ANTIFERTILITY SUBSTANCES

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I. INTRODUCTION

The development of antifertility drugs does not appear to have received due attention by the pharmacologist. Yet it is a field offering fascinating problems in fundamental research which carries with it tremendous biological potentialities—for both good and ill.

The object of this review is to present the problems involved in the pharmacological achievement of selective interference with the mammalian reproductive process, and to describe the progress made to date. So long and complex are the physiological events culminating in the production of the mammalian offspring, that there are very many possible points of interference by chemical agents. Unfortunately, knowledge of the natural controlling factors and the dynamics of the process is relatively rudimentary. Interference with reproduction may take the form of direct or indirect damage to the developing or mature gametes prior to copulation, or after this event but before fertilization. The union of sperm and ovum may be prevented or the fertilized ovum may be hindered during the process of implantation into the uterus. Finally the implanted embryo may be the focus of chemical attack in various ways at different stages of its development. It should not be overlooked that studies of drug action on reproduction may also furnish valuable information concerning reproductive physiology.

The control of population growth and the development of antifertility agents have, of course, been reviewed from time to time and reference may be made to the more important of these articles (65, 101, 119, 204).

II. SPERMATOGENESIS

Mammalian spermatogenesis is divisible into two main phases: the formation of morphologically mature sperm within the testis and the collection of these cells into the epididymis, a single tortuous tube of considerable length which terminates in the vas deferens.

The testicular phase. The principal stages of the extremely complex process of spermatogenesis are indicated in Table 1. Within the testis there are numerous

TABLE 1

*Outline of the development of spermatozoa**

Stage	Number of Cells†
Type A spermatogonia	1
"Intermediate type"	6
Type B spermatogonia	12
Primary spermatocytes	24
Secondary spermatocytes	48
Spermatids	96
Spermatozoa	96

* Adapted from work in the rat by Leblond and Clermont (84).

† The estimated number of cells originating from one spermatogonium is shown for each stage. The population of spermatogonia is believed to be maintained by one of 4 cells arising from the second division of type A cells.

minute individual seminiferous tubules, in each of which spermatogenic activity occurs as a continuous series of waves. The result is the liberation of morphologically mature spermatozoa into the lumen of the tubules. Very little is known concerning the dynamics of this intricate process or the factors which initiate and regulate its many phases. Recent estimates of the duration of the testicular phase in rats and mice indicates that upwards of 35 days is required for the conversion of spermatogonia into spermatozoa. During this time several further generations of spermatogonia have successively commenced the differentiation process, so that the histological appearance of the testis presents a varied series of cell associations, according to the stages of activity of the particular portion of the tubule examined. In the rat, during the development of 96 spermatozoa from each spermatogonium there are seven successive divisions of cells, six of which are normal (mitosis) whilst in one the number of chromosomes is reduced to one-half (meiosis). This latter process occurs during the conversion of primary to secondary spermatocytes and is unique among the body cells. The transformation of spermatids to spermatozoa, which occupies about 20 days, does not involve cell division but is a complex metamorphosis. It is important to note that all the spermatogenic cells are apparently in a similar environment and therefore presumably accessible to any substance which may gain entry to the tubules. Since there is a continuous passage of fluid and other materials towards the lumen of the tubule, cells at the periphery should be the first to be exposed to a drug. Although in some species spermatogenesis is subject to considerable variation, in rats and mice under laboratory conditions mitotic activity in the testis appears to be constant throughout the day (17, 29). Close agreement in the number of tubules containing cells at any given stage of the seminiferous cycles has also been observed in different rats (84). The picture of a continuous production of spermatozoa is in keeping with experimental evidence derived from the use of antifertility drugs (12) and the progressive maturation of germinal cells with final emptying of the testis and epididymis in unmated adult rats in which spermatogonia had been selectively destroyed by radiation (154, 171).

The epididymal phase. Morphologically mature sperm from the seminiferous tubules are collected by a number of ducts which discharge their contents into one main stream, the epididymis. This latter structure has long been regarded as a reservoir for spermatozoa, but its functions are not clearly understood. In keeping with the continuous production of spermatozoa by the testis, it has been demonstrated that, in the rat, injected radio-opaque material requires upwards of 14 days to traverse the epididymis (91). This movement was further shown to be due mainly to an independent secretion of fluid at the proximal end of the epididymis with a resorptive process towards the far end of this structure. Presumably this circulation of fluid is partly responsible for the onward progression of spermatozoa, assisted by peristalsis along the epididymis. The fate of sperm not utilized for insemination is not clear. The fact that spermatozoa spend so long traversing the epididymis is indicative of an important physiological role. In it the sperm are believed to undergo a maturation process and are nonmotile. During this phase, spermatozoa should be readily accessible to drug action in view of the adequate blood supply to this organ and the secretion of fluid by the epididymal cells. It is also possible that the vulnerability of spermatozoa to chemical agents will vary according to their location along the epididymal pathway. Thus the concept of spermatogenesis in rodents—in which the vast majority of drug studies have been carried out—is of a continuous process, with little or no hold-up of spermatozoa in the testis or epididymis.

