CHAPTER 15

Inhibitors of Steroid Actions and Cholesterol and Steroid Biosynthesis

R. I. Dorfman

I. INTRODUCTION

This chapter is concerned in part with those substances which interfere with the target organ action of steroid hormones. Also included is a consideration of some compounds which interfere with steroid hormone and cholesterol biosyntheses.

There is a reasonable possibility that inhibitors of biologically active steroids may be of real practical importance. Substances that interfere with the action of estrogens are considered to be of potential value in some forms of mammary cancer. Testosterone and 2α-methyldihydrotestosterone are known to be potent antiestrogens and effective agents in human mammary cancer. Similarly, it is believed that human prostatic cancer is stimulated by androgens and that antiandrogens may be effective drugs. Antiandrogens also may be valuable therapeutic agents in some forms of hirsutism and virilism in women, precocious puberty in boys, as well as effective therapy in acne. It is not impossible that antiandrogenic agents

could be of importance in the prevention and control of certain types of heart diseases. Anticorticoids could be effective agents in the treatment of certain types of Cushing's disease.

The association of hypercholesterolemia and atherosclerosis has been demonstrated in diabetes mellitus, in familial hypercholesterolemia, and familial xanthomatosis. It also has been reported that men with hypercholesterolemia who are neither obese nor hypertensive have a significantly higher chance to develop arteriosclerotic heart disease (*1*), while low serum cholesterol levels are usually associated with a low incidence of coronary heart disease (*2*). It is for these reasons that a lowered serum cholesterol level seems desirable. Since dietary restrictions frequently do not lower serum cholesterol, due to compensatory endogenous biosynthesis, it is particularly desirable to have an efficient nontoxic inhibitor of cholesterol formation. Some of these newer agents will be discussed.

II. INHIBITION OF ESTROGENIC ACTIVITY

Estrogenic activity in mammals is concerned with the stimulation of selected tissues pertaining to the reproductive tract in the female, including the Fallopian tubes, the uterus, and the vagina, as well as the mammary glands. In both males and females the estrogens have a highly specific effect on the formation and/or release of the gonadotropic hormones. In the fowl, oviduct stimulation by estrogens is well known. This section will deal with the various compounds that interfere with the estrogenic compounds on these target organs in the mammal and the fowl.

Inhibition of estrogen action can be effected by androgens (*3–8*), progestational substances (*3*, *6–13b*), certain estrogens (*14–16*), and corticoids (*5*, *13a*, *17–26*).

The substance MER-25, 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-*p*-methoxyethanol, is unique since it inhibits estrogens to the extent of 90–100% (*27*). The substance has a low uterotropic activity and antipituitary gonadotropin action. MER-25 is the best studied antiestrogenic substance, and it is effective in intact and castrated rats, mice, monkeys, chicks, and the rabbit. Lerner *et al.* (*27*) have further demonstrated that the compound is an effective inhibitor of steroidal and nonsteroidal estrogens.

A group of papers has appeared dealing with the antiestrogenic activity of certain synthetic steroids which show intense activity (*6*, *28–31*). Edgren *et al.* (*31*) reported that certain 17α-alkyl derivatives are extremely active, that the nature of the alkyl group has an important effect on the potency, and that the dihydro-19-nortestosterone as well as the $\Delta^{5(10)}$-isomers are much less active than the Δ^4-steroids.

Certain synthetic compounds such as (di-*p*-hydroxyphenyl)butane, -pentane, -hexane, and -1,4-pentanedien-3-one inhibit the proliferatioy and cornification in the vaginal epithelium of rats treated subcutaneousln with estradiol benzoate (*32*). Villee (*33*, *34*) has shown that certain antiestrogenic activity may be detected with an *in vitro* test employing the estrogen isocitric acid dehydrogenase reaction from human placental tissue.

A. *In vivo* Antiestrogen Studies

In vivo studies of antiestrogenic compounds have been described in rats, mice, and chicks, employing qualitative tests suitable to establish an approximate rank order of relative potency for a given set of compounds.

A rather wide range of compounds possessing this property has already been listed. In addition, it is known that folic acid is required for estrogen activity (*35–39*). No obvious interrelationship between the folic acid antagonists and the known antiestrogens of the steroid type is apparent.

