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MODIFIERS OF STEROID-HORMONE METABOLISM: A REVIEW OF THEIR CHEMISTRY, BIOCHEMISTRY AND CLINICAL APPLICATIONS

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SUMMARY

The *in vitro* and *in vivo* methods are described that are used in the investigation of the effects of drugs on the biosynthetic pathways of steroid hormones from cholesterol. The sub-cellular locations of the enzymes involved in steroidogenesis are also summarized and the effects on their activity of the following compounds are explained: o,p'-DDD, Amphenone B, Metyrapone, SU-8000, SU-9055, SU-10'603, Doriden®, o-aminoglutethimide, Elipten®, SKF-525A, SK & F-12185, Ba-40'028 and cyanotrimethyl-androstenolone. Some attempts are made to correlate their histological effects on mitochondria and endoplasmic reticulum with their inhibitory action on enzymes that are associated with these sub-cellular sites. The evidence for the binding of Metyrapone to cytochrome P-450, involved in the action of the "mixed function oxidases", is reviewed. Finally, the uses of the various inhibitors of steroid hormone biosynthesis in patients suffering from endocrine diseases are assessed.

It is now nearly a quarter of a century since Nelson and Woodard [1] reported the action of the insecticide DDD on adrenocortical function. Since that time, a large number of compounds have been synthesized and tested for activity by the pharmaceutical companies. Some of these drugs have been found to modify, to a greater or lesser degree, the biosynthesis of steroids, inhibiting or stimulating the activity of one or more of the enzyme-regulated steps of the biosynthetic pathways. Some, like o,p'-DDD, have been found to have undesirable side-effects and are now little used in clinical practice; some, like aminoglutethimide (Elipten®), are still under clinical investigation while others, like Metyrapone, are employed routinely by the endocrinologist in assessing pituitary function in patients. It is the purpose of this Review to draw together current knowledge of the chemistry, biochemistry, possible mode of action and clinical uses of some of these substances. The properties of triparanol (MER 29) and AY 9944, drugs that inhibit cholesterol biosynthesis, are not discussed. These have been reviewed earlier [2–4].

I. *Structure of modifiers*

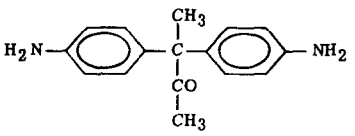
The compounds to be described here can be classified under four headings: (a) compounds of the diphenylmethane series such as the various isomers of DDD and Amphenone B. (b) pyridine derivatives such as drugs of the SU series, notably SU-4885 or Metyrapone (Metopiron®), SU-5482, SU-8000, SU-9055 and SU-10'603. (c) the α,α'-disubstituted glutaric acid imides such as glutethimide (Doriden®), *o*-aminoglutethide (SU-19120A) and *p*-aminoglutethimide (Elipten®). (d) triazines, hydrazides and thiosemi-carbazones such as drugs of the SKF- and Ba- series. The structures and names of these compounds are shown in Fig. 1.

II. *Methods of investigation of steroid metabolism*

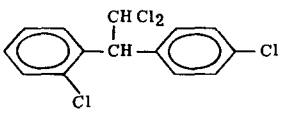
Before proceeding to a detailed description of the effects of drugs on steroid metabolism, it is necessary first to describe current views of the various biosynthetic pathways and the methods that are used to study them.

(a) In vivo *methods.* In early studies simple experiments were designed in which the drug under consideration was administered to groups of experimental animals in varying doses and the total urinary 17-oxosteroids (17-OS) and/or 17-hydroxycorticosteroids (17-OHCS) were measured. Later experiments have involved the fractionation of urine and plasma of animals and human subjects treated with various drugs so

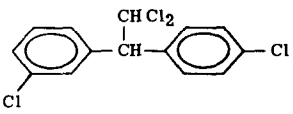
a. Diphenylmethane series



Amphenone B

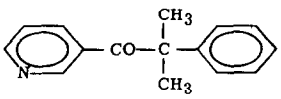


o, p' - DDD

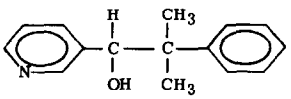


m, p' - DDD

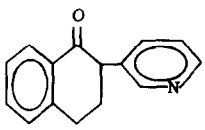
b. Pyridine derivatives



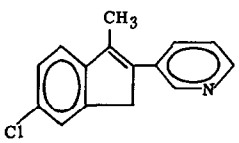
SU - 4885 (Metyrapone)



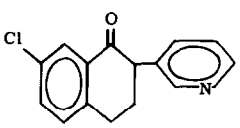
Reduced Metyrapone



SU - 9055

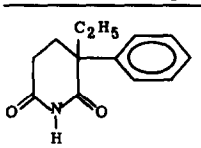


SU - 8000

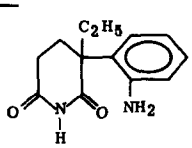


SU - 10'603

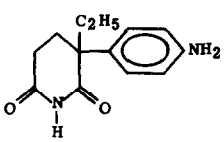
c. α, α' - disubstituted glutarimides



Glutethimide
("Doriden")

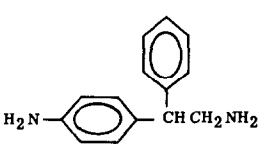


o-aminoglutethimide
(SU - 19120A)

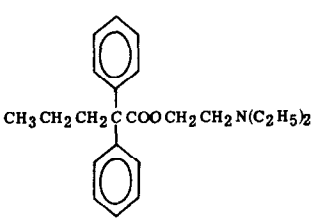


p-aminoglutethimide
("Elipten")

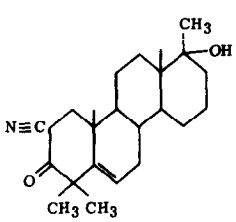
d. Miscellaneous



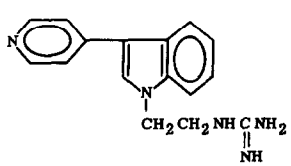
SK & F - 12185



SKF - 525A



Cyanotrimethylandrostenolone



Ba - 40'028

Fig. 1. Structures of some modifiers of steroid hormone biosynthesis.

that the effect on individual steroids may be investigated. Of particular interest in this connection is the "profiling" method, utilized by Voellman [5], in which a large number of steroid metabolites, extracted from urine and converted to the trimethyl silyl ethers plus methoxime derivatives, can be separated by temperature-programmed gas–liquid chromatography (g.l.c.). Using this technique, the diminution (or otherwise) in excretion of a particular steroid can be seen in comparison with control urines. A modification of the "profiling" method has recently been useful in attempting to assess the effect of Elipten® in some breast cancer patients [6] and will be described in more detail later.

Information concerning steroid biosynthesis by specific organs such as the testis can be obtained by infusion of isotopically-labelled steroid precursors while the venous blood supply is sampled at timed intervals both during and after the infusion. To investigate the effects of a modifier, a similar infusion can be performed with the drug included in the infusion mixture. Alternatively, as in the case of Elipten®, experimental rats can be given varying doses of the drug and corticosterone secretion measured by sampling adrenal venous blood from an indwelling catheter [7].

Finally, the metabolism of a steroid (preferably labelled with radioactivity) may be studied by its intravenous administration, either as a single injection or by continuous infusion. Venous blood is then sampled at intervals and the urine collected for a period of at least 36 h. The metabolic clearance rate, half-life in the circulation and volume of distribution of the steroid concerned can be calculated using the methods reviewed by Tait [8]. Any changes in one or more of these parameters can then be measured following the administration of a drug, such as Elipten® [9].

(b) In vitro *methods.* The limitations of an *in vitro* method have been pointed out on numerous occasions [10] but nevertheless, some useful information can be derived from such studies. However, it must be realised that the drug added to the tissue incubation may not necessarily pass into the cells or sub-cellular fractions; consequently, higher concentrations may be needed to obtain an effect on the enzyme system concerned than are actually needed *in vivo.*

The methods used entail the preparation of tissue minces, slices or homogenates; sub-cellular fractions may be made from the latter. Various isotopically-labelled steroids are then incubated with the tissue fraction in the presence or absence of the modifier. After addition of carrier, unlabelled steroids, the mixtures of metabolites are separated, conveniently by chromatography (paper partition, column or thin-layer or a combination of these). This is followed by localization of radioactive zones (scanning, radioautography, etc.), derivative formation and purification to constant specific radioactivity [11]. Comparison of the yields of metabolites obtained from various precursors, incubated in the presence or absence of the modifier, will then give some indication of the biosynthetic site(s) that may be affected.

III. *Pathways of biosynthesis of steroid hormones*

Using a wealth of *in vivo* and *in vitro* techniques, the biosynthetic pathways of corticosteroids, androgens and oestrogens have been worked out. These have been the subjects of numerous reviews in recent years [4, 12–14] and will not be described in detail here. By a series of enzymically-controlled reactions, the parent compound, cholesterol is hydroxylated at C-20α and C-22 before being converted to pregnenolone and progesterone [15] (Fig. 2). These C_{21} steroids are then transformed in the adrenal cortex into the 17-oxygenated corticosteroids (Fig. 3). The two pathways of androgen synthesis from pregnenolone (a 5-ene-3β-hydroxysteroid) and progesterone (a 4-en-3-oxosteroid) occur largely in the testis, but to a limited extent in the adrenals and ovaries (Fig. 3). In the latter, oestrogen formation occurs from the C_{19} steroids androstenedione and testosterone *via* the 19-hydroxylated intermediates. The properties and sub-cellular location of most of the enzymes involved are well-known [4, 16, 17] and it is on one or more of the enzyme-catalysed steps that the drugs to be described have their effect.

IV. *Histological effects of modifiers*

(i) o,p′-DDD [2,2-bis(2-chlorophenyl-4-chlorophenyl)-1,1-dichloroethane]. The effects of this insecticide (Fig. 1) on adrenal histology and on steroid metabolism were described by Nelson and Woodard [1]. The commercial material was shown to cause severe cytotoxic adrenal atrophy in dogs [18]. Subsequent experiments [19] revealed that it was a contaminant of DDD (the o,p′-isomer) that was responsible for most of the effects on the adrenal while the p,p′-isomer was ineffective in this respect. More detailed studies, in which dogs were fed with o,p′-DDD (50 mg/kg) for periods of 3–35 days [20] showed that, after 3–5 days,

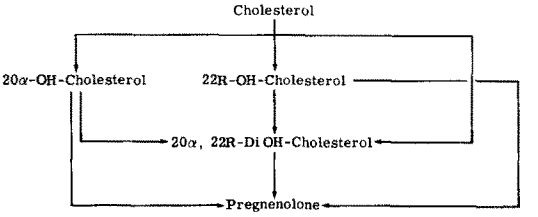


Fig. 2. Possible pathways of biosynthesis of pregnenolone (from [15]).

