SELECTED ASPECTS OF STEROID PHARMACOLOGY

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INTRODUCTION

Reviews dealing with the biological activities of steroids generally emphasize those actions which are classically hormonal in character and which fall within the conventional province of experimental and clinical endocrinology (384). The purpose of this report, however, is to describe a number of pharmacological effects produced by steroids, which are not customarily dealt with in such reviews, but which represent important, though not typically hormonal actions of these compounds. No fundamental distinction between hormonal and non-hormonal (29) properties of steroids is implied by this description.

The pharmacological actions summarized here represent a heterogeneous group of steroid effects described in the course of studies in many different fields of investigation, knowledge of which has often tended to be restricted to those with specialized interests. The majority of the reports reviewed have been solely descriptive in character and in most, mechanisms of action have largely been unexplored. For these reasons, as well as the desirability of making this review as comprehensive as possible with respect to lesser known pharmacological properties of steroids, critical evaluation of individual papers has been minimized. Instead, an attempt has been made, wherever possible, to indicate the potential significance of the steroid actions described to clinical pharmacology and medicine.

Certain major actions of steroids, such as those on cardiac and smooth muscle,

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and some of those on the central nervous system, have not been reviewed because they are so widely known; emphasis has been placed, however, on the pharmacological properties of steroid metabolites, since there is growing awareness of the biological importance of this class of compounds (110, 162, 201). With few exceptions, the influences of steroids on biochemical reactions or enzymes have not been considered to fall within the scope of this review.

1. ANESTHETIC ACTION OF STEROIDS

The powerful hypnotic or anesthetic properties of steroids were first demonstrated and studied in detail by Selye and co-workers in the 1940's, although earlier work by others had indicated that cholesterol was an anesthetic agent itself or potentiated the action of chemically unrelated anesthetics.

In 1927, Cashin and Moravek (49) reported that the intravenous administration of colloidal suspensions of cholesterol to cats induced anesthesia which, though transient, was sufficiently deep to permit major surgery. Injections of these colloidal suspensions, however, were associated with significant cardiopulmonary toxicity, part of which at least was probably accounted for by intravascular precipitation of the steroid. Addition of small amounts of lecithin to the sterol suspensions appeared to decrease toxicity without affecting anesthetic activity. The potentiating effect of cholesterol on the action of more conventional anesthetic agents was noted by Starkenstein and Weden some years later (364) in studies clearly demonstrating that intraperitoneal administration of the sterol produced a great increase in length and depth of anesthesia induced in rabbits by ether, chloroform, sodium barbital, urethane, and diphenylhydantoin. The possibility that cholesterol acted as a lipid "transport" material for the anesthetic in this action was suggested by the authors, but rejected in a subsequent report by Foldes and Beecher (103). In studying the effect of olive oil, lecithin, and cholesterol on barbiturate and ether anesthesia in mice and rabbits, these investigators showed that lecithin was without potentiating effect; that both olive oil and cholesterol enhanced ether anesthesia; and that only cholesterol potentiated barbiturate anesthesia. The differences in potentiating action of oil and cholesterol, and the lack of such action by lecithin despite ether-solubility comparable to that of cholesterol, were interpreted to mean that factors other than physical solubility or "transport" phenomena accounted for the enhancing effect of the sterol. A later report by Farson *et al.* (94) generally confirmed this action of cholesterol in other experimental animals but provided no direct evidence of mechanism of action.

In 1941, Selye (345) initiated a series of important investigations which clearly established the anesthetic action of steroids as a major pharmacologic property of certain natural hormones (342–344, 346, 347, 349) and related substances, and provided the theoretical and experimental background for the ultimate development of steroid anesthesia as an effective and safe clinical procedure.

In these studies steroids such as desoxycorticosterone, progesterone, testosterone, pregnandione, and androsterone, as well as many related compounds, were shown to induce profound anesthesia in laboratory animals when adminis-

tered by intraperitoneal or intravenous injection (345). Susceptible species included rats, mice, guinea pigs, rabbits, and at least one class of fish (*Notropis cornutus*, the red fin minnow). Invertebrates, however, were unresponsive to even the most potent steroid in this respect (349). Anesthesia produced by these compounds in experimental animals was characterized by a rapid (345) quiet induction period devoid of excitation, and subsequent recovery without ill effects. At the time of maximum anesthesia, generalized peripheral vasodilation and a slight decrease in body temperature were observed. Lethal amounts of steroid appeared to act primarily through depression of the respiratory center, since cardiac activity persisted long after breathing had ceased. Examinations *post mortem* in such animals revealed no morphologic evidence of toxicity except for some pulmonary extravasation (345).

Detailed study of the relation between chemical structure, anesthetic potency, and classical hormonal activity of steroids (folliculoid, testoid, corticoid, luteoid action) (346) revealed that a number of hormonally active compounds was capable of inducing anesthesia, but that this property was not restricted to steroids demonstrating conventional endocrine activity as had been thought previously (343). Among a total of 75 steroids examined for anesthetic action in partially hepatectomized rats (*i.e.*, rats sensitized to steroid-induced anesthesia), the estrogens as a group were the least active, while pregnandione was the most potent individual compound. Desoxycorticosterone and progesterone were powerful anesthetic agents as well, and testosterone somewhat less so, inducing a lighter anesthesia after a delayed induction period (345). Other active compounds included both metabolites of steroid hormones and synthetic steroid derivatives such as androsterone, 3β-etiocholanolone, etiocholanolone, 6-hydroxyprogesterone acetate, and acetoxypregnenolone (346). The most powerful anesthetic steroids were oxygenated only at the terminal ends of the molecule, and in the androstane and etiocholane series steric configuration of the C3 hydroxyl group did not influence anesthetic potency. Esterification did not suppress this action if the esterified steroid was made more soluble by this process (346).

A number of factors influenced the anesthetic response to steroids. As noted above, this activity appeared to be restricted to vertebrates since several varieties of crayfish were unresponsive to potent steroid anesthetics (349). There was considerable variation in individual susceptibility to this steroid action and, in rats and mice at least, females were generally more responsive than males (345, 414). Castration of females did not affect this sensitivity although castrate males became as susceptible as females to steroid anesthesia. Moreover, chronic administration of small amounts of androgens to females and castrate males diminished responsiveness to the level of intact males. Curiously, the order of activity of steroids was affected by size of the experimental animals—thus, progesterone was a more potent anesthetic than desoxycorticosterone in small rats, but the converse was true in animals weighing over 80 grams (343). Hypophysectomy, alone or combined with adrenalectomy, sensitized animals to the anesthetic action of steroids, although thyroidectomy was without effect. Bilateral nephrectomy was without significant influence except that desoxy-

corticosterone anesthesia was slightly enhanced (343, 345). Hepatectomy clearly heightened responsiveness to the anesthetic action of all steroids, presumably through failure of hepatic mechanisms for conjugating or detoxifying these compounds (342, 345). "Adaptation" to formaldehyde treatment and chronic forced muscular activity increased susceptibility to steroid anesthesia through unknown mechanisms, though possible exhaustion of "adaptation energy" was suggested (343). Brief pretreatment of animals with atropine antagonized progesterone anesthesia; chronic pretreatment had the opposite effect. Acetylcholine did not influence anesthesia in any way. Cervical vagotomy had the same influence on steroid anesthesia as chronic atropine pretreatment (343), but subdiaphragmatic vagotomy had only a slight effect. Hence it was uncertain whether the responses seen were specific effects of neural intervention or pulmonary complications secondary to cervical vagotomy. There appeared to be an acquired adaptation to the anesthetic action of steroids since pretreatment of animals with small doses of progesterone or desoxycorticosterone was able to confer a considerable degree of resistance to the anesthetic action of some steroids in rats (344). Progesterone was more effective in this regard than desoxycorticosterone. Cholesterol was without effect, and indeed appeared to have no direct anesthetic properties of its own, at least in the amounts administered (345). However, potentiation of the anesthetic action of other volatile anesthetic agents was noted following steroid pretreatment (343). These steroids also protected against the lethal action of metrazol overdosage, and conversely metrazol interrupted the anesthesia induced by such compounds (347). Anesthetic steroids also suppressed central nervous system excitation induced by steroid convulsants such as Compound S (161). Hypotension was no more prominent in anesthesia produced by steroids than in that by barbiturates (107); influences of these steroids on certain reflex phenomena in the throat appeared to be different (21).

Interest in this pharmacologic action of steroids seemed to subside for a number of years until 1954 when Merryman and colleagues reported on the anesthetic effect of progesterone in man (246), and Laubach, P'an, and Rudel (223) and P'an and associates (286) in 1955 demonstrated that the synthetic steroid 21-hydroxypregnandione sodium succinate (hydroxydione, or Viadril) induced pronounced central nervous system depression in experimental animals and appeared to have considerable promise for clinical application in basal or general anesthesia. In the Merryman report (246), progesterone administered intravenously in relatively large amounts (about 500 mg) over the course of one hour in man induced anesthesia which began a few minutes after the infusion started and lasted for 1 to 2 hours after termination of the injection. Lesser amounts given more rapidly produced sleep in pregnant women; and some tolerance to this action, manifested by a longer latent period and lighter anesthesia, was observed in subjects receiving repeated infusions. The likelihood would appear to be strong that this action of progesterone may account to a considerable degree for the lassitude and somnolence some women experience in pregnancy (97). The reports of Laubach, P'an, and associates (223, 286) showed that hydroxydione lacked the classical steroid hormone actions (117) but possessed marked anes-

thetic action in laboratory animals and in man (10, 66, 73, 112, 131, 147, 174, 176, 220, 258, 259, 379). Hydroxydione anesthesia could be induced experimentally in several species including mice, rats, dogs, rabbits, and monkeys whether administered intravenously or orally, although by the latter route, anesthesia required several times larger doses and onset was less rapid. Induction of anesthesia, as previously demonstrated by Selye for other steroids, was smooth and recovery was relatively rapid and marked by the lack of postanesthetic depression. Large doses produced hypotensive effects in cats but overall cardiovascular or respiratory toxicity was less than that induced by comparable non-steroidal agents such as the ultra-short-acting barbiturates. Bilateral nephrectomy did not influence the intensity of hydroxydione anesthesia, and, in contrast to Selye's observations with other steroids, liver damage had no significant influence on potency for production of this effect—probably because of differences in route of administration of steroids. In addition, no sex differences in response to anesthesia produced by this steroid were demonstrable, although such differences had been reported with steroids studied earlier (345, 414).

The general pharmacological properties of hydroxydione and its merits relative to barbiturates for clinical anesthesia have been discussed in previous reports (68, 286). Extensive experience with this compound has confirmed its usefulness as an anesthetic agent clinically and has brought to light several of the special problems related to its use in man. Its advantages include the ability to obtund reflexes sufficiently to permit easy tracheal intubation (258), ease of onset and maintenance of anesthesia by continuous infusion of the steroid (220), and its wide safety margin (220). The only serious problem associated with its use has been a variable incidence of venous inflammation or thrombosis at the injection site (147, 258, 259, 379), although attention has been called to the occasional delayed onset of hypotension and respiratory complications in some patients (176).