Outside the tubular structure of the testis lies the interstitial tissue, a connective tissue framework supporting the tubules and conveying the vascular, lymphatic and nerve supply; it contains also the cells of Leydig, producing testosterone and responsible for the maintenance of fertility via the accessory reproductive organs and secretions. Over-all control is exercised by the pituitary hormones; follicle-stimulating hormone maintains the continued activity of the seminiferous epithelium and luteinizing hormone the production of male hormone.

Turnover of spermatozoa. The determination of sterile periods after drug treatment may be useful in elucidating the duration of spermatogenic phases and in locating the cell types whose function is impaired by the substances concerned. For this purpose it is essential that the drug treatment should neither significantly interfere with the normal timing of spermatogenic transformations nor with the transport mechanisms in the seminiferous tubules or within the epididymis. A series of experiments with certain drugs have demonstrated decisively that in rats, at least, sperm turnover was largely independent of mating frequency, and that induced sterile phases were independent of varying delays after treatment before a series of matings was commenced (72).

III. CHEMICAL INTERFERENCE WITH SPERMATOGENESIS

There are clearly many opportunities for direct chemical interference with spermatogenic cells or spermatozoa, or with the local organization which must control this complicated, ordered series of transformations. The obvious means of detecting antifertility activity in a compound is by comparing the fertility of

treated and untreated males mated with normal females, preferably all of established fertility. Until recently, histological evidence of drug effects on the testis was the primary concern of investigators, with little mention of fertility studies. Since antifertility effects may be produced without obvious histological damage (75, 169), alterations in fertility should be the primary concern, with testicular histology as an ancillary investigation. Because spermatogenesis occupies at least six weeks it is necessary to test fertility for seven weeks or more from treatment. Separate matings of treated males with one female per week have been found to be a satisfactory method and produced consistent fertility patterns (12, 72). Instead of allowing impregnated females to go to term, additional information can be gained by examining the uterine contents at mid-term for viable and nonviable embryos and implantation sites, and determination of the number of corpora lutea in the ovaries (7). In this way a more comprehensive picture of drug action can be formed. Either method of investigation of fertility is tedious and requires considerable numbers of fertile animals. In the light of present knowledge sporadic tests of fertility are unsatisfactory.

Antifertility effects may be produced by interfering with the secretion of gonadotrophins or by directly antagonizing the action of testosterone. It is well known that reversible inhibition of spermatogenesis can be produced by administration of androgens and oestrogens. Testosterone propionate in low doses suppresses spermatogenesis in laboratory animals because of a fall in gonadotrophin production. High doses, although inhibiting this pituitary function, apparently stimulate the seminiferous epithelium directly (90). Administration of testosterone propionate to men (50 mg, 3 doses weekly) caused a progressive diminution in sperm count to very low levels during the course of several months (62, 63). Low counts persisted as long as treatment was maintained, after which recovery rapidly followed. Oestradiol and oestrogenic substances produced a severe suppression of testicular function in rats, which could be prevented by simultaneous injection of androgen (153). The oestrogenic substance genistein (4′,5,7-trihydroxyisoflavone) has been reported to produce subfertility and sterility in male mice (48). The dose required was large (15 mg per animal daily for 22 to 25 days), and some animals did not recover their fertility when treatment was discontinued. A hormonal approach to the control of male fertility seems likely to be unprofitable in the light of existing knowledge of endocrine control and duration of spermatogenesis. Certainly rapidity of action is unlikely to be achieved by such means, and administration of hormones and their synthetic analogues may not only interfere with sexual activity but also produce a more general disturbance of the endocrine system.

Nitrofuranes and related compounds. During the course of investigations into the potentialities of nitrofurane derivatives as tumour inhibitors, it was observed that continued high oral dosage of furadroxyl [5-nitro-2-furfuraldehyde-2-(2-hydroxyethyl)semicarbazone] exerted a specific effect on the testicular germinal epithelium of the rat (133). Other organs were unaffected, with the exception of the adrenal gland, in which reversible changes of a nonspecific nature were noted. In this interesting work the testicular changes were described as "necrosis fol-

