INTERACTIONS OF DRUGS WITH ENDOCRINES[1,2]

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In the last few years the field which might be called endocrine pharmacology has developed rapidly. Its historic aspects and future prospects have been considered by us elsewhere (70). As yet it is a field without commonly accepted boundaries. We prefer to think of work on hormones and hormone derivatives as belonging to the science of endocrinology and we think of endocrine pharmacology as the study of drugs, not chemically related to hormones, as they affect endocrine organs and functions. The recent rapid advances in this area have stemmed from two scores: (*a*) as a natural extension of the developing science of pharmacology, and (*b*) in response to need for tools to extend work both in theoretical and applied endocrinology. An inhibitory drug can dissect an endocrine process much more cleanly, broadly and surely than any scalpel. How else but with a drug, for instance, can one eliminate with some specificity certain steroid-synthesizing enzymes of the adrenal cortex? The use of drugs to block and thus study the action of neurohumors has been exploited previously with brilliant success in other areas, notably by students of the autonomic nervous system. Such techniques, originally limited in endocrinology largely to the thyroid, are now being applied widely. Witness the proceedings of the First International Pharmacological Meeting, Stockholm, 1961 (1).

The volume of literature is such that only a few limited aspects of the subject are being considered here.

Drugs Affecting Reproduction

A wealth of information has accumulated in the last few years concerning drugs which affect some aspects of reproductive endocrine functions. This involves the actions of chemically modified steroid hormones and miscellaneous substances with widely different points of action. The long and delicately integrated chain of neuroendocrine factors in procreative functions presents many susceptible links for pharmacological modification and control (148). We will deal here with nonsteroidal drugs.

Modern work in the field began with Markee, Everett & Sawyer (129, 176) using particularly atropine, morphine, and barbiturates as tools in a skillful analysis of rodent reproductive cycles. More recently the actions of drugs typified by reserpine and chlorpromazine have received special atten-

[1] The survey of the literature pertaining to this review was concluded in July 1962.

[2] Abbreviations used in this chapter include: ACTH (adrenocorticotropic hormone); ADH (antidiuretic hormone); DOC (11-desoxycorticosterone); FSH (follicle stimulating hormone); LH (luteinizing hormone); LSD (lysergic acid diethylamide).

tion, and intensive study is being given all drugs which might serve as antifertility agents.

Drugs Affecting Gonadotrophic Functions

Reserpine and its analogues.—It is now well established that reserpine exerts some kind of mild inhibitory action on pituitary function which can result in suppression of estrous or menstrual cycles, and block fertility in various species (72, 70, 186). Doses required, however, are large and outside the range used in conventional therapy. In rats, differences in colony sensitivity to reserpine (48) make quantitative comparisons of work from different laboratories difficult. Much of the animal work has been done with doses which provoke such toxicity as to obscure specific pharmacodynamic actions. That some specificity is involved, however, is shown by studies with various derivatives of methyl reserpate. Chemical alterations of the reserpine molecule can accentuate some of its reproductive effects relative to its other classic actions as an anti-hypertensive and sedative. Thus, tetrahydropyranyl methyl reserpate (Su-7064) will depress pituitary-gonad function in doses which do not cause depression in growth, appetite or overwhelming sedation (73). On the other hand, syrosingopine, which in laboratory situations has antihypertensive activity equal to reserpine but is less sedative, is also relatively weak in its reproductive effects (73, 166, 208a). Deserpidine has a pattern of action which differs in some details (208) including potency (166).

Specifically, the reported actions of reserpine-like alkaloids in females consist of : (*a*) Suppression of estrus, ovulation and fertility, which may be partial or total depending on dosage and sensitivity of test animals (72, 40, 50, 112). (*b*) Uterine and ovarian regression with histological ovarian changes characterized by lack of well-developed follicles and altered lutein tissue (206, 40, 112). Long-term treatment in mice, however, may produce different results in which initial suppression is succeeded by large interstitial ovaries with functional restoration of cycles (118). (*c*) Delayed sexual maturation and vaginal opening in infantile animals (112). (*d*) Histological changes in the pituitary (205), and inhibition of gonadotrophin excretion in menopausal women (112). (*e*) Induction of pseudopregnancy (9, 57), and modified decidual reactions. The pseudopregnancy-producing action can be blocked by some monamine oxidase inhibitors (20). (*f*) Implantation may be interfered with in rats (207) and rabbits (108), and abortion produced at limited times during pregnancy. (*g*) Inhibition of precocious growth of uterus and ovary induced in immature female rats by chronic electrical stimulation of the cervix (191). (*h*) Interference with menstrual phenomena in primates analogous to those in rodents (68). (*i*) Decreased response to exogenous gonadotrophins in immature female mice (166). (*j*) Stimulation of mammary glands and possible induction of lactation (discussed separately below). (*k*) Particular sensitivity of birds to the pituitary-gonad depressing effects of reserpine, which limits otherwise useful application of the drug in poultry husbandry (4, 84, 171).

In male mammals the reproductive effects of Rauwolfia alkaloids are much less easy to demonstrate than in females. Doses in a nontoxic range elicit few if any definite actions in our hands. Others report regression of testicular interstitial tissue and male sex accessories, in which case spermatogenesis may or may not be clearly affected (2, 112, 204), a delay in testis descent in immature animals (112), and associated changes in sex behavior (188).

No one hypothesis explains all available data as to how reserpine acts (9). At least in rodents it probably inhibits the release of both FSH (follicle stimulating hormone) and LH (luteinizing hormone), particularly the latter, and stimulates or withdraws an inhibition from the release of luteotrophin. The role of the concomitant marked effects of the drug on ACTH release (128) is unevaluated. The pituitary cytological effects involved are said to resemble those caused by androgens (159). Purshottam's (166) observation that reserpine and numerous pharmacologically related drugs blocked the ovulatory response to exogenous gonadotrophins suggests a confusing multiplicity of actions. Since many of the effects of reserpine are presumably due to the release from various tissues, including the brain, of catechol amines and serotonin, one must consider the relation of these substances to the gonadotrophic functions of the anterior pituitary. It has been noted that reserpine blocked the pituitary, but not the hypothalamic, uptake of administered norepinephrine (222). Also, monoamine oxidase inhibitors are said to block at least some of the reproductive effects of reserpine: iproniazid prevented reserpine-induced inhibition of estrus and decidual reactions (165), as it does ACTH release (73), while N′-1:4-benzodioxan-2-methyl-hydrazine (20) inhibited implantation and prevented reserpine from causing pseudopregnancy. Thus there is a strong suggestion that the endocrine actions of reserpine are related to amine metabolism in some way. The significance of the fact is not clear (200) that when serotonin was administered to the uterine cavity of the rat, it, like histamine, caused stimulatory changes resembling the early action of estrogens.

In a study of the electrical correlates of the effects of reserpine Barraclough & Sawyer (8) found that the drug changed EEG (electroencephalogram) patterns, particularly in hippocampal channels, but, unlike chlorpromazine, failed to alter arousal thresholds on stimulation of the midbrain reticular formation. It appeared to inhibit afferent transmission into the reticular activating system.

Chlorpromazine.—The actions of chlorpromazine on reproductive functions, which grossly resemble those of reserpine, have been reviewed recently by Batrinos & Verbizier (10). Like reserpine, the drug will block ovulation, suppress estrous cycles, cause infertility and pseudopregnancy, support the decidual reaction and induce lactation. It will similarly depress testis and reproductive accessory weight in males (192). Again, as with reserpine, the doses required to get consistent activity are high and in some circumstances reach toxic levels (66). Despite many similarities between the actions of

chlorpromazine and reserpine, it is well known that they differ in their mechanisms of action. A clean-cut example is that the two drugs had different effects on the electrical activity of the rat's brain (8, 90). Although it is difficult to make comparisons of different drugs from the literature of different laboratories, some of the pituitary-gonad effects of chlorpromazine seem to differ in detail from those reported for reserpine (5, 11, 110, 112, 163, 193).

Sulman (192) has attributed the growth-reducing activity of the phenothiazines to inhibition of growth hormone secretion, apparently on the basis that injections of growth hormone repair the drug-induced deficiency.