Emmens and his co-workers (*40*, *41*) have indicated that substances such as testosterone and progesterone inhibit the action of estrogens with respect to their cornification action on the vaginal epithelium but fail to interfere with the mitosis caused by estrogens. On the other hand, three stilbestrol derivatives including dimethylstilbestrol, ethylstilbestrol, and *n*-propylstilbestrol, do interfere with the mitotic stimulation caused by estrogens when the agents are instilled in the vagina.

Methods, by gavage and injection, involving the spayed rat vaginal response have been described by Lerner *et al.* (*27*). Other methods employing the uterus have been more popular and include the spayed rat (*42*), the hypophysectomized rat (*13a*, *13b*), and the immature mouse (*6–8*).

TABLE I

RELATIVE ANTIESTROGENIC ACTIVITIES OF VARIOUS STEROIDS (INJECTION)[a]

Steroid	Total no. of mice	Dosage range studied (μg)	Minimum dose to produce inhibition (μg)	Maximum inhibition (%)
2α,17α-Dimethyl-17β-hydroxyandrostan-3-one	81	2–4000	2	30
Norethisterone	180	1–4000	16	56
Testosterone	99	64–4000	500	36
Progesterone	129	10–4000	500	56
Deoxycorticosterone	245	2–4000	1000	21

[a] Data of Dorfman *et al.* (*7*).

Typical data using the Dorfman *et al.* (*7*, *8*) method are presented in Table I. The inhibitory effect of five steroids, administered by injection, is illustrated and demonstrates particularly that antiestrogenic activity is not necessarily correlated with any of the more classic activities of steroids, since androgens, progestational agents, and a minerocorticoid show this action. The steroid 2α,17α-dimethyl-17β-hydroxyandrostan-3-one was highly active, since 2 μg produced a statistically significant effect. The maximum effects of these compounds up to a dose of 4 mg was somewhat better than 50%. Table II indicates that steroids of various physiological classes are antiestrogenic when administered by gavage.

TABLE II

RELATIVE ANTIESTROGENIC ACTIVITIES OF VARIOUS STEROIDS (GAVAGE)[a]

Various steroids	Total no. of mice	Dosage range studied (μg)	Minimum dose to produce inhibition (μg)	Maximum inhibition (%)
Norethisterone	238	2–1000	32	40
17-Methyltestosterone	270	4–2000	250	36
Deoxycorticosterone	118	10–4000	2000	30
Ethisterone	103	50–4000	4000	20

[a] Data of Dorfman *et al.* (*8*).

MER-25 will produce 100% inhibition of the action of 17β-estradiol in the spayed rat using the vaginal response as the end point (Table III, *27*).

Table IV summarizes some of the data of Dorfman *et al.* (*7*, *8*) where various steroids were studied for their antiestrogenic action by gavage and by injection. The antiestrogenic activity of all steroids, with one exception, was significantly greater when injected subcutaneously than by the oral route. Some of these differences are indeed striking. For example, 17β-hydroxyandrostan-3-one was 40 times more active by injection and 17α-methyl-19-nor-17β-hydroxyandrostan-3-one was 20 times more active by the same route. One exception was the case of 17α-methyl-19-nortestosterone which produced a minimum effect at 32 μg subcutaneously and a similar effect at 40 μg by gavage. This difference in dosage is not significant. This may also be true for 17α-ethynyl-19-nortestosterone, where the subcutaneous dose of 16 μg and gavage dose of 32 μg are perhaps not significantly different. On the other hand, the relative potency of 17-ethyl-19-nortestosterone by the two routes shows a ratio of 5 for subcutaneous to gavage administration efficiency.

TABLE III

THE INHIBITION OF 17β-ESTRADIOL VAGINAL STIMULATION BY MER–25 IN THE SPAYED RAT (INJECTION)[a]

Total dose of MER–25 injected (mg)	Total dose of 17β-estradiol injected (μg)	No. of rats	Positive vaginal response (%)
0	0	10	0
0	0.6	10	100
0.04	0	10	0
0.2	0	10	0
1.0	0	10	0
0.04	0.6	10	90
0.2	0.6	10	10
1.0	0.6	10	0

[a] Adapted from Lerner *et al.* (*27*).

