EFFECTS OF SOME STEROID CONTRACEPTIVES ON SOME ENZYMES AND CARBOHYDRATE METABOLISM IN SOME MAMMALIAN SPECIES

A THESIS

Submitted by

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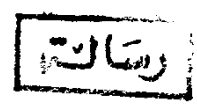

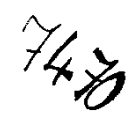

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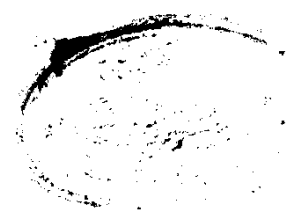

A C K N O W L E D G M E N T

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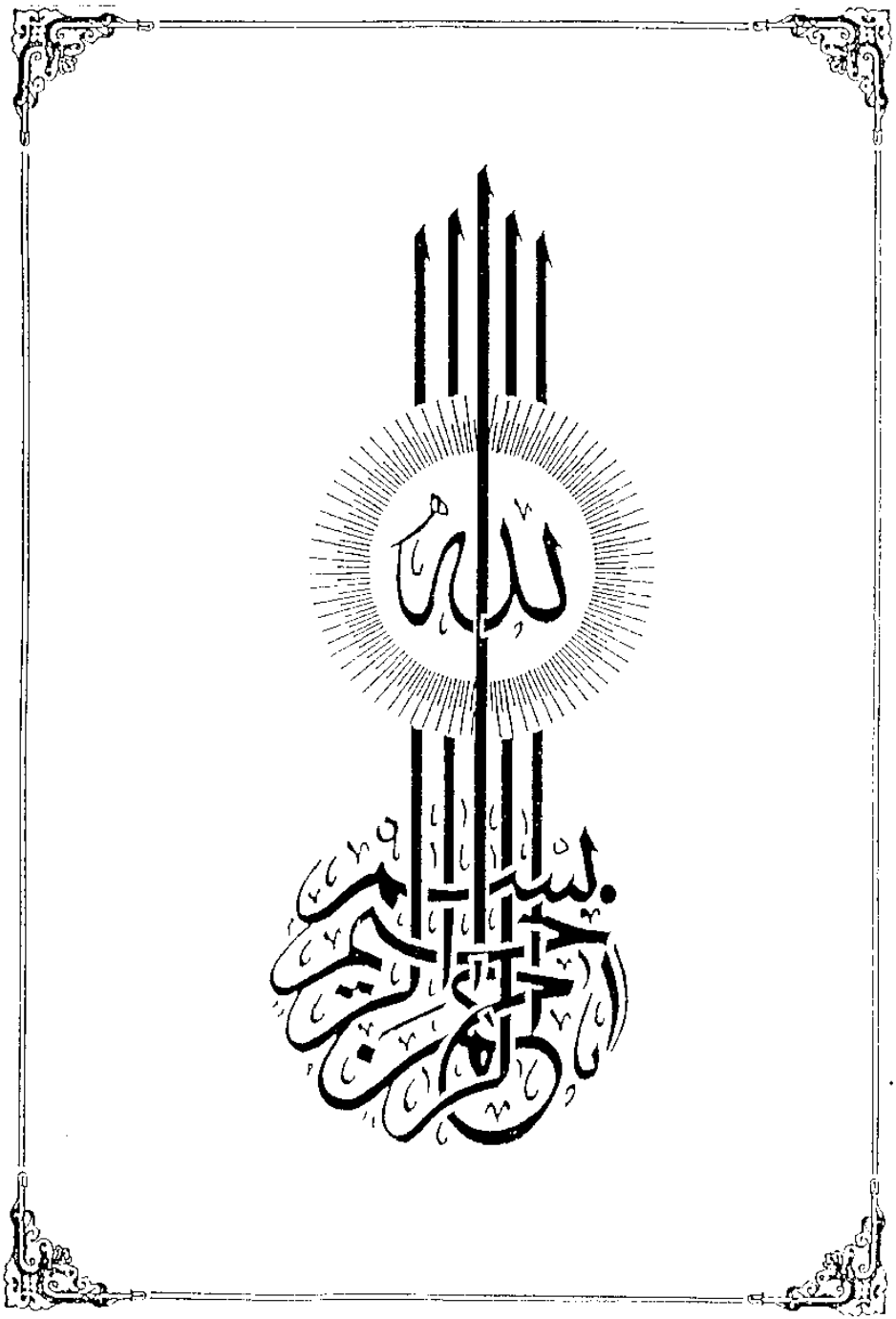

C O N T E N T S

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CHAPTER I

REVIEW OF THE LITERATURE

HISTORY OF HORMONAL CONTRACEPTION

The primary chemical studies towards the development of highly active antiovulatory steroids were initiated in 1930s with the isolation of progesterone. (Allen and Wintersteiner, 1934; Butenandt et al., 1934; Hartmann and Wettstein, 1934 and Slotta et al., 1934).

