PHARMACOLOGY OF REPRODUCTION AND FERTILITY[1]

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A third review of reproductive pharmacology in this series within four years reflects the importance of and accelerating progress in this field. Research, aimed at the control of fertility in the (human) female, continues to center around investigations of the mechanisms involved in modifications in the hormonal environment of the fertilized egg or developing ovum by steroidal and other chemicals. Male fertility control continues to attract great interest but relatively little active research has been reported in the mammalian field. However, the list of compounds producing relatively selective effects on spermatogenic cells is slowly increasing.

This review is not comprehensive but covers the more interesting research topics in this field. A previous survey by Fridhandler & Pincus (1) dealt mainly with developments in the use of steroidal agents; the review by Tyler (2) with clinical aspects and side effects, while Jackson (3) provided a comprehensive survey of progress in the development of antifertility substances. Two monographs cover the entire field in detail (4, 5), and symposia have dealt with fertility control in general (6) and ovarian regulatory mechanisms (7). Rudel & Kincl (8) discussed the biology of antifertility steroids. Biochemical mechanisms in spermatogenesis have recently been reviewed (9). Special research topics in reproductive physiology are now given annual coverage (10); this will doubtless extend to reproductive pharmacology.

Control of Spermatogenesis

The role of gonadotrophins and androgens in the control of spermatogenesis is not yet resolved. In the rat, testosterone from intertubular Leydig cells is considered to control post-meiotic events, that is, the metamorphosis of spermatids to spermatozoa; male hormone secretion is, in turn, controlled by interstitial cell stimulating hormone ICSH (LH), but the role of follicle stimulating hormone (FSH) is not clearly defined. The only stage of spermatogenesis in hypophysectomised rats which could not be restored to normal by testosterone propionate was the stage which produced type A spermatogonia (11). The number of these cells decreased progressively with time, suggesting that they are under direct pituitary control. Somatotrophin and thyroid hormone appear to contribute to the maintenance of spermatogenic function in hypophysectomised testosterone-treated rats (12), but did not restore their fertility to normal. Following inhibition of pituitary function by stilbestrol implants, spermatocyte development was progressively and grossly impaired and, after four months only types A and B spermatogonia

[1] The main survey of literature pertaining to this review was concluded in January 1967.

remained (13). In such rats, daily injection of purified ICSH had no effect, whereas FSH apparently restored spermatogenesis completely. Spermatogenesis may be regulated by local intratubular factors, perhaps via Sertoli cells which, in turn, may be controlled by FSH (14).

Clermont & Harvey (15) have carefully analysed germ cell population data of the rat testis following hypophysectomy and hormone replacement therapy. In hypophysectomised rats, the dynamics of the proliferative stages remains essentially normal, except for progressively increasing losses of the various cell types. Production of step 7 spermatids declined virtually to zero in about six weeks. Gonadotrophin preparations, testosterone, pregnenolone, androstenedione, and dehydroepiandrosterone supported the process with a normal proportionate yield of spermatids from spermatogonia type A. The population of the latter cells was not maintained at normal levels by hormones, singly or in combination. Spermatogenesis was not accelerated in hypophysectomised rats by excessive amounts of FSH, ICSH, or testosterone. Some investigators feel the data favor general support for spermatogenesis mediated by androgen from Leydig cell stimulation. The behaviour of the spermatogenic process suggests hormonal action upon the milieu in which the germ cells develop "provided mainly by the Sertoli cells" (15).

Both interstitial tissue and the contents of the seminiferous tubule in the rat can convert progesterone into testosterone (16). Steroids like norethandrolone (Nilevar) and progesterone injected into male rats reduce the pituitary gonadotrophin content to low levels, but because of their androgenic effect do not significantly inhibit spermatogenesis (17). Another study quantitatively evaluates the effect of the non-steroidal pituitary inhibitor methallibure (ICI 33828) on rat spermatogenesis and fertility (18). Daily administration was required to suppress spermatogenesis. The timing of the cycle was unaffected and the effect resembled that of hypophysectomy, the main action being arrest of spermatid metamorphosis (i.e. spermiogenesis) at step 8. Recovery occurred after discontinuation of treatment. The antifertility action appeared to result from a combination of loss of libido and arrest of spermatogenesis. In dogs, at similar doses (20 to 80 mg/kg/day), methallibure inhibited spermatogenesis at the primary spermatocyte stage (19). Much lower doses (0.75 to 1.5 mg/kg/day) soon resulted in total loss of libido with recovery in two to five weeks post-treatment; adverse effects on sperm morphology appeared at the 1.5 mg/kg level. Spermatogenesis in fish can also be inhibited by this compound (20).

Daily injection of rats with alphasone acetophenide (16α-17α-dihydroxyprogesterone acetophenide; in Deladroxate) reversibly suppressed spermatogenesis and sexual activity, possibly by direct action, since pituitary gonadotrophin levels were normal (21). In the rhesus monkey, the reported ineffectiveness of this compound might be consistent with direct gonadotrophic support for the seminiferous epithelium, as in man. Reversible arrest of spermatogenesis and loss of libido occurred with the enol ether of 17α-ethynyl-19-nortestosterone acetate (0.5 mg). In this case, pituitary

weight and gonadotrophin content were reduced, which could be due to estrogenic activity of this type of compound (22). By contrast, megestrol acetate (17α-acetoxy-6-dehydro-6 methyl progesterone) at 40 mg/kg/day for 30 days did not affect either spermatogenesis or the fertility of male rats (23).

Histological examination of the gonads has been used to test a variety of neutral steroids for ability to interfere with spermatogenesis (24) using testosterone and methyl testosterone as reference compounds. Four steroids—2α-hydroxymethyl-17β-hydroxy-5α-androstane-3-1; 2α-hydroxymethyl-5α-androst-2-en-17β-ol; 2α, 17α-dimethyl-5α-androst-2-en-17β-ol, and 2α-formyl-5α-androst-2-en-17β-ol–arrested spermatogenesis and caused decrease in weight of the accessory organs. Neither 19-nortestosterone nor chlormadinone produced convincing effects on the seminiferous epithelium. The antispermatogenic action of the four steroids mentioned was the result of a diminished output of FSH, while either ICSH inhibition, androgenicity, and possible anti-androgenicity might be responsible for effects on the ventral prostate and seminal vesicles.