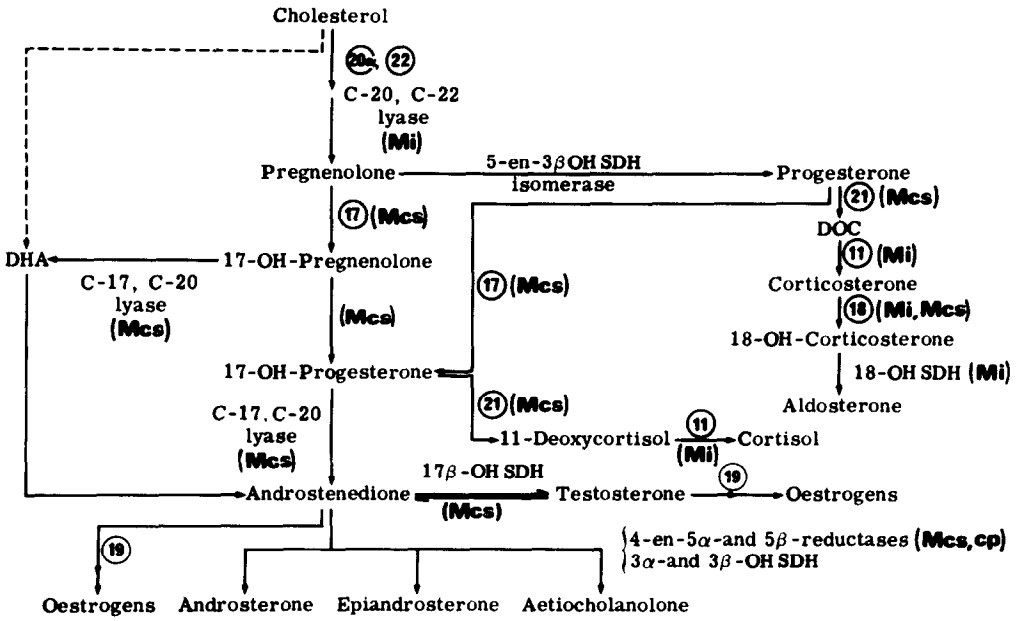


Fig. 3. Abbreviated scheme of biosynthetic pathways for corticosteroids, androgens and oestrogens. (17) represents position of hydroxylation; OH-SDH means hydroxysteroid dehydrogenase; Mi, mitochondria; Mcs, microsomes; cp, cytoplasm.

the cytoplasm of cells of the *zona fasiculata* and *zona reticularis* of the adrenal cortex became vacuolated due to the accumulation of lipid droplets. After 10 days, cells of the *zona reticularis* atrophied but at this time the *zona glomerulosa* was normal. However, on prolonged treatment (20 days), the cells of the *glomerulosa* showed moderate excess lipoid and eventually, after 35 days, all deeper zones of the cortex grossly atrophied and their stroma had condensed to a fibrous layer.

(ii) *Amphenone B.* The histological effects of Amphenone B [1,2-bis-(*p*-aminophenyl)-2-methyl propan-1-one], a drug first synthesized by Allen and Corwin [21], have been reviewed earlier [22]. In an extensive study of 24 patients (some with carcinoma of the breast, some with adrenal hyperplasia and other with thyrotoxicosis), Amphenone B was administered orally in dosages of 0·5–1·2 g/day for 4–22 days. At autopsy, the adrenal weights had increased two- to three-fold and the glands were a marked golden-yellow colour. Effects on the thyroid were also observed in that uptake of ^{131}I was inhibited and incorporation impaired. In similar studies using rats [22], the adrenals were found to contain three times their normal cholesterol content, the increased lipid being found in cells of the *zona fasiculata* and *reticularis*; the *glomerulosa* was unaffected. In the hypophysectomized animals, the effects on both the adrenals and the thyroid were abolished. In rabbits a marked progestational effect was noted [22], due presumably to an accumulation of progesterone which was not metabolized because of the inhibition of 17α-hydroxylation. In doses three times those required to produce endocrinological effects Amphenone B exhibits anaesthetic effects similar to Nembutal [22].

(iii) *Metyrapone.* The unpleasant side-effects of Amphenone B led to a search for a drug that retained the adrenocortical inhibitory effects yet without toxic side-effects. In 1959, Metyrapone was synthesized by Bencze and Allen [23] and studied by Chart *et al.* [24] in rats, dogs and rabbits *in vivo* and in adrenal preparations from rats, dogs and guinea-pigs. Although adrenal weights increased by 50% over controls, there was no atrophy of the adrenals, thyroid or liver. In rabbits at a dosage of 50 mg/kg/day, Metyrapone was not progestational nor was there any effect on the weight of seminal vesicles, testes or uterus in rats at 30–130 mg/kg orally or sub-cutaneously for 3–5 days. Due to its effect on (especially) corticosteroid biosynthesis (see later), this drug has found an established place in the investigations of pituitary reserve for ACTH secretion. A more recent study [25] in which male rats were injected with Metyrapone (20–50 mg/kg/day for 10 days) has shown that the volume of adrenal mitochondria was significantly less than in control animals, while the volume fraction of the endoplasmic reticulum (ER) and Golgi apparatus increased. Although the

intracellular lipid droplets seemed to become larger and more numerous in the treated animals, the differences were not found to be statistically significant.

(iv) *Elipten®*. This compound, one of a series of α,α'-disubstituted glutarimides [26], was used initially as a moderately successful anti-convulsant. In 1963, it became apparent that Elipten® had marked inhibitory effects on steroid biosynthesis. The literature on this aspect has expanded enormously during the past decade and has been reviewed by a number of writers [3, 27, 28].

(a) *Effect on adrenals.* The histological effects of Elipten® on human and animal adrenals were first described by Camacho *et al.* [29]. Children and adults were receiving 0·9–1·5 g/day of Elipten®, together with other anti-convulsant drugs. At autopsy, the weights of the adrenals were found to have increased markedly and there was loss of differentiation between the zona fasiculata and zona glomerulosa, the latter decreasing markedly in size. Moreover, many cells had become vacuolated. In the adrenals of rats used in the same study, there was an increase in the neutral lipid content (cholesterol or cholesterol ester as demonstrated by the Liebermann–Burchardt reaction); similar effects were noted in studies of the effects of Elipten® on patients with metastatic carcinoma of the breast who were awaiting adrenalectomy [30].

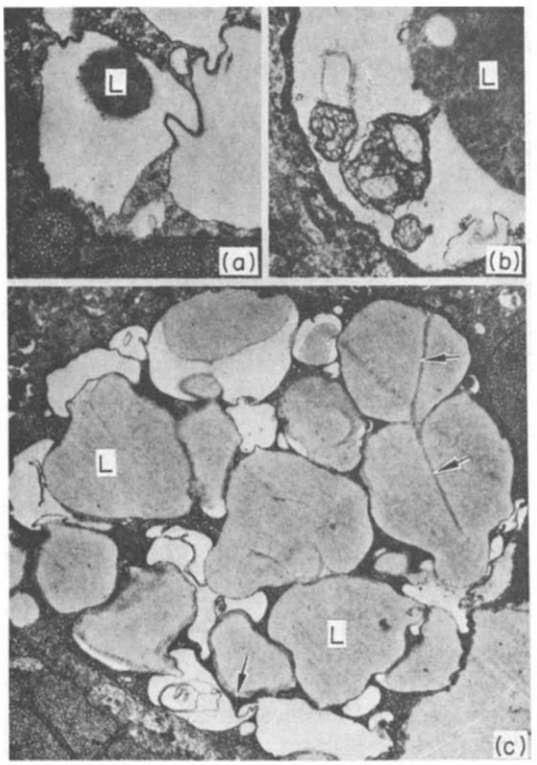


Fig. 4. Electronmicrograph of an adrenocortical section from a rat that had been treated with Elipten® (40 mg/day for 49 days). An accumulation of lipid can be seen inside mitochondrial vacuoles: (a) in the form of defined droplets; (b) in combination with reticular material; (c) shows the lipoid transformation of a mitochondrion: most vacuoles are filled with lipid; the grey stripes and borders (marked →) correspond to a periodic pattern. Magnifications (a) × 22,500; (b) × 17,200; and (c) × 14,000 (from [31]).

More recent light and electron microscopical studies have been published [31–34]. In the experiments by Marek *et al.* [31, 32], female albino rats were fed with Elipten® (30–40 mg/day) for periods of up to 49 days. In almost all the layers of the adrenal cortex, the mitochondria were enlarged with intra-mitochondrial membrane-bound cavities that were either empty or contained lipid material. After dissolution of the membranes, cytoplasmic lipid droplets (probably cholesterol or its esters) were visible (Fig. 4). The mitochondrial cavitation and hypertrophy of rat adrenals after Elipten® has also been described [35]. These results are particularly significant in view of the inhibitory effect of the drug on the conversion of cholesterol to pregnenolone, reactions that take place in mitochondria.

Further investigation of the adrenal microsomal fraction of rats fed with Elipten® for 14 days [32, 34] revealed that the smooth endoplasmic reticulum (S.E.R.) was almost exclusively tubular, in contrast to the normal vesicular or vesicular-tubular appearance (Fig. 5). After 49 days' treatment, the S.E.R. showed marked focal hyperplasia, some of the irregularly curved tubules themselves containing straight parallel tubules. These structures contained osmiophilic staining material. The significance of these structures is obscure but the effects of Elipten® on some steroid-transforming enzymes residing in the microsomal fraction of adrenal cells is now well-known (see section V).

(b) *Effect on ovaries.* Studies with rats [36] showed that administration of Elipten® stopped the oestrus cycle and caused sterility associated with follicular ovaries. This enlargement of ovaries by Elipten® had been reported previously [37]. In a similar study, Eversole and Thompson [38] showed that sub-cutaneous administration of 50–100 mg/day of Elipten® to immature female rats failed to alter ovarian weight although there was a modification in ovarian histology (markedly follicular). In adult female rats, however, the drug exhibited anti-fertility properties, suppressed ovulation and interfered with vaginal cycling. It also appears to reduce oestrogen secretion on long-term administration since uterine weight was decreased in intact, but not in castrate, animals and caused anomalous increase in body weight not seen in males, or in females after ovariectomy. It is presumed that these

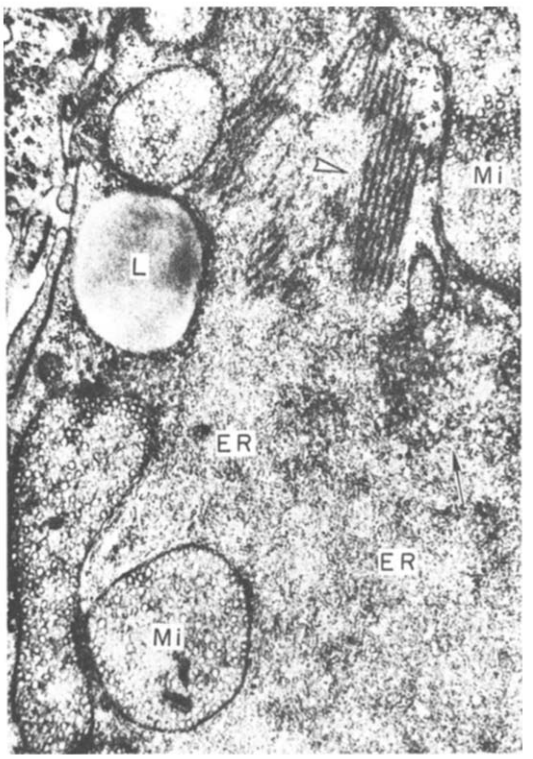


Fig. 5. Electronmicrograph of an adrenocortical section from a rat that had been treated with Elipten® (40 mg/day for 49 days). There is hypertrophy of smooth endoplasmic reticulum (ER), surrounded by mitochondria (Mi) and lipid droplets (L). Some areas contain moderate electron-dense material (→). At ▷, there are extended, parallel, ordered structures with strong osmiophilic content. Magnification × 32,000 (from [32]).

effects are due to the elimination by Elipten® of the growth depressing effect of oestrogen [3].