Other drugs affecting the nervous system. Other drugs affecting the nervous system can cause changes in reproductive physiology. Phenothiazines in general act like chlorpromazine but are generally weaker (50, 166, 191, 192, 218). Other tranquillizers, including meprobamate, have at least ovulation-suppressing effects in animals (52, 166) and suppress experimentally-induced precocious puberty (191). The actions of meprobamate can be potentiated by an antichloinergic, 3-diethylamine-1-cyclohexyl-1-phenyl-1-ethiodide, as can those of chlorpromazine (77). Such drugs, however, do not alter the "sub-estrous cycles" of ovariectomized mice (52).

Morphine, a known ovulation blocker under certain experimental conditions (7), also causes regressive changes in Leydig tissue and sex accessories of males, without reported inhibition of seminiferous tubules (91). This finding is not inconsistent with those of Rennels (167) who found morphine-induced estrous cycles in females associated with an increase in pituitary FSH, decrease in LH and profound changes in pituitary cytology.

It is of interest that chlordiazepoxide (Librium) is said to have no endocrine actions like those of pharmacologically related compounds (19).

While it is of theoretical importance to known that many commonly used drugs can and do have endocrine effects such as those outlined above under conditions of laboratory use, nonetheless such knowledge may be of only limited significance under the conditions of use and dosage in applied clinical medicine. Thus Ciprut *et al.* (41) fail to find that the following drugs affect gonadotrophin excretion, or can account for the high natural variability of such excretion in women: phenobarbital, tetracycline, reserpine, codeine chlorpromazine, acetylsalicylic acid, meralluride.

Miscellaneous Substances

Monoamine oxidase and histamine inhibitors.—The action of monoamine oxidase inhibitors has been noted above in antagonizing the effects of reserpine on pituitary-gonad function. When given alone, however, on a chronic basis, they may have important pituitary-gonad depressing actions of their own. Both iproniazid (181) and nialamide (209) alter ovarian and pituitary morphology and function in ways differing in detail from those of the tranquilizers, e.g., mammary glands were unaffected. Curiously, no related effects were seen in males (181).

Spector (190) found n-[1-methyl-2-phenoxyethyl] hydrazinium (1+) hydrogen maleate to block estrus, prevent implanatation and cause sterility in female rats. This monoamine oxidase inhibitor had marked anti-inflammatory actions said to be due to "inhibition of the enzymatic inactivation of an adrenaline-like substance that would otherwise occur at the site of injury." It was postulated that a similar mechanism accounts for the ability of the drug to interfere with estrus and implantation.

In that connection one is remainded of the better-known analogous blocking action of antihistamine drugs in certain reproductive processes (184, 199) suggesting that the study of tissue humoral mediators, and their inhibitors, may be a profitable route to understanding and controlling endocrine-regulated reproductive phenomena.

Adrenolytics and parasympatholytics.—Since the early work of Markee and co-workers (129) it is well known that autonomic blockers can interfere with ovulation if given at certain limited times during the estrous cycle of rodents, presumably due to blockade of LH release. In confirming this observation, Moore (139) has noted that the effect is also accompanied by ACTH release as measured by adrenal ascorbic depletion. With the continued administration of either dibenamine, phenoxybenzamine (Dibenzyline) or tridihexethyl chloride, refractoriness develops both to the ovulation-suppressing and ACTH-releasing actions of these drugs. He argues, therefore, that neither adrenergic nor cholinergic mediators have an indispensable function in spontaneous release of pituitary ovulation hormones, but that "stimuli which cause an increase in ACTH release prevent the release of LH." This concept, susceptible to verification in various ways, in effect predicts that any stressful procedure applied at the proper time in a reproductive cycle will block ovulation. It relates to the whole problem of the non-specificity of action of drugs on endocrine processes as discussed below (section on Drugs Affecting Mammary Glands, etc.).

Clomiphene.—This drug, formerly known as chloramiphene (MRL-41) (1-[*p*-*β*-diethylaminoethoxyphenyl]-1,2-diphenyl-2-chloroethylene) is a weak estrogen related to chlorotrianisene (TACE). Holtkamp *et al.* (94, 93, 217) found it to be a strong gonadotrophin inhibitor and hence an anti-fertility agent in rats. Doses which failed to prevent ovulation and fertilization still prevented pregnancy (180) when given while tubal eggs were present. The potential anti-fertility action seems, therefore, to be a dual one: ovulation may be suppressed or there can be a MER-25-like interference with implantation (178). In young male rats treatment resulted in reproductive effects like those of hypophysectomy (152).

In view of these animal results it was, therefore, a matter of some surprise that Greenblatt (80, 81) found clomiphene to cause ovulatory menstrual cycles in 28 of 36 amenorrheic women of various, but not all, etiologies. Tyler (213) obtained somewhat similar results, as had he and Kistner & Smith (113) previously with a related compound, MER-25 (*p*-2-diethylaminoethoxyphenyl-1-phenyl-2-anisyl ethanol). In human females the sub-

stance did not act as an estrogen, a progestin, nor as a glucocorticoid, but rather as a "pituitary modifier" which caused ovulatory menses and increases the functional life of corpora lutea (81). Tyler's group (213) considered that it acts only in amenorrheic patients having persistent estrogenic activity. Its anti-estrogenic functions were suggested by the occurrence of hot flashes in some treated patients (81, 213). In human males doses thus far tried have not markedly inhibited spermatogenesis (213).

Diamines.—The germinal epithelium of male mammals, characterized by the unusual meiotic cell divisions and cell metamorphosis, is susceptible to inhibition or injury by a wide variety of drugs. These include several of the anti-tumor agents (99, 100). An interesting new series of *bis*(dichloroacetyl) diamines, originally studied as amoebicides, was found by Coulston and co-workers (17, 47) to exert testicular changes with what seems to be an unusual degree of specificity. Spermatogenesis was blocked in animals and apparently also in men (87, 127) at the stage of primary spermatocytes by amounts of drug without other notable actions or preclusive toxicity. The mechanism of action (although undefined) seems to be identical with that exhibited by the nitrofurans (151). Detailed studies have been reported by Potts *et al.* (164), Moore *et al.* (140) Beyler & Potts (16).

Diphenylindenes.—A new series of compounds has recently been reported (122, 64) to have anti-fertility actions resembling those of the triphenylethanol derivative, MER-25 (179). This consists of a zygoticidal action limited to the time of passage of fertilized eggs through the oviducts. The compound most thoroughly studied, U-11555A (triethylamine, 2-[*p*-(6-methoxy-2[-*p*-methoxyphenyl]-indene-3-yl)-phenoxy]-hydrochloride) (64), 63), is itself weakly uterotrophic, an estrogen antagonist, interferes only mildly with estrous cycles, and only at high doses in males shows some inhibition of testicular and androgenic activity.

Other anti-fertility studies.—The worldwide interest in problems of population control and the attention given to it by various organized bodies is resulting in a wide search for new drugs of potential value in regulating reproduction (e.g., Epstein & Kupperman, 67). Much of the work mentioned above was stimulated by this program.

The greatest practical success has been achieved to date with steroids, primarily those with progestational properties, some of which can achieve fertility regulation with minimal disturbing consequences. This subject has been extensively reviewed elsewhere (62, 148, 149, 161, 197). Other non-steroids recently mentioned, but not adequately characterized for evaluations, are the following:

(*a*) An extractive of orange peel, called Cirantin, said to be a pyrone derivative (75); (*b*) 1-α-methylallylthiocarbarbamoyl-2-methyl-thiocarbamoylhydrazine, described as a reversible inhibitor of gonadotrophin secretion in rats, dogs and monkeys of ether sex but not in mice, guinea pigs, rabbits or horses (157); (*c*) polyphoretin phosphate, thought to inhibit

ovulation by inhibition of follicular hyaluronidase (224); (*d*) triethylene thiophosphoramide, a cytotoxic anti-tumor agent, with lytic effects on granuloma cells and ova; a functional antagonist to gonadotrophins, its own actions being antagonized by diethylstilbestrol (160); Jackson *et al.* (101) have continued their work on related substances (100) and have found compounds with improved therapeutic ratios; (*e*) various indigenous Indian plant extracts (82, 83); (*f*) nidroxyzone, a nitrofuran derivative known to inhibit spermatogenic functions, acting also as an ovarian inhibitor in mice (51).

The mode of action and chemical nature of extracts of *Lithospermum ruderale*, a subject of scientific interest for a decade, is still uncertain (149). Soffer *et al.* (187) have reported that the urine of children contains a substance, extractable by methods usually used for gonadotrophins, which specifically inhibits the action of gonadotrophin as tested in animals; it does not inhibit the action of estrogen.

