routine daily medication			20	10	0.1
Day before operation			20	10	0.1
Day of operation	10				
Day 1	5–7.5				
Day 2	2.5–5				
Day 3	2.5–5	*or*	40	20	0.1
Day 4	2.5–5	*or*	40	20	0.1
Day 5			40	20	0.1
Day 6			20	20	0.1
Day 7			20	10	0.1

[a] All steroid doses are given in milligrams. An alternative approach is to give 100 mg hydrocortisone as an intravenous bolus injection every 8 h on the day of the operation (see text).

TREATMENT

All patients with adrenal insufficiency should receive specific hormone replacement. These patients require careful education about the disease. Replacement therapy should correct both glucocorticoid and mineralocorticoid deficiencies. Hydrocortisone (cortisol) is the mainstay of treatment. The dose for most adults (depending on size) is 20 to 30 mg/d. Patients are advised to take glucocorticoids with meals or, if that is impractical, with milk or an antacid, because the drugs may increase gastric acidity and exert direct toxic effects on the gastric mucosa. To simulate the normal diurnal adrenal rhythm, two-thirds of the dose is taken in the morning, and the remaining one-third is taken in the late afternoon. Some patients exhibit insomnia, irritability, and mental excitement after initiation of therapy; in these, the dosage should be reduced. Other situations that may necessitate smaller doses are hypertension and diabetes mellitus. Obese individuals and those on anticonvulsive medications may require increased dosages. Measurements of plasma ACTH or cortisol or of urine cortisol levels do not appear to be useful in determining optimal glucocorticoid dosages.

Since the replacement dosage of hydrocortisone does not replace the mineralocorticoid component of the adrenal hormones, mineralocorticoid supplementation is usually needed. This is accomplished by the administration of 0.05 to 0.1 mg fludrocortisone per day by mouth. Patients should also be instructed to maintain an ample intake of sodium (3 to 4 g/d).

The adequacy of mineralocorticoid therapy can be assessed by measurement of blood pressure and serum electrolytes. Blood pressure should be normal and without postural changes; serum sodium, potassium, creatinine, and urea nitrogen levels should also be normal. Measurement of plasma renin levels may also be useful in titrating the dose.

In female patients with adrenal insufficiency, androgen levels are also low. Thus, some physicians believe that daily replacement with 25 to 50 mg of DHEA orally may improve quality of life and bone mineral density.

Complications of glucocorticoid therapy, with the exception of gastritis, are *rare* at the dosages recommended for treatment of adrenal insufficiency. Complications of mineralocorticoid therapy include hypokalemia, hypertension, cardiac enlargement, and even congestive heart failure due to sodium retention. Periodic measurements of body weight, serum potassium level, and blood pressure are useful. All patients with adrenal insufficiency should carry medical identification, should be instructed in the parenteral self-administration of steroids, and should be registered with a medical alerting system.

Special Therapeutic Problems During periods of intercurrent illness, especially in the setting of fever, the dose of hydrocortisone should be doubled. With severe illness it should be increased to 75 to 150 mg/d. When oral administration is not possible, parenteral routes should be employed. Likewise, before surgery or dental extractions, supplemental glucocorticoids should be administered. Patients should also be advised to increase the dose of fludrocortisone and to add salt to their otherwise normal diet during periods of strenuous exercise with sweating, during extremely hot weather, and with gastrointestinal upsets such as diarrhea. A simple strategy is to supplement the diet one to three times daily with salty broth (1 cup of beef or chicken bouillon contains 35 mmol of sodium). For a representative program of steroid therapy for the patient with adrenal insufficiency who is undergoing major surgery, see Table 321-8. This schedule is designed so that on the day of surgery it will mimic the output of cortisol in normal individuals undergoing prolonged major stress (10 mg/h, 250 to 300 mg/d). Thereafter, if the patient is improving and is afebrile, the dose of hydrocortisone is tapered by 20 to 30% daily. Mineralocorticoid administration is unnecessary at hydrocortisone doses >100 mg/d because of the mineralocorticoid effects of hydrocortisone at such dosages.

SECONDARY ADRENOCORTICAL INSUFFICIENCY ACTH deficiency causes *secondary* adrenocortical insufficiency; it may be a selective deficiency, as is seen following prolonged administration of excess glucocorticoids, or it may occur in association with deficiencies of multiple pituitary hormones (panhypopituitarism) (Chap. 318). Patients with secondary adrenocortical hypofunction have many symptoms and signs in common with those having primary disease but are *not hyperpigmented*, since ACTH and related peptide levels are low. In fact, plasma ACTH levels distinguish between primary and secondary adrenal insufficiency, since they are elevated in the former and decreased to absent in the latter. Patients with total pituitary insufficiency have manifestations of multiple hormone deficiencies. An additional feature distinguishing primary adrenocortical insufficiency is the *near-normal level of aldosterone secretion* seen in pituitary and/or isolated ACTH deficiencies (Fig. 321-11). Patients with pituitary insufficiency may have hyponatremia, which can be dilutional or secondary to a subnormal increase in aldosterone secretion in response to severe sodium restriction. However, severe *dehydration, hyponatremia*, and *hyperkalemia* are characteristic of severe mineralocorticoid insufficiency and favor a diagnosis of primary adrenocortical insufficiency.