lowed by a maturation arrest that is reversible." Experiments were carried out to show that inanition was not involved in the production of the changes observed, whilst pituitary extracts affected neither the degenerative process nor the subsequent restoration of the epithelium. Similarities were pointed out between the nitrofurane-induced changes and those caused by experimental cryptorchidism, severe malnutrition, irradiation and the administration of some nitrogen mustards. These comparisons are, however, only broad generalizations. The effects on male rat fertility of furadroxyl and two other nitrofurane derivatives, Furacin (5-nitro-2-furaldehyde semicarbazone) and Furadantin [n-(5-nitro-2-furfurylidene)-1-aminohydantoin], have since been investigated (112). All three substances when given by mouth produced a halt in spermatogenesis at the primary spermatocyte stage which was not countered by various supplementary measures. The minimal effective doses per kg of diet were: furacin, 0.2 g; furadroxyl, 0.75 g; and furadantin, 1.5 g. The action was apparently direct on the testis and developed only slowly, requiring several weeks to produce maximal effects, but the damage was not permanent. Even after 100 days of treatment, withdrawal of the drug was followed by complete recovery, assessed histologically and by fertile matings. No evidence was obtained of damage to other organs during treatment, and the body weight of animals was similar to that of controls. It is interesting that chronic under-feeding in young, adult animals produced spermatogenic arrest also at the primary spermatocyte stage (166); spermatogonia (and Sertoli cells) persisted except in very severe cases in which complete degeneration of the seminiferous epithelium occurred. Refeeding produced a rapid improvement in the testicular structure.

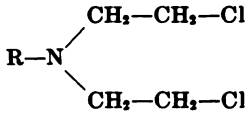
Nitrofuranes have been shown to interfere with carbohydrate metabolism and their effect on the testis appears limited to the meiotic division (108, 123); the metabolism of the testis becomes altered and the utilization of glucose and citric acid synthesis strongly inhibited (124). Other workers have shown that the seminiferous tubules, in similar circumstances, could not oxidize pyruvate *in vitro*; restoration of this function coincided with the reappearance of spermatids and spermatozoa in the tubules (50). Administration of furadroxyl to rats also caused a significant increase in the gonadotrophin content of the pituitary as well as "castration changes" in that organ; various gonadotrophin preparations failed to prevent the spermatocytic arrest induced by the compound (110). Furadantin is used in the treatment of urinary infections in man, but in the doses given it exerted only a slight inhibitory action on spermatogenesis (109). Daily ingestion by rats of Furacin (0.3 mg/kg), the most potent of these drugs, produced no effect on male rat fertility during a period of 7 weeks (74). Nor did a course of 5 injections of Furadantin [20 mg/kg intraperitoneally (i.p.)]. These results are not contradictory to those quoted earlier but emphasize the relative insusceptibility of the spermatogenic process to these substances and the prolonged time required for the effects on fertility to be manifest. On these grounds any practical application of this type of compound appears unlikely.

5-Chloro-2-acetylthiophene also induced effects in the male germinal epithelium very similar to those produced by nitrofurane derivatives. The compound

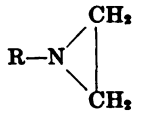
was also active by mouth (0.5 g/kg diet) and relatively slow degenerative changes followed, which were reversible. The depletion process again left spermatogonia and Sertoli cells unaffected (169).

Another related nitro-compound, 2-amino-5-nitrothiazole (Enheptin), used in the treatment of infectious enterohepatitis in the fowl, caused complete suppression of sexual function and of secondary sexual characters in the domestic fowl (69, 130). This effect, however, appeared to be due to a decrease in gonadotrophin output of the pituitary (131) and was alleviated by administration of pregnant mare serum (PMS) gonadotrophin. The compound did not produce such effects in rats; instead it had an antithyroid action similar to that of thiouracil (164). This is another example of the unpredictability of drug action in different species.

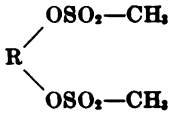
Radiomimetic alkylating chemicals. Several chemical varieties of alkylating agent are known which have biological effects more or less resembling those produced by exposure to ionizing radiations. They include nitrogen mustards (β-haloalkylamines), derivatives of ethyleneimine, and sulphonoxyalkanes:



Nitrogen mustards



Ethyleneimines



Sulphonoxyalkanes

In general the biological properties of these substances depend on the number of alkylating groups and the carrier molecule to which they are attached. Their varied biological properties include tumour inhibition and depression or destruction of normal proliferating tissues (bone marrow, lymphoid and germinal cells) duly ascribed to damage to cells engaged in division. In addition they are mutagenic and have carcinogenic properties. The biological activities of alkylating agents nevertheless present puzzling features, and the relative susceptibility of different normal and pathological proliferative tissues varies widely in the same and different species. The effects are generally believed to be due to the chemical reaction of these compounds with cell components essential to cell division—nucleoprotein is the most favoured material at present. Antifertility effects due to exposure to such substances are therefore not unexpected. After intraperitoneal injection into mice of a number of aliphatic nitrogen mustards, destructive changes in the testis were found similar to those resulting from metabolic deficiency, irradiation and many other chemical agents (83). Recovery of spermatogenic function was observed 3 to 4 weeks after nonfatal single or repeated doses. The results of injecting an aliphatic nitrogen mustard into rats have also been reported (56). Most males were sterile after injection of the maximum tolerated dose [1 mg/kg intravenously (i.v.)] owing to inhibition of spermatogenesis and testicular atrophy. In some animals regeneration occurred and potency returned after 2 to 4 months. Aliphatic nitrogen mustards are highly reactive and toxic chemicals with a very brief survival time after injection into the body. However, two aromatic nitrogen mustards, *p*-(N,N-di-2-chloroethyl)amino-L-phenylalanine (Melphalan, C.B. 3025) and *p*-(N,N-di-2-chloroethyl)amino-phenylbutyric