Drugs Affecting Mammary Glands and the Common Action of Diverse Drugs on Reproductive Processes

Interest in non-hormonal drugs which cause mammary growth and lactation has been kindled by many clinical and laboratory observations that reserpine and chlorpromazine have such effects. The relevant literature is now enormous (e.g., 10, 12, 114, 109). The phenomena involved were widely studied on the assumption that specific neuroendocrine mechanisms were being specifically affected. To some degree that must be the case. The perspectives of the problem have been changed, however, with the demonstration that the stimuli needed to initiate lactation are almost completely lacking in any intelligible specificity. This fact has emerged largely from the important work of Meites *et al.* (135, 156). They found that, in animals primed with estrogens so that mammary glands are responsive to prolactin, lactation can be induced not only by Rauwolfia alkaloids and phenothiazines, but in addition by: estrogens, progesterone, testosterone, cortisol, epinephrine, norepinephrine, acetylcholine, serotonin, pilocarpine eserine, atropine, amphetamine, morphine, reserpine, chlorpromazine, meprobamate, several carcinogens, rat hypothalamic extract, Guillemin's corticotrophin releasing factor, electrical stimulation of the head, nasal mucosa, nipples, lumbar region and uterine cervix, and stresses such as cold, heat, restraint and formalin injections (135). It therefore becomes of curious interest that this effect has thus far not been shown by oxytocin, vasopressin, histamine, dibenamine and LSD 25 (lysergic acid diethylamide) (135).

The scientific antecedent of this work was the report by Swingle's group (194, 195, 196) that a pot-pourri of stimuli would cause pseudopregnancy in the rat. Presumably this resulted from the release of luteotrophin with a consequent activation of the corpus luteum and secretion of progesterone. In the rat luteotrophin seems to be identical with prolactin and hence its re-

lease in animals with properly prepared mammary glands should cause lactation.

In general the same stimuli which cause pseudopregnancy or lactation cause ACTH release, and from old work [cited by Gaunt *et al.* (71); Nelson *et al.* (150)] it is also known that adrenal steroids are necessary for the full actions of lactogenic hormone. Hence the generalization seems warranted that stressful stimuli in general tend to result in an altered pattern of anterior pituitary activity characterized by decreased secretion of FSH and LH and increased outputs of luteotrophin (prolactin) and ACTH. If the prolactin is released when the mammary glands have been primed and adequately prepared morphologically by sex hormones, lactation may result. Similarly, if ovaries are in a receptive state of development, the same hormone can activate the rat's normally dormant corpora lutea to secrete progesterone and hence cause pseudopregnancy. The problem of primary interest, therefore, becomes not how any one particular drug may act, although identity of action could not be expected, but what is common to the spectrum of stimuli which result in common responses. The role of ACTH and the corticoids in inducing lactation may be more direct than suggested by the original hypotheses that they have only a permissive role. For instance, it has recently been shown that in pregnant or pseudopregnant rats or pregnant rabbits large doses of hydrocortisone can initiate lactation (202), as can ACTH in pseudopregnant rabbits (30). One emerging concept is that the secretion of prolactin and probably ACTH is held normally under some sort of inhibition by hypothalamic centers (86, 132). Additional evidence for this is that a transplanted pituitary secretes more prolactin than one *in situ* (136). Thus stressful stimuli might act in common by suppressing this inhibition with details of the response dependent on the status of receptor organs (ovaries, mammary glands, etc.). In turn it might be anticipated, as has been found with reserpine, for instance, that reproductive changes would be more difficult to induce with drugs in males than females. Males are probably less quickly sensitive to changes in the pituitary hormones primarily affected. In addition, luteotrophin has no known function in the male.

Drugs and Antidiuretic Hormone

The effect of drugs on the secretion rate of vasopressin is a subject of importance both from the standpoint of the interpretation of the action of the drugs themselves, and upon the reliability of their use as tools to regulate such secretion experimentally. Great uncertainty has attended work on this subject because of lack of methods with requisite sensitivity for the determination of the blood levels of this hormone.

Judged by varying but probably reasonably valid criteria, the secretion rate of vasopressin under normal conditions in man has been estimated at <7.5 to <50 milliunits per hour (121) and in the dog at one to five milli-

units per hour (182). Human plasma concentration figures have been obtained in the range of <10 to 150 (26) and (in both human and lower primates) <20 to <50 (36, 39) microunits per ml. It is clear that physiologically significant variations, unmeasurable by direct means, can and no doubt do occur at low blood levels of vasopressin. The methods now available can generally detect whether the activity of the many drugs which cause antidiuresis is associated qualitatively with release of excess vasopressin by direct determinations in blood. Possible inhibition of vasopressin secretion, on the other hand, can generally be inferred only by indirect means, e.g., pattern of renal activity.

Since the early work of Burn (27, 28), nicotine has become almost the standard for pharmacological stimulants of vasopressin secretion. It is used to test the physiological competence of the neurohypophyseal system under various experimental and clinical conditions (124, 60).

Other drugs reported to cause vasopressin release are: acetylcholine, yohimbine, hexamethonium, lobeline, morphine, several tranquilizers, many anesthetic and analgetic agents, dimercaprol, atropine, cinchoninic acid derivatives (175, 216). The antidiuretic activity of such drugs is not necessarily wholly due to the release of vasopressin. For instance, the mode of action of morphine may be complex (54, 177): one group maintains that it does not cause vasopressin secretion but, aside from renal vascular effects, only potentiates pre-formed vasopressin (54). This type of action has also been suggested for chlorpromazine (116).

An analogous situation probably obtains with resperine. Its acute effects on water and electrolyte excretion (72, 134) are associated according to Chaudhury *et al.* (38) with a transient release of ADH (antidiuretic hormone). In our hands this will not account entirely for the total renal actions of the drug (73). Possibly these are more dependent on the release of serotonin (111). Anomalously they are said to be associated with a decrease of antidiuretic activity in blood (219).

Gabe *et al.* (69, 206) found reserpine to deplete the neurosecretory material of the neurohypophysis in rats and to augment its expected depletion after administration of hypertonic saline. We, too, have seen such augmented depletion when reserpine was given with hypertonic saline (unpublished). We did not rule out the possibility that the effect was due to an observed inhibited excretion of the saline load and hence intensification of the osmotic stimulus, but the French workers apparently have done so (206). On the other hand, Moses (141) has found that reserpine, chlorpromazine, and diphenylhydantoin inhibit depletion of vasopressin content of pituitaries of rats dehydrated by water deprivation. This apparent discrepancy is likely due to differences in experimental techniques and parameters, and indicates the subtlety required for meaningful experimentation in the field.

Chlorpromazine, too, seems to have a multi-faceted action. Under certain but not all (49, 203) conditions of fluid or saline-loading or cardiovascular

disease it has a diuretic action in both animals and man (44, 116, 143, 158, 174, 198) and, along with many other drugs, blocks water imbibition by isolated muscle (65). These results are partly associated with hemodynamic actions and an inhibitory action on the neurohypophysis or ADH itself is strongly inferred but not established (10, 58, 116, 134, 157). It seems more certain that chlorpromazine can inhibit the release of oxytocin (37).

Many diuretic drugs will to some degree antagonize the antidiuretic effects of vasopressin, presumably by an algebraic summation of their various actions. Ethanol still stands, however, curiously alone as the only drug which, on the basis of unequivocal evidence, inhibits the secretion of ADH (70). Others probably exist as indicated above, but their identification is obscured by methodological limitations.

Drugs Affecting the Adrenal Cortex

Drugs which alter the secretory function of the adrenal cortex may be categorized according to their locus of action:

(*a*) There are those having a "central action," i.e., they affect the neural transmission of stimuli or the secretion of the neurosecretory substance responsible for the release of ACTH. In addition, some drugs probably act on the pituitary directly to block ACTH synthesis or secretion. (*b*) Drugs can interfere with the action of adrenal corticoids at end organ sites. (*c*) Various drugs exert their activity on the adrenal cortex directly either by cytotoxic action or by interfering with enzyme systems involved in the biosynthesis of corticoids.

Centrally acting drugs. Among these are ether, barbiturates, reserpine, chlorpromazine, phenoxybenzamine, other autonomic blocking agents, and the adrenal corticoids involved in the feedback control of ACTH secretion.