Patients receiving long-term steroid therapy, despite physical findings of Cushing's syndrome, may develop adrenal insufficiency because of prolonged pituitary-hypothalamic suppression and adrenal atrophy secondary to the loss of endogenous ACTH. These patients have two deficits, a loss of adrenal responsiveness to ACTH and a failure of pituitary ACTH release. They are characterized by low blood cortisol and ACTH levels, a low baseline rate of steroid excretion, and abnormal ACTH and metyrapone responses. Most patients with steroid-induced adrenal insufficiency eventually recover normal HPA re-

sponsiveness, but recovery time varies from days to months. The rapid ACTH test provides a convenient assessment of recovery of HPA function. Because the plasma cortisol concentrations after injection of cosyntropin and during insulin-induced hypoglycemia are usually similar, the rapid ACTH test assesses the integrated HPA function (see "Tests of Pituitary-Adrenal Responsiveness," above). Some investigators suggest using the low-dose (1 μg) ACTH test for suspected secondary ACTH deficiency. Additional tests to assess pituitary ACTH reserve include the standard metyrapone and insulin-induced hypoglycemia tests.

Glucocorticoid therapy in patients with secondary adrenocortical insufficiency does not differ from that for the primary disorder. Mineralocorticoid therapy is usually not necessary, as aldosterone secretion is preserved.

ACUTE ADRENOCORTICAL INSUFFICIENCY Acute adrenocortical insufficiency may result from several processes. On the one hand, *adrenal crisis* may be a rapid and overwhelming intensification of chronic adrenal insufficiency, usually precipitated by sepsis or surgical stress. Alternatively, acute hemorrhagic destruction of both adrenal glands can occur in previously well individuals. In children, this event is usually associated with septicemia with *Pseudomonas* or meningococcemia (Waterhouse-Friderichsen syndrome). In adults, anticoagulant therapy or a coagulation disorder may result in bilateral adrenal hemorrhage. Occasionally, bilateral adrenal hemorrhage in the newborn results from birth trauma. Hemorrhage has been observed during pregnancy, following idiopathic adrenal vein thrombosis, and as a complication of venography (e.g., infarction of an adenoma). The third and most frequent cause of acute insufficiency is the rapid withdrawal of steroids from patients with adrenal atrophy owing to chronic steroid administration. Acute adrenocortical insufficiency may also occur in patients with congenital adrenal hyperplasia or those with decreased adrenocortical reserve when they are given drugs capable of inhibiting steroid synthesis (mitotane, ketoconazole) or of increasing steroid metabolism (phenytoin, rifampin).

Adrenal Crisis The long-term survival of patients with adrenocortical insufficiency depends largely on the prevention and treatment of adrenal crisis. Consequently, the occurrence of infection, trauma (including surgery), gastrointestinal upsets, or other stresses necessitates an immediate increase in hormone. In untreated patients, preexisting symptoms are intensified. Nausea, vomiting, and abdominal pain may become intractable. Fever may be severe or absent. Lethargy deepens into somnolence, and hypovolemic vascular collapse ensues. In contrast, patients previously maintained on chronic glucocorticoid therapy may not exhibit dehydration or hypotension until they are in a preterminal state, since mineralocorticoid secretion is usually preserved. In all patients in crisis, a precipitating cause should be sought.

Rx TREATMENT

Treatment is directed primarily toward repletion of circulating glucocorticoids and replacement of the sodium and water deficits. Hence an intravenous infusion of 5% glucose in normal saline solution should be started with a bolus intravenous infusion of 100 mg hydrocortisone followed by a continuous infusion of hydrocortisone at a rate of 10 mg/h. An alternative approach is to administer a 100-mg bolus of hydrocortisone intravenously every 6 h. However, only continuous infusion maintains the plasma cortisol constantly at stress levels [>830 nmol/L (30 μg/dL)]. Effective treatment of hypotension requires glucocorticoid replacement and repletion of sodium and water deficits. If the crisis was preceded by prolonged nausea, vomiting, and dehydration, several liters of saline solution may be required in the first few hours. Vasoconstrictive agents (such as dopamine) may be indicated in extreme conditions as adjuncts to volume replacement. With large doses of steroid, i.e., 100 to 200 mg hydrocortisone, the patient receives a maximal mineralocorticoid effect, and supplementary mineralocorticoid is superfluous. Following improvement, the steroid dosage is tapered over the next few days to maintenance levels, and mineralocorticoid therapy is reinstituted if needed (Table 321-8).

ADRENAL CORTICOL INSUFFICIENCY IN ACUTELY ILL PATIENTS The physiology of the HPA axis is dramatically altered during critical illnesses such as trauma, surgery, sepsis, and shock. In such situations cortisol levels rise four- to sixfold, diurnal variation is abolished, and the unbound fractions of cortisol rise in the circulation and in target tissues. Inadequate cortisol production during critical illness can result in hypotension, reduced systemic vascular resistance, shock, and death.

A major area of controversy in presumably normal individuals is the correlation of clinical outcomes with the cortisol levels measured during critical illness. Subnormal cortisol production during acute severe illness has been termed "functional" or "relative" adrenal insufficiency. Conceptually, the elevated cortisol levels that are observed are viewed as insufficient to control the inflammatory response and maintain blood pressure. If such patients can be identified, treatment with supplementary cortisol could be beneficial.