acid (Chlorambucil, C.B. 1348), did not interfere with the fertility of male rats (72, 74). The doses used were adequate to produce transient leukopenia in the peripheral white cell count (49), and inhibition of tumour growth (9). Ability to inhibit tumour growth or suppress the activity of bone marrow does not therefore imply a similar ability to damage the germinal epithelium.

Among ethyleneimine derivatives the trifunctional triazine compound 2,4,6-triethyleneimonotriazine (triethylenemelamine, TEM, Tretamine) is well known. Although chemically relatively unreactive compared with aliphatic nitrogen mustards it is, nevertheless, highly active biologically. Its destructive action on the testis of the dog and rat was reported during investigations of the tumour-inhibiting properties of ethyleneimine derivatives (64). By means of a systematic mating procedure it was later shown that small doses of TEM produced well defined effects on male rat fertility (12), indicative of selective interference with certain stages of spermatogenesis. After a single dose of TEM (0.2 mg/kg, i.p.) a period of sterility occurred 4 weeks later (22 to 26 days from the dose), preceded by subfertility and succeeded by the return of normal fertility in the fifth week. This phase of infertility in rats is probably due to interference with spermatocytes (8, 73) and not to spermatogonial damage as was suggested earlier (12). The selective nature of the effect was emphasized by the rapid recovery of normal fertility a few days later. A short course of treatment (5 daily doses, 0.2 mg/kg, i.p.) resulted in a spread of drug action such that the males were completely sterile for several weeks; recovery of fertility occurred 5 to 6 weeks from treatment. The TEM thus affected earlier and later stages of spermatogenesis including mature sperm in the epididymis. Not only could TEM induce sterility very rapidly but the drug action was cumulative, for very small daily doses (0.05 mg/kg) soon produced and maintained the infertile state; full recovery occurred some weeks after treatment was discontinued (75). Other interesting features of these experiments were the absence of noticeable oligospermia and the retention by the sperm of motility and the ability to reach and penetrate ova; the "fertilized" ova did not develop to any significant degree. There was no evidence of interference with sexual behavior and the drug appeared to act directly on the germinal cells.

The absence of histological damage after continued low dosage with TEM has been confirmed (172). At the higher dose level (5 daily doses, 0.2 mg/kg) these investigators reported that "a radiomimetic type of damage to the germinal epithelium occurred characterized by apparent destruction of spermatogonia, the remaining cells continuing to develop into mature spermatozoa" (172). In rats a second brief phase of sterility occurs about 9 weeks after TEM (5×0.2 mg/kg) which is probably due to the destruction of spermatogonia (33a). The loss of these cells from the germinal epithelium is not necessarily associated with a prolonged infertile period. After radiation (300 r whole-body) it has recently been shown that a short period of infertility also occurs 9 to 10 weeks later (33a). Larger doses cause prolonged sterility. Male mice show a similar sensitivity to TEM. The maximum effect, 11 to 14 days after injection (2 doses, 0.4 mg/kg), was due to the induction of dominant lethals in spermatids (24, 25). After rapid

recovery of normal fertility, sterility again occurred in the fifth week associated with aspermia, attributed to spermatogonial destruction. These results in rat and mouse clearly indicate marked differences in the sensitivity of spermatogenic cells to this drug and in the timing of spermatogenic events in these animals. The male rabbit is also very sensitive to TEM. Sterility developed a few days following the commencement of a course of treatment (0.05 mg/kg, i.v. daily for 29 days) and persisted thereafter. Sperm were present at copulation in apparently normal numbers for about 50 days, after which mating became abruptly aspermic (74), although specimens of seminal fluid were of normal volume. Even a short course of treatment at a lower dose level (0.01 mg/kg, i.v.) produced definite periods of infertility.