TABLE IV

THE COMPARATIVE ANTIESTROGENIC ACTIVITY OF VARIOUS STEROIDS BY SUBCUTANEOUS INJECTION

[Data by subcutaneous injection (SI) and by gavage (G)][a]

Steroid	Minimum dose to produce inhibition (μg)		Maximum inhibition (%)	
	SI	G	SI	G
17α-Ethyl-19-nortestosterone	8	40	54	43
17α-Ethynyl-19-nortestosterone	16	32	56	40
17α-Methyl-19-nortestosterone	32	40	46	44
17α-Methyltestosterone	32	250	35	35
17α-Methyl-19-nor-17β-hydroxyandrostan-3-one	50	1000	38	24
17β-Hydroxyandrostan-3-one	100	4000	30	24
17α-Ethyl-19-nor-17β-hydroxyandrostan-3-one	250		27	
Androstane-3β,17β-diol	250	1000	22	36
Testosterone	500	4000	36	10
19-Nor-17β-hydroxyandrostan-3-one	500	4000	32	36
19-Nortestosterone	500	1000	33	17
17α-Ethynyl-19-nor-17β-hydroxyandrostan-3-one	500	4000	27	12
17α-Vinyl-19-nor-17β-hydroxyandrostan-3-one	500	>10,000	20	
17-Ethynyltestosterone	500	>4000	22	
17α-Ethynyl-19-norandrostane-3β,17β-diol	1000	500	14	34
19-Norandrostane-3β,17β-diol	>4000	4000		20

[a] Data of Dorfman *et al.* (*8*).

The data listed in Tables V, VI, and VII are presented on the basis of the enhancing ratio, which is defined as the minimum dose of a compound required to produce a statistically significant decrease in uterine size in the immature mouse stimulated with a total dose of 0.4 μg estrone divided by the minimum dose of the modified steroid to produce the same significant inhibitory effect. For example, in Table V, 500 μg of testosterone were required to produce a significant inhibition, while the Δ^4-reduced steroid 17β-hydroxyandrostan-3-one produced this effect at 100 μg. The enhancing ratio, therefore, would be 500/100 = 5.

TABLE V

COMPARATIVE ENHANCING RATIOS AS A RESULT OF Δ^4-REDUCTION (5α) BY SUBCUTANEOUS INJECTION (SI) AND GAVAGE (G)[a]

Steroid	Enhancing ratio	
	SI	G
Testosterone	5	1.0
19-Nortestosterone	1	<1
17α-Methyltestosterone		0.13
17α-Ethyltestosterone		
17α-Methyl-19-nortestosterone	0.5	
17α-Ethynyl-19-nortestosterone	0.03	

[a] Data of Dorfman *et al.* (*8*).

TABLE VI

COMPARATIVE ENHANCING RATIOS AS A RESULT OF LOSS OF C–19 (FORMATION OF 19-NOR DERIVATIVE) BY SUBCUTANEOUS INJECTION (SI) AND GAVAGE (G)[a]

Steroid	Enhancing ratio	
	SI	G
Testosterone	0.25	4
17α-Methyltestosterone	1.0	6.5
17α-Ethyltestosterone	62	>100
17α-Ethynyltestosterone	31	40
Androstane-3β,17β-diol	0.03	0.25
17β-Hydroxyandrostan-3-one	0.2	1.0

[a] Data of Dorfman *et al.* (*8*).

TABLE VII

COMPARATIVE ENHANCING RATIOS AS A RESULT OF 17α-METHYL, 17α-ETHYL, AND 17α-ETHYNYL SUBSTITUTION STUDIED BY SUBCUTANEOUS INJECTION (SI) AND GAVAGE (G)[a]

Steroid	17α-Methyl		17α-Ethyl		17α-Ethynyl	
	SI	G	SI	G	SI	G
Testosterone	7.8	16	1	<1	1	1
19-Nortestosterone	15.6	25	62	25	31	31
19-Nor-17β-hydroxyandrostan-3-one	7.8	—	2	—	1.0	1.0
17β-Hydroxyandrostan-3-one	0.2	2	—	—	—	—
Δ⁵-Androstene-3β,17β-diol	>2	<0.25	—	—	—	—
19-Norandrostane-3β,17β-diol	>1	0.1	—	—	>4	2.0

[a] Data of Dorfman *et al.* (*8*).