Cash *et al.* [39] have described the case of a 19-yr-old girl who had received Elipten® (1 g/day) for 6 yr together with other anticonvulsant drugs. During this period, there was gradual deepening of the voice and hirsutism. At laparotomy, the left ovary was found to be grossly enlarged and a histological section showed hypertrophy of luteal cells with neutral lipid (cholesterol and cholesterol esters) present in the *corpora lutea*. The large numbers of these present showed various stages of progressive involution.

(c) *Testis.* In 1968 Gaunt *et al.* [3] indicated that there was little evidence for a histological effect of Elipten® on the testis, and more recent investigations confirm this view. No changes were seen in germinal epithelium, interstitial cells or spermatic activity [40] of Elipten®-treated rats and Starka *et al.* [41] were likewise unable to detect any changes in testicular mitochondria or endoplasmic reticulum. However, some evidence is accumulating for some slight biochemical effects. The adrenal appears to be far more susceptible to the effects of the drug.

(d) *Prostate.* In a recent study [40] with rats that received 50 mg Elipten® twice daily for 4 weeks, the prostate weight decreased. There were no remarkable changes in histology except that secretory activity decreased, the glandular epithelium becoming flat and the interstitium scanty.

(e) *Thyroid.* It was partly on account of its goitrogenic activity that Elipten® was withdrawn from the market in the U.S.A. by the Food and Drugs Administration in February 1966; it was subsequently reinstated as a drug on clinical trial. Its anti-thyroid effects were first noted in children and subsequently studies using rats revealed that its action was similar to that of Amphenone B (see above) in that iodine uptake was diminished and thyroid weight markedly increased [37]. In hypophysectomized rats, however, these effects were not noticed. Later studies, reviewed by Hughes and Burley [27] showed that Elipten®, like Amphenone B, interferes with the incorporation of iodine.

(v) *Doriden®.* Single daily oral doses of 400 and 800 mg/kg in rats did not cause adrenal hypertrophy or cholesterol accumulation [42].

V. *Biochemical effects of modifiers*

(a) *Effects of o,p′-DDD.* Following the early studies with this drug showing that it caused adrenal atrophy, a number of workers provided evidence that it interfered with the production of corticosteroids [20, 43] and probably other major steroids (e.g. reference 44). However, the *in vitro* studies of Neher and Kahnt [45] revealed that o,p′- and o,m′-DDD suppressed the biosynthesis of some steroids in adrenal preparations only when used in very high concentrations; the production of corticosterone (involving 11β- and 21-hydroxylation) was unaffected. There is good evidence for an effect on the extra-adrenal metabolism of cortisol and on peripheral steroid transformation (Section VI).

(b) *Effects of Amphenone.* Early studies of the effects of this drug have been reviewed [22]. One effect of administration of Amphenone to dogs was a decrease in secretion of 17-hydroxycorticosteroids [46]. Trials with human subjects gave similar results in that Amphenone reduced plasma and urinary corticosteroids [47], although the inhibition of the synthesis of aldosterone was far more affected than that of other corticosteroids [48]. The excretion of 17-oxosteroids, however, was unaltered by Amphenone [49]. *In vitro*, the drug was shown to inhibit 11β-, 17, and 21-hydroxylation in perfused calf adrenals [50] and the

extensive investigations of Neher and Kahnt [51] confirmed these results. In addition, experiments with bovine adrenals *in vitro* [52] indicate that Amphenone may also interfere with the conversion of cholesterol to pregnenolone. A more recent study [53] has shown that Amphenone competitively inhibits rat testicular microsomal 17-hydroxylase and C-17, C-20 lyase.

Its effects on steroidogenesis in testicular microsomal preparations have been studied by Lynn and Brown [54] and the mechanism of its inhibition of 17α-hydroxylase activity has been elucidated. They showed that testis microsomes are surrounded by a layer of lipid which is essential for enzymic activity. Steroids are firmly bound to this layer but so also in Amphenone, thereby interfering with the contact of enzyme and substrate.

(c) *Effects of drugs of the SU-type* [(Metyrapone), SU-8000, SU-9055 *and* SU-10603]: *Metyrapone.* Early experiments with Metyrapone [24] showed that this drug markedly reduced the secretion of 17-hydroxycorticosteroids when administered (i.v.) to dogs at a concentration of 5 mg/kg. Experiments *in vitro* with adrenal slices taken from dogs, guinea-pigs and rats gave similar results, 50% inhibition of 17-hydroxycorticoid production being achieved with 50 mg Metyrapone/l. incubation fluid (0·095 M). Further studies both in man *in vivo* and in bovine and rat adrenal *in vitro* [55, 56] showed that Metyrapone was a relatively specific inhibitor of 11β-hydroxylase, an enzyme that resides in the mitochondria of all the zones of the adrenal cortex [57, 58]. Thus, the drug interfered with the final stage in the biosynthesis of cortisol from 11-deoxycortisol or of corticosterone from deoxycorticosterone (Fig. 3).

Although Metyrapone was originally thought to inhibit specifically 11β-hydroxylation, later studies showed that other steroid-transforming enzymes were also affected and Kahnt and Neher [59] were the first to show its inhibitory effect on 18- and 19-hydroxylation. In this brief review, however, it is not possible to mention the extensive literature on the effects of Metyrapone and other SU-type drugs; these have been described earlier [2, 51].

In summary, 17-hydroxylation [60], 18-hydroxylation (thereby reducing aldosterone biosynthesis) [59, 61, 62] and 19-hydroxylation [59, 63] are all inhibited by Metyrapone. The biosynthesis of aldosterone seems to be particularly affected, since, in both rats *in vivo* and in adrenal *zona glomerulosa* preparations, a dose of Metyrapone that had no effect on the synthesis of cortisol or corticosterone caused a considerable reduction in aldosterone production either from endogenous precursors or from added steroids *in vitro* such as progesterone, 11β-hydroxyprogesterone, DOC or corticosterone [64]. However, the drug failed to inhibit the side-chain cleavage of 17α-hydroxypregnenolone or 17-hydroxyprogesterone [51]. Of particular interest is the finding that Metyrapone causes an increase in the 21-hydroxylation of progesterone [65]. However, more recent results indicate a slight inhibition of the conversion of progesterone to DOC by duck adrenal microsomes [66].

In 1971, Bartova *et al.* [67] showed that Metyrapone (0·23 mM), added to rat adrenal glands *in vitro*, greatly reduced the synthesis of corticosterone both from endogenous sources and from exogenous DOC. However, it only slightly reduced glycolysis (lactic acid production), possibly because it caused only a slight reduction in tissue corticosterone (compare Elipten® below).

Other SU-type inhibitors

Intensive studies, particularly by Neher and Kahnt [51], have shown that other drugs such as SU-8000, 9055 and 10'603 also have profound effects on steroid biosynthetic pathways. They were all found to be inhibitors of steroid 17- and 18-hydroxylases [59, 61] but SU-9055 was particularly effective in suppressing aldosterone synthesis, presumably due to its effect on 18-hydroxysteroid dehydrogenase [61, 68]. In keeping with a block at the 18-hydroxylation of corticosterone, SU-8000, added to rat adrenal homogenates, produced a 50% drop in aldosterone production, a slight increase in corticosterone formation and a marked increase in DOC synthesis [69]. Other effects of SU- drugs on aldosterone biosynthesis have been reviewed [69, 70].

With testis tissue the inclusion of SU-8000 and SU-9055 in the incubation medium results in a depression of androgen synthesis [71, 72] through the inhibition of the side-chain cleavage enzyme and 17-hydroxylase [45]. 16α-Hydroxylation of progesterone is also markedly inhibited [73]. Shikita *et al.* [74] have also studied rat testicular microsomal 17-hydroxylase and the C-17, C-20 lyase and have shown that both SU-8000 and 10'603 competitively inhibited the 17-hydroxylation of progesterone, although the side-chain cleavage of 17-hydroxyprogesterone was inhibited non-competitively.

Recently, SU-10'603 has been shown to be an effective inhibitor of the 16α-hydroxylation of both pregnenolone and testosterone in rat and boar testis microsomal preparations [75]. The formation of testosterone and possibly also 17-hydroxypregnenolone were also markedly reduced, in keeping with the results [74] mentioned above. Lyne *et al.* [75] have also shown that SU-10'603 is an effective inhibitor (to the extent of 90–95%) of the enzyme that brings about the conversion of pregnenolone to androsta-5,16-dien-3β-ol and

Table 1. Effects *in vitro* of Elipten® on steroid-transforming enzymes

Tissue preparation	Substrates used	Concentration of drug (with % inhibition in parenthesis)	Reaction or specific enzyme inhibited	Ref.
Beef adrenal slices	Cholesterol	30 μg ml (25–50)	Cholesterol → pregnenolone (? 20α-hydroxylase)	[78]
Rat quartered adrenals	Cholesterol	$7{\cdot}6 \times 10^{-4}$ M (87)	Cholesterol → pregnenolone	
	Pregnenolone, Progesterone, DOC	$7{\cdot}6 \times 10^{-4}$ M (0)		[7]
Rat adrenal homogenate (acetone powders)	Cholesterol	$0{\cdot}76 \times 10^{-9}$ M (18)* $3{\cdot}8 \times 10^{-9}$ M (72) $7{\cdot}6 \times 10^{-9}$ M (80) 76×10^{-9} M (92)	C-20, C-22 lyase	[79]
Adrenal cell monolayers	Acetate, Pregnenolone, Progesterone	0·05 M	Cholesterol → pregnenolone	[86]
Rat and human adrenal homogenates (acetone powders)	Cholesterol	$15{\cdot}2 \times 10^{-6}$ M (66)	20α-Hydroxylase (competitive inhibition)	[80]
	20α-Hydroxy-cholesterol	$7{\cdot}6 \times 10^{-6}$ M (0)	No effect	
Bovine adrenal mitochondria (acetone powders)	Cholesterol	$0{\cdot}1 \times 10^{-3}$ M (40·2)	Cholesterol → pregnenolone	[87]
Murine adrenal cortex tumours (monolayer cultures)	Cholesterol	10 μg/ml	Various reactions	[82]
	Deoxycorticosterone	50 μg/ml	11β-Hydroxylase	
	Acetate	100 μg/ml	Various reactions	
Sheep adrenal homogenates	Corticosterone	$0{\cdot}5 \times 10^{-6}$ M (92) $1{\cdot}0 \times 10^{-6}$ M (97·4) $5{\cdot}0 \times 10^{-6}$ M (97)	18-Hydroxylase	[83]
Human placental microsomes	Androstenedione	$0{\cdot}1 \times 10^{-3}$ M (60)	Androstenedione → oestrone (aromatizing system)	[85]

* Percentage inhibition after 30 min incubation.

other 16-unsaturated C_{19} steroids in boar testis microsomes [76]. It may be that further studies of the effects of SU-10'603 may help to elucidate the mechanisms of formation of this group of steroids.