TEM is an alkylating substance possessing three reactive groupings, and a number of other ethyleneimino-compounds with 1 to 3 reactive groups have been examined for antifertility effects (72, 73, 76). These included triethylene-thiophosphoramide (thioTEPA) and the di-functional compounds 1,3-*bis*-(ethyleneiminosulphonyl) propane, 4-chloro-2,6-*bis*(ethyleneimino)pyrimidine, diethyleneurea and diethyleneiminosulphoxide. Three monofunctional ethylene-imino-compounds have also been tested, *viz.*, monoethyleneurea, carbethoxy-ethyleneimine and *p*-toluenesulphonylethyleneimine. With the exception of *p*-toluenesulphonylethyleneimine (which was inactive) all produced antifertility effects qualitatively similar to those induced by TEM (73, 77). The tri-functional compounds and the pyrimidine derivative were by far the most active and capable of producing selective effects. Increasing the dose of the monofunctional compounds above that causing minimal effects produced permanent destruction of the seminiferous epithelium. Not only were the polyfunctional compounds much more potent than the monofunctional ones but effective quantities of the latter were closer to the lethal dose. With one exception (diethyleneiminosulph-oxide) the tri- and di-functional ethyleneimines effectively inhibited the growth of the Walker tumour, while the monofunctional agents were inactive in this respect. Even sterilizing doses of monoethyleneurea produced only a transient fall in the white cell count. Selective pharmacological action on different systems of proliferating cells seems to be a distinct possibility.

The action of ethyleneimino-compounds on the seminiferous epithelium is undoubtedly complicated and may not be entirely due to direct action on the cells. The relative insusceptibility of spermatogonia to these substances contrasts with the fact that they are the germinal cells most susceptible to radiation (115, 154). In relation to the antifertility effect of certain derivatives of ethyleneimines it is interesting to recall that, not only is the effective dose small, but the compounds disappear rapidly from the circulation. Although the mouse possesses the ability to metabolize completely the ethyleneimino groups of injected compounds (33, 105), in the rat and man considerable proportions of ethyleneimino-compounds appear in the urine (31, 33, 94), including unmodified drug. This is consistent with their relative chemical stability.

Sulphonoxyalkanes form another group of alkylating agents of which 1,4-dimethanesulphonoxy butane (Myleran, busulphan) is well known in cancer

chemotherapy. It is a di-functional alkylating chemical able to inhibit tumour growth (9, 173a) and to cause a selective and profound depression of granulocytes in the peripheral blood. Its effects on the germinal epithelium are remarkable and different from those of TEM. According to a first report, 2 doses to male rats (11 mg/kg, i.p.), separated by an interval of 14 days, caused progressive and complete destruction of the germinal epithelium examined over a period of 150 days (13). After only one such dose, the testis histology was said to remain unaltered during the 30 days following the dose. However, it has since been shown that fertility remained normal until the eighth week after one similar dose of this drug, when sterility rapidly developed with associated oligo- or aspermia (32, 72). These results clearly indicated a blocking or destructive action of the drug on an early stage of spermatogenesis. This treatment interfered with spermatogenesis and fertility in a specific manner, for the development of new spermatogonia was prevented, while the germinal cells present at the time of treatment appeared to develop normally into mature sperm over a period of about 45 days. Thus the seminiferous epithelium became systematically depleted of spermatogonia, spermatocytes, spermatids and finally spermatozoa in this sequence (72). This accounted for the continuation of normal fertility after treatment, until the supply of spermatozoa was exhausted. The ensuing period of sterility persisted until repopulation of the testis tubules occurred (apparent at 45 days by the presence of numerous spermatogonia) and the sperm formed had traversed the epididymis. It is curious, however, that increased doses of Myleran did not result in a spread of action to adjacent spermatogenic cells which are also actively proliferating. There was no impairment of fertility with large and lethal doses of the drug (77). 1,4-Dimethylmyleran produced similar effects at a lower dose level (74). Mice are much less susceptible than rats to Myleran (173a); doses causing tumour inhibition (*e.g.*, 7 daily doses, 5 to 10 mg/kg, i.p.) caused an abrupt loss of fertility in the 5th week from the first dose (74a). The changes produced by these methane sulphonic esters can closely imitate the effect of radiation (33a, 171) and contrast with those due to ethyleneimines.

Spermatogenic cells thus vary in their susceptibility to different chemical types of alkylating agent according to the stage of their development. Species variations may well be connected with ability to metabolize or rapidly excrete the compounds administered. Modes of action are indicated for different groups of alkylating agent which are difficult to reconcile with the idea of a common nucleotoxic action based on the chemical reactivity of such drugs. In the seminiferous epithelium, cells of all kinds, dividing and differentiating, are present in a similar environment, so that the different antifertility effects produced by chemical types of alkylating agents suggest actions of a different character. Further investigations with these substances will contribute to knowledge of spermatogenesis but their exploitation as antifertility agents would be hazardous in view of the known carcinogenicity and mutagenicity of some of these substances, while the vulnerability of the bone marrow, especially in man, is a more immediate danger.