A level of cortisol in a critically ill patient below which replacement glucocorticoids may improve prognosis is not firmly established, although many have accepted a level of ≤441 nmol/L (15 μg/dL). On the other hand, a random cortisol >938 nmol/L (34 μg/dL) in the setting of critical illness is unlikely to be associated with relative adrenal insufficiency. In patients who have random cortisol levels between 441 and 938 nmol/L (15 and 34 μg/dL), a cosyntropin stimulation test may identify patients with diminished adrenal reserve [increment <255 nmol/L (9 μg/dL)] who may benefit from supplementary cortisol treatment. If the diagnosis of relative or functional adrenal insufficiency is considered in an acutely ill, hypotensive patient, treatment with supplementary cortisol should be initiated promptly following the measurement of a random cortisol level and/or performing a cosyntropin stimulation test. Supplemental cortisol may be particularly beneficial in patients with septic shock where glucocorticoids have been reported to reduce mortality and the duration of vasopressor therapy. Such patients should be treated with 50 to 75 mg of intravenous hydrocortisone every 6 h as bolus treatment or the same amount as a continuous infusion. Treatment can be terminated if the cortisol levels obtained at the outset are normal. On the other hand, those patients with abnormal testing should be treated for 1 week and then tapered. In surviving patients, adrenal function should be reevaluated after resolution of the critical illness.

HYPOALDOSTERONISM

Isolated aldosterone deficiency accompanied by normal cortisol production occurs in association with hyporeninism, as an inherited biosynthetic defect, postoperatively following removal of aldosterone-secreting adenomas, during protracted heparin administration, in pretectal disease of the nervous system, and in severe postural hypotension.

The feature common to all forms of hypoaldosteronism is the inability to increase aldosterone secretion appropriately in response to salt restriction. Most patients have unexplained hyperkalemia, which is often exacerbated by restriction of dietary sodium intake. In severe cases, urine sodium wastage occurs at a normal salt intake, whereas in milder forms, excessive loss of urine sodium occurs only with salt restriction.

Most cases of isolated hypoaldosteronism occur in patients with a deficiency in renin production (so-called hyporeninemic hypoaldosteronism), most commonly in adults with diabetes mellitus and mild renal failure and in whom hyperkalemia and metabolic acidosis are out of proportion to the degree of renal impairment. Plasma renin levels fail to rise normally following sodium restriction and postural changes. The pathogenesis is uncertain. Possibilities include renal disease (the most likely), autonomic neuropathy, extracellular fluid volume expansion, and defective conversion of renin precursors to active renin. Aldosterone levels also fail to rise normally after salt restriction and volume contraction; this effect is probably related to the hyporeninism, since biosynthetic defects in aldosterone secretion usually can-

not be demonstrated. In these patients, aldosterone secretion increases promptly after ACTH stimulation, but it is uncertain whether the magnitude of the response is normal. On the other hand, the level of aldosterone appears to be subnormal in relationship to the hyperkalemia.

Hypoaldosteronism can also be associated with high renin levels and low or elevated levels of aldosterone (see below). Severely ill patients may also have hyperreninemic hypoaldosteronism; such patients have a high mortality rate (80%). Hyperkalemia is not present. Possible explanations for the hypoaldosteronism include adrenal necrosis (uncommon) or a shift in steroidogenesis from mineralocorticoids to glucocorticoids, possibly related to prolonged ACTH stimulation.

Before the diagnosis of isolated hypoaldosteronism is considered for a patient with hyperkalemia, "pseudohyperkalemia" (e.g., hemolysis, thrombocytosis) should be excluded by measuring the *plasma* potassium level. The next step is to demonstrate a normal cortisol response to ACTH stimulation. Then, the response of renin and aldosterone levels to stimulation (upright posture, sodium restriction) should be measured. Low renin and aldosterone levels establish the diagnosis of hyporeninemic hypoaldosteronism. A combination of high renin levels and low aldosterone levels is consistent with an aldosterone biosynthetic defect or a selective unresponsiveness to angiotensin II. Finally, there is a condition that clinically and biochemically mimics hypoaldosteronism with elevated renin levels. However, the aldosterone levels are not low but high—so-called pseudohypoaldosteronism. This inherited condition is caused by a mutation in the epithelial sodium channel (see below).

Rx TREATMENT

The treatment is to replace the mineralocorticoid deficiency. For practical purposes, the oral administration of 0.05 to 0.15 mg fludrocortisone daily should restore electrolyte balance if salt intake is adequate (e.g., 150 to 200 mmol/d). However, patients with hyporeninemic hypoaldosteronism may require higher doses of mineralocorticoid to correct hyperkalemia. This need poses a potential risk in patients with hypertension, mild renal insufficiency, or congestive heart failure. An alternative approach is to reduce salt intake and to administer furosemide, which can ameliorate acidosis and hyperkalemia. Occasionally, a combination of these two approaches is efficacious.