When enhancing ratios for certain structural modification were compared for the subcutaneous and gavage routes, interesting differences were observed. As indicated in Table V, the dihydro (5α) derivative of testosterone is equal to that of testosterone by gavage, but by subcutaneous injection the dihydro derivative was 5 times more active. Excellent agreement for the two routes is seen for structural changes involving loss of carbon 19 for 17α-ethynyltestosterone and 17α-ethyltestosterone. The enhancing ratios by the two routes were quite similar for these compounds (Table VI). This was not the case for the other four compounds and their corresponding 19-nor derivatives. In all other instances a significantly higher enhancing ratio was found by gavage. The ratios of the enhancing ratios were as high as 16 for testosterone in favor of gavage, and the smallest advantage was found for androstane-3β,17β-diol, which was as high as 5.

Essentially similar enhancing ratios for both the subcutaneous and oral routes were found for all the 17α-alkyl-substituted derivatives of 19-nortestosterone and for 17α-methyltestosterone (Table VII). These compounds can also be characterized by the high individual enhancing ratios per se. Contrariwise, no change could be detected for the 17α-ethynyl derivative of 19-nor-17β-hydroxyandrostan-3-one. Values of unity for both the injection and gavage studies were recorded. Striking differences between the oral and injection routes were found in a number of instances, with usually the higher enhancing ratio found for the injection route. Tenfold differences in favor of the injection route were observed for the 17α-methyl derivatives of the following steroids: 17β-hydroxyandrostan-3-one, Δ⁵-androstene-3β,17β-diol, and 19-norandrostane-3β,17β-diol. A similar but less intense effect was recorded for 19-norandrostane-3β,17β-diol and its 17α-ethynyl derivative.

The relative antiestrogenic potencies of compounds reported by Dorfman *et al.* (*7*) and by Edgren *et al.* (*43*) are reported in Table VIII. All results have been referred to the same standard, progesterone. The results reported by Dorfman *et al.* (*7*) are consistently greater, by a factor of 2–5 times, than found by Edgren *et al.* (*43*). The reason for the discrepancy is not immediately apparent. It should be mentioned, however, that Edgren used a single oil solution for both the stimulating estrogen and the inhibitor, and the concentration of estrone was 0.3 μg. Estrone in an oil vehicle (total dose 0.4 μg) and the inhibitor were administered simultaneously at a different site in the Dorfman *et al.* study. The inhibitor was contained in an aqueous suspension. The strains of mice used in the two laboratories were also different.

TABLE VIII

COMPARATIVE RELATIVE ANTIESTROGEN POTENCY OF VARIOUS STEROIDS REPORTED BY TWO DIFFERENT LABORATORIES

Steroid	Edgren *et al.* (*43*)	Dorfman *et al.* (*7*)
Progesterone	1.0	1.0
19-Nortestosterone	0.4	1.0
17α-Methyl-19-nortestosterone	8.8	15.9
17α-Ethyl-19-nortestosterone	12.5	62.5
17α-Ethynyl-19-nortestosterone	8.0	31.3

B. *In vitro* Antiestrogen Tests

The results obtained with the *in vitro* method of Villee and Hagerman (*44*), using the placental dehydrogenase system, are not necessarily correlated with the *in vivo* studies. Estriol is an example of an estrogen in the classic sense; that is, it causes stimulation of female sex structures, which in the intact animal can also suppress the action of a more active estrogen such as 17β-estradiol. The *in vitro* enzyme studies show the same properties (Table IX). However, other substances, such as progesterone and cortisone, which are classified as antiestrogens on the basis of *in vivo* studies, neither stimulate the placental isocitric acid dehydrogenase system nor inhibit the action of 17β-estradiol.

TABLE IX

INFLUENCE OF ESTRIOL AND 17β-ESTRADIOL ALONE AND IN COMBINATION ON THE PLACENTAL ISOCITRIC DEHYDROGENASE SYSTEM[a]

Concentration of steroid added (μg/ml)	α-Ketoglutaric acid produced[b] per mg N/hour
Estriol alone	
0	0.22
0.33	0.29
3.3	0.34
33.0	0.38
Estriol plus 0.1 μg/ml 17β-estradiol	
0	0.42
0.33	0.41
3.3	0.38
33.0	0.35
Estriol plus 1.0 μg/ml 17β-estradiol	
0	0.47
0.33	0.47
3.3	0.44
33.0	0.40

[a] Villee and Hagermann (*44*).
[b] Mean of 8 determinations.