Inhibition of ovulation with progesterone was obtained in rabbits by Makepeace and Coworkers (1937), and in guinea pigs by Dempsey (1937). These fundamental observations were not extended to humans because progesterone had to be given by injections at short intervals since long acting preparations were not well tolerated. Inhoffen and Hohlweg (1938), found that a synthetic steroid (ethisterone) produced a progestational response in the rabbit when given orally. In 1954, Hertz and Associates reported a series of rabbit experiments using a compound similar in structure to ethisterone except it lacked an angular methyl group at C19. The compound (named norethindrone), was synthesized by Djerassi and Coworkers (1954) and Colton (1953, 1954). The progestational activity of norethisterone in human was described a year later by Tyler (1955). Soon thereafter, the antiovulatory effects of progesterone in human

In contraceptive therapy, where estrogen is the sole anti-fertility agent, ovulation inhibition or contraception could be affected, but tablet omission during the early phases of the cycle, might result in pregnancy (Pincus, 1960; Rock, 1961; and Liggins, 1967). Although estrogens inhibit ovulation, they promote the development of the uterine mucosa, and the endometrium remains proliferative, a state that can be readily transformed into the secretory type endometrium, capable of supporting pregnancy if there is ovulation. Under the influence of estrogens, the cervical mucus is also favourable to fertility, since it becomes thin, watery, and copious, providing a good enviroment for spermatozoal motility and penetration.

Combinations of estrogen and progestogen were used to inhibit ovulation (Pincus et al, 1958; Swyer and Little, 1962; Carter et al, 1964; and Goldzieher and Coworkers, 1968). The recent trend has been to reduce the quantity of the progestational component and to keep the amounts of estrogen below 0.08 mg per day. A number of combination products differing in their chemical composition, and proportion of ingredients are available.

The concept of producing anovulation in women by the use of an estrogen dose followed by a combination of estrogen with progestin (sequential type) was credited to Albright (1945). This sequential (or serial) hormonal contraoptive regimen was later investigated by Goldzieher and Coworkers (1963), and by Liggins (1964). The estrogen (generally mestranol or ethynylestradiol was given as a daily tablet for 15 days, starting on 5th day of the menestrual cycle, followed by a daily combination dose of estrogen mixed with progestin for further 5 days.

Original modes of delivery of hormonal contraceptives are being studied with the hope of finding the ideal contraceptive.

The 3-cyclopentyl ether derivative of etynylestradiol (Quinestrol) was used as long-acting estrogen in humans (Bombiani and Bubani, 1961; and Epstien et al, 1965) and animals (Giannina and Meli, 1969).

Quingestanol acetate (the 3-cyclopentylenol ether derivative of norethindrone acetate) had twice the progestational potency of norethindrone acetate and has the same biological profile (Giannina et al, 1969). A combination of 2 mg Quinestrol plus 2.5 mg Quingestanol acetate was found to be an effective oral contraceptive when given once a month (Berman, 1970; and Rubino and Berman, 1970).

The mechanism of action of injectable DMPA contraceptive was studied by Zanartu et al (1970) who found that spermatozoa had penetrated into cervical mucus but none were recovered from oviduct flushings after laparotomy. They also postulated that DMPA did not completely block the release of the gonadotropins required for follicular growth, but it inhibited preovulatory surge of luteinizing hormone that caused full follicle maturation and rupture.

Other progestational steroid, tested as long-acting contraceptive and given by intramuscular injection every 1,3 or 6 months, included Deladroxate, which consisted of 150 mg dihydroxyprogesterone acetophenide plus 10 mg estradiol enanthate (Lerner et al, 1961; Rutherford et al, 1964; Tymor et al, 1964; Pelton et al, 1965; Zanartu et al, 1966; Bernell, 1968; and Keifer et al, 1970).

All these were effective contraceptives, but the incidence of poor cycle control was high. However, suitable dose levels of these depot steroids may yet be obtained which will maintain an acceptable balance between effective long-term contraception and cycle control.