Elipten®

The effects of this drug on the biosynthesis of cholesterol from acetate in sheep adrenal cortex have been studied only recently [77] but much earlier than this, Kahnt and Neher [78] were the first to show that Elipten® inhibited the conversion of cholesterol to pregnenolone in bovine adrenal slices. This was confirmed shortly afterwards using rat and human adrenocortical preparations [7, 79]. It was shown that cholesterol accumulated in rat adrenal cortex in the presence of endogenous and exogenous ACTH but that the Elipten® had no effect on the conversion of pregnenolone to corticosterone. Since 1967, a number of workers have confirmed this finding (see Table 1); in particular, the experiments of Cohen [80] have shown that, although Elipten® had no effect on the conversion of 20α-hydroxycholesterol to pregnenolone, the conversion of cholesterol to pregnenolone was completely inhibited. This block of what is possibly the initial step in steroid biosynthesis (Fig. 2) would explain the accumulation of cholesterol in adrenal cells previously described. Furthermore, as the enzyme system involved is located in the mitochondria [81] the effect of the drug may be related to the ultrastructural changes produced in these sub-cellular organelles (see Section IVa). Further work *in vitro* has revealed that other steroid-transforming enzymes are also affected, notably 11β-, 18- and 21-hydroxylases [82, 84] and the aromatizing system involved in the conversion of androstenedione into oestrone [85].

The potency of Elipten® as a steroid biosynthesis inhibitor has been emphasized recently [88], for the drug almost completely inhibited the ACTH- and cyclic AMP-stimulated corticosterone production in rat adrenal preparations.

The effects of Elipten® on glycolysis (lactic acid production) in rat adrenals *in vitro* have been studied recently [67]. Glycolysis was stimulated by corticosterone or by steroids that could be converted to corticosterone, namely progesterone, 11β-hydroxyprogesterone and DOC. However, in the presence of Elipten®

(0·11 mM) glycolysis was reduced by 33% and the glycolytic response to ACTH was reduced by 63%. As anticipated, the steroidogenic response was completely blocked. These results again indicate an inhibitory effect on 11β- and 21-hydroxylation.

In contrast to the adrenal cortex, no definite inhibitory effect of Elipten® on testicular steroidogenesis has yet been described. Lyne *et al.* [75] have shown that cholesterol and pregnenolone were metabolized normally in rat testis homogenates, either in the presence or absence of the drug. The 16α-hydroxylation of both pregnenolone and testosterone was also unaffected but Elipten® may have some effect on the activity of the reductive enzymes involved in the deactivation of testosterone (Lyne and Gower, unpublished observations). Similar studies [41] also showed that the drug had no effect on the formation of progesterone, 17-hydroxyprogesterone, testosterone or androstenedione from cholesterol.

A number of workers [89, 90] have studied the effects of Elipten® on rat ovaries and have shown that it inhibited ovulation and interfered markedly with vaginal cycling. It also has marked effects on ovarian steroidogenesis and on cholesterol ester synthesis. Such esters are known to be hydrolysed to cholesterol which then gives rise to the steroid hormones. Luteinizing hormone and cyclic AMP inhibited the esterification of cholesterol in rabbit ovarian interstitial tissue [90] but Elipten® prevented this inhibition, the results being an accumulation of cholesterol

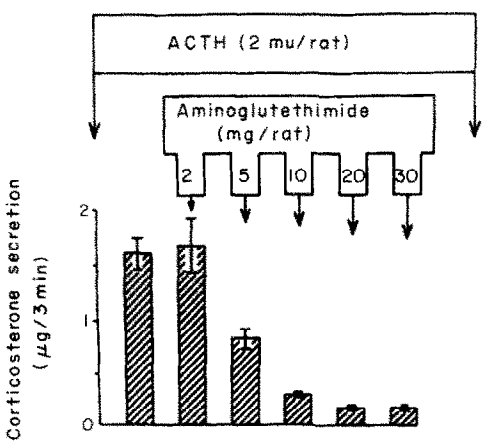


Fig. 6. Effect of Elipten® on the corticosterone secretory response to 2 mU ACTH in rats. A single dose of Elipten® (2 to 30 mg/rat) was injected subcutaneously and the corticosterone secretory response was measured 1 h later. Each column represents the mean adrenal venous corticosterone secretion (μg/adrenal/3 min) of a group of at least four animals. The vertical bars indicate one S.E. from the mean (from [7]).

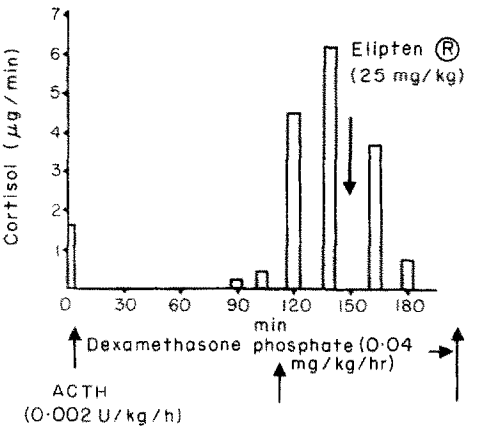


Fig. 7. Effect of Elipten® on cortisol secretion in dogs receiving ACTH by infusion. The animals also received a continuous infusion of dexamethasone phosphate as indicated. The average control cortisol secretion (1·68 μg/min) is shown at zero time (from [92] modified).

ester. In the same experiments, the cyclic AMP-stimulated biosynthesis of progesterone and 20α-hydroxyprogesterone from [^{14}C]-oleate was inhibited by Elipten®, thus causing an accumulation of cholesterol [90]. As both progesterone and 20α-hydroxyprogesterone were themselves shown to inhibit the formation of cholesterol ester, Flint *et al.* [90] suggest that the increases of ovarian cholesterol ester by Elipten® may be the results of the drug decreasing the concentrations of the two inhibiting steroids. These results are similar in some respects to the effects of Elipten® on the adrenal. Here, the ACTH-induced cholesterol ester depletion is inhibited [91] and the accumulation of adrenal cholesterol caused by Elipten® is well known (see Section IV). They also correlate well with the finding of large quantities of cholesterol ester in an ovary removed from a patient who had had long-term treatment with the drug [39].

Studies in vivo

The results of *in vivo* studies with Elipten® are in keeping with the *in vitro* effects described above. In rats, corticosterone secretion by the adrenals was unaffected by a dose of 2 mg Elipten®/day [7] but was progressively diminished by doses of up to 20 mg/day (Fig. 6). Numerous studies (Table 3) have confirmed this inhibition of corticosteroid secretion. That the adrenal cortex is the main site of action was indicated by experiments performed by Chart [92]. Dogs were given a continuous infusion of dexamethasone phosphate (0·04 mg/kg/h) to suppress endogenous ACTH production (Fig. 7) and, under these conditions, the adrenal cortisol secretion was considerably less than that found in

Table 2. Effects *in vitro* of Doriden®

Tissue preparation	Substrates used	Concentration of Doriden® (with % inhibition in parenthesis)	Reaction or specific enzyme inhibited	Ref.
Beef adrenals	Cholesterol	6 μg/ml (25–50)	Cholesterol → pregnenolone	[78]
Rat and human adrenal homogenate (acetone powders)	Cholesterol	$1{\cdot}5 \times 10^{-4}$ M (66)	20α-Hydroxylase	[80]
	20α-Hydroxycholesterol	$1{\cdot}5 \times 10^{-4}$ M (0)	No effect	
Rat adrenals	None	3×10^{-3} M (64)	Corticosterone production	[42]
	Pregnenolone, DOC, 11β-Hydroxyprogesterone	$1{\cdot}5 \times 10^{-3}$ M	11β-Hydroxylase and/or 21-hydroxylase, 21-Hydroxylase	

a group of control animals. ACTH was then infused continuously, the suppressive effect of the dexamethasone being overcome immediately and cortisol secretion increasing by 15- to 20-fold. Elipten® (25 mg/kg) was then administered i.v. and caused a marked diminution in cortisol secretion in spite of the continuing ACTH infusion. It was therefore concluded that the blockade of corticosteroidogenesis was at the adrenal level.

Other substituted glutarimides

Doriden®. It is of particular interest that this sedative, the parent compound from which Elipten® is derived (see Fig. 1), is much less active in inhibiting cholesterol side-chain cleavage and corticosteroidogenesis. This inhibiting effect was first shown by Kahnt and Neher [78] but it was 100 times less active than Elipten® in inhibiting the conversion of cholesterol to pregnenolone in acetone powders of human and rat adrenal homogenates [80]. Moreover, when 20α-hydroxycholesterol was incubated under the same conditions, in the presence of Elipten® or Doriden®, no inhibition of pregnenolone synthesis occurred, indicating that the enzyme affected was the 20α-hydroxylase. The work of Johnston *et al.* [42] has shown that Doriden® can also inhibit 11β- and 21-hydroxylases in rat adrenal glands *in vitro* but that a much higher concentration of the drug is required than the *p*-amino derivative, Elipten® (compare Tables 1 and 2).

Using dogs as experimental animals, Chart [92] has compared the effects of Elipten®, Doriden® and the ortho-amino derivative of glutethimide (SU-19120 A). Whereas Elipten® (25 mg/kg) reduced cortisol secretion to almost unmeasurable levels within 10 min, *o*-aminoglutethimide (at the same dosage) only reduced it by 46% and Doriden® had no effect. Only at a near lethal dose of 50 mg/kg did this compound have any effect on cortisol secretion. These results are amplified in Table 3.

It is beyond the scope of this Review to describe in detail the effects of all the existing modifiers of steroid hormone biosynthesis. However, three further types deserve brief mention:

(a) *Drugs of the SKF type.* SK & F-12185 [DL-2-(*p*-aminophenyl)-2-phenylethylamine] (Fig. 1) was first described by Saunders *et al.* [94] as an 11β-hydroxylase inhibitor in rat and guinea-pig adrenal preparations. It has been used subsequently by Gabrilove and collaborators [95–97] in the treatment of primary aldosteronism in Cushing's syndrome associated with non-tumourous adrenal hyperfunction and with adrenocortical carcinoma.

SKF-525A [2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride] was found to inhibit competitively rat testicular microsomal 17-hydroxylase and non-competitively the C-17, C-20 lyase [53].