Many, and perhaps most drugs affect the rate of ACTH secretion under some conditions of use or dosage. The effect may be that of stimulation, inhibition or a sequential combination of the two. Munson (145) suggests that morphine is the only non-steroid drug among the "centrally acting agents" which serves fairly consistently as a strong inhibitor of ACTH secretion. However, a recent report of interest suggests that ethionine inhibits the biosynthesis of ACTH in response to acute stresses (131). The results concerning the effects of chlorpromazine, reserpine, anesthetics, and analgesics are more variable. Munson has written recently a comprehensive and critical review on drugs such as those listed above which influence ACTH secretion (145); hence the subject is not considered further here.

Antagonism of corticosteroids at the end organ Although much has been written in past years concerning steroid antagonisms, it was only recently that practical application of these efforts was realized. In 1957 Kagawa *et al.* (104) reported that certain spirolactones antagonized the sodium-retaining activity of aldosterone on kidney tubules. According to the definition of Drill (61), they may be classified as specific antagonists of aldosterone.

Spironolactone (103) has been introduced as a diuretic for the treatment of diseases in which high aldosterone secretion is an etiologic factor. It blocks the effects of mineralocorticoids at other end organs as well as the kidney (45, 76).

The spirolactones have few, if any, direct hormone-like effects when given alone. The glucocorticoids (107, 214), testosterone (106), and progesterone (59, 104, 119, 169, 214) apparently have some true aldosterone antagonizing actions in addition to their other well-known effects.

Another group of compounds, the pteridines, were initially reported as antagonists of aldosterone (55, 120, 223). However, they do cause some sodium excretion in the adrenalectomized rat, and, therefore, cannot be classified with the spirolactones (125).

The thiazides and other diuretics also counteract the sodium-retaining properties of mineralocorticoids (15, 105, 168). Their diuretic activity, however, is apparent in the absence of sodium-retaining hormones [but perhaps not in the toad bladder (53)] and the results obtained when they are given in combination can generally as well be interpreted as an antagonism of the sodium-excreting properties of the thiazides by aldosterone.

Substances directly affecting the adrenal cortex. Since the first reports on suramin (98) many substances have been reported to inhibit the adrenal cortex. These have been reviewed by others (23, 31, 70, 144) and only selected ones are considered here. Most of them have received little attention, but exceptions are the DDD's amphenone B, triparanol, and some newer compounds described as 17α-hydroxylation inhibitors (35).

DDD's.—In 1949 Nelson & Woodward (147) first reported that the insecticide DDD, a tetrachlorodiphenyl ethane, caused cytotoxic atrophy of the adrenal cortex. Subsequent clinical studies confirmed an adrenal inhibition by the drug in man but toxic side effects prevented its widespread use. DDD was represented by the formula 2,2-*bis*-(para-chlorophenyl)-1,1-dichloroethane, but it was subsequently learned that the most active moiety was the o,*p'* isomer (56, 153).

In patients with adrenocortical carcinoma (13) or Cushing's Syndrome (74, 221), o,*p'*-DDD reduced the adrenal secretory rate but the side effects were severe. Perthane (2,2-*bis*-(para-ethylphenyl)-1,1-dichloroethane), a congener of DDD, shows activity like that of DDD in dogs (43) and man (201). Contrary to the situation with DDD, the *p,p'* isomer of Perthane was more active than o,*p'* (18).

Nichols (154) has shown that the *m,p'* isomer of DDD also is active in man. A dose of 5 g per day caused regression of metastatic pulmonary lesions of adrenocortical carcinoma.

The mode of action of the DDD's is still not clear. The older literature describes "adrenolytic" or "cytotoxic" atrophy of the adrenal cortex. In the dog this is generally true only following long term treatment. In our own laboratories we have observed necrosis of the adrenal cortex in dogs follow-

ing six days' treatment with 50 mg/kg o,*p'*-DDD orally in sesame oil. Three days of this treatment showed markedly decreased corticosteroid secretion, fatty deposition in the adrenal fasciculata and reticularis, some leucocytic infiltration and nuclear hyperchromatism but no frank necrosis.

In patients with Cushing's Syndrome, on the other hand, treated for months with 2 to 10 g of o,*p'*-DDD daily, a reduction of corticosteroid secretion occurred without adrenal necrosis (189, 221). As suggested by Vilar & Tullner (220) and by Nichols (155), necrosis of cortical tissue is not an absolute prerequisite for suppression of function. There still may be species differences in susceptibility to morphological changes and it appears that the dog is more sensitive in this respect. The adrenals of rats (22) and monkeys (211) are highly resistant to any action of the DDD's.

Laboratory testing of these compounds is not simple and is not very precise. Even when given intravenously, Tullner & Hertz (212) found that in the dog it was necessary to administer *o,p'*-DDD for at least three days in order to obtain consistent results. Cazorla & Moncloa (29), on the other hand, found responsiveness to ACTH of dog adrenal slices, tested *in vitro* reduced within two hours after the intravenous administration of o,*p'*-DDD. Addition of the drug directly to the incubation media, however, was without effect. This suggests either that problems of solubility or entrance of the drug into cells are limiting factors in its activity, or that some metabolite is the active moiety. In regard to the latter point, Moy (142), using human subjects, found that 60 per cent of administered o,*p'*-DDD was eliminated as such in feces, 10 per cent appeared as an unidentified metabolite in urine, and 20 per cent seemed to be retained and released slowly from tissue and fat stores.

The type of activity shown by the DDD's on the adrenal cortex could be of wide therapeutic use if and when more effective compounds with greater potency and specificity of action are discovered.

Amphenone.—Description of the biological activity of amphenone by Hertz, Allen & Tullner (3, 88) generated a marked interest in this compound. The literature from 1950 through 1959 has been reviewed (23, 31, 89).

A generalized decrease in the secretion of corticosteroids is observed after amphenone treatment, and *in vitro* studies (170) showed that amphenone blocks the 11-, 17- and 21-hydroxylation of pregnenolone, and very possibly interferes with the dehydrogenation of the 3-hydroxyl group. Clinical work confirmed that amphenone was an effective inhibitor of corticosteroid synthesis in man but various toxic side effects seriously limited its usefulness.

Hertz *et al.* (89) studied several derivatives of amphenone but none showed any substantial advantage over the parent compound. Recent studies (24) show that when the *p*-amino groups of amphenone are replaced by hydroxyl groups, the resulting compound has less toxicity both in animals and man but apparently also is a less effective adrenal inhibitor.

Further modifications of the amphenone molecule have been reported by Korman (115) who found the N,N′ diacetyl derivative to have reduced toxicity. We have studied this compound and also the triacetyl, tetracetyl, and dinicotinyl derivatives; although they appear to be less toxic than amphenone, they are also more selective in their inhibitory properties and preferentially inhibit 17α-hydroxylation of corticosteroids (35).

Methylenedianiline has effects like amphenone except for less anesthetic activity (210, 211).

Triparanol.—Recently triparanol (*p*-β-diethylaminoethoxy-phenyl-1-[*p*-tolyl]-2-[*p*-chlorphenyl] ethanol; MER-29) was shown to be an effective agent in reducing elevated serum cholesterol by inhibiting the conversion of desmosterol to cholesterol (6). In view of the recognized importance of cholesterol as a precursor in steroid biosynthesis, the use of triparanol as an inhibitor of corticosteroid biosynthesis was investigated.

Melby, St. Cyr & Dale (137, 138) treated patients with doses of one to two g per day and observed a reduction in basal urinary corticosteroid excretion, a reduction in the adrenal responsiveness to ACTH, clinical remission of Cushing's Syndrome, and a reduction in urinary excretion of tetrahydroaldosterone in a patient with hyperaldosteronism. In rats also it was found that triparanol caused depression of adrenal cholesterol and inhibition of corticosterone synthesis *in vivo* (92). Clinical studies by Marks *et al.* (130) showed that triparanol caused some reduction in basal 17-hydroxycorticosteroid excretion but little change in free plasma levels; only one of seven patients had a reduced response to ACTH, and four had normal adrenocortical response to surgery. No clinical adrenal insufficiency was observed. The discrepancy between the studies of Marks & Melby is difficult to explain, since in most cases dosage employed in the two studies was identical, 1.0 g per day.

Bethune & Nelson (14) agree that triparanol can be an effective adrenal inhibitor if given at approximately 10 times the recommended dose for reducing blood cholesterol, but at this level toxicity becomes a factor which makes its use for this purpose unacceptable.