Colchicine. This substance is a tropolone alkaloid and a spindle poison, arresting

cell division at metaphase; high concentrations cause pycnosis and cell degeneration. Its ability to inhibit the sexual stages in ferns (99) led to a study of its effects on spermatogenesis in rabbits (5). According to this work, 1.5 to 3 mg of the alkaloid subcutaneously twice weekly to adult male rabbits produced no toxic signs. Histologically there were no pathological lesions except in the testes, which were one-third the weight of those from control animals. Although various degrees of degenerative change were found, in most instances it was difficult to recognize the material as testicular tissue, although interstitial tissue and Leydig cells remained intact. No fertility studies were carried out, nor, apparently, were the gonads given the opportunity to carry out reparative processes. A number of colchicine derivatives have been prepared which are markedly less toxic than the parent compound while retaining antimitotic activity against mouse spermatogonia, *e.g.*, trimethylcolchicinic acid methyl ether (10, 52). Investigations on a mouse sarcoma (86) have shown that rather radical changes can be made in the colchicine structure without abolishing tumour-inhibitory activity. On the other hand relatively minor changes in molecular structure were found to alter markedly the abortifacient properties of two colchicine derivatives (200); these will be discussed later. There are no reports of the effects of colchicine and related compounds on male fertility in experimental animals.

Antimetabolites. There appears to be little published information concerning the effects on male fertility of compounds of the direct antimetabolite type, although, as will be seen later, this line of approach has been pursued with vigour in the female during attempts to discover abortifacient drugs. Testicular damage has been described after omission from the diet of essential amino acids including phenylalanine, threonine, histidine, leucine and tryptophane. Recently, ethionine (α-amino-γ-ethylmercaptobutyric acid), an analogue of methionine, has been shown to cause testicular damage in the rat (78). A synthetic, high protein diet was used to which 0.5% of ethionine was added; control experiments showed that the histological changes were not due to the diet used. It is interesting that the order of disappearance of the cellular components of the seminiferous tubules after treatment was spermatozoa and spermatids first, then spermatocytes and spermatogonia. This is the reverse of that following damage due to X-rays and Myleran. Atrophy of cells lining the seminal vesicles and prostate also occurred after ethionine administration, although no histological changes were detected in the cells of Leydig (which produce testosterone). No reference was made to the epididymis, nor were any fertility studies reported. It is not possible to say how far direct action of the drug on the seminiferous epithelium or the accessory glands was involved, although labelled material was localized in the testis when ethionine-S^{35} was fed (51). Pancreatic lesions also developed, but there was apparently no correlation between damage to this gland and the amount of testicular degeneration. The authors suggested, of course, that their results indicated that methionine has an essential role in spermatogenesis. Further studies of this type should be worthwhile, particularly in conjunction with fertility tests. It is difficult to prophesy how far selectivity of action on germinal tissues could be achieved by compounds whose activity is truly competitive with natural sub-

stances. The toxicity of antifolic compounds to spermatozoa has received some attention (174). A degree of correlation between spermicidal activity and antifolic activity suggested that the action on sperm may be due to displacement of folic acid from the cells.

Antihyaluronidases and spermicidal agents. The enzyme hyaluronidase is a component of seminal fluid. Its function is believed by some to be facilitation of penetration of the ovum by the spermatozoon. For this, it is possible that the male gamete possesses its own supply of the enzyme. In some mammalian species the recently ovulated ovum is surrounded by layers of cells, the zona folliculi, and it was thought that seminal hyaluronidase may encourage the dispersal of these cells and allow easier access of spermatozoa. However, it has been shown that this condition is not a prerequisite to fertilization (26). Since relatively few sperm reach the vicinity of the ovum (which also implies that only a minute amount of seminal fluid could reach this location) the over-all role of hyaluronidase remains in doubt. Nevertheless interesting data have emerged from studies of this enzyme and attempts to interfere with fertility by means of hyaluronidase inhibitors.

Estimation of the hyaluronidase content of the rat testis has been used as a criterion of the functional integrity of the germinal epithelium and provides a method of investigating drug action on the male germinal epithelium. After oestradiol or diethylstilboestrol, for example, the enzyme level showed an initial decrease about the 15th day and approached zero some 30 days after treatment (170). Testosterone propionate did not alter the enzyme levels in normal animals, but maintained it in hypophysectomized and hypophysectomized, oestrogen-treated animals. Sertoli cells and spermatogonia were not apparently concerned with the elaboration of testicular hyaluronidase; it had earlier been suggested that spermatocytes were the responsible cells (134). By means of the drug furadroxyl, the production of hyaluronidase has recently been correlated with the spermatid phase of spermatogenesis (171). Thus 10 days after treatment with this drug (which causes arrest of spermatogenesis at the primary spermatocyte stage) a significant drop in enzyme concentration occurred and there was an associated fall in spermatid number. By 15 days the enzyme concentration was negligible, with spermatids and sperm absent in most tubules. The numbers of primary spermatocytes remained relatively large even at 30 days, when there was complete lack of enzyme. It may eventually be possible to investigate the effects of antifertility drugs on other testicular biochemical systems and to correlate these with particular types of spermatogenic cell.