The structural basis of this central steroid action has been examined in an important study by Figdor *et al.* (97), and it appears that the anesthetic activity of steroids has a considerable structural specificity. Nuclear substitution in both saturated and unsaturated series of compounds had a deleterious effect on depressant activity; paradoxically, however, stereochemical requirements with respect to spatial conformation of the A ring were considerably less strict than those generally associated with more conventional "hormonal" actions of steroids. Steroids having the 5β-H configuration (A:B *cis*) were especially active, raising the interesting possibility that the anesthetic properties of compounds such as desoxycorticosterone and progesterone might be mediated by their biotransformation products (97) rather than by the unaltered hormones.

The mechanism of steroid anesthesia has not been investigated extensively, but Jakoby and Tomkins (184) have described hydrolytic and reductive transformations of hydroxydione which may represent inactivation processes. Inhibition of brain respiration and uncoupling of oxidative phosphorylation in brain tissue by steroid anesthetics have also been noted (103, 389); the latter effect has been shown to parallel the anesthetic potency of steroids. The precise site of action

of these compounds in suppressing brain respiration, however, is not known, but is thought to differ from that of other types of anesthetic agents (248).

2. GONADAL HORMONES AND IMMUNOLOGICAL REACTIVITY

Steroid hormones profoundly influence the course of infection and the intensity of immunologic or hypersensitivity phenomena in man and experimental animals. In recent years, the role of adrenocortical hormones in these processes has been emphasized (121), although there are many observations which indicate that gonadal steroids have interesting and important influences on these actions as well. This section of the review will be limited to a description of some effects on immunological phenomena induced by steroids related to hormones of the latter type. It will also deal briefly with certain reactions in which steroids participate directly as components of the immune complex, or in which they are presumed to act as "allergenic" agents. The effects of hormones on the general metabolism, involution, and growth of lymphoid tissue, and on the structure and number of circulating lymphocytes have already been described in detail (30, 78) and will not be reviewed here.

Excellent summaries of early work dealing with the influence of sexual maturity and endocrine secretions on "sero-maturity," infectious processes, and immunologic reactivity have been published (19, 75, 192, 398, 409). The effects of pregnancy and the menstrual cycle on these processes have attracted special interest because of their presumed relation to the incidence and severity of disease in females (93, 191–194, 362). Among these effects seen clinically and experimentally may be noted the occurrence of poliovirucidal activity in pregnancy serum in considerably higher titer than seen normally (191, 192); fluctuation in pneumococcal protective antibody titer during the menstrual cycle (314); enhancement of susceptibility to infection (387) or change in pattern of infectious disease (362) in the gravid state; sensitization to shocking doses of antigen in pregnant rabbits (182) but not rats (304); diminution of complement activity in the blood of pregnant guinea pigs (140) but not rabbits (388); and diminished blood levels of, or skin responses to, circulating or fixed cellular antibody (6, 37, 289) in pregnancy.

Mediation of these effects in part by gonadal hormones is suggested by the considerable influence which steroids of this type have on the pattern of experimental infectious disease (106, 409) and on immunologic phenomena. Estrogenic enhancement of natural or induced circulating antibody titers has been observed in several (239, 398, 399, 408) but not all studies (75). Weinstein (408) demonstrated increases in circulating *E. coli* agglutinin and sheep RBC hemolysin in rabbits following estrogen therapy, the degree of change being directly related to the dose of hormone. Small amounts of hormone induced selective increases in hemolysin levels without altering the agglutinin titer. Estrogenic potentiation of the circulating antibody response to pneumococcal antigen in other species was shown in the studies of Von Haam and Rosenfeld (398) and Mainwaring (239), and striking increases in the ability of rabbits to form heterophil antibody following this form of therapy has also been noted (399). Estrogens, however, failed

to alter the agglutinin response of young rats or rabbits to *B. pertussis* (75), a reflection possibly of the well-known immunologic unresponsiveness of immature animals. The immunopotentiating action of estrogens is reflected in morphological and functional alterations consistent with enhanced activity of the lymphatic system, as shown in the extensive studies of Nicol and colleagues (26, 52, 146, 263–268, 360, 403). In these studies, both natural and synthetic estrogens induced marked hyperplasia and proliferation in the reticuloendothelial systems of experimental animals; enhanced phagocytic activity, reflected by increased rate of disappearance of particulate material or dye from blood; reversed corticoid-induced suppression of phagocytosis and stimulated significant increases in both gamma-globulin levels and antibody-forming potential. Androgens either had no stimulatory effect or depressed the activity of the RE system in these experiments (26, 360).

The studies demonstrating the immuno-enhancing action of estrogenic steroids contrast with others indicating that gonadal hormones, when used in different experimental contexts, induce profound suppression of morphological development and functional activity of immunologically active tissues. These investigations have dealt largely with endocrine influences on the bursa of Fabricius, a lymphoid organ peculiar to birds.

The bursa of Fabricius consists of a blind, plicated, sac-like structure arising as a posterior diverticulum from the cloaca, with a parenchyma composed almost entirely of lymphoid follicles closely related to the lining epithelium of the bursa. In the chick the bursa begins development on about the tenth day of incubation and grows until the onset of sexual maturity at approximately four and one-half months, when it undergoes involution (1). In pigeons and doves, involution occurs somewhat earlier (about three months) (309), and in other species such as the blue goose, lesser snow goose, and yellow-billed loon, which require more than a year to reach sexual maturity, involution of the bursa is delayed (163). The functional significance of the bursa has been obscure until recently, when it has been implicated in a role comparable to that of the thymus (51, 125, 309, 404) in the development of the capacity of the host to respond to immunological challenge.

Parenthetically, it may be noted that this organ has important implications in another respect. Morphological and histochemical studies indicate that the lymphocytes of the lymphoid follicles in the bursa arise from undifferentiated epithelial cells through a series of direct transformations from primitive epithelial cells to small and medium sized lymphocytes (1). This histogenesis is highly significant, since theories of hemopoiesis almost universally hold that lymphocytes develop only from mesenchymal cells or their derivatives in vertebrates.

Involution of this lymphoid structure at the time of sexual maturity in birds had led to the suggestion that the organ is under gonadal hormone control (309), a suggestion that has been confirmed by several studies in recent years. Selye (348) reported in 1943 that a number of C-19 steroids such as dehydroisoandrosterone, Δ^4-androstenedione and Δ^5-androstene-3β,17α-diol, as well as methyltestosterone induced marked involution of the bursa in chicks, although

the organ tended to regain its normal size subsequently despite continued steroid treatment. A year later Kirkpatrick and Andrews (211) confirmed this effect of androgens and demonstrated that estrogens induced involution of the bursa as well. Gonadal hormone suppression of bursal development has since been studied in detail (247, 287, 303, 404), and it has been shown that injection of steroid into incubating chick eggs early in embryogenesis (5th day) completely arrests bursal morphogenesis, so that chicks are born bursa-less. Dipping of eggs into gonadal steroid solutions, or direct steroid injection also inhibits bursa formation (124). This response is graded so that appropriate doses of gonadal hormone at various intervals inhibit morphogenesis of the bursa at several different stages. Inhibition of bursa development takes place irrespective of sex and is associated with variable reduction in size of other lymphoid organs, such as spleen and thymus (247, 375). If steroid administration occurs early, permanent inhibition of bursa formation is noted; at a later stage, steroids can induce replacement of lymphoid follicles by epithelial tissue (404).

The significance of these gonadal steroid effects on the bursa of Fabricius lies in the pronounced functional alterations which accompany disturbed morphogenesis of this lymphoid organ. "Hormonally bursectomized" (404) birds have decreased immunological responsiveness, as reflected in failure of antibody production in response to antigenic challenge, with a high death rate occurring early in life (124, 255). Exposure to steroids in the embryonic period, moreover, diminishes the homograft response in young chicks and prolongs the embryonic stage of immunologic immaturity (287). The occurrence of significant though variable changes in other lymphoid tissues of fowl following hormonal treatment —especially persistence of a histologically normal thymus in a significant percentage of bursa-less chicks—has permitted dissociation between different types of immunological reactivity in such preparations. Hormonally bursectomized birds with intact thymuses have total failure of production of circulating antibody and absence of skin reactivity of the delayed hypersensitivity type, but their blood remains capable of producing chorio-allantoic membrane lesions, and the homograft rejection time is normal; in bursa-less birds with atrophic thymuses, diminution of the latter immune reactions is observed as well (375). This dissociation indicates that the influence of gonadal steroids is not manifest as a simple quantitative reduction of all immune responses but as a selective depression of some, leaving others, in less severely affected birds, intact (375). Other gonadal influences which may be consistent with suppression of immune reactivities of these types are reflected in the prolonged life of skin homografts in pregnant rabbits (27, 160), an effect which may be species-specific (245), and in the observations recorded in the extensive literature on the fate and immunologic consequences of ovarian transplants in experimental animals (54, 219, 234, 235, 415).

Modification of immune reactivity—either enhancement or suppression—by gonadal steroids represents an interesting and important pharmacological property of these compounds. Although emphasis in recent years has been placed largely on the role of cortisol-like hormones in immunity and hypersensitivity, the

investigations summarized above suggest that the influence of gonadal steroids on these phenomena may be profitably examined in more detail. Studies of gonadal hormone effects on immunological responsiveness, for example, may provide some insight into the mechanisms for the known differences in the incidence and character of disease between adults and children and men and women, and the possible relation of these gonadal influences to the sex incidence and time of onset of certain autoimmune diseases would appear to be of special interest.

3. STEROID ANTIBODIES AND STEROID ALLERGY

Apart from enhancing and suppressing immunological processes in the ways described above, gonadal and other steroids may participate in these phenomena directly, as components of the immune complex. Lieberman and co-workers have been pre-eminent in this line of investigation and their important studies have been summarized in a recent review (233). It had been suggested earlier that cholesterol, when injected into rabbits, had "antigenic" activity reflected in the production of specific "immune" sera demonstrable by complement fixation tests (354, 407), although technical aspects of this work leave much to be desired. Lieberman's studies showed that a variety of steroids coupled to protein, mainly through amide bonds, acted as haptens, leading, when injected into rabbits, to the production of antisera having considerable steroid specificity, demonstrable by precipitation reactions and by hapten inhibition tests. Steroid specificities of antisera were not absolute and cross reactions occurred with antigens having heterologous steroid haptens, although these cross reactions were minimized by appropriate immunological techniques (233). Protein steroid antigens had variable endocrinological activity which sometimes considerably exceeded that anticipated from the hormone content of the conjugate; antisera, however, were not shown unequivocally to have antihormonal action. The antihormonal action of steroid-specific antisera, however, has recently (261) been demonstrated by others, in experiments in which estrone, testosterone, cortisol, and aldosterone, coupled to bovine serum albumin, were used as antigens. The potential importance of these studies to methods of control of endocrine function by immunologic means is evident, but they may also have special relevance to the whole problem of steroid "allergy" or hypersensitivity (see discussion, 233).