Preferential enzyme inhibitors.—In 1958 the adrenal inhibitory activity of mepyrapone (Metopirone®, Su-4885) was first described (34) and in 1959 additional clinical and biological studies were reported which further clarified the activity of the compound (31, 46, 102, 126). The typical response to treatment with mepyrapone, a decrease in the secretion of cortisol and corticosterone and an increase in the secretion of the 11-desoxy compounds, 11-desoxycortisol and 11-desoxycorticosterone (DOC), indicated that this substance showed preferential inhibition of the 11β-hydroxylase system.

Other compounds have been described which have a preferential inhibitory activity, but of a type different from mepyrapone. These substances, Su-9055 (3-[1,2,3,4-tetrahydro-1-oxo-2-naphthyl]-pyridine) and Su-8000 (3-[6-chloro-3-methyl-2-indenyl]-pyridine), cause a decrease in the secretion

of cortisol and 11-desoxycortisol and an increase in the secretion of corticosterone and DOC; it is postulated that they are primarily 17α-hydroxylation inhibitors (32, 35, 185).

The type of activity shown by such compounds has not been applicable as yet to practical clinical treatment of adrenocortical hyperfunction but mepyrapone was adapted for use as a test for pituitary insufficiency (126). The development and application of mepyrapone for this purpose and its use in the study of adrenal physiology in animals and man comprises a bibliography of over 175 publications[3] and will not be described here in detail.

Insufficient time has elapsed since the initial reports (32, 185) on the 17α-hydroxylation inhibitors for extensive studies to be made. The proposed activity of these compounds has been confirmed by other laboratories and extended to show that inhibition of the 17α-hydroxylation reactions apply also in androgen synthesis *in vitro* (85) and *in vivo* in the rat (33). Neher (146) has reported that Su-9055 is a potent inhibitor of aldosterone synthesis *in vitro* and more specifically inhibits 18-hydroxylations in addition to its effects on 17α-hydroxylations. Sharma (183), using a water soluble 11β-hydroxylase prepared from beef adrenal, finds some inhibition of this system with Su-9055, but in this respect it is much less potent than mepyrapone which strongly inhibits the conversion of DOC to corticosterone. Neher's studies show that mepyrapone is also a strong inhibitor of 18- and 19-hydroxylation and of aldosterone synthesis. Such results are consistent with our original observations that in large dosage mepyrapone inhibited the biosynthesis of all the major corticosteroids (34).

Levy (123) perfused citrated bovine blood containing 50 mg/L of mepyrapone through beef adrenals; the conversion of DOC to corticosterone was still blocked when the perfusate was recycled five times. When cycled 30 times only 1.5 per cent of the DOC was converted to corticosterone. Two compounds appeared in the perfusate not hitherto isolated from such systems: 6β-OH-desoxycorticosterone and 3-oxo-etienic acid. Three other unknown substances were also present.

The enzymatic inhibition exerted by mepyrapone is reversible. Ungar (215) obtained adrenal tissue from a patient with Cushing's Syndrome treated daily with one gram of mepyrapone for four months. The 17-hydroxy steroid excretion had been reduced to normal along with only a slight rise in the 11-desoxy-steroids. Upon incubation of this tissue *in vitro* it synthesized corticosteroids normally.

Diphenylhydantoin reduced the response to mepyrapone (117) as did chlorpromazine, nialamide and meprobamate, but not reserpine (78). Both hypo- and hyperthyroidism seem to have similar effects (25, 79) as do hypothalamic lesions and non-functional pituitary tumors (79). Either exaggerated or normal responses may occur in Cushing's Syndrome (79). Male

[3] This bibliography will be supplied by the authors on request.

patients with congenital virilizing adrenal hyperplasia, having a defect in their 21-hydroxylating mechanisms, respond to mepyrapone by excreting large quantities of pregnantriol and 17-OH-pregnenolone (42). No increase in the secretion or excretion of any steroid occurred when mepyrapone was given a patient with adrenal hyperplasia associated with bronchogenic carcinoma (97). Presumably such tumors secrete ACTH-like substances unresponsive to normal controlling mechanisms.

Much discussion has revolved around the question whether pretreatment with corticoids or ACTH may lead to a false positive mepyrapone test for pituitary insufficiency (21, 25, 95, 96, 133, 162, 172, 173). From these reports it is also evident that there are differences of opinion concerning the effect of corticosteroid or ACTH treatment on the subsequent functional capacity of the anterior pituitary. Opinions also differ as to what effect exogenous corticosteroids have on adrenocortical sensitivity to ACTH. As further studies clarify this basic problem, a solution to the question concerning the effect of corticoid or ACTH pretreatment on the mepyrapone test should simultaneously emerge.

LITERATURE CITED

1. Abstr. and Titles, Intern. Pharmacol. Meeting, 1st Meeting, Stockholm, 1961, *Biochem. Pharmacol.*, **8** (No. 1)
2. Adams, A. E. and Fudge, M. W., *J. Exptl. Zool.*, **142**, 337 (1959)
3. Allen, M. J., Hertz, R., and Tuller, W. W., *Proc. Soc. Exptl. Biol. Med.*, **74**, 632 (1950)
4. Assenmacher, I., Tixier-Vidal, A., and Baylé, J. D., *Compt. Rend. Soc. Biol.*, **155**, 2235 (1961)
5. Audibert, A., Forgue, G. and Gage, C., *Compt. Rend. Soc. Biol.*, **150**, 173 (1956)
6. Avigan, J., Steinberg, D., Thompson, M. J., and Mosettig, E., *Federation Proc.*, **19**, 239 (1960)
7. Barraclough, C. A., and Sawyer, C. H., *Endocrinology* **57**, 329 (1955)
8. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, **61**, 341 (1957)
9. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, **65**, 563 (1959)
10. Batrinos, M. L., and de Verbizier, J., *Revue franç. Etudes Clin. Biol.*, **7**, 87 (1962)
11. Batrinos, M. L., de Verbizier, J., Moncuit, M., and Courjaret, J., *Revue franç Etudes Clin. Biol.*, **7**, 521 (1962)
12. Benson, G. K., *Proc. Soc. Exptl. Biol. Med.*, **103**, 132 (1960)
13. Bergenstal, D. M., Hertz, R., Lipsett, M. B., and Moy, R. H., *Ann. Internal Med.*, **53**, 672 (1960)
14. Bethune, J. E., and Nelson, D. H., *Disease-A-Month*, April, 3 (1962)
15. Beyer, K. H., *Ann. N. Y. Acad. Sci.*, **71**, 363 (1958)
16. Beyler, A. L., and Potts, G. O., *Federation Proc.*, **21**, 213 (1962)
17. Beyler, A. L., Potts, G. O., Coulston, F., and Surrey, A. R., *Endocrinology* **69**, 819 (1961)
18. Bleiberg M. J., *Endocrinology*, **69**, 13 (1961)
19. Boris, A., Costello, J., Gower, M. M., and Welsch, J. A., *Proc. Soc. Exptl. Biol. Med.*, **106**, 708 (1961)
20. Bovet-Nitti, F., and Bignami, G., *Biochem. Pharmacol.*, **8**, 3 (1961)
21. Bray, G. A., and Plager, J. E. Intern., Congr. Hormonal Steroids, Milan, 1962, *Excerpta Medica*, **51**, 150 (1962)
22. Brown, J. H. U., *Proc. Soc. Exptl. Biol. Med.*, **83**, 59 (1953)
23. Brown, J. H. U., *Nature*, **187**, 985 (1960)
24. Brownie, A. C., Hunter, R. B., and Sheppard; D. M., *Proc. Roy. Soc. Med.*, **52**, 819 (1959)
25. Brownie, A. C., and Sprunt, J. G., *Lancet*, **I**, 773 (1962)
26. Buchborn, E., *Endocrinology*, **61**, 375 (1957)