The assay methods developed for the determination of antiestrogenic action of steroidal and nonsteroidal compounds have not been developed to the point of desired precision, nor is there adequate information as to the meaning of the inhibition observed, except that in all the tests described it is believed but not proved that the effect is at the peripheral level. It should be emphasized that other alternatives are possible; for example, an antiestrogenic effect could be observed if the test compound produces a change in the effective concentration of the estrogen in the blood. More specifically, the shift of free "active" estrogen to a bound "inactive" form could be interpreted as an antiestrogenic effect. Similar results may be obtained if the test compound increases the rate of estrogen inactivation. Additional studies are indeed needed to answer this question more satisfactorily.

III. INHIBITION OF ANDROGENIC ACTIVITY

Antiandrogenic activity has been assessed primarily on the capon or chick comb with only a limited number of studies concerned with mammalian indicators. The methods have not been developed to quantitative precision, but at least one chick comb method is reasonably consistent. This method (*45*) involves the use of one- to three-day-old male or female white Leghorn chicks which are stimulated with testosterone enanthate by injection on the first day of the assay. The test compounds are applied to the comb. Typical results are indicated in Table X, using norethisterone and Ro 2–7239 (2-acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene). Significant reductions in comb ratios were found for both compounds, and, since the untreated comb ratio is of the order of 0.4, the inhibition by Ro 2–7239 was of the order of 50%.

TABLE X

THE INHIBITORY EFFECTS OF NORETHISTERONE AND 2-ACETYL-7-OXO-1,2,3,4,4a,4b,5,6,7,9,10,10a-DODECAHYDROPHENANTHRENE (RO 2-7239) INUNCTED ON THE COMBS OF TESTOSTERONE ENANTHATE-STIMULATED (INJECTION)[a] CHICKS[b]

Compound inuncted	Total dose (mg)	No. of chicks	Mean comb ratio[c] ± SE
None	0	24	1.08±0.07
Norethisterone	0.25	22	0.93±0.04
	0.5	21	0.92±0.04
	1.0	23	0.85±0.04
Ro 2-7239	0.1	17	0.97±0.034
	0.2	17	0.92±0.04
	0.4	17	0.73±0.06
	1.6	13	0.74±0.04

[a] All chicks received 0.5 mg of testosterone enanthate on the first day.
[b] Data of Dorfman (*45*).
[c] Comb ratio equals comb weight in mg/g of body weight.

Methods for the detection of compounds with antiandrogenic activity in the rat have been published by Randall and Selitto (*46*), Dorfman and Stevens (*47*), and Lerner *et al.* (*48*). These methods employ the testosterone-stimulated castrated rat, and the target organs studied were the seminal vesicles, prostate, and levator ani. Only two compounds, Ro 2–7239 (Table XI) and A-norprogesterone (Table XII) are known to produce consistent antiandrogenic activity under these experimental conditions.

TABLE XI

THE INHIBITION OF THE ACTION OF TESTOSTERONE BY RO 2-7239[a]

Testosterone injected (mg)	Ro 2-7239 injected (mg)	No. of rats	Mean Tissue Ratio ± SE		
			Seminal vesicle	Prostate	Levator ani
0	0	7	0.05±0.005	0.07±0.007	0.24±0.031
2	0	7	0.94±0.058	0.96±0.072	0.67±0.045
2	50	7	0.68±0.053	0.65±0.031	0.57±0.026

[a] Data of Dorfman and Stevens (*47*).

TABLE XII

THE ANTIANDROGEN ACTIVITY OF A-NORPROGESTERONE IN THE CASTRATED RAT[a,b]

Total dose of A-Nor-progesterone (mg)	Total dose of testosterone propionate (μg)	No. of rats	Mean tissue wt in MG ± SE		
			Seminal plus vesicle coagulating gland	Ventral prostate	Levator ani
0	0	42	11.2±0.3	11.3±0.3	21.6±0.7
0	175	42	52.7±1.7	58.1±1.8	31.2±0.9
7	0	6	11.1±0.7	11.6±0.8	22.3±2.3
35	0	4	8.6±0.2	12.6±1.5	18.9±1.4
7	175	6	38.6±3.6	36.2±1.7	29.0±2.0
35	175	4	24.6±1.2	31.9±1.7	20.3±1.3
175	175	3	13.1±0.5	13.1±2.3	17.3±1.6

[a] Seven-day assay.
[b] Data of Lerner *et al.* (*48*).