(b) *Cyanotrimethylandrostenolone.* This drug is known to inhibit the activity of 3α- and 3β-hydroxysteroid dehydrogenases and the steroid isomerase [98, 99]. Due to its inhibition of the 3β-hydroxysteroid dehydrogenase, the drug has been administered to rats at different stages of pregnancy [100], thereby simulating experimentally congenital adrenal hyperplasia in man. Using these experimental "models", Goldman has succeeded in studying the condition in detail and of experimenting with different kinds of treatment. Further references to the use of cyanotrimethylandrostenolone have been reviewed [3].

(c) The intensive studies of Kahnt and Neher [101] on the effects of a large number of synthetic 3- and 4-substituted pyridines have led to a six-fold classification, depending on whether they selectively stimulate or inhibit corticosterone or cortisol biosynthesis or, alternatively, produce total stimulation or total inhibition. Working particularly with Ba-40'028 (Fig. 1) and

Table 3. Comparison of the effects of glutarimides on cortisol or corticosterone secretion rates

Experimental subject or animals	Glutarimide administered	Dose administered	Percentage inhibition of cortisol (dogs) or corticosterone (rats) secretion rates	Ref.
Healthy men	Doriden®	1 g/day for 7 days	No effect on adrenal steroids	[92]
Dogs	Doriden®	10 mg/kg	None	[92]
		25 mg/kg	None	
		50 mg/kg	Small degree	
	Elipten®	25 mg/kg	Approx. 66% in 10 min	
	o-Aminoglutethimide	25 mg/kg	Approx. 46% in 10 min	
Rats	Doriden®	6·6 mg/kg	None	[92]
		16·5 mg/kg	39·0	
	Elipten®	10·0 mg/kg	73·7	
		25·0 mg/kg	80·8	
	o-Aminoglutethimide	10·0 mg/kg	30·0	
		25·0 mg/kg	40·0	
Rats	Elipten®	20 mg twice daily	80 after 1 h; 25 after 10 h	[7]
Rats	Elipten®	200 mg/kg twice daily (4 days) and 300 mg/kg (3 days)	60	[42]

bovine adrenocortical preparations it was shown [101] that cortisol was formed from pregnenolone, progesterone and their 17-hydroxylated derivatives (Fig. 3). However, the biosynthesis of corticosterone was inhibited when pregnenolone and progesterone were used as substrates but not when 11-deoxycorticosterone was used. 11β-Hydroxylation was unaffected in all cases. These experiments indicated that Ba-40'028 inhibited the 21-hydroxylation of pregnenolone and progesterone but not of the corresponding 17-hydroxylated derivatives, and strongly suggests that there may be two different 21-hydroxylases, one for 17-deoxy C_{21} and the other for 17-hydroxylated C_{21} steroids.

Figure 8 summarizes the sites of action of some modifiers of steroid biosynthesis.

Possible mechanism of action of modifiers. Many of the lyases and hydroxylases involved in the pathways of formation of corticosteroids, androgens and oestrogens are now known to require cytochrome P-450 for activity, in addition to NADPH and oxygen. This cytochrome, a haemoprotein of the cytochrome b_5 type, is distinct from the cytochromes of the respiratory chain. It has been called cytochrome P-450 on account of the absorption peak which occurs at wavelength 450 nm after the cytochrome is reduced and combined with carbon monoxide. Under these conditions, the haemoprotein is unable to participate in steroidogenic reactions; thus evidence for its involvement can be obtained if (i) the reaction concerned is inhibited in the presence of carbon monoxide and (ii) if the inhibition can be relieved maximally in the presence of light of wavelength 450 nm [102, 103]. The following enzymes are known to be cytochrome P-450 dependent: the C-20α,22, C-17,20 and C-10,19 lyases and various hydroxylases 6α, 16α, 17, 20α, 21 and 22. In addition, the hepatic microsomal 7α- and 12α-hydroxylases, involved in bile acid biosynthesis, also require this cytochrome for activity. In mammals its presence has been in adrenal, testis, *corpus luteum*, placenta, liver and kidney (see [104]).

A considerable amount of research, actively pursued during the past few years, has helped to elucidate the role played by cytochrome P-450 in "mixed-function" oxidase reactions. Two types of the Fe^{3+}-cytochrome P-450 are now recognized—the "low spin" type and the "high spin" type [105]. Most of the cytochrome is in the "low spin" form in a tissue such as adrenal cortex but, as interaction with substrate takes place, conversion to the "high spin" type occurs. This new form of cytochrome P-450 (Fe^{3+})-substrate complex is characterized by a spectral change (see below). After a one-electron reduction resulting in the cytochrome P-450 (Fe^{2+})-substrate complex, atmospheric oxygen is thought to oxygenate this and give rise to a ternary complex (Fig. 9). Transfer of an atom of oxygen to the substrate occurs finally by a mechanism that is not completely elucidated.

As indicated above, addition of substrate to a suspension of microsomes containing cytochrome P-450 results in spectral changes, one of these (type I change)

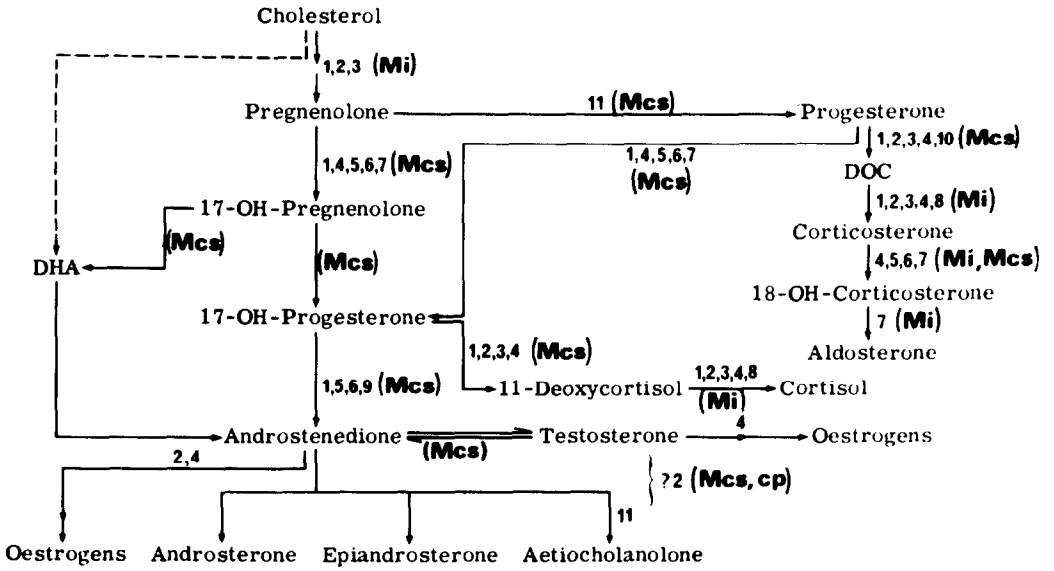


Fig. 8. Sites of action of some modifiers of steroid hormone biosynthesis (compare Fig. 3) 1. Amphenone B; 2. Elipten®; 3. Doriden®; 4. Metyrapone; 5. SU-8000; 6. SU-9055; 7. SU-10'603; 8. SK & F 12185; 9. SKF-525A; 10. Ba-40'028; 11. Cyanotrimethylandrostenolone.

being characterized by a decrease in absorbance at about 420 nm with an increase at 385 nm, and the other (type II change) characterized by an increase at about 430 nm and an associated decrease at about 400 nm [105]. Many steroids cause "type I" changes in optical absorption *viz*, cholesterol, and its 20α- and 22-hydroxylated derivatives, progesterone, DOC, corticosterone, cortisol. Pregnenolone, however, gives "type II" changes.

The mechanism of action of Metyrapone in inhibiting various hydroxylation reactions has been studied in great detail, and some of the work has been reviewed recently [106, 107]. Furthermore, the use of this drug has provided a useful tool in attempting to elucidate the mechanism of "mixed-function" oxidation reactions. There is no doubt that Metyrapone can bind to cytochrome P-450 producing a type II spectral change and interfering with interaction of the haemoprotein and its substrate [107]. The binding may occur at, or near, the haem group of the cytochrome, thus producing an altered ligand field. An absorption maximum of 442 nm occurs if cytochrome P-450 (Fe^{2+}) reacts with various pyridine bases as well as with Metyrapone [107] and, since the latter is itself a pyridine derivative (Fig. 1), this evidence may indicate that it is the nitrogenous part of the molecule that is involved in liganding to the iron ion. The dissociation constant for this [Metyrapone–cytochrome P-450 (Fe^{2+})] complex is $\sim 5 \times 10^{-5}$ M. This is much higher than that for the combination of the drug with the ferric form of the haemoprotein ($< 10^{-7}$ M) and furthermore, the reaction in this case produced chemically different species [107].

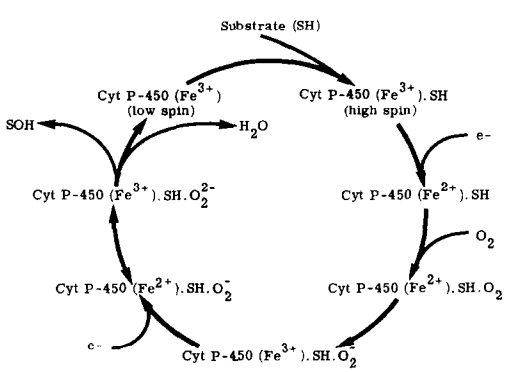


Fig. 9. Proposed scheme for the participation of cytochrome P-450 in steroid hydroxylations (modified from [104]).

The addition of SKF-525A to microsomal cytochrome P-450 results in a "type I" spectral change with an increase in absorbance at 386 nm and a decrease at 429 nm. When the cytochrome is treated with dithionite, however, the 429 nm peak disappears. So far as the inhibition of N-demethylation of ethyl morphine in liver microsomes is concerned, SKF-525A may well act as an alternative substrate, since the drug itself can be demethylated (Fig. 1). It is presumed that it inhibits enzymes involved in steroidogenesis by binding to

cytochrome P-450, but the mechanism remains to be elucidated.

There is now good evidence for the presence of cytochrome P-450 in testicular mitochondria [108] and microsomes [53, 108, 109]. Thus, it may be that drugs of the SU type (8000, 9055 and 10'603) inhibit steroid hydroxylation or side-chain cleavage by binding to cytochrome P-450. Figure 1 shows that the structures of these drugs have a pyridinium group in common, and it is conceivable that this may be responsible for an altered ligand field of the iron ion of the haemoprotein.

VI. *Effects of modifiers in patients suffering from endocrine diseases*

(a) *o,p'-DDD.* In some patients with adrenocortical carcinoma, the effect of o,p'-DDD (1–10 g, or even 20 g/day) was to markedly reduce the excretion of 17-hydroxycorticosteroids, 17-oxosteroids and, especially, 5β-pregnane-3α,17α,20α-triol (pregnanetriol) [110, 111]. Urinary pregnanetriol was affected even by doses of o,p'-DDD that were insufficient to cause a lowering of other steroid metabolites [111], indicating that this may provide a very sensitive indicator of the drug's action. An additional effect of o,p'-DDD appears to be the inhibition of the peripheral conversion of 5-en-3β-hydroxy-C_{21}-steroids to their 3α-hydroxypregnane analogues [112].