27. Burn, J. H., *Brit. Med. J.*, **II**, 199 (1951)
28. Burn, J. H., Truelove, L. H., and Burn, I., *Brit. Med. J.*, **I**, 403 (1945)
29. Cazorla, A., and Moncloa, F., *Science*, **136**, 47 (1962)
30. Chadwick, A., and Folley, S. J., *J. Endocrinol.*, **24**, xi (1962)
31. Chart, J. J., and Sheppard, H., *J. Med. Pharmacol. Chem.*, **1**, 407 (1959)
32. Chart, J. J., and Sheppard, H., *Endocrinology*, **68**, 20 (1961)
33. Chart, J. J. and Sheppard, H., Proc. Intern. Congr. on Hormonal Steroids, *1st Congr.*, Milan, 1962 (Academic Press, Inc., N. Y., in press)
34. Chart, J. J., Sheppard, H., Allen, M. J., Bencze, W. L., and Gaunt, R., *Experientia*, **14**, 151 (1958)
35. Chart, J. J., Sheppard, H., Mowles, T., and Howie, N., *Endocrinology* (In press)
36. Chaudhury, R. R., *Clin. Sci.*, **19**, 641 (1960)
37. Chaudhury, R. R., *Brit. J. Pharmacol.*, **17**, 297 (1961)
38. Chaudhury, R. R., Chaudhury, M. R., and Lu, F. C., *Can. J. Biochem. Physiol.* (In press)
39. Chaudhury, R. R., Chuttani, H. K., and Ramalingaswami, V., *Clin. Sci.*, **21**, 199 (1961)
40. Cheng, D. W., *Anat. Record*, **124**, 272 (1956)
41. Ciprut, S., Silverstein, J. N., Schwartz, H. L., Feldman, E. B., and Carter, A. C., *J. Clin. Endocrinol. and Metabolism*, **22**, 535 (1962)
42. Cleveland, W. W., Nikezic, M., and Migeon, C. J., *Journal of Clin. Endocrinol. Metab.*, **22**, 281 (1962)
43. Cobey, F. A., Taliaferro, I., and Haag, H. B., *Science*, **123**, 140 (1956)
44. Cohen, A. M., *Am. Heart J.*, **54**, 907 (1957)
45. Cole, D. F., *J. Endocrinol.*, **24**, vii (1962)
46. Coppage, W. S., Island, D., Smith, M., and Liddle, G. W., *J. Clin. Invest.*, **38**, 2101 (1959)
47. Coulston, F., Beyler, A. L., and Drobeck, H. P., *Toxicol. Appl. Pharmacol.*, **2**, 715 (1960)
48. Coupland, R. E., *Nature*, **181**, 930 (1958)
49. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koerschet, P., *Arch. intern. Pharmacodyn.*, **92**, 305 (1952)
50. Cranston, E. M., *Proc. Soc. Exptl. Biol. Med.*, **98**, 320 (1958)
51. Cranston, E. M., *Endocrinology*, **69**, 331 (1961)
52. Cranston, E. M., *Proc. Soc. Exptl. Biol. Med.*, **108**, 514 (1961)
53. Crawford, J. D., Frost, L., Meara, P., and Terry, M. L., *Am. J. Diseases Children*, **102**, 731 (1961)
54. Crawford, J. D., and Pinkham, B., *J. Pharmacol. Exptl. Therap.*, **133** 431 (1955)
55. Crosley, A. P., Jr., Ronquillo, L., and Alexander, F., *Federation Proc.*, **20**, 410 (1961)
56. Cueto, C., and Brown, J. H. U., *Endocrinology*, **62**, 334 (1958)
57. De Feo, V. J., *Anat. Record*, **127**, 409 (1957)
58. de Wied, D., and Jinks, R. *Proc. Soc. Exptl. Biol. Med.*, **99**, 44 (1958)
59. Dimick, D. F., Dietz, A. A., and Bernstein, L. M., *J. Lab. Clin. Invest.*, **58**, 812 (1961)
60. Dingman, J. E., Despointes, R. H., Laidlaw, J. C., and Thorn, G. W., *J. Lab. Clin. Med.*, **51**, 690 (1958)
61. Drill, V. A., in *Biological Activities of Steroids in Relation to Cancer*, 25 (Pincus, G., and Vollmer E. P., Eds., Academic Press, Inc., New York, 530 pp. 1960)
62. Drill, V. A., *Japan. J. Pharmacol.*, **11**, 88 (1962)
63. Duncan, G. W., and Lyster, S. C., *Federation Proc.*, **21**, 437 (1962)
64. Duncan, G. W., Stucki, J. C., Lyster, S. C., and Lednicer, D., *Proc. Soc. Exptl. Biol. Med.*, **109**, 163 (1962)
65. Eckhardt, E. T., and Govier, W. M., *Proc. Soc. Exptl. Biol. Med.*, **97**, 124 (1958)
66. Eisler, M., Neri, R., Russo, M., and Perlman, P. L., *Federation Proc.*, **17**, 366 (1958)
67. Epstein, J. A., and Kupperman, H. S., *Clin. Pharmacol. Therap.*, **3**, 216 (1962)
68. Erikson, L. B., Reynolds, S. R. M., and De Feo, V. J., *Endocrinology*, **66**, 824 (1960)
69. Gabe, M., Tuchmann-Duplessis, H., and Mercier-Parot, L., *Compt. Rend.*, **252**, 1857 (1961)
70. Gaunt, R., Chart, J. J., and Renzi, A. A., *Science*, **133**, 613 (1961)
71. Gaunt, R., Eversole, W. J., and Kendall, E. C., *Endocrinology*, **31**, 84 (1942)
72. Gaunt, R., Renzi, A. A., Antonchak, N., Miller, G. J., and Gilman, M.,

Ann. N. Y. Acad. Sci. **59,** 22 (1954)

73. Gaunt, R., Renzi, A. A., and Chart, J. J., *Endocrinology* (In press)
74. Geyer, G., *Acta Endocrinol.*, **40,** 332 (1962)
75. Ghosh, B. P., and Bose, S. N., *Med. Expt.*, **5,** 130 (1961)
76. Gilder, H., McSherry, C., and Besl, J. M., *Federation Proc.*, **19,** 166 (1960)
77. Gitsch, E., *Endocrinology*, **62,** 533 (1958)
78. Gold, E. M., DiRaimondo, V. C., Kent, J. R., and Forsham, P. H., *Ann. N. Y. Acad. Sci.*, **86,** 178 (1960)
79. Gold, E. M., Kent, J. R., and Forsham, P. H., *Ann. Internal Med.*, **54,** 175 (1961)
80. Greenblatt, R. B., *Fertility Sterility*, **12,** 402 (1961)
81. Greenblatt, R. B., Barfield, W. E., Jungck, E. C., and Ray, A. W., *J. Am. Med. Assoc.*, **178,** 101 (1961)
82. Gujral, M. L., Varma, D. R., Sareen, K. N., and Roy, A. K., *Indian Journal of Medical Research*, **48,** 52 (1960)
83. Gujral, M. L., Varma, D. R., and Sareen K. N., *Indian J. Med. Res.*, **48,** 46 (1960)
84. Hagen, P., and Wallace, A. C., *Brit. J. Pharmacol.*, **17,** 267 (1961)
85. Hall, P. F., and Eik-Nes, K. B., *Federation Proc.*, **21,** 197 (1962)
86. Haun, C. K., and Sawyer, C. H., *Acta Endocrinol.*, **38,** 99 (1961)
87. Heller, C. G., Moore, D. J., and Paulsen, C. A., *Toxicol. Appl. Pharmacol.*, **3,** 1 (1961)
88. Hertz, R., Allen, M. J., and Tullner, W. W., *Proc. Soc. Exptl. Biol. Med.*, **75,** 627 (1950)
89. Hertz, R., Tullner, W. W., Schricker, J. A., Dhyse, F. G., and Hallman, L. F., in *Recent Progress in Hormone Research*, XI, 119 (Pincus, G., Ed., Academic Press, Inc., New York, 518 pp., 1955)
90. Himwich, H. E., *J. Neuropsychiat.*, **3,** 279 (1962)
91. Hohlweg, W., Knappe, G., and Dörner, G., *Endokrinologie*, **30,** 152 (1961)
92. Holloszy, J., and Eisenstein, A. B., *Proc. Soc. Exptl. Biol. Med.*, **107,** 347 (1961)
93. Holtkamp, D. E., Davis, R. H., and Rhoads, J. E., *Federation Proc.*, **20,** 419 (1961)
94. Holtkamp, D. E., Greslin, J. G., Root, C. A., and Lerner, L. J., *Proc. Soc. Exptl. Biol. Med.*, **105,** 197 (1960)
95. Holub, D. A., Jailer, J. W., Kitay, J. I., and Frantz, A. G., *J. Clin. Endocrinol. Metab.*, **19,** 1540 (1959)
96. Holub, D. A., Wallace, E. Z., and Jailer, J. W., *J. Clin. Endocrinol. Metab.*, **20,** 1294 (1960)
97. Hudson, B., and Evans, J., *J. Clin. Endocrinol.*, **22,** 494 (1962)
98. Humphrey, E. M., and Donaldson, L., *Am. J. Pathol.*, **17,** 767 (1942)
99. Jackson, H., *Pharmacol. Rev.*, **11,** 135 (1959)
100. Jackson, H., *Phar. J.*, **184,** 151 (1960)
101. Jackson, H., Fox, B. W., and Craig, A. W., *J. Reprod. Fertility*, **2,** 447 (1961)
102. Jenkins, J. S., Meakin, J. W., Nelson, D. H., and Thorn, G. W., *Science*, **128,** 478 (1958)
103. Kagawa, C. M., *Endocrinology*, **67,** 125 (1960)
104. Kagawa, C. M., Cella, J. A., and Van Arman, C. G., *Science*, **126,** 1015 (1957)
105. Kagawa, C. M., and Drill, V. A., *Arch. intern. pharmacodyn.*, **136,** 283 (1962)
106. Kagawa, C. M., and Jacobs, R. S., *Proc. Soc. Exptl. Biol. Med.*, **102,** 521 (1959)
107. Kagawa, C. M., and Van Arman, C. G., *Proc. Soc. Exptl. Biol. Med.*, **94,** 683 (1957)
108. Kehl, R., Audibert, A., Gage, C., and Amarger, J., *Compt. Rend. Soc. Biol.*, **150,** 2196 (1956)
109. Kehl, R., Czyra, J. C., and Becache, A., *Compt. Rend. Soc. Biol.*, **155,** 807 (1961)
110. Khan, M. Y., *Anat. Record*, **142,** 248 (1962)
111. Khazan, N., Adir, J. Pfeifer, Y., and Sulman, F. G., *Proc. Soc. Exptl. Biol. Med.*, **109,** 32 (1962)
112. Khazan, N., Sulman, F. G., and Winnik, H. Z., *Proc. Soc. Exptl. Biol. Med.*, **105,** 201 (1960)
113. Kistner, R. W., and Smith, O. W., *Fertility Sterility*, **12,** 121 (1961)
114. Kivikoski, A., Grönroos, M., and Kyöstilä, J., *Ann. Med. Exptl. et Biol. Fenniae (Helsinki)*, **40,** 8 (1962)
115. Korman, J., *Chem. Abstr.*, **54,** 1438 (1960)