GENETIC CONSIDERATIONS **Glucocorticoid Diseases** ■ *CONGENITAL ADRENAL HYPERPLASIA* Congenital adrenal hyperplasia (CAH) is the consequence of recessive mutations that cause one of several distinct enzymatic defects (see below). Because cortisol is the principal adrenal steroid regulating ACTH elaboration and because ACTH stimulates adrenal growth and function, a block in cortisol synthesis may result in the enhanced secretion of adrenal androgens and/or mineralocorticoids depending on the site of the enzyme block. In severe congenital virilizing hyperplasia, the adrenal output of cortisol may be so compromised as to cause adrenal deficiency despite adrenal hyperplasia.

CAH is the most common adrenal disorder of infancy and childhood (Chap. 328). Partial enzyme deficiencies can be expressed after adolescence, predominantly in women with hirsutism and oligomenorrhea but minimal virilization. Late-onset adrenal hyperplasia may account for 5 to 25% of cases of hirsutism and oligomenorrhea in women, depending on the population.

Pathology Enzymatic defects have been described in 21-hydroxylase (CYP21A2), 17α-hydroxylase/17,20-lyase (CYP17), 11β-hydroxylase (CYP11B1), and in (3β-HSD2) (Fig. 321-2). Although the genes encoding these enzymes have been cloned, the diagnosis of specific enzyme deficiencies with genetic techniques is not practical because of the large number of different deletions and missense mutations. CYP21A2 deficiency is closely linked to the HLA-B locus of chromosome 6 so that HLA typing and/or DNA polymorphism can be used to detect the heterozygous carriers and to diagnose affected individuals in some families (Chap. 296). The clinical expression in the different disorders is variable, ranging from virilization of the female (CYP2/A2) to feminization of the male (3β-HSD2) (Chap. 328).

Adrenal virilization in the female at birth is associated with ambiguous external genitalia (*female pseudohermaphroditism*). Virilization begins after the fifth month of intrauterine development. At birth there may be enlargement of the clitoris, partial or complete fusion of the labia, and sometimes a urogenital sinus in the female. If the labial fusion is nearly complete, the female infant has external genitalia resembling a penis with hypospadias. In the *postnatal* period, CAH is associated with virilization in the female and isosexual precocity in the male. The excessive androgen levels result in accelerated growth, so that bone age exceeds chronologic age. Because epiphyseal closure occurs early, growth stops, but truncal development continues, the characteristic appearance being a short child with a well-developed trunk.

The most common form of CAH (95% of cases) is a result of impairment of CYP21A2. In addition to cortisol deficiency, aldosterone secretion is decreased in approximately one-third of the patients. Thus, with CYP21A2 deficiency, adrenal virilization occurs with or without a salt-losing tendency due to aldosterone deficiency (Fig. 321-2).

CYP11B1 deficiency causes a "hypertensive" variant of CAH. Hypertension and hypokalemia occur because of the impaired conversion of 11-deoxycorticosterone to corticosterone, resulting in the accumulation of 11-deoxycorticosterone, a potent mineralocorticoid. The degree of hypertension is variable. Steroid precursors are shunted into the androgen pathway.

CYP17 deficiency is characterized by hypogonadism, hypokalemia, and hypertension. This rare disorder causes decreased production of cortisol and shunting of precursors into the mineralocorticoid pathway with hypokalemic alkalosis, hypertension, and suppressed plasma renin activity. Usually, 11-deoxycorticosterone production is elevated. Because CYP17 hydroxylation is required for biosynthesis of both adrenal androgens and gonadal testosterone and estrogen, this defect is associated with sexual immaturity, high urinary gonadotropin levels, and low urinary 17-ketosteroid excretion. Female patients have primary amenorrhea and lack of development of secondary sexual characteristics. Because of deficient androgen production, male patients have either ambiguous external genitalia or a female phenotype (*male pseudohermaphroditism*). Exogenous glucocorticoids can correct the hypertensive syndrome, and treatment with appropriate gonadal steroids results in sexual maturation.

With 3β-HSD2 deficiency, conversion of pregnenolone to progesterone is impaired, so that the synthesis of both cortisol and aldosterone is blocked, with shunting into the adrenal androgen pathway via 17α-hydroxypregnenolone and DHEA. Because DHEA is a weak androgen, and because this enzyme deficiency is also present in the gonad, the genitalia of the male fetus may be incompletely virilized or feminized. Conversely, in the female, overproduction of DHEA may produce partial virilization.

Diagnosis The diagnosis of CAH should be considered in infants having episodes of acute adrenal insufficiency or salt-wasting or hypertension. The diagnosis is further suggested by the finding of hypertrophy of the clitoris, fused labia, or a urogenital sinus in the female or of isosexual precocity in the male. In infants and children with a CYP21A2 defect, increased urine 17-ketosteroid excretion and increased plasma DHEA sulfate levels are typically associated with an increase in the blood levels of 17-hydroxyprogesterone and the excretion of its urinary metabolite pregnanetriol. Demonstration of elevated levels of 17-hydroxyprogesterone in amniotic fluid at 14 to 16 weeks of gestation allows prenatal detection of affected female infants.

The diagnosis of a *salt-losing form* of CAH due to defects in CYP21A2 is suggested by episodes of acute adrenal insufficiency with hyponatremia, hyperkalemia, dehydration, and vomiting. These infants and children often crave salt and have laboratory findings indicating deficits in both cortisol and aldosterone secretion.