A study by Bledsoe *et al.* [113] revealed that, in Addisonian patients (maintained on cortisol) and in Cushing's patients, o,p'-DDD (6–9 g/day) produced a marked decrease in urinary 17-hydroxycorticosteroids that was related to an altered extra-adrenal metabolism of cortisol and not to a fall in plasma 17-OHCS nor to a lowered cortisol secretion rate. Of particular interest was the increase in the excretion of 6β-hydroxycortisol in these patients. Similar results were obtained by Southren *et al.* [114] who administered o,p'-DDD orally in doses of 7–10 g/day (total dose 140–146 g) to a woman with Cushing's syndrome, due to non-tumourous adrenocortical hyperfunction and to a bilaterally adrenalectomized, castrated woman (maintained on cortisol). It was concluded that the drug affected both the adrenal secretion and the extra-adrenal metabolism of cortisol but that, in the early stages of therapy, it was the latter effect that predominated. Like the patients of Bledsoe *et al.* [113], mentioned above, these patients also excreted large quantities of free corticosteroids, notably 6β-hydroxycortisol. In 1969, Temple *et al.* [115] treated four Cushing's patients with low doses (3 g/day) of o,p'-DDD and showed successful reduction of hypercortolism without aldosterone deficiency. In keeping with these results, the *zona glomerulosa* of one patient's adrenal was unaffected by the drug even though there were marked degenerative changes in the mitochondria of the *zona fasciculata.*

It is also conceivable that o,p'-DDD may interfere with the sulphoconjugation of 5-pregnene-3β,17α,20α-triol, as the excretion of this conjugate decreased to 25% of pre-therapy levels whereas the excretion of the same steroid conjugated as glucosiduronate remained unaffected [110].

In summary it seems that o,p'-DDD is helpful in treating cases of adrenocortical carcinoma [44, 116] and Cushing's disease [115]. Combined therapy of such cases with o,p'-DDD and Elipten® has also been utilized [117, 118]. The usefulness of o,p'-DDD, however, has been limited by its toxic side-effects. At a dosage of 6–10 g/day these include nausea, vomiting, intermittent diarrhoea, somnolence, lethargy and mental confusion [118].

(b) *Amphenone B.* The effects of this drug on normal subjects have been referred to earlier. Administration reduced plasma and urinary corticosteroids [47] but the inhibition of aldosterone synthesis was more marked [48]. The drug has been used in the treatment of patients with carcinoma of the breast [119], with adrenal hyperplasia [119] and adrenal carcinoma [120], but its severe side-effects have limited its usefulness. For example, in an extensive study of 24 patients [119], 18 became drowsy, 11 had gastrointestinal symptoms (nausea, etc.), while a few suffered from methaemoglobinaemia and impaired liver function.

(c) *Metyrapone.* The ability of this drug to inhibit 11β-hydroxylation at once pointed to a potential clinical use, because the lowered plasma cortisol would result in an increased secretion of ACTH from the anterior pituitary in an attempt to increase the plasma cortisol to normal values. The pituitary reserve of a patient could then be estimated simply if the plasma cortisol or corticosteroid metabolites were measured before and after the administration of a standard dose of Metyrapone (usually 750 or 1000 mg) [46, 121, 124]. Similar inhibition of steroid 11β-hydroxylation has been noted in adrenal carcinoma patients [126].

However, the ability of Metyrapone to inhibit the 11-hydroxylation of 11-deoxycortisol is greatly diminished in patients being treated with drugs such as diphenylhydantoin or phetharbital [126]. It is possible that the induction of hepatic microsomal enzyme activity by these drugs may result in a rapid metabolism of Metyrapone and an increased hepatic clearance thereby reducing the effect on 11-hydroxylation. Oestrogen therapy also reduced the effect of Metyrapone on this enzyme. Thus, in these situations, the interpretation of pituitary response to Metyrapone is questionable.

As a result of its great usefulness, the Metyrapone test has found an established place in routine clinical investigations. These have been reviewed earlier [2] and will not be detailed here. Since that time, the plasma and urinary 11-hydroxycorticosteroid response to Metyrapone in various disease states has been described, e.g. in pituitary, renal and hepatic disease [127]. The potent inhibition of 11β-hydroxylation by the drug has been strikingly demonstrated by the experiments of Goldman [100, 128] in which administration of Metyrapone to pregnant rats caused symptoms of congenital adrenal hyperplasia (CAH) of the foetuses. The CAH, produced experimentally in this way, has enabled Goldman and Winter [129] to make a close study of this condition.

Recently, with methods of measuring plasma ACTH levels becoming available, various workers have been able to determine the extent of the increase in plasma ACTH during the Metyrapone test [130–133]. Donald *et al.* [133] studied 20 patients who had a normal oxogenic steroid response and showed that the administration of the drug (1 g orally at 0800 h and 6 hourly thereafter for 48 h) resulted in a greater than three-fold increase in plasma ACTH (Fig. 10). The normal diurnal rhythm in ACTH release persisted despite administration of the drug.

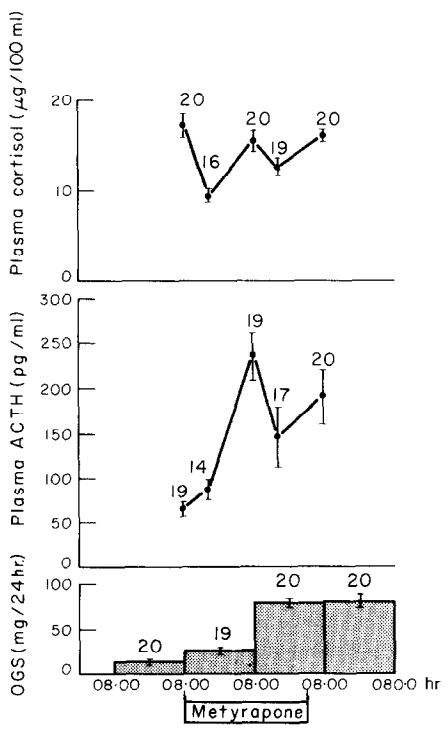


Fig. 10. Plasma cortisol, ACTH and urinary oxogenic steroid (OGS) excretion during the administration of Metyrapone (1 g 6-hourly for eight doses) in subjects with a normal urinary oxogenic steroid response. The vertical bars indicate one S.D. from the mean. The numbers refer to the number of individuals studied (from [133]).

In addition to reducing cortisol and corticosterone biosynthesis, Metyrapone reduces aldosterone production [134] and oestrogen excretion in normal women [135, 136]. These results are in keeping with *in vitro* work already described. A recent study [137] showed that Metyrapone greatly reduces the oestrogen-like (uterotropic) effect of DHA in pubertal rats with closed vaginae, although in prepubertal animals, the DHA effect was unaltered by the drug. It seems possible, therefore, that Metyrapone has an anti-oestrogen effect in pubertal rats.

(c) *Elipten®*. Although the side-effects of this drug, notably rashes, drowsiness, respiratory depression and ataxia, are severe, it has nevertheless been used extensively.

Effects on normal subjects. Fishman *et al.* [138] studied the effects of Elipten® (2 g/day for 3–4 days) on four normal subjects. The cortisol secretion rate and plasma 17-OHCS were virtually unaffected but there was concomitant seven-fold or more increase in plasma ACTH. This indicated that adrenal cortisol secretion had, in fact, been inhibited by Elipten®. Urinary 17-OHCS decreased as did aldosterone secretion, the latter being accompanied by a rise in urinary sodium and a fall in urinary potassium.

Initially, 17-OS excretion falls after Elipten® but later returns to the original values [84, 149]. The decrease involves primarily the 11-oxy-17OS, while androsterone and aetiocholanolone are either unaffected or slightly increased. A more detailed study [140] with ten subjects treated with 750 mg/day for 5 to 25 days, confirmed these results and also showed that there was a marked tendency for the 11-oxy- and 11-deoxy-17OS to increase at the end of the period of drug treatment (Fig. 11). It seems likely that the initial decrease in urinary 11-oxy-17OS results from a diminution in 11β-hydroxyandrostenedione production by the adrenals (11β-hydroxylase being inhibited by Elipten®), or from a decreased peripheral metabolism of corticosteroids.

When a patient with primary hypogonadism was treated with testosterone propionate there was a considerable increase in urinary 17-OS excretion; this was much less, however, when Elipten® was administered at the same time as the testosterone derivative. Thus, the drug interferes in some way with the peripheral metabolism of androgens [140, 141].

Effects in patients with adrenal tumours. A number of patients treated with Elipten® (1–1·5 g/day for periods of 15 days to 9 months) all showed a marked reduction

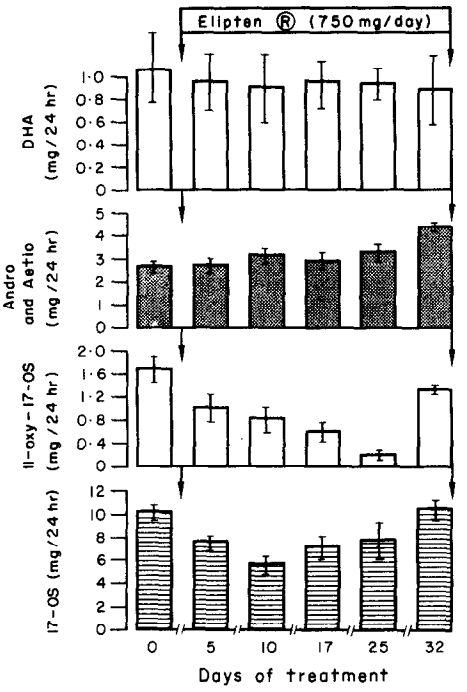


Fig. 11. Average values (±S.E.M.) of urinary total 17-oxosteroids, 11-oxygenated-17-oxosteroids, aetiocholanolone plus androsterone and DHA excretion in 10 patients before and during Elipten® administration (750 mg/day) (from [141]).

in cortisol secretion rates, urinary 17-OHCS and 17-OS, and Cushingoid symptoms disappeared [117, 118, 142–144]. In two cases [117, 118] o,p'-DDD was also administered (6–10 g/day) but Elipten® appeared more effective in causing clinical and biochemical adrenal insufficiency in as short a time as 5 days. On the other hand, o,p'-DDD caused the pulmonary metastases to shrink in the patient of Bochner *et al.* [118] but Elipten® had no effect on the cancer development itself (Fig. 12). In general, it appears that patients with adrenal carcinoma do reasonably well when treated with this drug [142].

In adrenal hypofunction. Four children with adrenal hypofunction, who were receiving Elipten® and other anti-convulsants, rapidly exhibited adrenal insufficiency, with serum electrolyte changes and corticosteroids of plasma and urine decreasing to low values [29].

Effects in Cushing's syndrome due to non-tumorous adrenal hyperplasia. Fishman *et al.* [138] studied nine Cushing's patients and concluded that Elipten® was of limited usefulness in pituitary-dependent Cushing's syndrome, although it was able to correct the hypercorticolism in seven of the nine patients, and was of modest therapeutic value in four patients over several months of treatment.