Allergic phenomena occurring periodically with the menstrual cycle or in pregnancy have been reported for many years (see 22, 93, 98, 251, 274, 310, 356), but the view that endocrine secretions or their derivatives served as a source of endogenous allergenic substances was first clearly proposed by Zondek and Bromberg in 1945 (420). These authors reported that a large number of women reacted with erythema and wheal formation at the site of intracutaneous injections of small amounts of steroid hormones or metabolites such as estradiol, estrone, progesterone, pregnandiol, testosterone, and androsterone. These local reactions were elicited by amounts of steroid as small as 0.1 μg, and in time of development and appearance resembled delayed hypersensitivity skin responses. Passive transfer of skin reactivity by serum of sensitive patients (Prausnitz-Küstner reaction) was thought to confirm the presence of "specific antibodies,"

though specificity was not established in other ways. In some patients direct intracutaneous injection of serum itself was capable of eliciting positive reactions. Skin reactions to hormones were particularly prominent in certain pathological conditions related to menstruation or the menopause (421). Recurrent reactions could be evoked at the site of initial skin testing by injection of steroid later at another site, and certain patients responded to steroid injections by serious generalized reactions such as asthma, urticaria, angioneurotic edema, and fever (421). Constitutional or familial predisposition to skin reactivity of this type was observed, as was relief of hypersensitivity by specific "desensitization" procedures (421–423). The concept of endogenous allergy to steroid hormones, with special emphasis on its relation to disorders of ovarian function, was studied by Heckel and colleagues (149–154), who confirmed in all essential details the earlier investigations of Zondek. In Heckel's studies, particular emphasis was placed on the role of pregnandiol as an allergenic agent in the etiology of various disorders such as premenstrual distress, painful breasts, chronic cystic mastitis, and dermatoses associated with the menstrual cycle. The majority of such patients reacted in a "hypersensitive" manner to pregnandiol injection and most obtained relief of symptoms or signs of illness by specific desensitization with this compound. Interestingly, pregnandiol desensitization occurred even when the steroid was administered orally, although subcutaneous injection was more effective. Delayed skin reactions to the steroid could also be demonstrated in rabbits and guinea pigs following several immunization procedures (151, 154). The role of the autonomic nervous system was felt to be of fundamental importance in the genesis of these reactions, but the interesting speculation was made that failure of conjugation of pregnandiol produced endogenously was the basis for the allergy to this steroid observed in patients. The occurrence of allergic skin responses to gonadal hormones has been confirmed in other reports (13, 74, 297) and it is interesting that the induction of local or generalized hypersensitivity reactions may extend to anti-inflammatory adrenal hormones as well (214, 273).

The concept of allergy to endogenous steroid hormones does not appear to have gained widespread acceptance, a reflection possibly on the vague assortment of ills to which these allergies have been etiologically related and the tenuous grounds for assuming the immunological nature of such disorders. For example, Heckel's observations that the hormone metabolites, pregnandiol and etiocholanolone, evoke by far the highest percentage of allergic skin reactions when injected intradermally, may not be unrelated to the fact that both steroids have powerful pyrogenic and inflammatory actions—properties which are manifest in nearly all subjects and for which there is no present evidence of an immunologic basis (200, 201, 203). Nevertheless, the phenomenon of steroid antibody formation in animals (233) and the clear demonstration of untoward clinical responses resembling hypersensitivity reactions to certain steroid hormones and bile acids (22, 55, 69, 70, 88, 214, 232, 273, 356, 372, 406), especially those lacking pyrogenic or inflammatory properties, provide considerable justification for assuming the rare occurrence of true allergic reactions to endogenously produced steroids in man.

4. STEROID FEVER

The alterations in temperature which accompany the menstrual cycle and pregnancy have attracted the interest of clinical investigators for more than a century, and for most of this time the dependence of these cyclic temperature variations on ovarian activity has been known. Excellent reviews of early work in this field have been published (14, 15, 100, 210, 279) and the general features of this relationship have been confirmed repeatedly (15, 42, 145, 319, 320). Basal body temperature usually falls slightly during or immediately preceding the menstrual flow, tends to remain depressed until the mid-period, rising at or near ovulation and remaining elevated during the luteal phase of the cycle, until the succeeding menstrual decline. During the early part of pregnancy, and perhaps longer, there is a slight, sustained elevation in basal body temperature as well. The hormonal basis for the diphasic character of the temperature cycle in women has been studied extensively and it is usually conceded that despite the occasional disparity in time between the observed temperature increase and the appearance of pregnandiol in the urine (100, 311), the post-ovulatory rise in temperature parallels, and is probably caused by, ovarian secretion of progesterone. Confirmation of the thermogenic action of this luteal hormone awaited its availability in a pure form, but since then this property of progesterone has been repeatedly demonstrated (16, 41, 70, 105, 122, 138, 139, 180, 238, 269, 270, 280, 292, 293, 315, 316, 417). Intramuscular injection of this steroid in small amounts (5 to 10 mg or more per day) consistently induces small but significant elevations of temperature in man. Repeated injection of the hormone results in slight, sustained elevations which subside shortly after injections cease. Local inflammatory reactions and sometimes "allergic" symptoms may accompany the rises in temperature. Although there is considerable individual variation, increases in temperature are usually proportional to dose; elevations exceeding 1°C are rare, however. The hormone is slightly thermogenic when administered by mouth in large amounts (139) or when applied by vaginal suppository (138). Several animal species apart from man are responsive to this hormonal effect (122, 269, 270, 417). Synthetic progestational agents also appear to be slightly thermogenic (56, 105, 288, 292, 293), and a single report indicates that desoxycorticosterone acetate may have similar properties (195).

The theoretical and practical importance of this property of progesterone has been outlined in several studies (41, 70, 100) and the influence of other gonadal hormones on this thermogenic phenomenon has been examined in man and in experimental animals (180, 238, 269, 315). A detailed description of these hormonal interactions is beyond the scope of this review, but it is of interest to record that estrogenic hormones have slight hypothermic action when injected alone and may inhibit the temperature-elevating effect of progesterone (16, 41, 180, 269, 280, 292). Androgens, however, have no significant influence on thermogenesis induced by progesterone (280, 315). The possibility that the temperature variations characteristic of the menstrual cycle and pregnancy depend upon complex quantitative relationships between estrogen and progesterone rather than simply

reflecting alterations in progesterone secretion, has been raised in an important study by Magallon and Masters (238), but apart from this report, and the suggestion that progesterone thermogenesis is mediated through the central nervous system (100), no information relating to the basic mechanism of this action is available. Moreover, the significance of this progesterone effect has not been considered to extend beyond its relation to ovarian activity. Recent studies, however, originating with Gallagher and associates (198), and describing a class of steroids having fever-producing activity of an entirely different order of magnitude from that of progesterone, have broadened the chemical basis and potential clinical importance of this pharmacologic property of steroids.

The intense pyrogenic activity of the steroid hormone metabolite, etiocholanolone, was first described by Kappas, Hellman, Fukushima, and Gallagher in 1956 (198), and subsequent studies by this group and others have confirmed the powerful fever-producing action of this 5β-H steroid and related compounds (32, 38, 126, 196, 197, 199–205, 283, 285, 335, 336). Intramuscular injection of this steroid metabolite in man evokes a pyrogenic response typifying, except for variations in intensity, the febrile reaction induced by all structurally related fever-producing steroids. Four to eight hours, on the average, after injection of etiocholanolone a pronounced, rapid increase in temperature begins, reaching a peak approximately twelve hours after injection and usually subsiding within twenty-four hours. Temperature elevation is dependent on dose and may exceed 4°C at the height of the response. Repeated injections induce consistent temperature elevations of the same magnitude; following large single injections, small secondary rises may occur for two to three days. The febrile responses of men and women are comparable. Local inflammatory reactions frequently occur at the site of injection and their severity is generally proportional to dose. However, in many subjects there is considerable disparity in time between the peak of fever and the height of the inflammatory reaction, the latter occurring or reaching its maximum intensity one or more days after the temperature has reverted to normal. A pronounced polymorphonuclear leucocytosis and a variety of constitutional symptoms including malaise, myalgia, and arthralgia, usually accompany the pyrogenic response. In contrast to progesterone, etiocholanolone does not induce fever when administered orally in amounts many times larger than those evoking intense fever when injected intramuscularly (284), and there is a high degree of species specificity to the pyrogenic action of this and related steroids (202, 285).

The 5β-H configuration (A:B ring junction *cis*) of etiocholanolone characterizes a variety of C19, C21, and C24 steroids derived from the biotransformation of cholesterol and adrenal and gonadal hormones, and a number of these steroid metabolites also have powerful fever-producing activity in man. These pyrogenic steroids include the C19 compounds, 17β-hydroxyetiocholane-3-one (336), etiocholanedione (203), 11β-hydroxyetiocholanolone (203), as well as etiocholanolone; the C21 steroids, pregnanolone (205), pregnandiol and 11-keto-pregnanolone (205); and the C24 steroids, lithocholic acid, its physiological conjugate, glycolithocholic acid, and its synthetic 3-acetate and 24-methylether derivatives (282).

The taurine conjugate of lithocholic acid has intense inflammatory properties without significant fever-producing action, and the bile acids, ursodeoxycholic and hyodeoxycholic acids, have variable but slight thermogenic activity in man (282).

A number of structural transformations or chemical substitutions known to occur *in vivo* significantly influence the fever-producing activity of steroids (203). Of these the influence of isomerization at C3 and C5, and of synthetic and physiological conjugation on pyrogenicity are of special interest. As noted above, synthetic as well as physiological conjugates of the bile acid pyrogen, lithocholic acid, retain powerful inflammatory and thermogenic properties. In contrast, synthetic esters and physiological conjugates of neutral steroid pyrogens, including such compounds as etiocholanolone and pregnanolone acetates, etiocholanolone sulfate, and the glucosiduronate derivatives of etiocholanolone and 11-ketopregnanolone, are without significant fever-producing action in man (203, 284). The reasons for these differences are not clear, but these findings emphasize that conjugation processes *per se* do not always inactivate steroid hormones or their derivatives. In earlier studies on the influence of C3 and C5 isomerization on pyrogenic activity, it was noted that the 3β-OH, C5 α-H derivative of pregnanolone (3β-hydroxyallopregnane-17-one) and the related C3 and C5 stereoisomers of etiocholanolone (3β-etiocholanolone, *iso*androsterone, androsterone) failed to produce fever when single injections of large amounts of steroid were made in man (200). Subsequent study has shown that the 5α-H steroid, androsterone, injected repeatedly induced small to moderate increases in temperature in some subjects (156). Recently, under comparable circumstances irregular temperature increases have been observed in this laboratory following intramuscular injection of the synthetic 5α-H steroid, SH567 (Schering: 1-methyl-Δ'-androstene-17β-ol-3-one-17-acetate) (221). The 5α-H steroid, stanolone (17β-hydroxyandrostane-3-one), has also been reported to produce occasional small temperature elevations in man (206, 290) though the great majority of studies indicate that 5α-H steroids of natural or synthetic origin lack significant pyrogenicity (28, 36, 118, 337–341, 380, 412).