With the *hypertensive form* of CAH due to CYP11B1 deficiency, 11-deoxycorticosterone and 11-deoxycortisol accumulate. The diagnosis is confirmed by demonstrating increased levels of 11-deoxycortisol in the blood or increased amounts of tetrahydro-11-deoxycortisol in the urine. Elevation of 17-hydroxyprogesterone levels does not imply a coexisting CYP21A2 deficiency.

Very high levels of urine DHEA with low levels of pregnanetriol and of cortisol metabolites in urine are characteristic of children with 3β-HSD2 deficiency. Marked salt-wasting may also occur.

Adults with *late-onset adrenal hyperplasia* (partial deficiency of CYP21A2, CYP11B1, or 3β-HSD2) are characterized by normal or moderately elevated levels of urinary 17-ketosteroids and plasma DHEA sulfate. A high basal level of a precursor of cortisol biosynthesis (such as 17-hydroxyprogesterone, 17-hydroxypregnenolone, or 11-deoxycortisol), or elevation of such a precursor after ACTH stimulation, confirms the diagnosis of a partial deficiency. Measurement of steroid precursors 60 min after bolus administration of ACTH is usually sufficient. Adrenal androgen output is easily suppressed by the standard low-dose (2 mg) dexamethasone test.

℞ TREATMENT

Therapy in CAH patients consists of daily administration of glucocorticoids to suppress pituitary ACTH secretion. Because of its low cost and intermediate half-life, prednisone is the drug of choice except in infants, in whom hydrocortisone is usually used. In adults with late-onset adrenal hyperplasia, the smallest single bedtime dose of a long- or intermediate-acting glucocorticoid that suppresses pituitary ACTH secretion should be administered. The amount of steroid required by children with CAH is approximately 1 to 1.5 times the normal cortisol production rate of 27 to 35 μmol (10 to 13 mg) of cortisol per square meter of body surface per day and is given in divided doses two or three times per day. The dosage schedule is governed by repetitive analysis of the urinary 17-ketosteroids, plasma DHEA sulfate, and/or precursors of cortisol biosynthesis. Skeletal growth and maturation must also be monitored closely, as overtreatment with glucocorticoid replacement therapy retards linear growth.

Receptor Mutations *Isolated glucocorticoid deficiency* is a rare autosomal recessive disease secondary to a mutation in the ACTH receptor. Usually mineralocorticoid function is normal. Adrenal insufficiency is manifest within the first 2 years of life as hyperpigmentation, convulsions, and/or frequent episodes of hypoglycemia. In some patients the adrenal insufficiency is associated with achalasia and alacrima—Allgrove's, or triple A, syndrome. However, in some triple A syndrome patients, no mutation in the ACTH receptor has been identified, suggesting that a distinct genetic abnormality causes this syndrome. *Adrenal hypoplasia congenita* is a rare X-linked disorder caused by a mutation in the *DAX1* gene. This gene encodes an orphan nuclear receptor that plays an important role in the development of the adrenal cortex and also the hypothalamic-pituitary-gonadal axis. Thus, patients present with signs and symptoms secondary to deficiencies of all three major adrenal steroids—cortisol, aldosterone, and adrenal androgens—as well as gonadotropin deficiency. Finally a rare cause of hypercortisolism without cushingoid stigmata is *primary cortisol resistance* due to mutations in the glucocorticoid receptor. The resistance is incomplete because patients do not exhibit signs of adrenal insufficiency.

Miscellaneous Conditions Adrenoleukodystrophy causes severe demyelination and early death in children, and adrenomyeloneuropathy is associated with a mixed motor and sensory neuropathy with spastic paraplegia in adults; both disorders are associated with elevated circulating levels of very long chain fatty acids and cause adrenal insufficiency. Autosomal recessive mutations in the steroidogenic acute regulatory (STAR) protein gene cause congenital lipoid adrenal hyperplasia (Chap. 328), which is characterized by adrenal insufficiency and defective gonadal steroidogenesis. Because STAR mediates cholesterol transport into the mitochondrion, mutations in the protein cause massive lipid accumulation in steroidogenic cells, ultimately leading to cell toxicity.

MINERALOCORTICOID DISEASES Some forms of CAH have a mineralocorticoid component (see above). Others are caused by a mutation in other enzymes or ion channels important in mediating or mimicking aldosterone's action.

Hypermineralocorticoidism ■ *LOW PLASMA RENIN ACTIVITY* Rarely, hypermineralocorticoidism is due to a defect in cortisol biosynthesis, specifically 11- or 17-hydroxylation. ACTH levels are increased, with a resultant increase in the production of the mineralocorticoid 11-deoxycorticosterone. Hypertension and hypokalemia can be corrected by glucocorticoid administration. The definitive diagnosis is made by demonstrating an elevation of precursors of cortisol biosynthesis in the blood or urine or by direct demonstration of the genetic defect.