Interest has been aroused recently in the possibility of delivering long-acting hormonal contraceptives with a jet

acetate as depot-minipill enclosed within a sealed capsule of silastic. Tatum et al (1969); and Coutinho (1970) established the minimal number of silastic capsules implants containing megestrol acetate that permitted normal function of the pituitary hypophyseal-ovarian axis and ovulation. Larger scale clinical tests of the contraceptive efficacy of this mode of administrating low-dose progestins are in progress, but it has already been possible to obtain contraceptive efficiency lasting for one year (Coutinho 1970).

The initial studies with the subcutaneous silastic implants incorporating contraceptive steroids led to the investigation for the intrauterine or intravaginal drug delivary. The intrauterine silastic capsules containing progesterone and other steroids as potential contraceptives were studied by Doyle and Clewe (1968); Lifchez and Scommegna (1970), and Scommegna et al (1970). They postulated that, if such steroids were placed directly at site of terminal action (e.g., the uterine endometrium), only small drug dose would be required to achieve a local hormonal contraceptive effect, and that these doses would not produce hypothalamic-pituitary inhibition or undesirable systemic effect. Scommegna et al (1970), inserted silastic capsules containing 30 mg progesterone directly into the uterine cavity of rhesus monkeys

and human volunteers. These produced endometrial changes consistant with a progesterone effect. The length of the menstrual cycle was unchanged, implying a lack of systemic action. Studies are continuing with more potent long acting synthetic steroids (Vickery et al 1970).

A more convenient chemical contraceptive depot is an intravaginal ring; a patient can, if she wishes, insert and remove the ring herself. The intravaginal ring is made of silastic rubber in a design similar to the conventional intravaginal diaphragms. The steroid compounds are contained within the ring in such a way that the release rate at the site of application is constant over a prolonged period of time. Medroxygrogesterone acetate was impregnated within the silastic rubber ring during the process of molding and curving the device. Mishell (1970) and Mishell and coworkers (1970) showed that the amount of medroxyprogesterone acetate released in situ by intravaginal silastic rings was sufficient when absorbed systemically to produce an elevation of the basal body temperature, a shift in the maturation index, abolition of the mid-cycle LH peak, and alteration of the endometrial histology. Similar studies were done by Zanartu and Guerrero (1971) with progesterone and several progestogens. A more detailed investigation was carried out by Mishell et al (1971).

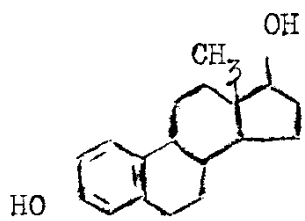
CHEMISTRY OF HORMONAL CONTRACEPTIVES

During 1951, a series of major discoveries in steroid chemistry greatly assisted progress towards hormonal contraception. Djerassi et al (1951, 1954) synthesized a highly potent progestin, 19-norprogesterone like the isomer synthesized earlier by Ehrenstein (1944), and was found to be more potent than progesterone. The same year, these workers also accomplished the synthesis of 19-norethisterone (norethindrone). Shortly thereafter, Colton (1953) succeeded in the synthesis of norethynodrel. The orally active progestin ethisterone and the orally active estrogen ethynylestradiol was also prepared.

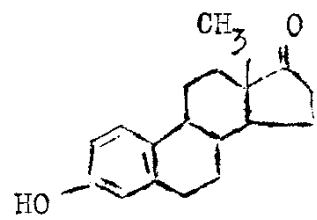
Although steroid hormones belong to the same class of compounds, there are important clinical differences between them. Progesterone and estradiol are good examples; progesterone is more lipid soluble, it can be easily extracted from biological fluids with solvents such as aliphatic hydrocarbons (petrolium ether or hexane). Estrogens are much more polar, the phenolic hydroxyl group at C-3 is sufficiently acidic to form salts as alkali metals, and estradiol may be removed from solutions by extraction with dilute aqueous sodium hydroxide.

E S T R O G E N S

The structural configurations of the three main estrogens: estrone, estradiol-17β and estriol are shown. Three other chemically related estrogens that have been isolated from natural sources are α-hydroxyestrone, 16-oxoestradiol-17β and 16-epistriol.