The mechanism by which steroid pyrogens act to provoke fever in man is not known. A comparison of the biological properties of bacterial endotoxins and steroid pyrogens has indicated a number of major differences, suggesting that the modes of action of these two classes of fever-producing agents are different (196, 197). The role of inflammation in thermogenesis induced by steroids remains uncertain, but for a number of reasons noted earlier the inflammatory and pyrogenic activities of these compounds may represent independent biological properties (205). The recent demonstration of dissociation between these activities in taurolithocholic acid (281, 282) strongly supports this view and indicates that nonspecific inflammation *per se* does not explain the mechanism of fever. However, highly specific interaction between pyrogenic steroids and tissue components or local products of inflammation may play an important role in the mechanism of hyperpyrexia. For example, recent studies from this laboratory (284) have shown marked inhibition of fever following simultaneous local injection of steroid

pyrogen and small amounts of the antipyretic, anti-inflammatory hormone, cortisol. Much larger amounts of cortisol or cortisone, administered orally or injected at distant sites at the time of steroid pyrogen administration, may fail to influence fever production, as noted previously (199). Direct central effects, however, cannot be excluded, and this mode of action would be consistent with other well-known central activities of steroids (97). Moreover, access to sites of action in the central nervous system by these compounds is evident from studies indicating passage of radioactive steroid pyrogens across the blood-brain barrier into the cerebrospinal fluid (284). Finally, whether or not the substance active centrally is in fact injected steroid or a further transformation product is not known. The high recovery of injected etiocholanolone in earlier metabolic studies suggested that this metabolite, and presumably other steroid pyrogens, were not significantly transformed further *in vivo* (200). Recently, however, further metabolic alteration of etiocholanolone has been observed in man (108), and the possibility that pyrogenic activity resides in transformation products of the injected steroid metabolites cannot be excluded.

The endogenous origin of these powerful thermogenic agents has stimulated interest in their possible participation in the mechanism of fever observed clinically (18, 85, 201)—an interest which has found timely substantiation in the important investigations of Bondy and colleagues (32, 33, 58) on the disorders collectively termed periodic disease, or periodic fever (12, 305). These studies demonstrated the presence, during febrile episodes, of unconjugated steroid pyrogen (etiocholanolone) in plasma of patients with one form of the disease and its disappearance during afebrile periods of the disorder. Comparable levels of free steroid pyrogen were not observed in other febrile diseases; hence, this steroidal abnormality, which appears to be specific for this variant of periodic disease, may be etiologically related to the occurrence of the febrile episodes. Steroid pyrogen participation in the mechanism of fever in other clinical disorders has been postulated, though not established with certainty (44, 115, 385). The large number of steroid pyrogens already described, together with the unavailability of techniques for isolating and quantitating trace amounts of most of these substances in biological fluids, makes study of their relation to clinical fever difficult at present. This would appear, however, to represent a fruitful area of future research on the pathogenesis of fever in man.

5. HEMOLYTIC AND CYTOTOXIC PROPERTIES OF STEROIDS

The lytic effects of steroid hormones and related compounds on lymphocytes have been reviewed previously (78), but their actions on other types of cells have received less attention and are summarized in this section.

The hemolytic effect of bile was first recognized in 1813, and during the 19th century it became evident that the bile salts themselves, among other components of bile, were potent cytolytic agents. A comprehensive review of the literature up to 1937 dealing with these cytolytic actions can be found in Horrall's book on the toxicity and relation of bile to disease (172). While these cytolytic effects extend to a number of cell types, including leucocytes, spermatozoa, and protozoa,

the mechanism of this action has generally been studied using erythrocyte hemolysis as a convenient experimental model. Conclusions drawn from many early as well as more recent studies dealing with this phenomenon must be regarded with caution, however, because of uncertainties attributable to trace contamination of bile acids with related steroids, *etc.* For example, for a number of years it was not clear whether cholesterol enhanced or inhibited erythrocyte hemolysis *in vitro* (318).

Recent work dealing with the relation of chemical structure to the hemolytic action of bile acids and sterols stems largely from the classic paper by Berliner and Schoenheimer in 1938 (24), in which cholesterol, purified by repeated recrystallization, was shown conclusively to inhibit the hemolytic action of bile acids. The hemolytic activity of these steroid acids was found to depend mainly on the spatial configuration of the substituents at carbons 3 and 5. In general, 3α-OH,5β-H acids were strongly hemolytic; 3β-OH,5β-H acids were only moderately active, and 3β-OH,5α-H or Δ^{5-6} compounds were inert in this respect. Thus, lithocholic, deoxycholic, and hyodeoxycholic acids were potent hemolytic agents while cholic, dehydrocholic, and dehydrodeoxycholic acids were weaker. No sterols were found to have hemolytic properties. Inhibition of bile acid hemolysis occurred with bile acids and sterols whose steric configuration was opposite to that of the hemolytic compounds, *i.e.*, which had 3β-OH,5α-H, or Δ^{5-6} structures. Thus, cholesterol, dihydrocholesterol, and 3β-hydroxyallocholanic and 3β-hydroxy-Δ^{5-6}-cholenic acids all inhibited erythrocyte hemolysis induced by lithocholic acid.

Further aspects of the structural basis for cytolytic action have been examined in other studies. Chenodeoxycholic acid (3α,7α-dihydroxy) has been shown to be a more potent hemolysin than ursodeoxycholic acid (3α,7β-dihydroxy) (181), and surprisingly, 3β-hyodeoxycholic acid has been found to be more hemolytic than 3α-hyodeoxycholic acid (209). In another study, deoxycholic and apocholic acids were strongly hemolytic, and glycocholic, cholic, and taurocholic acids were less so (142), though Rothlin and Schalch (317) found the last mentioned acid to be an extremely potent hemolysin. This result seems to have been due to impurities, however, since Pethica and Schulman (295), using chromatographically purified taurocholic acid, were unable to demonstrate hemolytic activity with concentrations of steroid several times larger than those used by Rothlin and Schalch. Oxidation of cholic and deoxycholic acids to dehydrocholic acid and dehydrodeoxycholic acids, respectively, results in a decrease in hemolytic activity (142).

Sterol inhibition of hemolysis produced by bile acids and other hemolytic agents has been examined in a number of studies and, in general, the conclusions of Berliner and Schoenheimer have been confirmed. Cholesterol is a potent inhibitor of hemolysis induced by bile acids, digitonin, and streptolysin O (24, 173, 299, 318). Inhibition of digitonin hemolysis is favored by 3β-OH,5α-H compounds such as cholestanol; conversely, epicoprostanol (3α,5β-H) is without inhibitory activity. Similarly, cholesterol and cholestanol (3β,5α-H) strongly inhibit erythrocyte hemolysis by streptolysin O; coprostanol (3β,5β-H) is less effective,

and epicholesterol (3α), epicoprostanol (3α,5β-H), and androsterone (3α,5α-H) have no inhibitory activity. Interestingly, the 3α-configuration appears to be more effective than the 3β- in protecting against saponin-induced hemolysis; however, the greater inhibitory effects of 5α-H compounds over 5β-H are similar to those observed with other hemolytic agents.

Curiously, there has been little study of the possible hemolytic effects of neutral steroids, possibly because investigations with these compounds are complicated by solubility problems and by the inhibitory as well as hemolytic action of solvents used in such experiments (300). Sporadic clinical observations, however, indicate that neutral steroids may be potent hemolytic agents. For example, Segaloff (334) has reported that the water-soluble compound, testosterone 17β-diethylamino-ethyl-carbonate·HCl, induced severe hemoglobinuria following its intravenous injection in one patient. Although no experimental details were given, subsequent studies *in vivo* and *in vitro* with this compound and sodium testosterone sulfate indicated that both were extremely potent hemolytic agents. Antoniades *et al.* (11) have also reported hemolysis in one subject induced by intravenous injection of 100 mg of crystalline estrone dissolved in 35 ml of propylene glycol. No hemolysis was noted subsequently when the same amount of estrogen was dissolved in 35 ml of propylene glycol and 80 ml of 5% serum albumin. A systematic examination of steroid and stilbestrol hemolysis has been made by Tateno and Kilbourne (378), and stilbestrol and progesterone were shown to have strong hemolytic properties. Desoxycorticosterone acetate was moderately hemolytic, whereas testosterone, estradiol, and compounds A, B, E, and F were practically inert. Using stilbestrol hemolysis as an experimental model, this phenomenon was shown to be inhibited by calcium ions and serum, and enhanced by a decrease in pH or an increase in temperature.

The hemolytic properties of a number of steroids and bile acids have been examined in this laboratory, utilizing a 20% methanol solvent system to ensure steroid solubility (284). Among the bile acids, the potent pyrogen, lithocholic acid-3-acetate, was the most active hemolytic substance. Lithocholic, glycolithocholic, taurolithocholic, and deoxycholic acids all had lesser and generally comparable activity; chenodeoxycholic acid was considerably less active, and cholic acid was essentially inert. Among the neutral steroids, progesterone was the most potent hemolysin, with an activity comparable to that of chenodeoxycholic acid; lesser hemolytic effects were induced by etiocholanolone, androsterone, 11-ketopregnanolone, and testosterone, in that order.

The mechanism of steroid-induced hemolysis is poorly understood. There is general agreement, however, that the stability of the red cell membrane is of fundamental importance, and that prelytic loss of potassium and decreased osmotic resistance result in some way from interference with this stability (299, 300). Solubilization of red cell membrane cholesterol has attracted attention as one mode of action because of the well-known lipid- and cholesterol-solubilizing effects of bile salts (87). Ruyssen (324) has reported that in preparations of red blood cells incubated with C^{14}-cholesterol, labeled sterol exchanged with the cholesterol of the erythrocyte membrane and was not removed by saline washes.

When such cells were incubated with deoxycholate, labeled cholesterol was detected in the supernatant in amounts proportional to the degree of hemolysis. However, cholesterol also appeared in the supernatant in the same manner when erythrocytes were lysed by hypotonic saline; with butanol, a great deal of cholesterol was solubilized before appreciable hemolysis developed; with sodium dodecyl sulfate, solubilization began only after 25 % hemolysis had occurred; and with digitonin, hemolysis proceeded without any loss of cholesterol into the supernatant—as expected. Thus, direct solubilization of cholesterol by these compounds did not seem to be a causative factor in the hemolytic reaction, but rather a concomitant phenomenon paralleling it in a general way.

Hemolysis has also been related to the ability of bile acids to lower surface tension and penetrate cholesterol films, although the correlation of these effects is not entirely satisfactory (317, 324, 363). Studies with labeled bile acids indicate, however, that a certain number of molecules of the lytic substance must be bound to red cells prior to lysis (65), and it is possible that specific molecular interactions (as suggested by the structural relationships discussed earlier) may result in local changes in surface tension, affecting in turn the physical characteristics of lipoprotein molecules and consequently membrane stability.

Mediation of steroid hemolysis through "metabolic" effects of these substances has also been postulated, since bile acids depress O_2 consumption of tissues (86, 144), and glucose has been reported to inhibit the hemolytic action of bile acids (299, 324). The nature of these "metabolic effects" is obscure, but the absence of increased methemoglobin levels during etiocholanolone-induced hemolysis *in vitro* (284) suggests that oxidative mechanisms are not involved. It should be noted that bile acids and neutral steroids may produce hemolysis by different mechanisms, since under identical conditions, cholesterol inhibits bile acid but not neutral steroid hemolysis, and albumin is much more effective in inhibiting hemolysis produced by bile acids (284).