Glucocorticoid administration can also ameliorate hypertension or produce normotension even though a hydroxylase deficiency cannot be identified (Fig. 321-9). These patients have normal to slightly elevated aldosterone levels that do not suppress in response to saline but do suppress in response to 2 days of dexamethasone (2 mg/d). The condition is inherited as an autosomal dominant trait and is termed *glucocorticoid-remediable aldosteronism* (GRA). This entity is secondary to a chimeric gene duplication whereby the 11-β hydroxylase gene promoter (which is under the control of ACTH) is fused to the aldosterone synthase coding sequence. Thus, aldosterone synthase activity is ectopically expressed in the zona fasciculata and is regulated by ACTH, in a fashion similar to the regulation of cortisol secretion. Screening for this defect is best performed by assessing the presence or absence of the chimeric gene. Because the abnormal gene may be present in the absence of hypokalemia, its frequency as a cause of hypertension is unknown. Individuals with suppressed plasma renin levels and juvenile-onset hypertension or a history of early-onset hypertension in first-degree relatives should be screened for this disorder. Early hemorrhagic stroke also occurs in GRA-affected individuals.

GRA documented by genetic analysis may be treated with glucocorticoid administration or antimineralocorticoids, i.e., spironolactone, triamterene, or amiloride. Glucocorticoids should be used only in small doses to avoid inducing iatrogenic Cushing's syndrome. A combination approach is often necessary.

HIGH PLASMA RENIN ACTIVITY *Bartter syndrome* is characterized by severe hyperaldosteronism (hypokalemic alkalosis) with moderate to marked increases in renin activity and hypercalciuria, but normal blood pressure and no edema; this disorder usually begins in childhood. Renal biopsy shows juxtaglomerular hyperplasia. Bartter syndrome is caused by a mutation in the renal Na-K-2Cl co-transporter gene. The pathogenesis involves a defect in the renal conservation of sodium or chloride. The renal loss of sodium is thought to stimulate renin secretion and aldosterone production. Hyperaldosteronism produces potassium depletion, and hypokalemia further elevates prostaglandin production and plasma renin activity. In some cases, the hypokalemia may be potentiated by a defect in renal conservation of potassium.

Gitelman syndrome is an autosomal recessive trait characterized by renal salt wasting and as a result, as in Bartter syndrome, activation of the renin-angiotensin-aldosterone system. As a consequence affected individuals have low blood pressure, low serum potassium, low serum magnesium, and high serum bicarbonate. In contrast to Bartter syndrome, urinary calcium excretion is reduced. Gitelman syndrome results from loss-of-function mutations of the renal thiazide-sensitive Na-Cl co-transporter.

Increased Mineralocorticoid Action *Liddle syndrome* is a rare autosomal dominant disorder that mimicks hyperaldosteronism. The defect is in the genes encoding the β or η subunits of the epithelial sodium channel. Both renin and aldosterone levels are low, owing to the constitutively activated sodium channel and the resulting excess sodium reabsorption in the renal tubule.

TABLE 321-9 A Checklist for Use Prior to the Administration of Glucocorticoids in Pharmacologic Doses

Presence of tuberculosis or other chronic infection (chest x-ray, tuberculin test)
Evidence of glucose intolerance or history of gestational diabetes mellitus
Evidence of preexisting osteoporosis (bone density assessment in organ transplant recipients or postmenopausal patients)
History of peptic ulcer, gastritis, or esophagitis (stool guaiac test)
Evidence of hypertension or cardiovascular disease
History of psychological disorders

A rare autosomal recessive cause of hypokalemia and hypertension is 11β-HSD II deficiency, in which cortisol cannot be converted to cortisone and hence binds to the MR and acts as a mineralocorticoid. This condition, also termed *apparent mineralocorticoid excess syndrome*, is caused by a defect in the gene encoding the renal isoform of this enzyme, 11β-HSD II. Patients can be identified either by documenting an increased ratio of cortisol to cortisone in the urine or by genetic analysis. Patients with the 11β-HSD deficiency syndrome can be treated with small doses of dexamethasone, which suppresses ACTH and endogenous cortisol production but binds less well to the mineralocorticoid receptor than does cortisol.

The ingestion of candies or chewing tobacco containing certain forms of licorice produces a syndrome that mimics primary aldosteronism. The component of such agents that causes sodium retention is glycyrrhizinic acid, which inhibits 11β-HSD II and hence allows cortisol to act as a mineralocorticoid. The diagnosis is established or excluded by a careful history.

Decreased Mineralocorticoid Production or Action In patients with a deficiency in aldosterone biosynthesis, the transformation of corticosterone into aldosterone is impaired, owing to a mutation in the aldosterone synthase (CYP11B2) gene. These patients have low to absent aldosterone secretion, elevated plasma renin levels, and elevated levels of the intermediates of aldosterone biosynthesis (corticosterone and 18-hydroxycorticosterone).

Pseudohypoaldosteronism type I (PHA-I) is an autosomal recessive disorder that is seen in the neonatal period and is characterized by salt wasting, hypotension, hyperkalemia, and high renin and aldosterone levels. In contrast to the gain-of-function mutations in the epithelial sodium channel in Liddle syndrome, mutations in PHA-I result in loss of epithelial sodium channel function.