The many actions of androgens on the CNS as shown by effects on libido and gonadotrophin secretion, in the control of spermatogenesis, and in maintenance of accessary structures might involve different end-organ mechanisms with the possibility of pharmacological dissociation. Perhaps the wide range of compounds which show anti-androgenic properties (25) is a manifestation of the complexity of end-organ processes.

On the other hand, these different functional activities may be related to chemoreceptors in the CNS. Testosterone-sensitive centers in the rat hypothalamus have been reported, judged by the response to implants in the basal tuberal median eminence but not in the pituitary or elsewhere (26). These caused atrophy of the testis, prostate, and seminal vesicles.

Human studies.—In man, as judged by cell labeling with thymidine, neither injection of gonadotrophin nor completely blocking later stages of spermatogenesis by norethandrolone altered the rate constancy of the proliferative stages (27). While pituitary gonadotrophins constitute the efferent pathway for the control of testicular function, the afferent mechanism is still unknown. Clinical studies of hypogonadic males and the effect of gonadotrophin on their germinal epithelium led to the concept that the testicular-hypophyseal feed-back mechanism involves a pituitary inhibitor liberated from the late stages of spermatid development (28).

MacLeod, Pazianos & Ray (29) established that total hypophysectomy in a human male caused loss of the seminiferous epithelium as far back as spermatogonia, in about three months. Administration of menopausal gonadotrophin (mainly FSH) daily for 67 days restored all stages of spermatogenesis qualitatively, but chorionic gonadotrophins were also required for a quantitative recovery, including return of the ejaculate and the appearance of normal spermatozoa. They suggested that FSH acted primarily at the level of the spermatogonium. Earlier, Gemzell & Kjessler (30) restored spermatogenesis in a partially hypophysectomized patient. Paulsen (31) failed to

achieve a clinical response with human menopausal gonadotrophin (HMG; Pergonal) after three months of daily treatment. From long-term administration of gonadotrophins in two cases of persistent infantilism, Johnsen (32) found no effect on testicular histology or on hormone excretion using either human chorionic gonadotrophin (HCG) or HMG. The two preparations combined, however, produced complete gonadal maturation, but rapid regression followed discontinuation of injections. These observations are endorsed by another study in which, commencing from aspermia, motile sperm appeared 60 days from start of treatment with FSH-rich pituitary extracts. The sperm count reached 25×10^6, although pregnancy was not achieved. In this case, HCG did not stimulate spermatogenesis significantly beyond the spermatocyte stage (33). Unlike in the rat, androgen does not stimulate tubular development since testicular growth and tubular function occur before initiation of Leydig cell secretion (32). In the "fertile eunuch", tubular development and spermatogenesis occur in the complete absence of Leydig cells. Control of spermatogenic processes thus appears to be remarkably different in rat and man.

The anti-estrogen, clomiphene, which stimulates ovulation in human females, although it is inhibitory in the rat, augmented urinary FSH and ICSH in most human males with normal pretreatment levels (34). Variable sperm counts were observed in oligospermic individuals and in no case was fertility demonstrated. Harkness et al. (35) reported increased excretion of various steroids on 100 mg/kg/day of clomiphene, but no consistent effect occurred on gonadotrophin excretion. In a patient with Klinefelter's syndrome, clomiphene stimulated production of morphologically normal, motile spermatozoa within three days, but had no marked effect on hormone output (36). Areas of spermatogenesis are rare in this condition, and it appears that clomiphene prevented degeneration of spermatids in stage 1 of the cycle. This seems unlikely since spermatid metamorphosis into spermatozoa requires three weeks even in the rat, with a further two weeks for sperm transport through the epididymis. Probably, spermatozoa were continually produced by this patient and clomiphene somehow enabled them to survive a hostile transport environment. From a recent study in the rat, the inhibitory effects of long-term clomiphene treatment seem due to its estrogenicity, mediated via the hypothalamo-hypophysial axis. The drug effect was manifest at the primary spermatocyte level while spermatogonial mitoses appeared unaffected. Testis and accessory glands recovered within 30 days post-treatment (37).

If control of germ cell development in man is mediated directly by gonadotrophins, fertility control via steroids could utilize selective pituitary inhibition by a compound possessing sufficient residual androgenicity to maintain libido. MacLeod (38) discussed the prolonged suppression of spermatogenesis in men by injected testosterone oenanthate (Delalestryl, 250 mg. weekly) and by 6α-methyl-17α-hydroxyprogesterone acetate (MPA, 1 g). The former caused loss of sperm after about 70 days, which was maintained as long as treatment continued, with a post-treatment recovery of 75 to 100

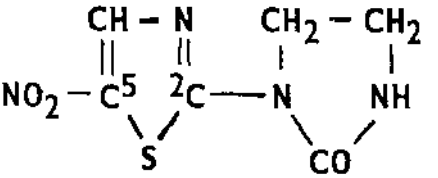
days. These experiments epitomize the steroidal approach mentioned above and were accomplished with only a mild disturbance in sperm morphology. In three subjects, the progestational steroid, MPA, produced near-aspermic levels by 70 days, persisting to about 125 days, with a recovery time of 175 to 360 days, although the original sperm count levels were not attained. Loss of sperm motility and severe aberrations in sperm morphology accompanied the preliminary phase.

Antispermatogenic Chemicals

Direct interference with the seminiferous epithelium as an approach to male fertility control should avoid the problem of depressing sexual activity. Substances suppressing the development of reproductive cells are conveniently designated 'anti-spermatogenic', but modes of action are little understood. Of three potentially useful types of compounds;—'alkylating' chemicals, nitro-aromatic compounds, and dichloroacetyldiamines—there are few recent reports on the two latter groups and unfortunately, details of structure/activity relationships have not been published. Nelson & Patanelli (39) discussed nitro-aromatic compounds and diamines, indicating that all inhibited at the stage of the primary spermatocyte and had minimal effects on the endocrine function of the testis. Gonadotrophin is said to be necessary for the antispermatogenic effects of nitrofuranes, nitropyrroles, and diamines. One compound, 1-N,N-diethylcarbamyl-methyl-2,4-dinitropyrrole, mentioned in a previous review (1) was considered to be by far the most effective of these agents (40). Judged by testis weight, 20 mg/kg daily to rats orally, induced a pharmacological action and twice this dose reduced testis weight by nearly 50 per cent in 30 days. One treatment of 500 mg/kg every four weeks maintained sterility indefinitely; in man a corresponding dose would be 35 g presuming comparable susceptibility. Oral administration to adult rhesus monkeys (9 mg/kg daily for 30 days) was recently reported to have no effect on testicular function (41).