It appears that patients with adrenal hyperplasia do less well when treated with Elipten® than those with adrenal tumours. Only partial suppression of steroid secretion was achieved by the drug (even at dosage levels of 1·5 g/day) in five patients with adrenal hyperplasia [84, 143]. When treating a 59-yr-old woman, suffering from bilateral adrenal hyperplasia, with Elipten® (750 mg/day for 36 days), Faglia *et al.* [145] obtained evidence for an 11β-hydroxylase block (compare section V). Plasma and urinary 17-OHCS decreased as did urinary 11-oxy-17-OS. The urinary 11-deoxy-17-OS, however, showed only a slight reduction and recovered subsequently (compare the results obtained with normal individuals [140]). The fact that the urinary tetrahydro-11-deoxycortisol increased four-fold in response to Elipten® indicated that 11β-hydroxylation was being inhibited.

Effects in ectopic ACTH syndrome. The attempted control by o,p'-DDD of adrenal hyperfunction due to

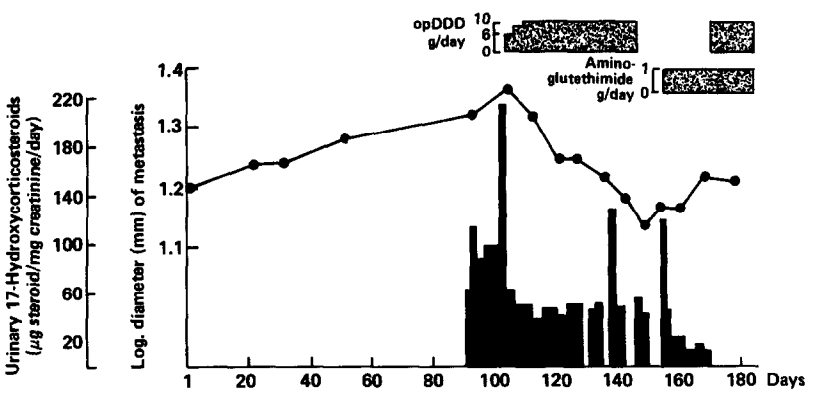


Fig. 12. Effects of o,p'-DDD and aminoglutethimide (Elipten®) on 17-hydroxycorticosteroid excretion and size of a pulmonary metastasis in a female patient (from [118]).

ACTH secreted by non-endocrine tumours has been alluded to earlier. However, the undesirable toxic effects of this drug [116, 119] indicated the use of Elipten® in the hope that it would correct the Cushingoid features (electrolyte balance disturbance, hypertension, etc.) characteristic of the ectopic ACTH syndrome. Often the patients are too ill for adrenalectomy and the primary ACTH-secreting tumour may be inoperable.

Two patients with ACTH-secreting tumours of the lungs and liver were treated with Elipten® (1 g/day for 1 day or 7 days) [146]. However, the stimulatory effect of the ACTH being secreted was not overcome by the limited period of therapy. Other workers have had more success. A patient of Gordon *et al.* [147] responded well to 1 g Elipten®/day for 15 weeks: urinary and plasma 17-OHCS decreased dramatically while electrolyte balance, diabetic tendencies and blood pressure were all corrected. Other workers [84, 139] have also studied the effect of Elipten® administration in the ectopic ACTH syndrome and have shown that, in general, although there was temporary inhibition of cortisol secretion and relief of Cushingoid symptoms, there was no effect on the tumour tissue.

A detailed study of a man who had an ACTH-secreting bronchial carcinoma has been published recently [148]. During 12 days of treatment (the dose of Elipten® being initially 1 g/day and gradually increased to 2 g/day), there was a marked improvement in the patient's condition. The plasma cortisol, urinary 17-OHCS, 17-OS and oestriol all decreased, although the excretion of testosterone was unaffected. Of the individual 11-deoxy-17-OS, androsterone and aetiocholanolone were greatly reduced, the former more so than the latter. This suggests that the drug had some selective inhibitory effect on the 4-ene-5α-reductase involved in androsterone formation from androstenedione. It is significant that the patient's condition deteriorated when the drug was withdrawn temporarily, and the plasma cortisol rose rapidly from 46 to 136 μg/100 ml. There was a further significant decrease when Elipten® (4 g/day) was administered subsequently.

Effects in patients with carcinoma of the breast. Hall *et al.* [149] treated 9 patients with Elipten® (1–3 g/day) together with dexamethasone and flurocortisone. There was no uniform suppression of urinary 17-OS, 17-OHCS or oestrogens, although three patients showed regression of tumour tissue for up to 9 months. In a similar study using prednisolone therapy followed by Elipten®, it was found that Elipten® caused a decrease in urinary 11-deoxy-17-OS and oestrogen excretion, except in a case with severe liver involvement where values rose [6]. Prolonged therapy resulted in a further reduction in urinary oestrogens but 11-deoxy-17-OS secretion was unaffected. The effect on plasma 11-OHCS was much more noticeable, the resting level decreasing markedly after Elipten® and the response to $\beta^{1\ 24}$-ACTH (Synacthen®) being abolished. Estimation of individual 11-deoxy-17OS [6] revealed that prednisolone therapy caused a marked decrease in urinary DHA while Elipten® therapy caused only a small decrease (Fig. 13). These findings, which are like those obtained earlier [140] with the urinary 17-OS of normal individuals, were obtained using a "profiling" method by g.l.c. Another preliminary Elipten® trial [167], published recently, has shown closely similar results to those described by Lyne *et al.* [6].

The reason for the "escape" of androgens and androgen metabolites is still unknown. However, there is some evidence [150, 151] that cholesterol may be converted directly to the C_{19} steroid, DHA, particularly in the ovaries and testes. If the inhibitory effects of Elipten® on this pathway are less marked than on the conversion of cholesterol to pregnenolone, then it would be possible for DHA and other C_{19} steroids to be synthesized *via* this route, even if Elipten® had caused a block in the usual biosynthetic pathways. It is noteworthy that one of the breast cancer patients in the group studied by Lyne *et al.* [6] has been treated with varying doses of prednisolone and Elipten® for some 4 yr and remains well.

Effects in patients with primary and secondary hyperaldosteronism. Elipten® has a profound effect on aldosterone biosynthesis; this is known to be reduced consistently [138, 139, 142, 152] irrespective of any increase in plasma renin. In three patients with primary and one with secondary hyperaldosteronism, the drug inhibited both cortisol and aldosterone secretion [138], the fall in the latter being accompanied by increased urinary sodium, a decrease in urinary potassium, a rise in plasma renin activity and a lowering of blood pressure, in hypertensive patients. Horky *et al.* [139] also noticed the diuretic effect of Elipten® which was useful in lowering the blood pressure of patients with hyperaldosteronism, secondary to idiopathic oedema and ascitic cirrhosis of the liver. In a more extensive study of 37 patients with secondary aldosteronism and oedema due to congestive heart failure, cirrhosis of the liver and idiopathic oedema [152], it was shown that Elipten® (0·75–1 g/day for 10 days) was used with advantage and was better in some ways than treatment with spironolactones. Thirty-two out of the 37 patients responded to therapy with marked sodium diuresis (secondary to decreased tubular reabsorption), a diminution in aldosterone secretion and an increased plasma renin.

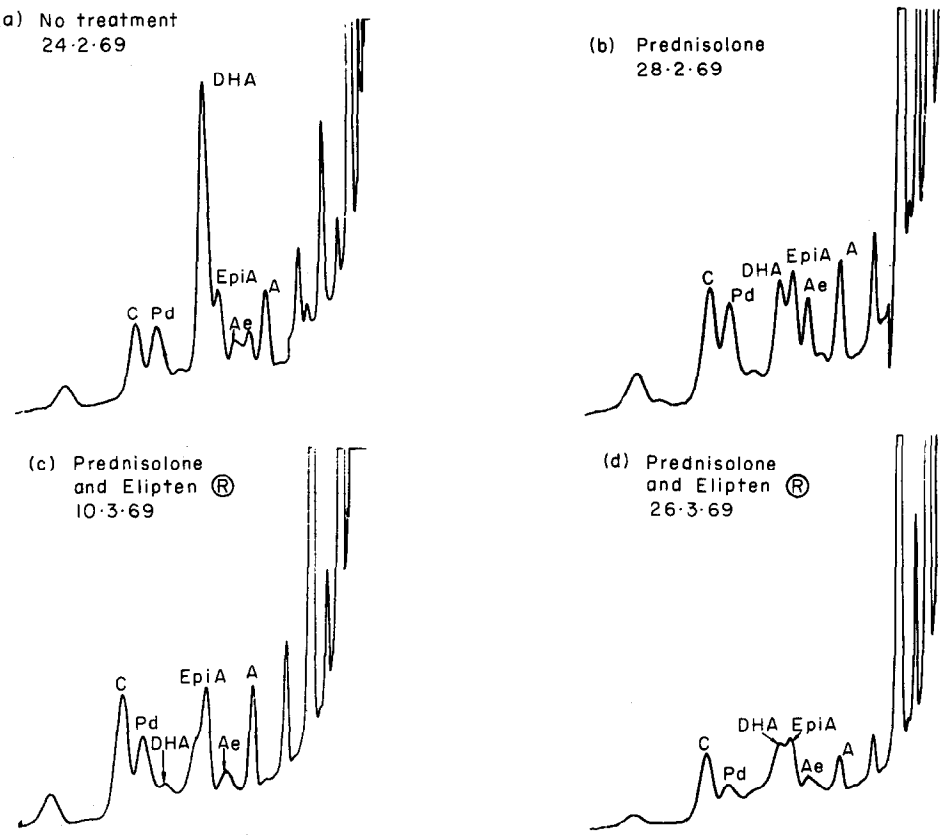


Fig. 13. Urinary steroid g.l.c. "profiles" of a patient with carcinoma of the breast before (a) and after (b) administration of prednisolone and both prednisolone and Elipten® (c,d). A, androsterone; Ae, aetiocholanolone; epi-A, epi-androsterone; Pd, pregnanediol; C, cholestane (internal standard). G.l.c. column maintained at 200°C (from Lyne & Gower, unpublished results).

Recent studies [153] have shown that, although Elipten® treatment caused a diminution in aldosterone secretion rates of normal subjects and hypertensive patients, the secretion rates of deoxycorticosterone (DOC) and of 18-hydroxyDOC were increased (in the case of DOC the increase was not consistent). Sodium diuresis was also noted at this time. When dexamethasone was administered together with Elipten® to suppress the compensatory ACTH production, the DOC and 18-hydroxyDOC secretion rates decreased to their initial values, with further sodium diuresis, although there was no consistent reduction in blood pressure in the hypertensive patients. The authors conclude that the drug may have a greater inhibitory effect on the aldosterone-producing *zona glomerulosa* of the adrenal, while the production of 18-hydroxyDOC and, in part, also of DOC, increases due to ACTH stimulation in the *zona fasciculata*.