PHARMACOLOGIC CLINICAL USES OF ADRENAL STEROIDS

The widespread use of glucocorticoids emphasizes the need for a thorough understanding of the metabolic effects of these agents. Before adrenal hormone therapy is instituted, the expected gains should be weighed against undesirable effects. Several important questions should be addressed before initiating therapy. First, how serious is the disorder (the more serious, the greater the likelihood that the risk/benefit ratio will be positive)? Second, how long will therapy be required (the longer the therapy, the greater the risk of adverse side effects)? Third, does the individual have preexisting conditions that glucocorticoids may exacerbate (Table 321-9)? If so, then a careful risk/benefit assessment is required to ensure that the ratio is favorable given the increased likelihood of harm by steroids in these patients. Supplementary measures to minimize undesirable metabolic effects are shown in Table 321-10. Fourth, which preparation is best?

THERAPEUTIC CONSIDERATIONS The following considerations should be taken into account in deciding which steroid preparation to use:

1. *The biologic half-life.* The rationale behind alternate-day therapy is to decrease the metabolic effects of the steroids for a significant part of each 48 h period while still producing a pharmacologic effect durable enough to be effective. Too long a half-life would defeat the first purpose, and too short a half-life would defeat the second. In general, the more potent the steroid, the longer its biologic half-life.

TABLE 321-10 Supplementary Measures to Minimize Undesirable Metabolic Effects of Glucocorticoids

Monitor caloric intake to prevent weight gain.
Restrict sodium intake to prevent edema and minimize hypertension and potassium loss.
Provide supplementary potassium if necessary.
Provide antacid, H_2 receptor antagonist, and/or H^+,K^+-ATPase inhibitor therapy.
Institute alternate-day steroid schedule if possible. Patients receiving steroid therapy over a prolonged period should be protected by an appropriate increase in hormone level during periods of acute stress. A rule of thumb is to *double* the maintenance dose.
Minimize osteopenia by
Administering gonadal hormone replacement therapy: 0.625–1.25 mg conjugated estrogens given cyclically with progesterone, unless the uterus is absent; testosterone replacement for hypogonadal men
Ensuring high calcium intake (should be approximately 1200 mg/d)
Administering supplemental vitamin D if blood levels of calciferol or 1,25$(OH)_2$ vitamin D are reduced
Administering bisphosphonate prophylactically, orally or parenterally, in high-risk patients

2. *The mineralocorticoid effects of the steroid.* Most synthetic steroids have less mineralocorticoid effect than hydrocortisone (Table 321-11).
3. *The biologically active form of the steroid.* Cortisone and prednisone have to be converted to biologically active metabolites before anti-inflammatory effects can occur. Because of this, in a condition for which steroids are known to be effective and when an adequate dose has been given without response, one should consider substituting hydrocortisone or prednisolone for cortisone or prednisone.
4. *The cost of the medication.* This is a serious consideration if chronic administration is planned. Prednisone is the least expensive of available steroid preparations.
5. *The type of formulation.* Topical steroids have the distinct advantage over oral steroids in reducing the likelihood of systemic side effects. In addition, some inhaled steroids have been designed to minimize side effects by increasing their hepatic inactivation if they are swallowed (Chap. 236). However, all topical steroids can be absorbed into the systemic circulation.

TABLE 321-11 Glucocorticoid Preparations

Commonly Used Name[a]	Estimated Potency[b]	
	Glucocorticoid	Mineralocorticoid
SHORT-ACTING		
Hydrocortisone	1	1
Cortisone	0.8	0.8
INTERMEDIATE-ACTING		
Prednisone	4	0.25
Prednisolone	4	0.25
Methylprednisolone	5	<0.01
Triamcinolone	5	<0.01
LONG-ACTING		
Paramethasone	10	<0.01
Betamethasone	25	<0.01
Dexamethasone	30–40	<0.01

[a] The steroids are divided into three groups according to the duration of biologic activity. Short-acting preparations have a biologic half-life <12 h; long-acting, >48 h; and intermediate, between 12 and 36 h. Triamcinolone has the longest half-life of the intermediate-acting preparations.

[b] Relative milligram comparisons with hydrocortisone, setting the glucocorticoid and mineralocorticoid properties of hydrocortisone as 1. Sodium retention is insignificant for commonly employed doses of methylprednisolone, triamcinolone, paramethasone, betamethasone, and dexamethasone.

ALTERNATE-DAY STEROID THERAPY The most effective way to minimize the cushingoid effects of glucocorticoids is to administer the total 48-h dose as a *single* dose of *intermediate-acting steroid* in the morning, *every other day*. If symptoms of the underlying disorder can be controlled by this technique, it offers distinct advantages. Three considerations deserve mention: (1) The alternate-day schedule may be approached through transition schedules that allow the patient to adjust gradually; (2) supplementary nonsteroid medications may be needed on the "off" day to minimize symptoms of the underlying disorder; and (3) many symptoms that occur during the off day (e.g., fatigue, joint pain, muscle stiffness or tenderness, and fever) may represent relative adrenal insufficiency rather than exacerbation of the underlying disease.

The alternate-day approach capitalizes on the fact that cortisol secretion and plasma levels normally are highest in the early morning and lowest in the evening. The normal pattern is mimicked by administering an intermediate-acting steroid in the morning (7 to 8 A.M.) (Table 321-11).