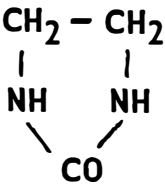
The unfortunate antabuse-like side-effect complicating administration of dichloroacetyl diamines (WIN 18,446 and 13,099) to human subjects apparently has not been overcome. Like tetraethylthiuram disulphide, both these compounds inhibit liver nicotinamide adenine dinucleotide linked aldehyde dehydrogenase (42) and drug metabolism (e.g. of barbiturates and N- and O-demethylation processes) is inhibited *in vivo* and *in vitro* (43). A closely related ethoxy-derivative, although amoebicidal *in vivo* and *in vitro*, was not antispermatogenic and did not inhibit drug metabolism. The antispermatogenic changes induced by the bis-(dichloracetyl) diamines was reported to be basically similar in rat, monkey, and dog, although there were marked differences in the effective dose levels (44).

Nearly 15 years ago, 2-amino-5-nitrothiazole (Enheptin) was shown to suppress sexual function completely in the domestic fowl, but not in the rat by an anti-gonadotrophic mechanism (45). It is interesting that the antifertility activity of nitrothiazole should be implicated in the treatment of schistosomiasis(bilharziasis). Niridazole (Ambilhar) which has an imidazoli-



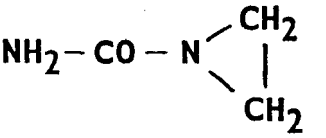
Niridazole (Ambilhar)

1-(5-nitro-2-thiazolyl)-2-imidazolidinone



Imidazolidinone

N, N' - ethyleneurea



N, N - ethyleneurea

FIG. 1.

dinone ring in position 2 was selected for trial from a number of 5-nitrothiazole derivatives (Fig. 1). Toxicity tests with niridazole in mice provided abnormal findings only in the testis. Administration to host mice halted spermatogenesis in the male schistosomes and cell structure in the gonad was completely destroyed; female worms were even more sensitive to-the compound (46).

Studies with C^{14}-labeled niridazole (47), demonstrated accumulation of the drug within the schistosomes; the concentration within schistosome ova was greater than in any host tissue, except for gastric mucosa. The drug is rapidly broken down within the parasite and metabolites accumulate (48). Since the C^{14} label used in the schistosome experiments involved C_4 on the imidazolidinone ring, this part of the molecule may be involved in the damage to the gonads. Thus N,N'-ethyleneurea (synonymous with 2-imidazolidinone and not to be confused with aziridinyl urea, N,N-ethyleneurea in Fig. 1) is an insect growth inhibitor and chemosterilant (49). Female houseflies only were sterilized but both sexes of milkweed bug were affected.

Alkylating chemicals.—Recently, a number of publications have dealt with the actions, metabolism and possible mode of action of certain alkylating chemicals on spermatogenesis and reproduction in rodents. Jackson (50) discussed the antifertility effects of different alkylating chemicals. Cumula-

tive damage to different stages of this complex proliferating and differentiating cell system appears to be correlated with the total dose administered. It has also been suggested that some aspects of these actions might include pharmacological events corresponding to the premalignant change of chemical carcinogenesis as encountered in other proliferating cell systems of the body (51).

This recalls the selective destruction of rat spermatogonia and resting spermatocytes following a small dose of the carcinogen 7,12-dimethylbenz (α) anthracene (DMBA) (52) but not after several other powerful carcinogens. DMBA produced total sterility for 9 to 12 weeks after a smaller intravenous dose of 0.25 mg/kg (51). A progressive decline in fertility from the time of treatment indicates the damage inflicted was more widespread than the histological picture revealed although the endocrine functions of the gonads were not disturbed (53). Changes in enzyme systems within the testis have been followed after administration of DMBA (52) and myleran (54), as well. Myleran also inhibits early stages of the spermatogenic process. Numerous derivatives of the cyclic base ethyleneimine (aziridine) have been tested as male chemosterilants in insects. In rodents, limited structure/activity tests support the view that the overall actions of these agents on the range of spermatogenic cell types are qualitatively similar (50). Spermatids, spermatozoa, and spermatogonia are the most susceptible to damage. Metabolic studies with triethylenephosphoramide (TEPA), now extended to male insects (55), still have not provided any clue to the nature of the "functional" chemosterilant action via post-meiotic cells. The initial reaction of potent compounds like TEPA and triethylene melamine (TEM) probably involves opening of the aziridine ring and attachment of the molecules at biological sites. The efficiency of the pharmacological effect is related to the number of aziridine rings and the structure of the carrier molecule.

An important discovery was that the analogues of TEPA and TEM, hexamethylphosphoramide (HEMPA) and hexamethylmelamine (HMM), were also chemosterilant against male house flies (56). Much higher doses were required but these are stable compounds without aziridine rings. They are thought to be nonalkylating but because of the availability of methyl groups, this may not be true. In rodents too, HEMPA and HMM are antispermatogenic (51, 57). HEMPA is relatively nontoxic and can induce prolonged and possibly permanent sterility. Aspermia with continued production of semen occurred in the rabbit. The spectrum of action of spermatogenic cells is different from that of TEM and TEPA. A metabolic study in rats and mice with P^{32}-labeled HEMPA showed that much of the material was rapidly cleared apparently unchanged (57) with little or no breakdown to phosphate, in contrast to TEPA. However, refinements in technique have since indicated metabolic breakdown (58). A detailed study with C^{14}-labeled HEMPA in the house fly has proved that the major metabolite is pentamethyl phosphoric triamide, i.e. one methyl group is lost (59). It is still possible that the antifertility action of HEMPA may be due to a potent

metabolic product. Structure/activity relationships of analogues of TEPA and HEMPA against house flies have been presented (60).