IV. INHIBITION OF CORTICOID ACTIVITY

Effective inhibitors of glucocorticoids have not been reported. The action of mineralocorticoids, however, can be inhibited. Kagawa *et al.* (*49*), using the adrenalectomized rat, and Liddle (*50*), in humans, showed that steroidal spirolactones are effective competitive inhibitors of deoxycorticosterone and aldosterone. Other studies have indicated that certain gonadal hormones have a similar action (*51*).

Concentrations of progesterone which do not influence sodium excretion in adrenalectomized rats when administered together with a sodium-retaining dose of deoxycorticosterone inhibit the action of the latter steroid. Specifically, 4 mg of progesterone and a ratio of progesterone to deoxycorticosterone of 666:1 and 160:1 produced 55% and 36% inhibition, respectively, of the deoxycorticosterone effect. When the ratios were decreased to 80:1, 40:1, and 20:1 at a 4-mg dose of progesterone, the blocking effect was not observed (*52*). These results confirm the studies of Kagawa *et al.* (*49*), who found that 1800 μg of progesterone could inhibit 12 μg of deoxycorticosterone to the extent of 50%. The mechanism of this inhibition has not been elucidated, but Rosemberg and Engel (*52*) have suggested that it may be due to inhibition of tubular reabsorption of sodium, to increased glomerular filtration rate, or to a combination of both.

V. INHIBITION OF STEROID BIOSYNTHESIS

A recurring theme in steroid biosynthesis is the hydroxylation reaction. The initial pathway to steroid hormones, cholesterol to pregnenolone, requires 20α-and 22ξ-hydroxylation (*53*, *54*). The route from pregnenolone to cortisol involves three different hydroxylation steps at carbon atoms 11β, 17α, and 21. An 18-hydroxylation reaction is obligatory in aldosterone formation, while 19-hydroxylation is related to estrogen biosynthesis from androgens (*55*). It is not surprising, therefore, that agents which inhibit hydroxylation reactions decrease over-all steroid hormone formation. The initial demonstration by Hertz *et al.* (*56*) that amphenone B produces, among other effects, an inhibition of adrenal cortical function was demonstrated in the rat. The report of Hertz *et al.* (*56*) contained a description of a preliminary experiment showing that amphenone added to a bovine adrenal perfusion caused almost total arrest of corticoid biosynthesis. This effect has been studied in detail and confirmed. In bovine perfusion studies hydroxylations at 11β, 17α, and 21 were significantly inhibited (*57*). Inhibition of androgen, estrogen, and corticoid biosynthesis in experimental animals and man are well documented (*58–63*).

Perhaps more interesting than amphenone is the related compound metopirone (Su–4885) which relatively specifically inhibits 11β-hydroxylase. This compound, when administered to humans or experimental animals, inhibits the formation of 11β-hydroxylated corticoids, such as aldosterone, corticosterone, and cortisol. As a consequence of decreased amounts of circulating cortisol, the blood ACTH concentration is elevated, causing in turn an excessive rate of adrenal biosynthesis of "unfinished" corticoids such as 11-deoxycortisol and deoxycorticosterone (*64*, *65*).

Metopirone is effective as an *in vitro* inhibitor. This effect has been observed in adrenal tissues from rats and guinea pigs (*66*). Similar studies by Roche *et al.* (*67*) using rat adrenal slices indicated reductions in corticosterone biosynthesis up to 50% of the controls.

A preliminary report by Chart and Shephard (*68*) announces two compounds which appear to be relatively specific as inhibitors of 17α-hydroxylase. The administration of Su–8000 [3-(chloro-3-methyl-2-indenyl)pyridine] and Su–9055 [3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)pyridine] to a dog caused the following changes in the steroids of the adrenal venous blood: increased corticosterone, decreased cortisol, and no change in deoxycorticosterone. The possible value of the compound in Cushing's disease is intriguing on the basis of exchange of the highly potent corticoid, cortisol, for the relatively inactive corticoid, corticosterone.

Decreased production of 17-ketosteroids and corticoids in adrenal hyperfunction results from the administration of *o,p'*-DDD (2-*o*-chlorophenyl-2-*p'*-chlorophenyl-1,1-dichloroethane) (*69*). These authors found an almost uniform suppression of steroid production by adrenal tumors, as well as objective regression of the tumors in 5 of 14 patients. Marked decreased production of corticoids resulted when dogs were treated with *o,p'*-DDD *in vivo*. These studies involved steroid analyses of adrenal venous blood (*70*). Specifically, the intravenous infusion of 5 mg/ml decreased the corticoid production from 7 to 0.3 μg per minute.