In considering the roles of surface tension and metabolic effects in steroid-induced hemolysis, studies on the bile solubility of pneumococci may be of great importance. An exhaustive review of this problem has been published previously (363). Pneumococci are solubilized (lysed) with variable ease by bile acids (262). Deoxycholic is the most active solubilizing steroid acid (79), cholic acid is moderately active, and dehydrocholic and dehydrodeoxycholic acids are inactive. Downie *et al.* (79) suggested that the bacterial lysis resulted from effects of bile acids on intracellular enzymatic processes since heat-killed pneumococci were not solubilized. When lysed cultures of pneumococci were added to heat-killed bacteria, solubilization readily occurred, presumably due to enzymes added from the lysed cultures. Furthermore, certain strains of pneumococci grown in bile media became quite resistant to the solubilizing action of bile—a phenomenon suggesting the possibility of enzymatic adaptation (79). However, in the review of Stacey and Webb (363), the conclusion was drawn that factors other than enzymatic ones were probably involved in bacterial lysis by steroids, since pneumococci were readily lysed by deoxycholate at 0°C, a temperature at which enzymatic processes would be expected to be retarded. Moreover, it was noted

that in contrast to pneumococci, heat-killed *H. influenzae* retained their bile solubility. Therefore, if bacterial lysis represents a reasonable facsimile of steroid-induced erythrocyte hemolysis, surface tension effects, influences on intracellular enzymatic processes, or both may be responsible for cytolysis in different experimental contexts.

In an interesting study of the effect of steroids on marine eggs, Leonard and Rutstein (224) showed that progesterone and desoxycorticosterone acetate produced characteristic changes in the cells, consisting of cytoplasmic budding, separation of the cell membrane, and lysis with ghost formation. Testosterone produced a unique type of budding, but no raised membranes or ghosts (lysis) were seen. Compounds E and F produced no significant changes and actually seemed to protect the cells. The parallelism of these cellular effects with the order of hemolytic activity reported by Tateno and Kilbourne (378) is remarkable.

The deleterious effects of steroid acids on the epithelial cells of the gallbladder, stomach, and intestine are well known (172). Concentrated whole bile and bile salts injected into the gallbladder of dogs through the common duct produce varying degrees of inflammatory changes in the epithelial mucosa of this organ (8, 416), often followed by bile peritonitis and death. Cytolytic effects of bile on the gastric and duodenal mucosae have also been noted (137). Deoxycholic acid was the most toxic bile acid for dog gallbladder mucosa in the study of Andrews and Aronsohn (8); on the other hand, the cholecystitis and cholelithosis induced by feeding dihydrocholesterol to rabbits (25, 253) have been inhibited by deoxycholic acid as well as by cholic acid. Choluresis induced by these bile acids probably played an important role in this inhibition, although differences in species susceptibility or experimental technique may also have been involved. The cytolytic activities of deoxycholate, and to a lesser extent of cholate, on rat intestinal mucosa have been described recently (71, 302); the important inhibitory effects of conjugation on the activity of these compounds have been emphasized by Dawson and Isselbacher (71), who found taurodeoxycholate, taurocholate, and glycocholate to be nontoxic. On the other hand, Palmer *et al.* (281, 282) have reported that both lithocholic acid and its conjugates have pronounced inflammatory effects in man, an action undoubtedly reflecting the cytotoxic activity of these compounds, thus demonstrating that conjugation does not necessarily inhibit these effects of bile acids.

The possible relation of the cytolytic actions of steroids to clinical disorders in man is not known, but it is not unlikely that red cell hemolysis in particular may be influenced at any given time by the total balance of antihemolytic and hemolytic factors, including neutral steroids and bile acids, in plasma. The studies of Zieve and associates (418, 419) are especially interesting in this regard. These authors have described a special type of hemolytic anemia associated with hyperlipemia and liver disease, which differs from the anemia of hypersplenism. Patients with hyperlipemic anemia generally have mild liver disease with prominent fatty infiltration, and hepatomegaly without splenomegaly. Symptoms include upper abdominal pain, anorexia, jaundice, nausea, malaise, and low grade fever; during the hemolytic phase of the disease, the osmotic fragility of red

blood cells is increased. In contrast to the anemia of advanced cirrhosis and hypersplenism, hyperlipemic anemia tends to be brief and improves rapidly with treatment of the hepatic disorder. Zieve has suggested that abnormal lipids, such as lysolecithin, may be responsible for hemolysis in this syndrome. Previous studies, however, have shown that the serum bile acids are elevated in liver disease and that the ratio of dihydroxy to trihydroxy acids is altered, with a relative increase in the more actively hemolytic dihydroxy fraction (46, 276, 321, 353, 358). Moreover, certain clinical features of this hyperlipemic disorder, such as anorexia, malaise, abdominal pain, and, particularly, fever, are reminiscent of those observed in periodic disease (305), and of the reactions produced by injection of pyrogenic steroids and bile acids in man (200, 203, 205, 282). For these reasons, detailed analysis of plasma steroids and bile acids of the types described in this report may be extremely informative in elucidating the pathogenesis of hyperlipemic anemia.

Another abnormality of erythrocytes gives some indication of involving abnormal steroids or steroid metabolism. Acanthrocytosis, a genetically determined disorder characterized by neurological disturbances, retinitis pigmentosa, and malformed erythrocytes, was first described by Bassen and Kornzweig in 1950 (17), and other cases have since been reported (80, 185, 215, 249, 357). The original description of the red blood cells in this disease stated in part that "they resembled spherocytes from which buds or pseudopods were protruding" (17)—cellular changes strikingly similar to those described by Leonard and Rutstein (224) in their studies of the effects of steroids on marine eggs. Other aspects of this syndrome pertinent to this discussion include markedly diminished serum cholesterol levels (40 to 60 mg%), frequent diarrhea and steatorrhea, menstrual irregularities and hirsutism, shortened red cell survival time and, in one case, a greatly increased urinary copper excretion in the absence of proteinuria or aminoaciduria (249). To our knowledge, the last mentioned abnormality has not been reported in any other condition except Wilson's disease, and is of particular interest in view of the cupruric effects of certain steroids to be described in a later section of this review. The erythrocyte abnormalities have been shown by Switzers and Eder (374) to be reversed by non-ionic detergents *in vitro*; ionic detergents caused normal erythrocytes to pass through a stage, indistinguishable from acanthrocytotic cells, in the course of hemolysis. Ponder (299) has indicated that this stage in the hemolysis produced by a number of lysins can be reversed by cholesterol and other substances. Bile acids also reverse this phase temporarily, apparently by displacing the lytic molecules attached to the red cell membrane, but the changes recur during the ensuing hemolytic process induced by the bile acids themselves. Thus, a number of clinical features, biochemical abnormalities, and morphological alterations of erythrocytes characteristic of acanthrocytosis suggest the possibility that abnormal steroids or disordered steroid metabolism may be importantly involved in this syndrome.

The studies summarized here on the inflammatory effects of bile acids and neutral steroids correlate well with those on the hemolytic properties of these compounds, thus permitting certain generalizations to be made concerning the

relation of structure to cytotoxicity. Cellular toxicity seems to be especially characteristic of steroids having a 3α-OH and a 5β-H configuration—structural features common to the potent fever-producing steroids as well. Cytotoxicity is diminished by epimerization of the C3 hydroxyl group, by its oxidation to a ketone, or by alteration of the nature of the A:B ring junction. In the bile acid series, introduction of an additional hydroxyl group in the steroid nucleus diminishes toxicity; in this respect a 7α-OH may be more effective than a 12α-OH, while the influence of equatorially oriented hydroxyl groups, such as 6α and 7β, is less pronounced. In the neutral series of steroids, a 17α-OH markedly suppresses toxicity; 11β-OH and 11-ketone substitutions are less effective in suppressing pyrogenic or inflammatory properties; and 11β- and 17α-hydroxyls together impart protective activity. The influence of conjugation varies with the type of steroid and conjugating substance concerned, so that no generalizations can be made.

While the structural aspects of cytotoxicity are of interest, one of the particularly important implications of these studies is that certain steroids, especially bile acids, commonly found in the gastrointestinal tract may have profound effects on mucosal morphology, function and, indeed, viability. The nature of these steroids is dependent in part on species-specific patterns of steroid metabolism, but the role of the bacterial flora in determining the qualitative steroidal composition is of the greatest importance. In man, for example, the two most cytotoxic steroids, lithocholic and deoxycholic acids, are both probably formed to a large extent as a result of bacterial transformation of less toxic bile acids, and local effects of these cytotoxic compounds may seriously impair intestinal activity and structure. Thus, the possibility that the toxic properties of the steroids reviewed in this section may find clinical expression can be seriously entertained, and study of the possible relation of these compounds to various hemolytic or intestinal disorders may prove to be highly rewarding.

6. STEROID-INDUCED CIRRHOSIS OF THE LIVER

Although the effects of steroids on the liver might properly be described in the section dealing with cytotoxicity of these compounds, the subject merits special attention because of the clinical implications of recent work, especially that of Holsti and collaborators (31, 166–170). The recently described jaundice-producing effects of methylated steroids are well known. Steroid-induced liver injury, however, received some mention in relation to early work on the choluretic action of dehydrocholic acid; Horrall (172) has referred to several descriptions of parenchymatous liver damage and necrosis in animals which received large amounts of this compound. The subject in general lay dormant, however, until interest in the pathogenesis of atherosclerosis stimulated a number of investigations on tissue responses to cholesterol and its derivatives. Among these investigations, several demonstrated hepatotoxicity of certain steroids. Altschul and Spencer (4) noted such toxicity with 7-ketocholesterol, 7-hydroxycholesterol, and cholestenone in several species. In rabbits, administration of these compounds induced thinning of liver cords, progressing to frank cirrhosis within periods of

four to eight weeks. Bile duct proliferation was marked; connective tissue overgrowth was less prominent, and fatty degeneration and necrosis were minimal. Guinea pigs reacted with foci of necrosis without frank cirrhosis; in rats and hamsters, the hepatic changes consisted solely of thinning of liver cords without cirrhosis or infarction. Changes produced by all three oxidation products of cholesterol were similar, although those produced by 7-ketocholesterol were most marked.

Cholestenone has also been reported to impair bromosulphophthalein (BSP) excretion in man (365), but it is not clear whether this reflects actual hepatic damage or the relatively specific interference with BSP uptake induced by bile acids and other detergents (9). The possibility that the hepatotoxic effects of the sterols, cholestenone, 7-ketocholesterol, and 7-hydroxycholesterol, may be mediated through bioconversion products (bile acids) should be entertained, since studies on the structural basis of steroid hemolysis and cytolysis would suggest that sterols themselves are not particularly harmful to cells, although cholesterol and its esters do have some inflammatory properties (361).