Much interest was aroused some years ago by the claim that hesperidin—a flavonoid substance and a powerful inhibitor of hyaluronidase—was an active antifertility substance when injected into rats and mice (97), and was effective by mouth in man (165). These claims have apparently not been substantiated (28, 190). Some of the most powerful antihyaluronidases are not lethal to sperm and exert little or no effect on sperm motility *in vitro*. Many organic compounds have been tested for inhibitory action against this enzyme (59, 60), and one of the most potent was rehibin, a polymerization product of gentisic acid with

formaldehyde. It was nevertheless devoid of antifertility activity (118). Apparently inhibition of seminal hyaluronidase does not render sperm infertile. From an investigation of the antifertility activity of antihyaluronidases chemically related to rehibin (137), one polymer, 53 D/K, derived from hydroquinone sulphonic acid and added to rabbit semen before artificial insemination, inhibited both hyaluronidase and fertilization. Sperm motility was however unaffected *in vitro* by concentrations 10 times greater, indicating the possibility of developing spermicidal compounds which permit the retention of sperm motility. It seems unlikely that after oral administration, antihyaluronidases could reach secretions of the male genital pathway in concentrations adequate to interfere with the natural function of spermatozoa. This applies particularly to complex molecules which are primarily hyaluronidase inhibitors.

The dose of X-radiation needed to interfere with sperm motility is far in excess of that required to produce infertility. Sterilization of sperm by irradiation evidently does not involve damage to biochemical processes connected with motility; damage to nuclear material of the head is much more likely (1, 95). Sperm from TEM-treated (sterile) male rats also retained motility and were able to reach and penetrate ova but subsequent development did not occur (12). Certain other ethyleneimino-compounds also render sperm in the epididymis infertile, being the only substances yet known to affect mature sperm in this situation. They, too, are believed to react preferentially with nuclear components of the cell. The persistence of sperm motility after *in vivo* or *in vitro* exposure to a drug does not eliminate a sterilizing action.

A great deal of work has been carried out over many years in the search for *in vitro* spermicidal substances, with a view to their application as direct contraceptive agents. The general method is to determine the ability of a substance to immobilize spermatozoa—a rational approach since immobile sperm are infertile. The laboratory assessment of spermicidal activity, however, is a very different matter from the measurement of antifertility activity. Results based on *in vitro* tests are by no means indicative of potential activity *in vivo*, for local conditions in the female reproductive pathway are far removed from those used in laboratory tests. Although sperm can be rendered infertile without interference with motility, the recognition of such an effect requires artificial insemination of sperm after exposure to drugs *in vitro*.

For discussions on the chemical nature of spermicidal agents other articles should be consulted (54, 65, 100). Of more interest is the attempt to unravel details of the mode of action of these substances and to classify them accordingly (95, 95a). The sperm head apparently contains all the deoxyribonucleic acid and is relatively inert metabolically; the tail is the active part of the cell. It is now established that various mechanisms exist whereby chemical agents produce lethal effects on spermatozoa, on the basis of which a general classification of spermicidal compounds is possible, *e.g.*, electrolytes, enzyme inhibitors, SH-binding substances and surface-active agents (95). The importance of SH— groups in sperm metabolism and motility is well established. Inhibitors of this type fall into 3 groups according to the mechanism of inactivation, *e.g.*, oxidizing, mer-

captide-forming and alkylating substances. Oxidizing substances function by converting SH— groups into S—S linkages. Hydrogen peroxide, *o*-iodosobenzoate and quinones are examples of oxidizing spermicides, whereas heavy metals, arsenite, selenite and phenyl mercuric acetate are spermicidal due to mercaptide formation. Iodo-acetate is an alkylating spermicide which acts by blocking SH— groups in intermediary enzymes. Further details, and references to the activities of detergents and soaps, *i.e.*, surface-active compounds, are given elsewhere (95a). There are other groups of compounds which might interfere with essential biochemical mechanisms in spermatozoa. The spermicidal activity of radiomimetic compounds would be an interesting study. Investigations of this type may provide valuable contributions to knowledge of the biochemistry of spermatozoa and seminal fluid.

IV. OVOGENESIS

The development of the ovum, its fertilization, implantation into the uterus and subsequent gestation, provide natural subdivisions convenient for a discussion of antifertility substances. So far as possible these sections are adhered to in the account which follows, with a brief preliminary description of the anatomical and physiological aspects of the phase under consideration.