VI. INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Cholesterol biosynthesis may be blocked by a variety of compounds of diverse structures. Some of these inhibitors have been studied in reasonable detail and their locus of action in the biosynthetic sequence defined. Among the better-studied compounds is triparanol (MER-29, 1-[4-(diethylaminoethoxy)phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)-ethanol) which was synthesized by Palopoli *et al.* (*71, 72*). This compound inhibits cholesterol formation *in vivo* at reactions 11, 12, and 14 (Fig. 1). The inhibition of the reduction of the Δ^{24}-double bond results in accumulation of desmosterol (*73–77*). *In vitro* studies involving liver tissue triparanol produced major blocks somewhere between isopentenyl pyrophosphate and squalene, probably between lanosterol and zymosterol, and definitely between desmosterol and cholesterol (*95*).

Since steroid hormones are derived from cholesterol, it may be expected that an agent such as triparanol also influences steroid hormone biosynthesis. At a dosage of 750 mg/day in humans only a suggestive reduction in the rate of biosynthesis was observed (*78*). However, when rats

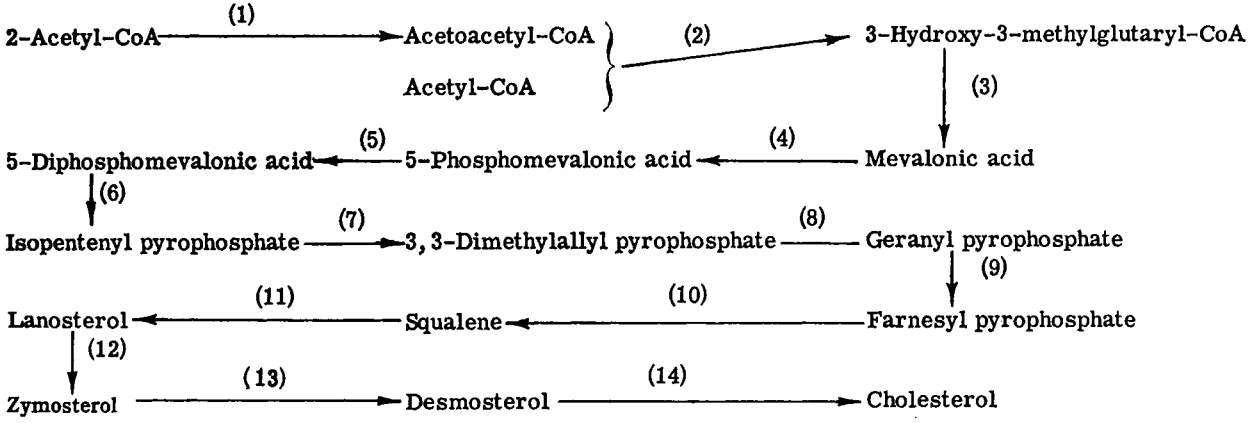

FIG. 1. Abbreviated representation of biosynthetic pathway of cholesterol from acetate.

were pretreated *in vivo* with triparanol, the adrenals showed a significantly decreased ability to synthesize corticosterone, deoxycorticosterone, and aldosterone. ACTH could not overcome this inhibition (*79*).

Feeding Δ^4-cholestenone at the level of 1% of the diet inhibits the synthesis of cholesterol and produces adrenal hypertrophy (*80, 81*). The feeding of Δ^4-cholestenone to dogs and chickens (*82*) and to man (*83*) lowers plasma cholesterol. The adrenal hypertrophy is accompanied by a decrease in adrenal venous corticoids (*84*). Treated rats had mean plasma corticosterone levels 43% lower than the controls, while the steroid production expressed per gram of tissue per minute was decreased 78%.

Singer *et al.* (*85*) found that Δ^1-dehydrotestololactone, Δ^4-androstene-17α-ol-3-one-17β-oic acid, and fluoromevalonic (β-hydroxy-β-fluoromethyl-δ-valerolactone) acid inhibited the conversion of acetate to cholesterol. The steroid acid and fluoromevalonic acid also were effective inhibitors of mevalonic acid to cholesterol. Fluoromevalonic acid was the most potent of these inhibitors. These studies were carried out using a rat liver homogenate according to the method of Bucher (*86*).