Hexamethylmelamine (HMM) shows tumor-inhibitory activity and its antifertility activity in rodents is less well defined (51). A survey of numerous derivatives of melamine as sterilants of male house flies correlates methylated substituents as a requisite for this pharmacological property (61). Size and spatial distribution of substituents on the triazine ring affected the sterilizing activity, rather than their electronic characteristics. Introduction of cyclic substituents other than aziridine, did not result in active compounds.

Among alkylating compounds of the sulphonic ester type, the delayed antifertility action of myleran (Busulphan) correlates with a selective suppression of early stages of spermatogonial development (62). The time of onset of sterility with aspermia after a minimal dose is an estimate of the duration of spermatogenesis. Among homologous esters of this series, methylenedimethane-sulphonate, which is unstable in solution, produces immediate sterility, due to some action mediated on mature spermatozoa in the epididymis (63). In addition, it has a less obvious but important spermatogonial action. Like myleran, the methylene ester produces marked depression of bone marrow function (64, 65). Surprisingly, the homologue, ethylenedimethanesulphonate exerts quite a different antispermatogenic action and does not depress bone marrow. Spermatids and spermatocytes rapidly degenerate under its action (5, 51). Cumulative actions of myleran and ethylene dimethanesulphonate are mentioned in a discussion of the toxic actions of alkylating chemicals on reproductive cells (51). Isotopic studies with these compounds in the rat and mouse do not account for the relatively high tolerance shown by the latter species to these chemicals.

Single doses of the simple methyl and ethyl esters of methanesulphonic acid do not affect cell morphology but produce predictable episodes of sterility in rodents, due to spermatid damage. High cumulative potency even by the oral route is correlated with the dose rate and total amount ingested (50). This type of sterility was maintained through the life span of the males.

Dominant lethal mutation mechanisms are involved in the antifertility process with substerilizing doses of methyl alkane sulphonates (66, 67). Heritable damage in mice after a substerilizing dose of methylmethanesulphonate was indicated by the fact that about 4 per cent of male offspring were partially sterile (68).

Two other substances are known to produce selective testicular damage. Fluoroacetamide in the diet (50 mg/kg food) caused a disappearance of germinal elements in the rat testis; after 64 days only spermatogonia and Sertoli cells remained. The action appeared first on the more mature cells of the seminiferous epithelium, not on the proliferating cells. The intestinal epithelium showed normal proliferation (69).

Procarbazine (Natulan), a hydrazine derivative, also produces a drastic effect on the spermatogenic epithelium. Bollag (70) first commented on this action on the testis, while Fox (71) and Jackson & Craig (72) referred to its antifertility effect in the rat.

Hypothalamic and Pituitary Control of Ovulation

Gorski (73) has published an interesting review on localization and sexual differentiation of the nervous structures which regulate ovulation.

The classical feed-back theory, which assumes that levels of estrogens and progesterone impede further secretion of FSH or LH [or, in certain species, leuteotrophic hormone (LTH)] by the anterior pituitary gland, cannot be accepted as the sole controlling mechanism. McCann and co-workers (74) have shown that implantation of estrogen or testosterone in the median eminence of the brain is followed by a decrease in hypothalamic content of luteinizing hormone releasing factor (LHRF). Ramirez & McCann (75) demonstrated that estradiol implanted into the median eminence or anterior lobe of the pituitary of rats induced diestrus and development of the mammary glands. The anterior lobe was enlarged and contained increased LTH. Corpora lutea in these animals were also enlarged and the vagina mucified, suggesting increased synthesis and release of LTH.

Implants of estradiol in the region of the amygdaloid complex modified the mammary response of pseudopregnant rabbits (76). The possibility exists, therefore, that hypothalamic control of LTH secretion may be modified by a fine control from limbic structures. However, in recent years, evidence has accumulated that a shorter feed-back system is also involved, viz, that pituitary gonadotrophins may themselves feed back into hypothalamic centers and thus modify their own secretion rate. Further evidence for this has been supplied by Corbin et al. (77) with the demonstration that LH implants into the median eminence of female rats effectively lowered the content of pituitary LH, while similar implants of FSH or ACTH were ineffective.

Studies in the rat (78) indicate that estrogen and progesterone may interact in the same CNS area in that species. Döcke & Dörner (79) implanted estradiol benzoate into the hypothalamus or anterior pituitary of immature female rats and found that a smaller dose of the drug was required in the adenohypophysis to induce corpus luteum formation. One difficulty in interpreting the results of stereotaxic drug implantation is that drug may spread from its implantation site. After implantation of labeled estradiol in the median eminence of adult female rats some radioactivity was found in the anterior pituitary (80).

McCann and associates (81) examined the LH-releasing activity of hypothalamic extracts in a number of experimental situations. In immature female rats pretreated with gonadotrophins, the extract given intravenously caused a release of LH. This occurred in adult female rats or ovariectomised

females pretreated with estrogen, or estrogen plus progesterone, but not in animals simply ovariectomised. The authors postulate that in the untreated gonadectomised female (where plasma LH is already elevated) pituitary cells are responding maximally to endogenous LHRF and are incapable of further response to exogenous releasing factor. Some progress is reported towards separating FSHRF and LHRF by column chromatography. However, LHRF is still closely associated with a prolactin inhibiting factor after Sephadex G25 treatment. From these and similar studies, it appears that these releasing substances are small basic polypeptides (molecular weight 1200 to 2000) somewhat larger than vasopressin. They are stable at room temperature and are not broken down by thioglycollate. Endocrizi & Hilliard (82) have studied the LH-releasing effects of extracts of rabbit and dog brain injected into the anterior pituitary of pregnant or pseudopregnant rabbits. While extracts of median eminence, posterior hypothalamus, reticular formation, central grey matter, anterior hypothalamus, and amygdala had marked LHRF activity, extracts of thalamus, hippocampus, white matter, and caudate nucleus were ineffective. The agent(s) responsible for these effects have not been defined. Saito and associates (83) reported a modified procedure for estimating FSH in castrated male rats pretreated with testosterone propionate. Using this technique, they found that hypothalamic extracts of stalk and median eminence of rat or pig contained FSH-releasing material. A number of substances known to occur in CNS extracts (epinephrine, norepinephrine, acetylcholine, spermine, and extracts of human cerebral cortex) failed to deplete pituitary FSH in this preparation. Histamine, spermidine, and lysine vasopressin had some activity at high dose levels.