In patients with essential hypertension, those 20 per cent who had suppressed plasma renin levels responded to Elipten® (750 mg/day) and prednisone (7·5 mg/day for 1 month) with significant decreases in blood pressure [154]. They differed from the 80 per cent of patients, also with essential hypertension but who had normal, or near-normal, plasma renin levels. It was concluded [154] that there might be large quantities of an unidentified mineralocorticoid present in the blood of the suppressed renin patients, or, alternatively, that even normal levels of aldosterone itself might play some supporting role in the hypertensive process.

Effects in patients with congenital adrenal hyperplasia (CAH). Hamilton and collaborators have studied the effects of Elipten® at a dosage of 30 mg/kg body weight on five cases of CAH [155, 156]. Prednisolone (1 mg nocte), also given to suppress nocturnal release of corticotrophin, resulted in the patients losing their Cushingoid features. It was found that the urinary 17-OHCS and 17-oxogenic steroids were well-controlled within the normal range and only in one case were the

urinary 17-OS greater than normal. Urinary DHA excretion was generally lower than normal. Androsterone excretion exceeded that of aetiocholanolone in some patients (as in normal children) while in others the reverse was found. In all five cases there was increased linear growth velocity and inhibition of osseous maturation. Of particular interest was the finding that, in one case with the hypertensive form of the disease, Elipten® produced a hypotensive effect (c.f. [139]).

Horky *et al.* [157] have recently made an intensive study of treatment with Elipten® of five adult women who were suffering from CAH (incomplete 21-hydroxylase deficiency). In the control period, urinary 17-OS were raised (due especially to high androsterone, aetiocholanolone and DHA) and pregnanetriol was raised, while tetrahydro-21-deoxycortisol (THS) was low (a characteristic feature of compensated 21-hydroxylase deficiency). In response to therapy (Fig. 14), urinary excretion of 17-OHCS and of 11-oxy-17-OS decreased, the latter finding indicating inhibition of 11β-hydroxylation by the drug. Urinary DHA also decreased, although androsterone and aetiocholanolone increased as did pregnanetriol. The latter finding is in direct contrast to normal and hypertensive subjects when pregnanetriol is reduced by Elipten® [158]. Urinary THS increased from the 16th day of therapy but subsequently returned to pre-treatment levels, even though Elipten® was continued. Horky *et al.* [157] attempted to explain these results by speculating that, with incomplete 21-hydroxylase deficiency and high ACTH secretion, the drug caused a further decrease in glucocorticoid production. This would lead, in turn, to overproduction of ACTH, thus overcoming the partial blockade by Elipten® and leading to an accumulation of 17-hydroxypregnenolone and 17-hydroxyprogesterone. The latter would give rise to the raised pregnanetriol. Moreover, the raised production of 17-hydroxypregnenolone would result in raised DHA and androstenedione, leading to increased urinary androsterone and aetiocholanolone. It appears that Elipten® treatment may enhance and make more obvious an incomplete 21-hydroxylase deficiency, as shown when it was administered to a second group of patients in whom the diagnosis of 21-hydroxylase defect was not entirely evident. Horky *et al.* [157] conclude from their study that the Elipten®-induced increase in urinary pregnanetriol may prove to be a useful tool in discovering a hidden defect in 21-hydroxylation.

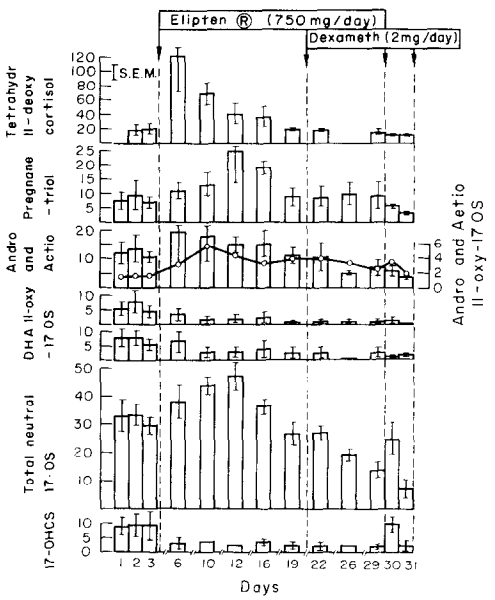


Fig. 14. Mean urinary excretion of individual 17-oxosteroids (± S.E.) in three patients with congenital adrenal hyperplasia and 21-hydroxylase defect during a control period and after administration of Elipten® and dexamethasone (from [157]).

Effects on ovarian function. It appears that, at least in the short-term, Elipten® has little or no effect on ovarian function. It was shown that it caused no changes in the menstrual cycle of six women, neither were there any significant changes in urinary pregnanediol or oestrogens of three hirsute women who had normal ovarian function [142]. However, long-term treatment does appear to affect the ovaries. Cash *et al.* [39] have described the case of a 19-yr-old girl who had been treated with Elipten® (1 g/day) for 6 yr. She presented with deepening of the voice, increasing hirsutism, coarse skin, recession of hair-line and clitoral enlargement. Laparotomy revealed that the left ovary was grossly enlarged and contained a large *corpus luteal* cyst as well as numerous *corpora lutea*. After wedge biopsies of both ovaries had been performed, the histological findings revealed the presence of excessive quantities of cholesterol and cholesterol ester, thus suggesting inhibition of the normal conversion to C_{21} steroids.

VII. *Further metabolism of modifiers*

A small amount of information is now available which shows that some modifiers of steroid hormone metabolism are themselves metabolized after administration to human subjects or animals.

Metyrapone. As this drug is a ketone, it is readily reduced to the corresponding secondary alcohol [2-methyl-1,2-bis(3′-pyridyl)-1-propanol, metyrapol, Fig. 1] by sodium borohydride [159], by adrenal or liver tissue [160, 161] and *in vivo* [162]. Sprunt and Hannah [160] have further shown that the reduction of Metyr-

apone in liver occurs in the microsomal fraction and is NADH- or NADPH-dependent.

When Metyrapol was incubated with quartered rat adrenals, it behaved differently to Metyrapone [163]. It had no effect on 11β-hydroxylation, inhibited aldosterone biosynthesis as effectively as the parent compound and increased by two- to three-fold the yield of 18-deoxycorticosterone formed from deoxycorticosterone.

In 1968, Sprunt *et al.* [162] showed that, in human subjects, an oral dose of Metyrapone was rapidly cleared from the plasma, the half-life being 20–26 min. It was excreted in the urine mostly during the day of administration and occurred largely as Metyrapol, both free and conjugated as glucosiduronate. The unchanged drug and some more polar metabolites were also excreted. Being more polar than Metyrapone itself, Metyrapol could be separated by t.l.c. in the system benzene–acetone (35:65, v/v). Both compounds were detected with U.V. light (wavelength 254 nm) [162].

Doriden®. The metabolism of Doriden® has been studied after administration to dogs and rats [164]. The drug was extensively modified, metabolites occurring in the urine largely conjugated as glucosiduronates (88–90%) and to a small extent (6–8%) in the unconjugated form. Furthermore, 88–90% of the administered dose was eliminated in urine and faeces in 48 h, a faster rate of elimination than the medium-acting hypnotics, such as phenobarbitone or amytal. Undoubtedly, the extensive metabolism and rapid excretion of Doriden® results in its lack of cumulative effects, side-effects and after-effects.

Elipten®. Unlike the parent compound Doriden® Elipten® is excreted virtually unchanged. This was shown by Douglas and Nicholls [165] who administered 250 and 500 mg of Elipten® to two men, and subsequently extracted urine, excreted during the next 48 h, with dichloromethane. The partition was found to be pH-dependent and was 99·8% in favour of the organic phase at pH 6·1. Using this method, 39% of the 250 mg dose and 54% of the 500 mg dose was excreted in 48 h. On the basis of these results, Fishman *et al.* [138] have advocated the preliminary extraction with dichloromethane of urine samples taken from patients treated with this drug; this prevented the drug interfering with some steroid assays, although no interference with the Zimmerman reaction has been noted [166].

Elipten® is readily extracted from incubations of adrenocortical or testis tissue to which it had been added previously (Lyne and Gower, unpublished observations). These workers also showed that, when the tissue extract was subjected to t.l.c. in e.g. di-isopropyl ether–formic acid (99:1, v/v), seven yellow zones were visible, one being due to Elipten® itself and the others due, presumably, to breakdown products of the drug. However, when freshly-spotted onto a t.l.c. plate, the drug fluoresces purple-pink, but on standing for a few hours in air, a yellow colour develops. It seems likely then that it is progressively oxidized in air to several products, whose nature is as yet undetermined. Nevertheless, it still retains its effectiveness as an inhibitor.

VIII. *Conclusion*

The intensive investigations of the enormous number of drugs synthesized since 1949 have clearly shown that some caused pronounced effects on steroid hormone biosynthesis and catabolism. Many of these drugs have been helpful in elucidating the mechanism of steroid hydroxylation and side-chain cleavage. Some have been used to advantage in the clinical management of patients with various disorders of steroid hormone metabolism. Metyrapone has been used in a relatively simple manner to estimate pituitary reserve. Aminoglutethimide, or Elipten®, the most recent compound to be included in the list of steroid biosynthesis modifiers, is a potent inhibitor and must be used with care [27], otherwise a patient may rapidly pass into an Addisonian-type crisis, unless cortisol is administered promptly. Nevertheless, it has proved beneficial in such conditions as adrenal carcinoma and congenital adrenal hyperplasia. Undoubtedly, further research will produce other compounds that, hopefully, may be used to advantage in the treatment of endocrine disorders.

NOTES ADDED IN PROOF

Recently, the effect of Elipten® on adrenal medulla has been studied [168]. Administration of the drug to rats in doses of 150 mg/kg twice daily for 5 consecutive days resulted in an increase in adrenal weight, a depletion (by 58%) of medullary adrenaline, no change in noradrenaline and an increase (by 136%) in dopamine contents. Furthermore, the relative ratio adrenaline/noradrenaline storing cells decreased. These changes could be reversed by daily administration of betamethasone (1 mg/kg for 5 consecutive days).

The inhibition by Elipten® (25 μM) of cholesterol side-chain cleavage [79] has been confirmed recently [169] in rat adrenal mitochondria. That the inhibition was non-competitive was indicated by (a) the constancy of K_m for the reaction and (b) the almost four-fold decrease in maximum velocity (as compared with control experiments).

The virilizing effects of Elipten® administration have already been described [39]. A case of pseudohermaphodism in a female child has also been reported [170], thought to be due to administration of Elipten® to the mother during pregnancy (up to the 8th month). These results are in keeping with those of Goldman [171, 172] who showed that severe virilization of female foetuses could be produced by administration of Elipten® to pregnant rats. This was accompanied

by increased weight of adrenals and ovaries of both mothers and foetuses, as well as an accummulation of cholesterol in these organs.

Following preliminary experiments [165], the metabolism of Elipten® has been investigated in men [173]. A metabolite, acetamidoglutethimide, was isolated from the urine. When this compound was administered to mice, it caused polyuria, diarrhoea and weight loss, as well as an increase in adrenal weight. It is possible that his metabolite may be responsible for the side-effects of Elipten® described earlier.

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