Estradiol-17 (β-Estradiol)



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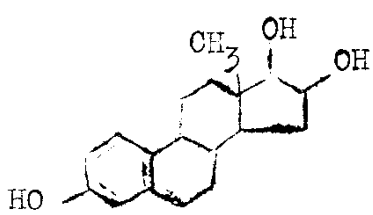

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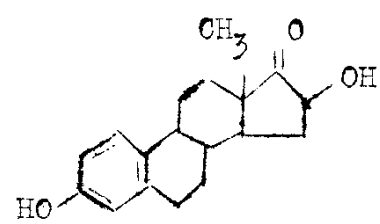
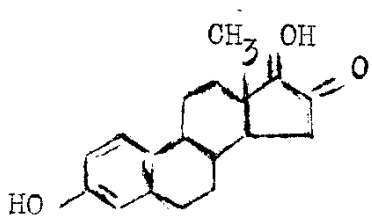
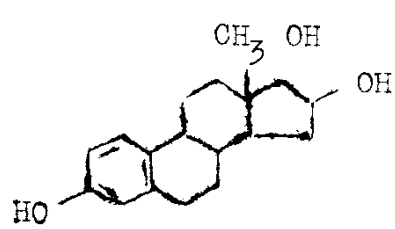


α-Hydroxyestrone



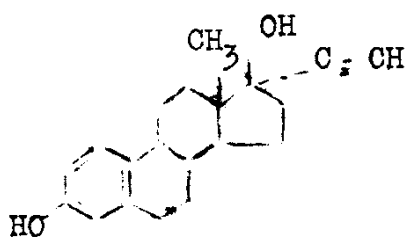
16-Oxoestradiol-17



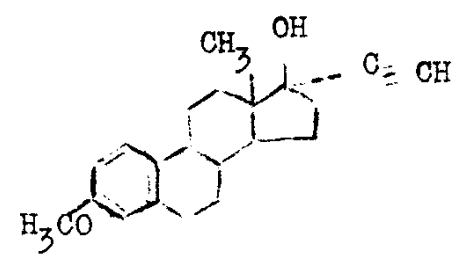
16-Epistriol

Scheme (1) Estrogens

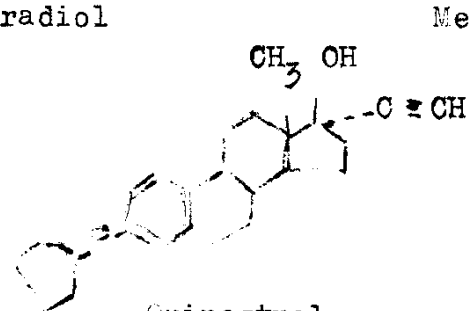
A variety of oral and injectable preparations of estrogens are available. Ethynylestradiol, mestranol and quinestrol are the most important estrogenic compounds involved in oral contraceptives. All have an ethinyl (-C≡CH) group in the α-position at C-17, which is thought to be responsible for their effectiveness when taken orally. Quinestrol (3-cyclopentyl ether of ethynylestradiol), has a greatly prolonged estrogenic effect when administered orally to human subjects. The reason for this seems to be storage in and release from body fat, which prolongs the estrogenic activity of the compound to such an extent that following injection of a single dose, metabolites are excreted in the urine over a period of 4 months or more (Williams et al, 1967).



Ethynylestradiol



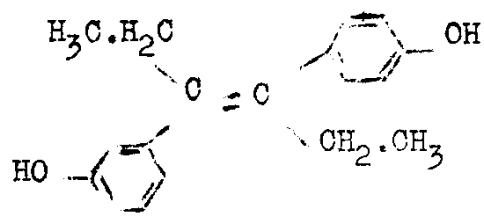
Mestranol



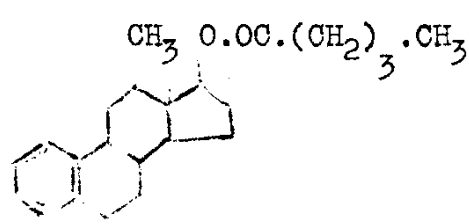
Quinestrol

Scheme (2) Estrogens

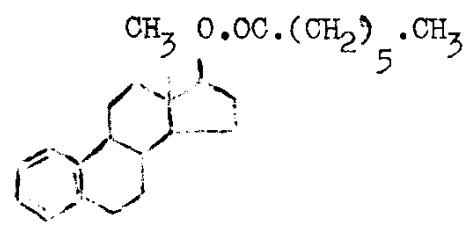
Another orally active estrogenic compound is stilbestrol (Middleton, 1966). Estradiol valerate (Siegel, 1963) and estradiol enanthate (Felton et al, 1965; Reifenstein, 1965 and Kizkallah and Tymor, 1966), were used as long acting injectable estrogens.



Stilbestrol



Estradiol valerate



Estradiol enanthate

Scheme (3) Estrogens