Initially, the steroid regimen often requires daily or more frequent doses of steroid to achieve the desired anti-inflammatory or immunity-suppressing action. *Only after this desired effect is achieved is an attempt made to switch to an alternate-day program.* A number of schedules can be used for transferring from a daily to an alternate-day program. The key points to be considered are flexibility in arranging a program and the use of supportive measures on the off day. One may attempt a gradual transition to the alternate-day schedule rather than an abrupt changeover. One approach is to keep the steroid dose constant on one day and gradually reduce it on the alternate day. Alternatively, the steroid dose can be increased on one day and reduced on the alternate day. In any case, it is important to anticipate that some increase in pain or discomfort may occur in the 36 to 48 h following the last dose.

WITHDRAWAL OF GLUCOCORTICOIDS FOLLOWING LONG-TERM USE It is possible to reduce a daily steroid dose gradually and eventually to discontinue it, but under most circumstances withdrawal of steroids should be initiated by first implementing an alternate-day schedule. Patients who have been on an alternate-day program for a month or more experience less difficulty during termination regimens. The dosage is gradually reduced and finally discontinued after a replacement dosage has been reached (e.g., 5 to 7.5 mg prednisone). Complications rarely ensue unless undue stress is experienced, and patients should understand that for ≥1 year after withdrawal from long-term high-dose steroid therapy, supplementary hormone should be given in the event of a serious infection, operation, or injury. A useful strategy in patients with symptoms of adrenal insufficiency on a tapering regimen is to measure plasma cortisol levels prior to the steroid dose. A level <138 nmol/L (5 μg/dL) indicates suppression of the pituitary-adrenal axis and implies that a more cautious tapering of steroids is indicated.

In patients on high-dose daily steroid therapy, it is advised to reduce dosage to ~20 mg prednisone daily as a single morning dose before beginning the transition to alternate-day therapy. If a patient cannot tolerate an alternate-day program, consideration should be given to the possibility that the patient has developed primary adrenal insufficiency.

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322 PHEOCHROMOCYTOMA

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Pheochromocytomas produce, store, and secrete catecholamines. They are usually derived from the adrenal medulla but may develop from chromaffin cells in or about sympathetic ganglia (extraadrenal pheochromocytomas or paragangliomas). Related tumors that secrete catecholamines and produce similar clinical syndromes include chemodectomas derived from the carotid body and ganglioneuromas derived from the postganglionic sympathetic neurons.

The clinical features are due predominantly to the release of catecholamines and, to a lesser extent, to the secretion of other substances. Hypertension is the most common sign, and hypertensive paroxysms or crises, often spectacular and alarming, occur in over half the cases.

Pheochromocytoma occurs in approximately 0.1% of the hypertensive population but is, nevertheless, an important correctable cause of high blood pressure. Indeed, it is usually curable if diagnosed and treated, but it may be fatal if undiagnosed or mistreated. Postmortem series indicate that most pheochromocytomas are unsuspected clinically, even when the tumor is related to the fatal outcome.

PATHOLOGY ■ Location and Morphology

In adults, approximately 80% of pheochromocytomas are unilateral and solitary, 10% are bilateral, and 10% are extraadrenal. In children, a fourth of tumors are bilateral, and an additional fourth are extraadrenal. Solitary lesions inexplicably favor the right side. Although pheochromocytomas may grow to large size (>3 kg), most weigh <100 g and are <10 cm in diameter. Pheochromocytomas are highly vascular.

The tumors are made up of large, polyhedral, pleomorphic chromaffin cells. Fewer than 10% of these tumors are malignant. As with several other endocrine tumors, malignancy cannot be determined from the histologic appearance; tumors that contain large numbers of aneuploid or tetraploid cells, as determined by flow cytometry, are more likely to recur. Local invasion of surrounding tissues or distant metastases indicate malignancy.

EXTRAADRENAL PHEOCHROMOCYTOMAS Extraadrenal pheochromocytomas usually weigh 20 to 40 g and are <5 cm in diameter. Most are located within the abdomen in association with the celiac, superior mesenteric, and inferior mesenteric ganglia. Approximately 10% are in the thorax, 1% are within the urinary bladder, and <3% are in the neck, usually in association with the sympathetic ganglia or the extracranial branches of the ninth or tenth cranial nerves.

Catecholamine Synthesis, Storage, and Release Pheochromocytomas synthesize and store catecholamines by processes resembling those of the normal adrenal medulla. Little is known about the mechanisms of catecholamine release from pheochromocytomas, but changes in blood flow and necrosis within the tumor may be the cause in some instances. These tumors are not innervated, and catecholamine release does not result from neural stimulation. Pheochromocytomas also store and secrete a variety of peptides, including endogenous opioids, adrenomedullin, endothelin, erythropoietin, parathyroid hormone–related protein, neuropeptide Y, and chromagranin A. These peptides contribute to the clinical manifestations in selected cases, as noted below.

EPINEPHRINE, NOREPINEPHRINE, AND DOPAMINE Most pheochromocytomas produce both norepinephrine and epinephrine, and the percentage of norepinephrine is usually greater than in the normal adrenal. Most extraadrenal pheochromocytomas secrete norepinephrine exclusively. Rarely, pheochromocytomas produce epinephrine alone, particularly in association with multiple endocrine neoplasia (MEN). Although