The anti-androgen cyproterone (1,2-methylene-6-chloro-pregna-4-6-diene-17α-ol-3,20-dione) prevented the inhibition of ovulation in rats induced by testosterone propionate or 6α-methyl-17α-hydroxyprogesterone acetate (MPA) (84). Ovulation inhibition by estradiol and progesterone was not affected. Presumably, cyproterone blocks androgen receptors in the hypothalamus. This steroid also induced development of a vagina in a male foetus. Pellets of testosterone propionate (2 μg or 6 μg) placed bilaterally in the hypothalamus of four-day-old female rats advanced vaginal opening and most rats showed persistent vaginal oestrus (85) and repeated acceptance of the male.

Pheromones.—The blocking effect of strange male scent on pregnancy in female mice was reviewed in 1964 (1, 3). In that year, Bruce (86) reported that administration of prolactin during exposure to the strange male scent prevented this effect. The olfactory block to pregnancy could also be produced in wild rats. Strain, however, appears important as the effect was absent in tame rats and alien male mice from strain CBA/J did not inhibit the pregnancy of newly mated females of strain C57BL/6J (87).

Dominic (88) reported that urine from alien male mice caused olfactory block to pregnancy but that urine from castrated mice was less effective indicating that excretion products of androgen or the products of androgen-dependent glands might be responsible. Urine from androgen treated spayed female mice but not from normal or spayed females proved as effective as that of normal males in producing pregnancy block. Dominic showed that pregnancy inhibition by strange male urine could be antagonised by reserpine (89). Since exogenous prolactin was also effective, the mechanism postulated is that reserpine acts on the hypothalamus, overcoming the inhibition of prolactin release from the anterior pituitary. Histological evidence suggests that pregnancy block is due to failure to maintain the corpus luteum, and it has been reported that progesterone also allowed pregnancy to continue (90). Chipman & Fox (91) showed that mice at two or three months of age are more sensitive to pregnancy blocking by males than at six months of age, possibly due to a lack of neuroendocrinological stability. To retain its effectiveness in blocking pregnancy urine of alien males should be collected in the presence of a mixture of antibiotics and an antioxidant (92).

Steroidal Antifertility Substances

It it not certain whether contraceptive steroids act via central or peripheral mechanisms. France & Pincus (93) submitted evidence favoring action at the ovarian level, for estradiol-17β, estrone, and norethynodrel interfered with pregnant mare serum (PMS), human chorionic gonadotrophin (HCG) induced superovulation in the hypophysectomized, immature rat (relative doses 1:100:5000). Similar evidence demonstrates the relative failure of estradiol, mestranol, progesterone, 19-norprogesterone and norethindrone, even in high doses, to suppress ovulation following PMS-, HCG-stimulation in the intact immature rat (94, 95). Progesterone and norethynodrel had previously been reported effective in 21-day-old immature mice treated with gonadotrophin (96). Ten μg of chlormadinone acetate inhibited ovulation in the mated rabbit but ten times that amount failed when HCG was used (97). Much higher doses (300 μg and more) were also ineffective when PMS and HCG were used (95).

Depressants of the CNS (reserpine, chlorpromazine, perphenazine, promazine) inhibited superovulation in immature mice (96). In the PMS-stimulated intact rat, phenobarbital, and chlorpromazine were only partially successful (98, 95). Psychotic, normally menstruating women developed amenorrhoea during phenothiazine treatment (95). Increase in rat prolactin secretion occurs (mammary growth and lactation) following treatment with reserpine, chlorpromazine, meprobamate, and other CNS depressants (99).

Ovulation inhibition by reserpine, as well as its sedative action, was antagonized by DOPA (3,4-dihydroxy-phenylalanine) (100). α-Methyl-

DOPA antagonized only the latter action. Methoserpidine was ineffective in inhibiting ovulation although it has many properties in common with reserpine.

Most data strongly support the view that steroid hormones directly inhibit production or secretion of gonadotrophin-releasing factors by the hypothalamus. Pincus (101) believed that progestational steroids operate on the hypothalamo-pituitary axis, preventing release of LH and that natural estrogens influence the ovary directly and possibly via an estrogen-labile hypothalamic factor also. In immature rats, courses of MPA appeared to block LH synthesis and leave sensitivity to gonadotrophin unimpaired (102). Nilevar (17α-ethyl-19-nortestosterone) did not affect growing ovarian follicles in the guinea pig, but prevented the action of gonadotrophin on the mature follicle, either by central or peripheral action (103). In women, too, ethinyl estradiol with either norethindrone acetate or medroxyprogesterone acetate did not abolish the ovarian response to injected gonadotrophin (104).

Diczfalusy (105) suggests that the classical contraceptive tablet (progestational steroid and estrogen) inhibits LH release and renders cervical mucus 'hostile' to sperm. The 'sequential' combination (estrogen first, followed by estrogen and progestogen) inhibits FSH and LH release. The low dosage 'luteal supplement' (progestational steroid only) interferes with cervical mucus. All types may also render the endometrial environment unfavorable to chance blastocysts from 'escape' ovulations.

Rudel & Kincl (95) point out that low doses of chlormadinone alter cervical mucus and possibly the endometrium, thus controlling fertility without ovulation inhibition. Earlier observations on the effectiveness of this method of contraception have been substantiated (106) and extended (107). The progestogen can act without inhibiting ovulation and normal menstruation occurs if the pituitary-gonadal axis is not affected. This type of regime suggests that adequate sperm hostility in cervical mucus can be produced without interference with normal secretory endometrial development (95).

Interference with Post-Ovulatory Events

Estrogenic and progestational hormones are included in post-ovulatory events, so that the pharmacological approach to post-ovulatory contraception is to disturb their essential relationships by administering natural or synthetic hormones or antagonistic chemicals—antiestrogens or antiprogestational substances. Study of mechanisms involved is difficult since these may overlap. Furthermore, *in vivo*, progestational substances may be converted into estrogen; a possibility which has caused much controversy in past years.