116. Kovács, K., Kovács, G. S., Kovács B. M., and Petri, G., *Arch. Intern. Pharmacodyn.*, **109**, 1 (1957)
117. Krieger, D. T., *J. Clin. Endocrinol.*, **22**, 490 (1962)
118. Lacassagne, A., Duplan, J.-F., and Buu-Hoi, N. P., *Compt. Rend.*, **247**, 729 (1958)
119. Landau, R. L., and Lugibihl, K., *J. Clin. Endocrinol. Metab.*, **18**, 1237 (1958)
120. Laragh, J. H., Reilly, E. B., Stites, T. B., and Angers, M., *Federation Proc.*, **20**, 410 (1961)
121. Lauson, H. D., *Am. J. Med.*, **11**, 135 (1951)
122. Lednicer, D., Babcock, J. C., Lyster, S. C., Stucki, J. C., and Duncan, G. W., *Chem. Ind. (London)*, Dec. **23**, 2098 (1961)
123. Levy, H., *Program Meeting Endocrine Soc.*, 44th meeting, **39**, (1962)
124. Lewis, A. A. G., and Chalmers, T. M., *Clin. Sci.*, **10**, 137 (1951)
125. Liddle, G. W., *Metab., Clin. Exptl.*, **10**, 1021 (1961)
126. Liddle, G. W., Island, D., Lance, E. M., and Harris, A. P., *J. Clin. Endocrinol. and Metab.*, **18**, 906 (1958)
127. MacLeod, J., *Anat. Record*, **139**, 250 (1961)
128. Maickel, R. P., Westermann, E. O., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **134**, 167 (1961)
129. Markee, J. E., Everett, J. W., and Sawyer, C. H., in *Recent Progress in Hormone Research*, VII, 139 (Pincus, G., Ed., Academic Press, Inc., New York, 527 pp., 1952)
130. Marks, L. J., Doiron, J. C., and Oyama, H. T., *New Engl. J. Med.*, **266**, 965 (1962)
131. Marks, B. H., and Vernikos-Danellis, J., *Nature*, **195**, 85 (1962)
132. McCann, S. M., and Friedman, H. M., *Endocrinology*, **67**, 597 (1960)
133. Meakin, W., Tantongco, M. S., Crabbé, J., and Bayles, T. B., *Am. J. Med.*, **29**, 459 (1960)
134. Meier, R., Bruni, C., and Tripod, J., *Arch. Intern. Pharmacodyn.*, **104**, 137 (1955)
135. Meites, J., *Biochem. Pharmacol.*, **8**, 27 (1961)
136. Meites, J., and Hopkins, T. F., *Proc. Soc. Exptl. Biol. Med.*, **104**, 263 (1960)
137. Melby, J. C., St. Cyr, M., and Dale, S. L., *J. Lab. Clin. Med.* **56**, 927 (1960)
138. Melby, J. C., St. Cyr, M., and Dale, S. L., *New Engl. J. Med.*, **264**, 583 (1961)
139. Moore, W. W., *Am. J. Physiol.*, **200**, 1293 (1961)
140. Moore, D. J., Roscoe, R. T., Matson, L. J., and Heller, C. G., *Clin. Res.*, **10**, 88 (1962)
141. Moses, A. M., *Proc. Ann. Vet. Admin. Res. Conf.* 1962 (In press)
142. Moy R. H., *J. Lab. Clin. Med.*, **58**, 296 (1961)
143. Moyer, J. H., Kent B., Knight, R., Morris, G., Huggins, R., and Handley, C. A., *Am. J. Med. Sci.*, **227**, 283 (1954)
144. Munson, P. L., in *Ann. Rev. Pharmacol.*, 1, 315 (Cutting, W. C., Dreisbach, R. H., and Elliott, H. W., Eds., Ann. Rev., Inc., Palo Alto, Calfornia, 479 pp. 1961)
145. Munson, P. L., *Prog. Neuroendocrinol.*, Univ. of Illinois Press, Urbana, Illinois (In press)
146. Neher, R., Discussion of J. J. Chart paper in Proc. Intern. Congr. Hormonal Steroids, *1st meeting*, Milan, Italy (In press)
147. Nelson, A. H., and Woodward, G., *Arch. Pathol.*, **48**, 387 (1949)
148. Nelson, W. O., *J. Prosthetic Dentistry*, **11**, 382 (1961)
149. Nelson, W. O., *Fertility and Sterility*, **12**, 109 (1961)
150. Nelson, W. O., Gaunt, R., and Schweizer, M., *Endocrinology*, **33**, 325 (1943)
151. Nelson, W. O., and Patanelli, D. J., *Federation Proc.*, **20**, 418 (1961)
152. Nelson, W. O., and Patanelli D. J., *Federation Proc.* **21**, 437 (1962)
153. Nichols J., and Hennigar, G., *Exptl. Med. Surg.*, **15**, 310 (1957)
154. Nichols, J., Prestley, W. F., and Nichols, F., *Current Therap. Res.*, **3**, 266 (1961)
155. Nichols, J. and Richardson, A. W., *Proc. Soc. Exptl. Biol. Med.*, **104**, 539 (1960)
156. Nicoll, C. S., Talwalker, P. K. and Meites, J., *Am. J. Physiol.*, **198**, 1103 (1960)
157. Paget, G. E., Walpole, A. L., and Richardson, D. N., *Nature*, **192**, 1191 (1961)
158. Parrish, A. E., and Levine, E. H., *J. Lab. Clin. Med.*, **48**, 264 (1956)
159. Pasteels, J. L., *Ann. Endocrinol.*, **22**, 257 (1961)
160. Payne, R. W., and Whitsett, T. L.,