In this connection, the studies of Holsti (31, 166–171), in Finland, represent important contributions to the field of experimental cirrhosis. In a detailed series of investigations, Holsti and collaborators have examined the phenomenon of liver cirrhosis which occurred in rabbits fed desiccated bile during the course of other experiments. In these animals, liver injury was noted within several days, and, depending on dose and experimental technique, pronounced hepatic changes appeared in one week to three months. These changes consisted first of increased lobulation with degeneration of liver cells at the periphery of the lobule and often, but not always, necrosis of groups of liver cells. Connective tissue appeared first between the cells at the periphery and then became prominent in portal areas. In advanced stages, fibrous septa extended to the centers of the lobules, separating groups of liver cells into smaller nodules. Lymphocytes, plasma cells, and fibroblasts became more noticeable as the connective tissue increased. Central fibrosis was not common; as a rule, cirrhosis was not preceded by fatty infiltration. The peripheral fibrosis and the absence of fatty infiltration during development of the cirrhosis distinguished these hepatic changes from those seen in nutritional deficiency. Further study of the factors responsible for these effects indicated that hog bile was active, whereas cow bile was not; furthermore, an alcoholic extract of hog bile, consisting mainly of bile acids, was as effective as whole bile (168). In 1958, Holsti reported (167) that bile acids in very high concentration could produce slight liver changes; subsequently, the monohydroxycholanic acid, lithocholic acid, was shown to be highly effective in inducing experimental cirrhosis in rabbits (168). Large amounts (300 mg/day), suspended in water and administered by gastric tube, produced rapid, severe liver damage, and most animals died within three weeks. Smaller doses (50 mg/day) produced severe cirrhosis of the liver, accompanied by jaundice and ascites, in approximately three months. A number of other bile acids (cholic, taurocholic, hyodeoxycholic, 3α-hydroxy-6-ketocholanic, 3α-hydroxy-6-ketoallocholanic, and dehydrocholic) were investigated in amounts up to 800

mg/day and found to be inactive. Deoxycholate, 300 mg/day, produced some liver necrosis and slight fibrosis but no frank cirrhosis, while larger amounts were lethal.

Recently, Holsti has reported (170) that conjugation of lithocholic acid with glycine does not abolish its cirrhogenic effect, and that chenodeoxycholic acid is as potent as lithocholic acid in producing cirrhosis. These important observations of Holsti have been confirmed by Stolk (367), who was able to produce cirrhosis of the liver in the iguana with small doses (15 mg/day) of lithocholic acid administered orally over a period of three months. Higher doses caused extensive liver damage and death. Since cirrhosis of the liver can be more easily induced in rabbits than in many other species, these confirmatory studies of Stolk, in a reptile, emphasize the general significance of the bile acid cirrhosis phenomenon of Holsti. Sugihara (373) has also noted degenerative changes in the livers of rabbits fed bile acids; these changes, more marked for deoxycholic than for cholic acid, may have been similar to those reported by Holsti.

The pyrogenic and inflammatory actions of lithocholic acid in man (281, 282), together with the structural similarity of this compound to neutral steroid pyrogens (203), suggested that the latter compounds may have powerful cirrhosis-producing action as well. Preliminary studies in this laboratory have confirmed this suggestion, and indicate that the neutral steroid pyrogen, etiocholanolone, like lithocholic acid, evokes cirrhotic lesions in rabbits (284); it is entirely possible that other steroid pyrogens may act similarly. Moreover, production of cirrhosis by the physiological conjugate, glycolithocholic acid, confirms in a different experimental context the observations of Palmer *et al.* (282) that in the bile acid series of compounds, at least, conjugation processes do not protect against either the inflammatory or the pyrogenic activity of free steroid acids.

The earlier suggestion (see above) that sterol-induced liver damage might be mediated through bile acid metabolites has special relevance to the interesting observation by Holsti that chenodeoxycholic acid is as effective as lithocholic acid in producing cirrhosis in rabbits. In the rat, Norman and Sjövall (272) have shown that only a small amount of labeled chenodeoxycholic acid introduced into the cecum remained unchanged, and that more than 50% was converted to lithocholic acid. More importantly, Hellström and Sjövall have shown that in the rabbit, chenodeoxycholic acid is mainly converted to lithocholic acid (158). Hence, if the cirrhogenic activity of chenodeoxycholic acid is mediated through lithocholic acid and its derivatives, the hepatotoxic effects of bile acids are in complete accord with their hemolytic cytotoxic, and pyrogenic activities, as reviewed in previous sections.

Holsti's investigations have important implications for the pathogenesis of human cirrhosis, although species differences must be kept continually in mind. It is of interest that there are many reports indicating that quantitative and qualitative changes in serum bile acids in human cirrhosis are in the direction of increased amounts of the more cytotoxic dihydroxy bile acid fraction (46, 276, 321, 353). Chenodeoxycholic acid constitutes the principal, if not the only, component of this fraction in man (45), in whom it is also metabolized to lithocholic

acid (159). While the presence of the latter steroid in the blood has not been investigated carefully, its occurrence in significant amounts in the bile and feces of normal subjects (155, 159, 254, 312) indicates that its eventual detection in plasma, and in the portal circulation in particular, may be anticipated.

Thus, alterations in the normal degradation of cholesterol to bile acids, or the influences of intestinal bacteria on transformations of steroids, especially with respect to conversion of chenodeoxycholic to lithocholic acid, may have important pathological consequences. Apart from the possible role these toxic steroids may play in initiating liver injury in man, the qualitative and quantitative changes in plasma bile acids which accompany hepatic damage may, as suggested by Holsti, in themselves be involved in the progression of the disease in a self-perpetuating fashion.

7. ANTIBACTERIAL ACTIVITY OF STEROIDS

Interactions between steroids and microorganisms are reflected in steroidal transformations by these organisms (89, 376) and by the profound influence which steroids exert on the growth of microbiological agents. This influence may be stimulatory or suppressive in nature, but the latter, or antibacterial effect, of steroids will be emphasized in this section of the review.

The occurrence of steroids in certain microorganisms is well known although their physiological significance is obscure. In some cases, however, absolute nutritional requirements for these compounds have been established (62). There are numerous examples of the growth-promoting action of steroids under aerobic and anaerobic conditions for various microorganisms and for certain varieties of insects and crustacea as well (7, 40, 59, 62, 63, 186, 188, 227, 306, 359, 395, 396). Growth-promoting steroids of this type include ergosterol (7), cholesterol, Δ^4-cholestenone, β-sitosterol, fucasterol (63, 395, 396), stigmasterol (61), brassicasterol (62), dehydroisoandrosterone (109), estrone, estradiol, Δ^4-androstenedione, testosterone, androsterone (40), and many other structurally related compounds (40, 62). Comprehensive papers describing the growth-promoting effects of steroids on microorganisms as well as the relationships between steroid molecular structure and these effects have been published (7, 40, 62, 359, 396) and will not be summarized here. It is useful to emphasize, however, that steroid requirements of different microorganisms vary widely, and that the profound influences which minor structural alterations of steroids have on bacterial cell growth relate strictly to the test organism and experimental circumstances described; these considerations make generalizations hazardous. The same consideration probably applies also to studies on the mechanism of such growth stimulation. Thus, although *P. testosterone* may utilize steroids as the sole carbon source in nutrient media and metabolize these compounds actively (376), another organism, *E. gracilis* var. *bacillaris*, though stimulated to increased growth by many steroids, fails to transform them in any substantial way (40). A "catalytic" role for steroids in the mechanism of growth promotion in the latter instance has been postulated. Other suggested mechanisms of steroid action include mediation of growth stimulation through influences on processes concerned with generation

of high energy phosphate bonds (59) or through the as yet unconfirmed PGA-like activity of dehydroisoandrosterone (109). In a detailed investigation on testosterone stimulation of growth of *E. gracilis*, Buetow and Levedahl (40) have also suggested that the steroid effect is primarily on the permeability of cells to nitrogen-containing compounds, or perhaps on enzyme systems involved in the synthesis of proteins from simple nitrogen-containing sources.

Antibacterial effects of steroids represent a major aspect of the interaction between these compounds and microorganisms and there is considerable evidence indicating that, *in vitro*, a large number of steroids have profound inhibitory effects on the growth of a variety of bacteria, yeasts and molds. Bile and its component steroid acids have significant antibacterial properties (172, 363) which are reflected in part in their incorporation in culture media for the selective isolation of enteric organisms (326). Additionally, bile acids have an influence on the severity of experimental infections, and have both inhibitory and enhancing effects on the action of certain antibiotics on microorganisms. The paper of Stacey and Webb (363) provides an excellent review of much of the work dealing with effects of bile salts on bacteria, and discusses the bacteriostatic properties of bile acids and derivatives with particular reference to their relative activities in solubilizing the gram-positive complex from yeast. It had been thought earlier that the solvent action of bile salts on the magnesium nucleoproteins which constitute this complex was related to the antibacterial potency of these substances, but no correlation between these properties of the steroids was observed. However, bacteriostatic action of these compounds on growth of *Staphylococcus aureus* was related to their surface-acting effects on the nutrient medium—a relationship which held only within this class of substances of closely related molecular structure—and, as described in the section on hemolysis, does not represent a general mechanism of bacterial or other cell lysis.

The means by which bile acids effect their antibacterial actions are unknown, as is the basis for the special resistance of gram-negative bacteria to this property of the compounds. Association of this resistance with increased virulence in *Salmonella typhimurium* strains and *Salmonella paratyphi B* has been noted, however (381), and under certain circumstances these steroid acids may convert ordinarily innocuous infections with gram-positive organisms into lethal ones (5, 333). Paradoxical effects of bile acids on the antimicrobial action of conventional antibiotic agents have been noted. Bile salts enhance the susceptibility of coagulase-positive, hemolytic staphylococci and of enterococci to the antibiotic action of neomycin (331). They also enhance the activity of penicillin against penicillin-resistant staphylococci but have no effect on the potency of this antibiotic against penicillin-sensitive strains of enterococci. Conversely, bile salts inhibit the actions of polymyxin, ristocetin, and vancomycin on both gram-positive species, and generally do not influence the response of a number of gram-negative organisms to the usual antibiotics employed against them (331). They also inhibit the antifungal action of nystatin, amphotericin B, chlorquinadol, and gentian violet to a variable degree, depending on the antimycotic agent (332). Mechanisms for these effects are not known, although it has been suggested that

they are mediated *via* the chemotherapeutic agents rather than through actions on culture media or organisms directly.

Hormonal steroids, their metabolites, and related compounds have also been shown to have substantial growth-inhibiting action on a wide variety of microorganisms *in vitro*. Several studies have demonstrated the bactericidal action of stilbestrol on a number of gram-positive bacteria, an activity which extends to the tubercle bacillus but not to gram-negative organisms (39, 95, 96). The structural basis of this action has been examined by Brownlee *et al.* (39), in a paper indicating that deoxydiethylstilbestrol and its dihydro derivative had the most potent antibacterial action among a number of stilbene compounds. In this investigation, the natural steroid estrogens were also found to have antibacterial properties although variable results with these compounds have been obtained in other studies. Thus, these estrogens produced no inhibition of growth of *T. piriformis* (60), stimulated growth of *P. puberulum* and *A. niger* (186, 227), and inhibited growth of *T. utilis* (187). Progesterone has shown bactericidal or bacteriostatic activity against a variety of microbial agents including *N. catarrhales*, *N. intracellulares*, and *N. gonorrhea* (213); *T. piriformis* (60); *A. niger* and *T. utilis* (186, 187); *N. crassa* (228); *P. puberulum* (227) and *S. fragilis* (243). Other hormonal steroids or derivatives shown to have antibacterial action against these or related microorganisms, include such compounds as cortisone, corticosterone, desoxycorticosterone, 17α-hydroxyprogesterone, testosterone, dihydrotestosterone, androstanedione, Δ^1-androstenedione, Δ^4-androstenedione, and etiocholanedione (60, 186, 187, 225–229, 243). Pronounced antifungal and antibacterial effects of a number of synthetic aminosteroids have also been described (216). These steroids or derivatives can also block the inhibitory action of antibiotics against certain microorganisms in a manner analogous to the action of bile acids (350); antibacterial effects of steroids can also be antagonized in certain systems by related compounds; thus, for example, the marked inhibiting effect of desoxycorticosterone on *P. puberulum* may be reversed by estradiol, an action extending to some but not all steroids which suppress growth of this organism (227). The possibility that similar interactions among steroids native to other microbial agents might serve to regulate growth processes in such organisms has been suggested (227). An amebicidal action of certain hormonal steroids has been noted (136) as has inhibition of early cell cleavage in embryonic development of sea urchin eggs. The latter action was presumed to result from suppression of DNA synthesis at the time it would ordinarily begin (3). The mechanism of desoxycorticosterone inhibition of *N. crassa* has been studied in some detail by Lester and Hechter (226). This steroid, which significantly inhibits growth of a variety of gram-positive microbial agents (225), interferes with permeability processes in *N. crassa* for uptake of essential nutrients, such as amino acids, sugars, and ions. Studies utilizing rubidium-uptake as an experimental model of ion transport suggested that desoxycorticosterone inhibition of uptake probably involved an effect on some binding event in the bacterial cell, postulated as representing a specific cytoplasmic system for rubidium binding. This inhibiting action might involve effects on dissociation of rubidium from its postulated

binding site, or the formation or alteration of the latter in some fashion, but the data did not permit a more definitive conclusion regarding this mechanism.