Action of estrogen at the cellular level.—The mode of action of estrogen in the reproductive pathway at the cellular level is relevant to the topic post-ovulatory antifertility chemicals. The substantial progress in this area admir-

ably described by Segal & Scher (108), must surely be the forerunner of much similar research using synthetic hormones and their antagonists. RNA synthesis in the endometrium is stimulated by estrogen, initially in the nucleus and apparently affecting all RNA fractions (109). The hormonal stimulus is antagonized by specific inhibitors of DNA-dependent RNA synthesis (110, 109). Thus, there is the likelihood of hormone-gene interaction comparable to that occurring with the steroid hormone, ecdysone, which specifically controls the moulting process in insects (111).

Estrogen-like phenomena in the rat uterus follow treatment with RNA extracted from estrogen-stimulated rat uteri (112–114). Introduced into the lumen of the uterine horns of ovariectomized rats, the purified RNA causes endometrial stimulation comparable to that produced by estrogen alone. The possibility that contaminant estrogen was involved is considered to be minimal, and the activity was destroyed by RNase or diphosphoesterase, but not by DNase or trypsin (113). RNA from other locations in the estrogen-treated rat was ineffective. Similar results were obtained using the restoration of uterine alkaline phosphatase in the ovariectomized mouse as a criterion (114).

Blastocyst nidation in the rat is estrogen-dependent and likewise responds to uterine—RNA. The implantation process can be greatly delayed by ovariectomy on day four postinsemination, provided progesterone maintenance is given. In such test animals, estradiol (0.01 μg) or the prepared RNA (0.75 μg) injected into the parametrial fat, will induce implantation (115). It is suggested that the primary and perhaps sole role of the hormone is to evoke a pattern of RNA biosynthesis in target cells. As yet, there is no indication that the estrogenic effects are correlated with any particular uterine RNA species.

Egg transport.—The crucial time relationship between the fertilized ovum and its environment as it progresses towards and into the uterus has been emphasized by ovum transfer techniques. As in the mouse, transfer of fertilized rat ova which were 'older', relative to mating time, than the uterus of recipients was much more successful than the reverse process (116). Beyond day five in the rat, both ova and endometrium rapidly lose their ability to take part in an implantation process. The overdeveloped endometrium is particularly inhospitable, if not maintained on progesterone. Recovery of rabbit ova, after administration of graded doses of various estrogens following ovulation induction, supported the conclusion that interference with their normal transport was the detrimental mechanism (117). Subcutaneous progesterone (2 mg/day) or oral (MPA) before ovulation and ethinyl estradiol given post-ovulation induced complete degeneration of eggs due to enhanced rates of passage (118). The recovery rates of transferred one-day rabbit eggs were very low in recipients subsequently treated with ethinyl estradiol. Progesterone counteracts the detrimental effect of estrogen by slowing egg transport.

Toxic actions on eggs.—Cleavage of mouse or rabbit eggs *in vitro* was inhibited by 10^{-4} and 10^{-5} *M* estradiol respectively, although after a critical time, mitosis occurred in spite of addition of hormone (119, 120). The inhibitory action was prevented by addition of amino acids or raising the serum content of the medium above 30 per cent. Blastocysts of the mouse but not of the rabbit still showed sensitivity to progesterone. Norethindrone and norethynodrel had no effect on cleavage of rabbit eggs, although norethindrone acetate, MPA, ethinyl estradiol, and its 3-methyl ether were detrimental (121).

The antifertility action of clomiphene in the rat has been attributed to a direct anti-zygotic action (122). Antifertility mechanisms mediated by direct effects on fertilized egg or altered transport rate will clearly require detailed studies of individual compounds in several species for their elucidation.

Termination of Pre-Implantation Stages

The interdependent, yet antagonistic, roles of estrogen and progesterone in this process have been known and probed for a considerable time. One of the hypotheses concerning the mode of action of contraceptive steroids concerns a possible action affecting the viability of the early zygote.

The availability of relatively simple synthetic estrogens has led to the development of antiestrogenic compounds. Because all progestational substances are steroids, antagonists capable of interfering with post-ovulatory events must come from modifications of the steroid structure. Conventional steroid derivatives, as well as compounds produced by deletion of a carbon atom from ring A (A-nor steroids) and the introduction of heteroatoms (e.g. N or S) at appropriate points in the steroid structure are being explored. Steroid derivatives inhibiting early pregnancy included a 19-nor steroid diacetate and an A-nor steroid (Fig. 2A and 2B). Blastocysts recovered from the rat on day four post-insemination appeared normal and the antifertility action was not reversed by progesterone (123). Twenty-three additional steroids were similarly assayed (124) and it was evident that potent compounds acted as estrogens. A-nor-androstane-2α, 17α-diethynyl-2β, 17β-diol, was highly effective; its primary action is to cause expulsion of pre-implantation phase ova from the rat reproductive tract. Such ova implanted and developed in pseudopregnant recipients. An unexpected finding was estrogenic activity associated with a saturated ring A. Nevertheless, the implantation inhibition was not antagonised by progestogens. Mode of action will have to be carefully defined before the term 'antiprogestational' may properly be applied.

Antifertility studies in the rat using 53 steroids administered from the day of proestrus for seven days (125), again showed that activity frequently correlated with estrogenicity. Progestational compounds were ineffective.

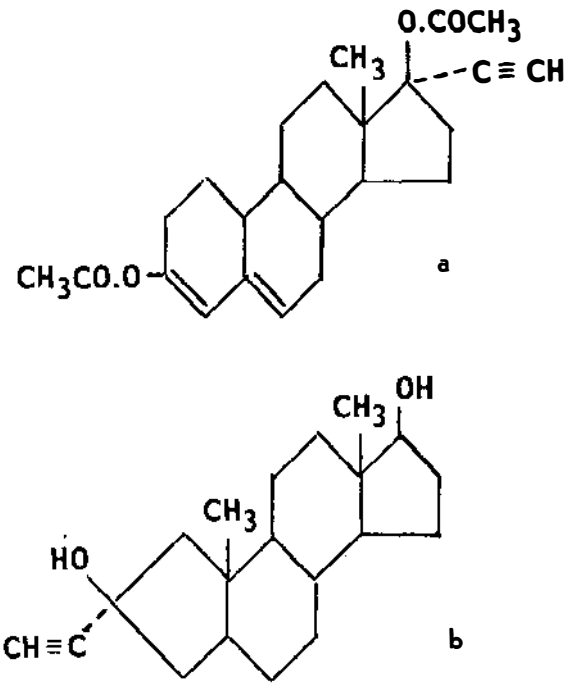


FIG. 2. Inhibitors of early pregnancy.