Schön (*87*) has suggested that nicotinic acid inhibits hepatic cholesterol biosynthesis in the rat due to a lack of acetyl coenzyme A, which is needed for both cholesterol and fatty acid biosynthesis. This interference was confirmed by Schade and Saltman (*88*) using rabbit liver slices from animals fed nicotinic acid. The authors conclude that inhibition of cholesterol biosynthesis is due to the limiting amount of coenzyme A (CoA) in the liver cells since the CoA is used in the formation of the reaction

$$\text{Nicotinic acid} + \text{glycine} \xrightarrow[\text{ATP}]{\text{CoA}} \text{nicotinuric acid.}$$

The authors further point out that this hypothesis is consistent with the findings of Wagner-Jauregg (*89*), who demonstrated that acetylation of sulfanilamide by pigeon liver extract is also inhibited by nicotinic acid and other compounds requiring CoA for detoxification.

Benzmalecene, *N*-(1-methyl-2,3-di-*p*-chlorophenylpropyl)meleamic acid (α-isomer) is an efficient noncompetitive inhibitor of cholesterol biosynthesis (*90*). In a cell-free rat liver homogenate system this compound produced up to 100% inhibition of cholesterol biosynthesis when DL-mevalonic acid was employed. When added to the diet at the 0.4% level a significant drop in plasma cholesterol from 60.1 to 36.4 mg % was observed in rats.

Benzmalecene at dose levels of 500 to 1000 mg/day is an effective hypocholesteremic agent in man (*91*). At these dosage levels an average decrease of 18% (11.4–25.3%) was reported. However, there were de-

creases in serum alkaline phosphatase in 3 of 9 patients, and in 2 of this group eosinopenia was observed. The authors felt that doses of 0.5–1.0 gm may prove to be too toxic for clinical use.

The hypocholesteremic agent, β-diethylaminoethyl diphenylvalerate hydrochloride (SKF 525A) has been studied in dogs, mice, and monkeys (*92*) and in rats (*93*). In the dog, significant reduction in plasma total cholesterol and aortic cholesterol was a constant finding. After five months of treatment, marked fatty infiltration of liver was observed, which was rapidly reversed upon withdrawal of the compound. It has been suggested that the liver toxicity may be due to interference with the function of compounds such as coenzyme Q, which leads to fatty liver formation. The compound (SKF 525A) was also an effective inhibitor of mevalonate conversion to cholesterol when studied in a rat liver homogenate system *in vitro*. The inhibition was uncompetitive and did not interfere with decarboxylation of mevalonic acid. The inhibitor did prevent the conversion of C_5-alcohol pyrophosphates to nonsaponifiable lipids (*94*).

Holmes and DiTullio (*95*) summarize the *in vivo* action of SKF 525A as producing major blocks somewhere between isopentenyl pyrophate and squalene and at reaction 11 (Fig. 1), the cyclization of squalene. A minor inhibition was observed at reaction 12, lanosterol to zymosterol. *In vitro* studies have shown inhibitory action of SKF 525A at reactions 7 and 8.

The compound 2,2-diphenyl-1-(β-dimethylethylaminoethoxy)pentane (SKF 3301) inhibits cholesterol formation at a variety of sites, both *in vitro* and *in vivo* (*95*). A minor inhibitory action on reaction 12, a major inhibition of the cyclization of squalene and a major block between isopentenyl pyrophosphate and squalene, has been reported for SKF 3301 by *in vivo* methods. This inhibitor was effective at reaction 12 and somewhere between reactions 7 and 10 in the *in vitro* studies. By *in vitro* methods SKF 7732 [tris(2-dimethylaminoethyl)phosphate] and SKF 7997 [tris(2-diethylaminoethyl)phosphate] have been shown to produce a major block between lanosterol and zymosterol (*95*).

Beher and Baker (*96*) have studied the biosynthesis of cholesterol from C^{14}-labeled acetate and mevalonic acid in the presence of administered cholic acid in the rat. This bile acid retards cholesterol formation to the extent of 65% from acetate and 25% when mevalonic acid was used as the substrate.

Farnesoic acid (*97*, *98*) and related analogues of this acid (*99*) can inhibit the biosynthesis of cholesterol from mevalonate. Two of these analogues (3,7,11-trimethyldodecanoic acid and 3-hydroxy-3,7,11-trimethyldodeca-6,10-dienoic acid) were especially efficient inhibitors of mevalonic kinase, however, but not particularly effective in lowering liver and plasma cholesterol of mice (*100*).

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