The steroid antimicrobial effects described above represent actions of these compounds *in vitro* for which there is little evidence of a counterpart *in vivo*. Estrone has shown some protective action for mice infected with Type 1 pneumococci (400), but other hormonal substances with powerful antibacterial activity *in vitro* failed to demonstrate this action in experimental animals (39, 400). An extensive earlier interest in the antifungal properties of gonadal hormones (53, 230, 231, 301, 306–308) probably originated in clinical preoccupation with the influence of pregnancy and menstruation on the incidence and severity of infectious diseases in man. There appears, however, to be little value in the use of steroids for this purpose, although the possible future development of clinically effective steroid antibacterial agents cannot be excluded.

The significance, if any, of these steroid-microorganism interactions for human pathophysiology is obscure, but they may play a role in bacterial homeostasis in the intestinal tract. In this way endogenous steroids, such as the bile acids, could, through stimulation or inhibition of different microorganisms, in turn influence bacterial transformation of these or related steroids, with resultant implications for the production of toxic compounds such as those described in the previous section.

8. HEMATOPOIETIC EFFECTS OF GONADAL HORMONES

This section of the review summarizes certain effects of gonadal hormones on erythropoiesis, the relevance of which to clinical medicine has been emphasized by recent studies from several laboratories. A number of excellent reviews dea ing with other aspects of the significant relation between endocrine secretions and the hemopoietic system, has been published in recent years (69, 132, 133, 394).

Hemoglobin levels of men tend to be significantly higher than those of women, and male blood donors are known to regenerate blood losses faster than female donors (104, 241). This sex difference is not observed until after puberty, is not due to menstrual blood loss, and grows less pronounced after the age of 60 (393, 413). Comparably higher hemoglobin levels are seen in the males of a number of species of mammals and birds (77, 329), and the evidence that these sex differences are mediated by gonadal hormones is unequivocal (72, 77, 99, 135, 366, 397).

Numerous studies have shown that estrogens and androgens have reciprocal influences on hematopoiesis, the former tending to depress, and the latter to stimulate erythroid organs. Prolonged administration of estrogens such as stilbestrol or estradiol to experimental animals leads to profound suppression of bone marrow function, aplastic anemia, and death (50, 64, 391). Dogs are highly sensitive to this action of estrogens; mice, rats and guinea pigs are less so, and monkeys are quite resistant (64, 391, 392). Estradiol is a more potent bone marrow depressant than synthetic estrogens (50). The usual pattern of toxic response to chronic administration of estradiol in susceptible animals is a transient hyperplasia of myeloid marrow elements, associated with a significant peripheral

neutrophilia which may become leukemoid in character. Subsequent depression in levels of peripheral leucocytes and red blood cells occurs, with the marrow characterized by scattered areas of focal hypoplasia. Thrombocytopenia is common terminally, and decreases in number, with abnormalities in morphology and staining characteristics, of megakaryocytes may be noted earlier. Decreases in the various blood cell elements may not parallel each other, and severe depression of erythrocytes may be seen during the initial transient leucocytosis (391). Neither testosterone nor liver feeding protects against this effect of estrogens (391).

It is not clear whether these compounds suppress erythroid tissue by the same mechanism in the several susceptible species. Prolonged estrogen treatment leads, through endosteal bone deposition, to partial or complete obliteration of the bone marrow space in several species, and this process alone could lead, ultimately, to anemia in these species (116, 296, 410). However, Jacobson has demonstrated that the partial replacement of bone marrow space by endosteal new bone formation may not necessarily result in any marked alteration in the peripheral blood picture (183), and in the case of dogs, which are highly sensitive to this action of estrogens, a direct toxic effect is likely (391). This possibility is supported by the studies of Dukes and Goldwasser (83, 84) who demonstrated that estradiol in physiological amounts depressed incorporation of Fe^{59} into red blood cells and inhibited erythroid stimulation induced by erythropoietin. Reciprocal influences of the two hormones on hemopoiesis could thus represent an important mechanism for regulating the levels of peripheral red blood cell elements in man (84).

The significant alterations in blood cell components which accompany cyclic activity of the ovaries in normal menstruating women (67, 119, 252, 291, 298) may be a physiological counterpart of these experimental observations, and it is likely that certain thrombopenic disorders represent pathological expressions of endogenous hormone actions (250, 406).

Androgens have effects opposite to those of estrogens on hemopoiesis; and their administration to man and experimental animals is regularly associated with increase in red cell elements peripherally and in marrow depots (2, 77, 123, 208, 237, 244, 366, 377, 397, 405). Much evidence of this effect, particularly in man, has accumulated from studies during the administration of natural and synthetic steroids for therapy of metastatic carcinoma of the breast in women. Such therapy almost invariably leads to small but significant increases in peripheral hematocrit values and early bone marrow changes consistent with enhanced activity of the erythroid system. These effects are usually manifest following administration of suitable doses of androgenic steroids, although significant erythroid response to much smaller amounts of androgen has been noted in anemia associated with endocrine disease (123).

Curiously, except for isolated reports such as those of Rosenthal and Erf (313) and Erf and Herbut (90) in the early 1940's, little interest seems to have been shown until recently in the use of androgens in the therapy of non-endocrine anemias in man. In the past several years, however, attention has been called,

by two groups in particular, to the impressive value of androgen administration in the treatment of certain intractable anemias. Shahidi and Diamond reported that testosterone induced a significant remission of aplastic anemias, of both the acquired and congenital types, in children (351, 352), and Gardner and Pringle (113, 114) also showed that testosterone and related androgenic steroids induced significant hemopoietic responses in adults with anemias associated with osteoporosis, hypogonadism and hemochromatosis, malignancy, drug-induced marrow depression, reticuloendothelioses, myeloid metaplasia, and other disorders. In most patients who responded to this therapy, androgenic steroids induced significant reticulocytosis, increases in peripheral red blood cells of all types, sometimes increases in leucocytes and thrombocytes, and repopulation of bone marrow, leading to diminution or disappearance of requirements for transfusions and other forms of supportive therapy. The mechanism of these androgen effects is uncertain, but their mediation in part through stimulation of renal erythropoietin production is a possibility which merits consideration in view of the pronounced renal hypertrophy which these compounds produce experimentally. The delayed onset of beneficial responses, as well as the large doses of steroid required to induce remissions, would be consistent with such a mode of action; preliminary observations on the kinetics of this androgen effect could be similarly interpreted (92, 260).

9. ESTROGENS AND COPPER METABOLISM

Estrogens alter significantly the blood levels of copper in man, an action which was recognized in the early studies of Krebs and others; reference to these investigations may be found in the paper by Locke *et al.* (236). There is a significant difference in blood levels of copper between women and men, with the latter having consistently lower values (47, 217, 236, 256, 257, 271). The likelihood of a hormonal basis for this difference is indicated by the pronounced increase in blood levels of copper observed during the course of pregnancy (48, 143, 218, 236, 240, 330, 383). Cyclic alterations in serum copper levels coincident with the menses are not observed consistently (236, 271, 325), however, although there is some difference of opinion in this regard (326). The copper elevations noted in pregnancy develop early, may reach levels considerably higher than normal, and decline rapidly in the postpartum period. It has been suggested that the blood copper rises to particularly high levels in pre-eclamptic toxemia, although there is no apparent relationship between the level attained and severity of the toxemia (383). Significant differences are found between maternal and fetal blood levels of copper at term (330), and during pregnancy erythrocyte copper concentrations do not rise concomitantly with serum levels (218). These findings are consistent with several studies indicating that serum copper elevations in pregnancy represent predominantly increases in the fraction bound to the copper-binding α-globulin, ceruloplasmin (143, 164, 165, 240, 330). The predominant role played by estrogenic steroids in mediating this increase in blood copper in pregnancy is indicated by the marked copper-elevating activity of estradiol in normal subjects as well as in patients with Wilson's disease (20, 120, 322, 323, 327).

During estrogen administration, "total" and "indirect" (ceruloplasmin bound) blood copper levels may reach values even higher than those observed in the last trimester of pregnancy (322), and, although copper responses in Wilson's disease are not consistent (323), enhanced cupruria may occur (120). The response to estrogens in Wilson's disease is of interest, since elevations in serum copper may occur in the absence of concomitant increases in the copper-binding protein (120). This type of response suggests that estrogens affect copper metabolism directly, as well as indirectly through nonspecific increased synthesis of copper-binding protein. Although estrogens may elevate serum copper and ceruloplasmin concentrations in some patients with Wilson's disease, to levels comparable to those seen in normal subjects, such treatment is not without hazards. Pronounced deleterious effects have been observed in several patients following this form of therapy, a response which may (120) or may not (323) be related to selective elevation of serum copper in the absence of associated increases in ceruloplasmin. The possibility that certain patients with Wilson's disease who have normal serum copper and ceruloplasmin values represent subjects with high endogenous estrogen levels, secondary to inadequate hepatic degradation of normally secreted hormone, is an intriguing one (327) and warrants further investigation. Study of the influence of other gonadal hormones on blood copper levels, especially confirmation of androgen-induced elevation of this trace metal (189), would also be of interest.

10. HYPOCHOLESTEROLEMIC ACTION OF ANDROSTERONE

Alterations in blood cholesterol levels follow the administration of several bile acids and steroid hormones in man; a newly recognized hypocholesterolemic agent of this general structural type has been identified in recent studies by Gallagher and associates (111, 156, 157). This compound, androsterone, is a C19 17-ketosteroid metabolite derived from the degradation *in vivo* of testosterone and related compounds such as dehydroisoandrosterone and Δ^4-androstenedione. Intramuscular injection of the 5α-H steroid in amounts of 50 mg/day for periods of three days to five weeks resulted in significant lowering of serum cholesterol in a variety of patients, although the effect was most striking and consistent in myxedematous subjects (156). Decreases were evident in free as well as in esterified fractions of cholesterol. Blood levels of the sterol returned to pretreatment values in nearly all patients following cessation of steroid therapy. Species specificity of this action was suggested by failure of androsterone to induce hypocholesterolemia in cholesterol-fed rabbits (111); it is of interest that species specificity of the pyrogenic activity of etiocholanolone, the C5-epimer of androsterone, has also been observed (285).