However, these parameters were separated by several tetrahydropyranyl ethers derived from estradiol lacking either a 3 or 17 oxygen atom. The most active oral compound compared with mestranol was 3-methoxy-17β-cyanethoxyoestra-1, 3, 5 (10)-triene which was sixtyfold more potent in antifertility activity than in estrogenicity. Thus, an effective steroidal antifertility compound seems possible.

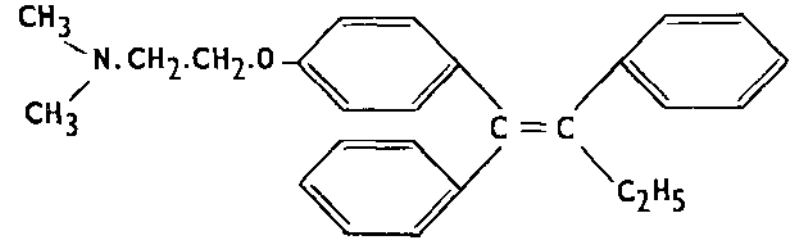
Banik & Pincus (126) inhibited implantation in the rat by estrone (20 μg) on day one post-insemination, which caused premature transport of ova into the uterus and their expulsion. The action was not countered by daily treatment with progesterone or two other potent, progestational compounds–1, 2α-methylene-6-chloro-6-dehydro-17α-hydroxyprogesterone acetate, and 3β–17α-diacetoxy-6α-methylpregn-4-en-20-one. Greenwald (127) found that the stated doses of estradiol cyclopentylpropionate administered shortly after mating, interrupted pregnancy in most animals of the following species: guinea pig (10 μg); hamster (25 μg); mouse (1 μg); rabbit (50 μg); rat (10 μg). These doses accelerated egg transport except in the hamster and guinea pig, in which four and ten times the amount was

required to speed up passage of the zygote. 'Tube locking' of eggs was not demonstrable in the rat. In hamsters and guinea pigs, other mechanisms must be involved in the termination of pregnancy, e.g. luteolytic action in corpora lutea of pregnancy has been demonstrated (128).

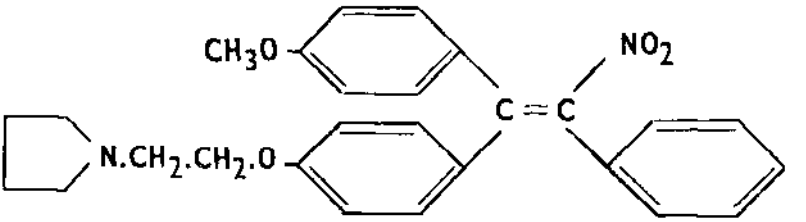
Reviews of the antiestrogens, ethamoxy-triphetol, clomiphene, and two 'cyclized' structural versions are available (129, 5). Perhaps the most unexpected phenomenon was that clomiphene inhibited ovulation in the rat, but stimulated the process in women, although this does not preclude an effective post-coital action also. In further studies in the rat, clomiphene was found to terminate the early stages of gestation in different ways, including direct action on the blastocyst, depending upon dose and time of administration (130). An antigonadotrophic effect need not be involved in this mode of action and normal ovarian steroid production was maintained in effectively treated animals, as indicated by induction of the decidual cell response (DCR). On the other hand, treatment of blastocysts held viable by the delayed implantation technique indicated that clomiphene had no direct toxic action on the early embryo (131). Estrogen failed to induce implantation of such blastocysts held in the ligated uterus.

The antifertility action of 'cyclized' triphenylethylene compounds, 1-[2-[*p*-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-ethyl]-pyrrolidine (U-11,100A) and 1-[2-[*p*-(3-hydro-5-methoxy-2-phenylindyl)phenoxy]-ethyl]-diethylamine (U-11,555A) is also restricted in the rat to the post-ovulatory, preimplantation interval. Prolongation of this phase by MPA with concurrent administration of U-11,100A up to 10 days post-insemination did not reduce the number of blastocysts recovered. These remained viable as shown by transfer to untreated recipient females (132). Direct protection by the progestational compound was deemed unlikely. Some acceleration of passage through the oviducts was demonstrable, but was not considered a major factor. In exploring the possible estrogen-antiestrogen mechanism, these authors stress the critical concentration of estrogen required for implantation to occur. The action and potency of U11,100A and U11,555A are considered due to interference with the particularly susceptible estrogen dependent implantation process of the rat. Shelesnyak (133) believed that U-11,555A blocked the action of the estrogen surge, thus preventing nidation. On the other hand, the antifertility activity of U-11,100A, in the mouse (given on days one to three or four to six of gestation) was ascribed to its estrogenicity (134). The biological manifestations of interactions between this compound and estradiol are described as 'complex.'

The effectiveness of topically applied progestational compounds (135, 136) has been extended to nonsteroidal compounds applied in a 'vanishing' cream base (137). Comparable antifertility actions were obtained topically and subcutaneously. Clomiphene was ten times as potent as U-11,555A by either route, using single or multiple exposures.



ICI - 46,474



A nitro-triphenylethylene derivative

FIG. 3.

Other variations on the triphenylethylene theme include *trans*-1-(*p*-*β*-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene (ICI-46,474) (138) and a nitroethylene derivative (139) (Fig. 3). The former compound emphasizes the importance of the geometrical disposition of groups about the basic ethylene bond. Thus the *cis*-isomer (ICI-47,699), behaved like a conventional estrogen, whereas the *trans* substance (ICI-46,474) was very effective in preventing implantation when given up to the fifth day of pregnancy in the rat. The principal mechanism is probably counteraction of the essential estrogen-release required for implantation (140); the compound is believed to persist for some days in the uterus.