Proc. Soc. Exptl. Biol. Med., **106**, 189 (1961)
161. Pincus, G., and Merrill, A. P., in *Control of Ovulation*, **37** (Villee, C. A., Ed., Pergamon Press, Ltd., Oxford, England, 251 pp., 1961)
162. Plager, J. E., and Cushman, P., *J. Clin. Endocrinol.*, **22**, 147 (1962)
163. Polishuk, W. Z., and Kulscar, S., *J. Clin. Endocrinol. and Metab.*, **16**, 292 (1956)
164. Potts, G. O., Burnham, D. F., and Beyler, A. L., *Federation Proc.*, **21**, 436 (1962)
165. Psychoyos, A., *Compt. Rend. Soc. Biol.*, **152**, 1086 (1958)
166. Purshottam, N., *Am. J. Obstet. Gynecol.*, **83**, 1405 (1962)
167. Rennels, E. G., *Texas Rept. Biol. Med.*, **19**, 646 (1961)
168. Renzi, A. A., Chart, J. J., and Gaunt, R., *Toxicol. Appl. Pharmacol.*, **1**, 406 (1959)
169. Rosemberg, E., and Engle, I., *Endocrinology*, **69**, 496 (1961)
170. Rosenfeld, G., and Bascom, W. D., *J. Biol. Chem.*, **222**, 565 (1956)
171. Rudolph, J. W., Gilbreath, J. C., and Morrison, R. D., *Poultry Sci.*, **41**, 184 (1962)
172. Savage, O., Chapman, L., Copeman, W. S. C., Wells, M. V., and Treadwell, B. L. J., *Lancet*, **I**, 232 (1962)
173. Savage, O., and Treadwell, B. L. J., *Lancet* **I**, 911 (1962)
174. Savlov, E. D., *Surgery*, **45**, 229 (1959)
175. Sawyer, W. H., *Pharmacol. Rev.*, **13**, 225 (1961)
176. Sawyer, C. H., Critchlow, B. V., and Barraclough, C. A., *Endocrinology*, **57**, 345 (1955)
177. Schnieden, H., *Brit. J. Pharmacol.*, **15**, 510 (1960)
178. Segal, S. J., and Davidson, O., *Anat. Record*, **142**, 278 (1962)
179. Segal, S. J., and Nelson, W. O., *Proc. Soc. Exptl. Biol. Med.*, **98**, 431 (1958)
180. Segal, S. J., and Nelson, W. O., *Anat. Record*, **139**, 273 (1961)
181. Setnikar, I., Murmann, W., and Magistretti, M. J., *Endocrinology*, **67**, 511 (1960)
182. Shannon, J. A., *J. Exptl. Med.*, **76**, 387 (1942)
183. Sharma, D. C., *Program Meeting Endocrine Soc., 44th Meeting*, **38** (1962)
184. Shelesnyak, M. C., in *Recent Progress in Hormone Research*, XIII, 269 (Pincus, G., Ed., Academic Press, Inc., New York, 603 pp., 1957)
185. Sheppard, H., and Chart, J. J., *Biochem. Pharmacol.*, **8**, 128 (1961)
186. Smelik, P. G., and Sawyer, C. H., in *Ann. Rev. of Pharmacol.*, **2**, 313 (Cutting. W. C., Dreisbach, R. H. and Elliott, H. W., Eds., Ann. Rev., Inc., Palo Alto, California, 477 pp., 1962)
187. Soffer, L. J., Futterweit, W., and Salvaneschi, J., *J. Clin. Endocrinol. Metab.*, **21**, 1267 (1961)
188. Sonlairac, A., and Sonlairac, M. L. *Compt. Rend. Soc. Biol.*, **155**, 1010 (1961)
189. Southren, A. L., Weisenfeld, S., Laufer, A., and Goldner, M. G., *J. Clin. Endocrinol. and Metab.*, **21**, 201 (1961)
190. Spector, W. G., *J. Reprod. Fertility*, **2**, 362 (1961)
191. Stedmann, H., and Ströbele, P., *Archiv für Gynakol.*, **192**, 423 (1960)
192. Sulman, F. G., *Arch. Intern. Pharmacodyn.* **118**, 298 (1959)
193. Sulman, F. G., and Winnik, H. Z., *Lancet* **I**, 161 (1956)
194. Swingle, W. W., Fedor, E. J., Barlow, G., Jr., Collins, E. J., and Perlmutt, J., *Am. J. Physiol.*, **167**, 593 (1951)
195. Swingle W. W., Seay, P., Perlmutt, J., Collins, E. J., Barlow, G., Jr., and Fedor, E. J., *Am. J. Physiol.*, **167**, 586 (1951)
196. Swingle, W. W., Seay, P., Perlmutt, J., Collins, E. J., Fedor, E. J., and Barlow, G., Jr., *Am. J. Physiol.*, **167**, 599 (1951)
197. Symposium on Fertility-Controlling Steroids, *Federation Proc.*, **18**, 1039 (1959)
198. Szabo, G., Solti, F., Rev, J., and Megyesi, K., *Brit. Med. J.*, **1**, 865 (1957)
199. Szego, C., in *Recent Progress in the Endocrinology of Reproduction*, 331 (Lloyd, C. W., Ed., Academic Press, Inc., New York, 532 pp., 1959)
200. Szego, C. M., and Sloan, S. H., *Gen. Comparative Endocinol.*, **1**, 295 (1961)
201. Taliaferro, I., and Leone, L., *New Engl. J. Med.*, **257**, 855 (1951)
202. Talwalker, P. K., Nicoll, C. S., and Meites, J., *Endocrinol.*, **69**, 802 (1961)
203. Tiwari, N. M., Jindal, M. N., and Jaiswal, C. L., *Arch. Intern. Pharmacodyn.*, **128**, 383 (1960)

204. Tuchmann-Duplessis, H., *Compt. Rend.*, **242**, 1651 (1956)
205. Tuchmann-Duplessis, H., *Presse Med.*, **64**, 2189 (1956)
206. Tuchmann-Duplessis, M., Gabe, M., and Mercier-Parot, L., *Ann. Endocrinol.*, **23**, 65 (1962)
207. Tuchmann-Duplessis, H., and Mercier-Parot, L., *Compt. Rend.*, **243**, 410 (1956)
208. Tuchmann-Duplessis, H., and Mercier-Parot, L., *Compt. Rend. Soc. Biol.*, **152**, 29 (1958)
208a. Tuchmann-Duplessis. H., and Mercier-Parot, L., *Compt. Rend.*, **251**, 800 (1960)
209. Tuchmann-Duplessis, H., and Mercier-Parot, L., *Compt. Rend.*, **252**, 3882 (1961)
210. Tullner, W. M., *Endocrinology*, **66**, 470 (1960)
211. Tullner, W. W., *Excerpta Med.*, **51**, 36 (1962)
212. Tullner, W. W., and Hertz, R., *Endocrinology*, **66**, 494 (1960)
213. Tyler, E. T., Winer, J., Gotlib, M., Olson, H. J., and Nakabayashi, N., *Clin. Res.*, **10**, 119 (1962)
214. Uete, T., and Venning, E. H., *Endocrinology*, **67**, 62 (1960)
215. Ungar, F., Doe, R. P., and Moran, W. H., *Program Meeting Endocrine Soc., 44th Meeting*, **87** (1962)
216. van Dyke, H. B., in *Renal Function, Trans. of 2nd Conf., Josiah Macy, Jr., Foundation*, 48 (Bradley, S. E., Ed., Corlies, Macy and Co., Inc., New York, 178 pp., 1951)
217. Van Maanen, E. F., Greslin, J. G., Holtkamp, D. E., and King, W. M., *Federation Proc*, **20**, 419 (1961)
218. Velardo, J. T., *Fertility and Sterility*, **9**, 60 (1958)
219. Vial, S. U., Croxatto, H., and Barnafi, L., *J. Appl. Physiol.*, **11**, 227 (1957)
220. Vilar, O., and Tullner, W. W., *Endocrinology*, **65**, 80 (1959)
221. Wallace, E. Z., Silverstein, J. N., Villadolid, L. S., and Weisenfeld, S., *New Engl. J. Med.*, **265**, 1088 (1961)
222. Weil-Malherbe, H., Whitby, L. G., and Axelrod, J., *J. Neurochem.*, **8**, 55 (1961)
223. Wiebelhaus, V. D., Weinstock, J., Brennan, F. T., Sosnowski, G., and Larsen, T. J., *Federation Proc.*, **20**, 409 (1961)
224. Wohlzogen, F. X., *Acta Endocrinol.*, **37**, 298 (1961)

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