The hypocholesterolemic effect of androsterone has been confirmed in several laboratories (57, 76, 82), but it is not clear whether the steroid is active orally. Dingman and Jenkins (76) reported decreases in cholesterol of 12 to 22% in three androgen-deficient patients given androsterone orally in amounts of 100 to 200 mg/day, although the response to androsterone feeding in one subject was not reproducible. Similar decreases have been observed by Hellman *et al.* (157)

in patients receiving small amounts of androsterone and *p*-chlorophenoxyisobutyrate by mouth. Cohen *et al.* (57), however, failed to observe decreases in serum cholesterol in any of 13 male patients receiving androsterone orally in amounts of 100 mg/day for 30 days. Examination of this aspect of androsterone action is important, if only because of the incidence of local reactions accompanying intramuscular injection of the steroid (156).

Hypocholesterolemic effects of steroids structurally related to this 5α-H metabolite have been reported in other studies. Dihydrotestosterone, the 5α-H reduction product of testosterone, is potent in lowering cholesterol in man (76) and 3β-(β-dimethyl-aminoethoxy)-androst-5-ene-17-one like dehydroisoandrosterone (386), markedly inhibits cholesterol biosynthesis in rats (134). Although the latter compounds are not 5α-H steroids, their C5-6 double bonds result in planar A:B ring junctions resembling those of 5α-H steroids.

The implications of this effect of androsterone with respect to the pathogenesis of certain manifestations of thyroid deficiency, and the possible mechanism of action of the thyroid hormone in this disorder have been discussed in detail elsewhere (35, 111, 156). This action of androsterone represents, in addition, a further and major demonstration that hormonal metabolites possess important independent biological properties which may be quite distinct from those characterizing their precursor hormones.

11. PROGESTERONE STIMULATION OF RESPIRATION

It has been known since the beginning of the century that significant alterations in the pattern of respiration accompany pregnancy and the menstrual cycle (127–129, 302a, 390). In normal ovulating women these patterns are cyclic and reproducible, and are characterized by depression of alveolar pCO_2 during the luteal phase of the cycle, with lowest levels occurring at the time of menstruation. A rise in alveolar pCO_2 follows menstruation and is maintained until the subsequent luteal phase (127, 390). During pregnancy alveolar pCO_2 remains depressed, although in some subjects, periodic increases may occur at intervals corresponding to prepregnancy ovulatory cycles (129, 390). These periodic alterations probably reflect cyclic or sustained production of progesterone, since intramuscular administration of this hormone in man stimulates respiratory activity. The stimulation reaches its maximum approximately twelve hours after injection of the steroid (390), although depressions in alveolar pCO_2 may last considerably longer (128). Estrogen is without effect on respiratory activity, although combined administration of estrogen and progesterone produces more significant and prolonged depression of alveolar pCO_2 than does the latter hormone alone (128). Increased respiration is not a consequence of thermogenesis induced by progesterone (128); moreover, this respiratory action of progesterone is not related to its luteal activity, since synthetic agents such as anhydroxyprogesterone and 19-nor-ethinyl-testosterone, which have marked progestational action, do not stimulate ventilation (390). Respiratory stimulation with lowering of arterial pCO_2 following progesterone injection in patients with chronic pulmonary disease has been shown by Tyler (390); although the mechanism of this effect is uncertain,

failure of progesterone to alter the normal response of the respiratory center to CO_2 suggests that other areas of the brain, possibly the hypothalamus, may represent the site of the action of the hormone (390).

12. ANTIHYPERTENSIVE STEROIDS

Production of hypertension by adrenal steroids, administered in several experimental contexts, is a well-known phenomenon. Several studies have indicated, however, that steroids and steroid-like substances have antihypertensive properties as well. Among the latter compounds, 2,3-*bis*(*p*-hydroxyphenyl)valeronitrile and the corresponding propionitrile were shown by Sturtevant *et al.* (371) to have significant hypotensive action in normotensive and hypertensive animals. Decreased peripheral resistance and cardiac output both appeared to be involved in this action. A number of steroids, structurally related to androgens and estrogens, also have depressor activity in hypertension produced in rats by combinations of DCA, salt feeding, and nephrectomy. Among the C19 antihypertensive steroids, 11β-OH androstenedione is most potent, testosterone and 11-ketotestosterone are less so, androstenedione and androenosterone have slight activity, and 11β-OH testosterone is inert (368). Replacement of the 11β-OH function by a ketone decreased the hypotensive action of 17-ketosteroids in this study, but the converse was noted when 17-hydroxy-compounds were altered in the same way. Depressor activity of these steroids showed no relation to their androgenic or anabolic properties (368). Among several synthetic and natural estrogens studied for depressor activity, the two synthetic compounds, 16-epiestriol-3-methyl ether and 16-oxo-estradiol-3-methyl ether induced significant lowering of blood pressure in hypertensive laboratory animals (369); estradiol, estrone, and estriol had minimal antihypertensive properties. The synthetic steroid, 17α-propyl-4,5β-dihydro-19-nor-testosterone (SC-6584, G. D. Searle and Co.), also was a potent depressor in several forms of experimental hypertension (370), although this compound is lacking in other endocrinological effects. Bile salts produced hypotension and bradycardia as well, the latter presumably through direct effects on the heart, since these responses were observed in the absence as well as the presence of autonomic innervation of the heart (401). Oral administration of Δ5-androsten-3β,16β-diol in amounts of 10 to 60 mg/day has been reported to lower blood pressure in human subjects (43), but there has not been, to our knowledge, any extensive study of the possible use of this or related steroids in the clinical management of hypertensive disease.

13. MISCELLANEOUS STEROID EFFECTS

Glycolithocholic acid administered in large amounts by subcutaneous injection to rats produces a "pernicious anemia-like (PA-like) syndrome" characterized by macrocytic anemia, skin lesions, and neurological disturbances; the disorder responds to injections of liver (179). Whole bile inhibits absorption of vitamin B_{12} (190) (an effect which is prevented by gastric juice), and a high molecular weight, glucosamine-rich component has been shown to bind this vitamin (275). Thus, the PA-like syndrome produced by glycocholic acid may be related in part to

enhancement of bile flow produced by this steroid. Recently, bile has also been shown to facilitate iron absorption in iron-deficient dogs, but the mechanism of this action is not known (411).

Certain steroids appear to protect laboratory animals against experimental thyrotoxicosis; these compounds include several bile acids (81, 277, 278) and cholesterol (91, 242, 278), although this property of cholesterol is open to question (277). Among the bile acids, deoxycholic, and to a lesser extent cholic, acid antagonized the weight loss and lethality of administered thyroid hormone; lithocholic and hyodeoxycholic acids had a deleterious effect, an interesting action consistent with the previously described toxicity of these compounds.

Progesterone, its derivatives, pregnanolone and pregnandiol, and a number of related steroids inhibit glucuronosyl transferase activity, thus suppressing conjugation of *o*-aminophenol, phenolphthalein and body constituents such as bilirubin (175). This steroid action, for which the structural basis has been examined in detail (222), undoubtedly accounts for the inhibiting effects on glucosiduronate formation exhibited by sera from pregnant women; it may also explain the jaundice which occurs with the prolonged administration of certain steroids, and may in addition account in part for the physiological hyperbilirubinemia of the newborn (175).

Progesterone also alters galactose metabolism in certain patients with congenital galactosemia (294). In control periods, no significant amounts of $C^{14}O_2$ were found in the expired air of these patients after intravenous injection of galactose-1-C^{14}; after 6 days on small doses of progesterone (10 to 20 mg/day, i.m.), expired $C^{14}O_2$ levels increased considerably—a response not associated with detectable decreases in galactose-1-phosphate levels of red cell hemolysates. In rats, progesterone also delays the onset and decreases the incidence of cataract on a high galactose diet. Progesterone probably acts by antagonizing the toxic effects of galactose feeding in these animals, since it had no effect on the development of cataracts due to xylose (294). The mechanism of action of progesterone in galactosemia is not clear. Although an effect *in vitro* is demonstrable on the uridine diphosphogalactose-4-epimerase reaction (355), effects *in vivo* may be due to entirely different mechanisms (294).

Estrogens, in suitable doses, exert therapeutic benefit in patients with recurrent bleeding due to hereditary hemorrhagic telangiectasia. This action of estrogens was first reported in 1952 by Koch *et al.* (212) and has since been confirmed in other studies (148, 177, 402) which have generally indicated relief of bleeding episodes after this form of therapy. The mechanism of this action probably involves alterations in the friability of epithelial tissues overlying the telangiectatic lesions (148); demonstrated shrinkage in size of the lesions themselves, however, speaks for a direct estrogenic action as well (212).

Estrogens may also exert considerable protective action against the toxic effects of digoxin on the myocardium. This protective effect is probably reflected in the relatively high sensitivity of male and castrate female dogs to the toxic actions of cardiac glycosides, and the high degree of resistance to digoxin toxicity exhibited by pregnant animals (141). Clinical application of this pharmacological

effect of estrogens has not been made to our knowledge, but this approach to therapy of digitalis toxicity in man may prove rewarding.

Several minor but interesting effects of estrogens include their protective action against amidopyrine intoxication in man (34); their pronounced stimulation of water-intake in certain species of mice (382); and (with progesterone) their enhancing effects on production of gallstones in rabbits (178). The latter effect may be related to the high incidence of cholelithiasis in women who have had multiple pregnancies (178). A number of other extragenital effects of estrogens have been reviewed by Fisher (101).

14. CONCLUDING REMARKS

The effects summarized in this review comprise, to our knowledge, the principal known pharmacological activities of steroids apart from their conventional endocrine or hormonal effects and the exceptions noted in the introduction. The number and diversity of these actions attest both to the manifold extragenital influences which steroids may exert and to the probable existence of further unrecognized pharmacological properties of these compounds as well. The clinical implications of many of these steroid actions have been alluded to in each of the preceding sections; the heterogeneous nature of the material summarized in this review precludes further generalizations.

One aspect of the review which merits particular emphasis, however, is the repeated demonstration that metabolic transformations of steroid hormones and related substances *in vivo* may lead not to "inactivation" of these compounds, but to the formation of potent new derivatives having independent biological properties. In this respect even conjugation processes themselves may not terminate the biological activity of steroids, and termination can be assured only when ultimate excretion of these substances takes place. The properties which characterize steroid metabolites may be related to those of the precursor substances, may be antagonistic to them, or may be entirely novel. Moreover, alternative metabolic pathways for the same precursor can favor formation of derivatives having various combinations of activities, which may then express themselves by discrete physiological manifestations or by profound alteration of the biological action of the original hormonal substance (23, 102, 110, 162, 201). Certain steroid transformations *in vivo* can, in particular, result in excessive production of highly toxic metabolites which may participate actively in the pathogenesis of disease or its manifestations in man (198). Further investigations in this direction hold special promise of yielding information of considerable significance to clinical medicine.

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