The so-called 'estrogen surge' in the rat is postulated as the final link in a timed chain of events normally initiated by the stimulus of mating, which induces gonadotrophin secretion via the hypothalamus and pituitary to stimulate ovarian estrogen output. The LH outflow occurs about 18 hours before the uterus achieves maximum sensitivity to the estrogenic decidualizing action. Thus, daily administration of reserpine, chlorpromazine, or trifluoperazine after mating will delay implantation in rats but not if treat-

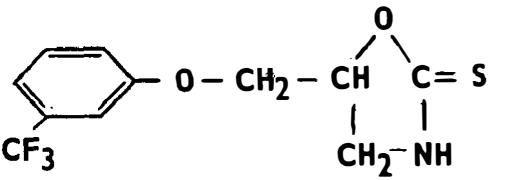


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FIG. 4.

ment is postponed to day four post-coitum, when the hypothalamic-pituitary interaction has occurred (141). Mayer (142) presented an interesting discussion on a series of phenothiazine tranquilizers and nidation. The delay in implantation may be terminated, by a minute dose (0.1 μg) of injected estradiol, as well as by local application of hormone to the uterus. A similar hormonal action has been shown in rats treated with trifluoperazine (143).

Evidence against the 'estrogen surge' hypothesis has been provided by De Feo (144). Pentobarbital, which blocks LH release in rats, did not affect the time of maximal sensitivity to decidualization. Either treatment was inadequate, or the CNS surge mechanism did not occur. Further, in castrated animals without cervical stimulation, the development of maximal uterine susceptibility was produced by administration of estrogen/progesterone in a fixed ratio (145). The phenomenon appeared to date to the preceding ovulation rather than to stimulation of the uterine cervix.

The nitro-triphenylethylene compound (1-[2-[*p*-[-α-(*p*-methoxyphenyl)-β-nitrostyryl]-phenoxy]ethyl]pyrrolidine) (Fig. 3) is hypocholesterolaemic and shows antifertility activity in the mouse, rat, and dog (139). In the rat, complete effectiveness is claimed for 25 μg/kg daily from days zero to eight post-coitum or for a single dose of 500 μg/kg in a post-coital test. The compound showed antiestrogenic properties. Chronic administration was tolerated at 1000 times the therapeutic level.

Pregnancy inhibition has also been reported with a 2,3-diphenylbenzofurane derivative (146), and recently, by a quite different type of nonsteroidal chemical, an oxazolidinethione (Fig. 4) (147). In rats, pregnancy was completely prevented by single oral doses on day four only, but mice and hamsters were insusceptible by this route. Effective doses in the rat were not antiestrogenic, antigonadotrophic or toxic to the blastocyst or fetus. The ability to prevent the decidual cell response was not reversed by progesterone or estradiol.

One report of human fertility control with estrogen alone has recently appeared. Middleton (148) described the control of ovulation with stilbestrol in 86 selected patients for up to three years.

Implantation

Contact between the blastocyst and endometrium under appropriate physiological circumstances leads to embedding of the zygote by active invasion, engulfment by the endometrium, or both. The mechanisms involved are still controversial. The role of estrogen in nidation has been discussed at length (149), and McLaren (150) has provided a digestible, condensed account of maternal factors involved in nidation. Unlike the situation in the rat and mouse, implantation in the ovariectomized guinea pig or rabbit does not require exogenous estrogen. Is this true in the human, and if so, are compounds which inhibit implantation via the estrogen surge in the rat likely to be ineffective in women for this reason? The non-specific nature of uterine implantation is emphasized by the fact that blastocysts readily implant and develop in a variety of extrauterine sites (anterior chamber of the eye, kidney, peritoneal cavity) irrespective of sex and hormonal status of the host (150).

The Shelesnyak school believes that contact between blastocyst and estrogen-sensitized endometrium induces decidualization in the latter, with histamine as a possible chemical mediator (149). Depletion of uterine mast cells (the presumed histamine source) prevents decidualization and, hence, nidation (151). The artifically induced decidual cell reaction in the pseudopregnant rat and mouse is often utilized in seeking mechanisms. According to Marcus and associates (152), the hormonal circumstances in pseudopregnancy and pregnancy are not identical. No histamine release occurred after the estrogen surge of pseudopregnancy, even when supplemented by additional estrogen. Others have failed to show that histamine produced a greater decidual cell reaction than saline alone (153), and the antihistamine, mepyramine, failed to antagonize the response (154). Reduction of the mast cell population of the rat uterus by a chemical designated '48/80' did not affect pregnancy incidence in rats, though it was effective in mice (155).

The ergocornine effect on pregnant rats (156) has also been exploited in studies of nidation mechanisms. Indefatigable attempts to elucidate the mode of action of ergocornine in interrupting early pregnancy and pseudopregnancy have led to the conclusion that the compound interferes with the action of progesterone, thus disturbing the requisite hormonal balance. There is no evidence of direct progesterone-ergocornine interplay nor of direct action of the alkaloid on uterus, ovary, pituitary, or hypothalamus (157). Delayed implantation is induced by hypophysectomy and autotransplantation of the pituitary. Nidation did not occur in such animals treated with ergocornine, in spite of an appropriate implantation-triggering dose of estrogen. Progesterone countered this effect of ergocornine in a high percentage of animals, suggesting that the alkaloid acts by reducing progesterone availability (158). It had previously been shown that pregnancy-terminating doses of ergocornine were not toxic to the blastocysts of rats

using delayed implantation produced by hypophysectomy (159). For four ergot alkaloids, the relative effectiveness for termination of rat pregnancy by single injection (s.c.) on day four was: ergokryptine, 175 μg; ergocornine, 335 μg; ergosine, 505 μg; and ergovaline, 945 μg. The highest activity was obtained when the cyclopeptide moiety had two *β-iso*-propyl-5*α-iso*-butyl substituents (160).

The pharmacological basis has been established for the development of post-coital agents for human use, and within the next few years, clinical progress in this field may occur. In fact, trials of at least one estrogenic agent given post-coitally to women have been reported (161). There is much to be said for mere transient disturbance of endocrine mechanisms by occasional dosage post-coitum as opposed to sustained therapy.

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