The formation of the mature ovum ready for penetration by a spermatozoon corresponds to the process of spermatogenesis. Whereas the production of morphologically mature sperm is carried out completely within the testis, the development of the ovum within the ovary is regarded as the counterpart of spermatogenesis only so far as the primary spermatocyte stage. After ovulation, while the ovum and its attendant cells are free in the oviduct, the critical meiosis occurs, *i.e.*, reduction division followed by a single mitosis. Each time, one of the products of division (the "polar bodies") degenerates leaving the mature ovum (now the homologue of the spermatid) ready for fertilization. Within the ovary of the adult mammal the number of potential ova (primordial follicles) is of the order of several hundreds of thousands; in fact, during the reproductive life of the female only a few hundred or a few thousand, depending upon the species, reach maturity. The remainder of the primordial follicles gradually disappear. This "atresia" is said to begin in intrauterine life and continue thereafter. Why so few follicles reach maturity and rupture, while so many degenerate at various stages of their development, is unknown. Why certain ova only are selected for development during each female cycle is equally obscure. It is difficult to imagine a pituitary hormonal control which can act in a sufficiently discriminative fashion unless the ovarian germ cells are in reality progressing in a predetermined order of development. The discovery of chemical agents which act selectively on the ovarian stages would make a valuable contribution to this obscure phase of reproductive physiology. The primitive follicle, when stimulated to develop, undergoes a complicated period of growth in which the ovum does not apparently divide. It lies surrounded by follicular cells, which increase in number by mitosis as the follicle grows and becomes cyst-like owing to the secretion of fluid (Graafian follicle). Ultimately the follicle approaches the surface of the

ovary and rupture occurs (ovulation); this may happen spontaneously or may require the stimulus of coitus, according to the species. The follicular cells are said to fulfil a secretory and nutritional function to the developing ovum.

V. CHEMICAL INTERFERENCE WITH THE OVARIAN STAGE

Suppression of the gonadotrophic activity of the pituitary. a) *Steroid hormones.* Follicular development is due to the secretion of FSH and in animals its administration can produce "super-ovulation." Inhibition of ovulation occurs during pregnancy—a natural period of sterility resulting from combined influence of corpus luteum and embryo on pituitary gonadotrophin output. In rodents, mating with vasectomized males or artificial stimulation of the uterine cervix results in pseudopregnancy, when ovulation is inhibited for approximately two-thirds of the normal duration of pregnancy—the combined effect of neural and hormonal influences on gonadotrophin secretion. Ovulation may be suppressed for a considerable proportion of reproductive life, according to the number of pregnancies, without maternal harm. The development of antifertility compounds along these lines may be a highly successful and harmless procedure provided the mechanism approaches the normal one. Antifertility effects due to prevention of ovulation can be obtained by chemical antagonism to the secretion of gonadotrophin. This may be accomplished by means of oestrogens, androgens and progestational steroids. It is well known that androgens and oestrogens inhibit maturation of ovarian follicles in lower animals and in women. The periodic use of stilboestrol for the control of fertility in women has been suggested (65) *viz.*: 1 mg daily by mouth commencing the first day of menstruation to inhibit ovulation, followed by progesterone to induce menstruation. The effect of continuous administration to adult rats and mice of stilboestrol (in the drinking water) at several different concentrations has recently been reported (4). Prevention of ovulation required dose levels well above those producing the usual oestrogenic effects. Large doses of testosterone propionate suppressed ovulation in women (55, 88, 116). These effects are presumably mediated by inhibition of gonadotrophin secretion.

Experimental evidence supports the view that progesterone can hinder the release of luteinizing hormone from the pituitary; it also suppresses ovulation in rats and mice (2, 121), guinea pigs (38), rabbits (92), ewes (44), gilts and heifers (194). In the rat, the inhibitory effects of oestrogen and progesterone on gonadotrophin secretion may be additive and the amounts required are said to be within physiological limits (23). Oral progesterone reproduced the characteristic effects of the corpus luteum hormone (192), and its ability to inhibit ovulation (*i.e.*, to produce antifertility effects) has been examined in rabbits, rats and human subjects (126). One dose to rabbits [1 to 2 mg per animal, subcutaneously (s.c.) or intravaginally] was effective if administered at least 5 hr before copulation. The minimal effective dose by mouth was 2 to 5 mg. The duration of inhibition depended upon the dose, *e.g.*, more than 24 days after 30 mg, while a subcutaneous pellet could prevent ovulation for many months. Intravenous injection of gonadotrophin into such rabbits produced ovulation in 10 hr, as in normal rabbits,

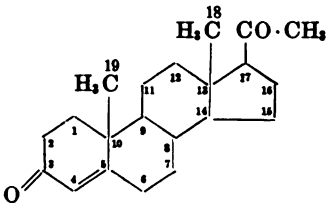
suggesting that the effect was due to prevention of gonadotrophin secretion. In rats, which ovulate spontaneously every 5 days, the effect of progesterone was measured by the prolongation of time between pairing and successful mating. For controls this was about 4.7 days, but increased to between 2 and 4 weeks after single injections (5 to 50 mg, s.c.). The hormone was less effective by mouth with little indication of a dose-response relationship (5 to 50 mg increased the time for successful matings to about 10 days only). Human studies necessitated knowledge of the duration of the menstrual cycle and the determination of the time of ovulation. Using combined diagnostic criteria for the latter, a significant decline in the frequency of ovulation occurred when oral progesterone (300 mg) was administered from days 5 to 25 of the menstrual cycle (126).

b) *Other steroids and miscellaneous substances.* The safety of progesterone, even in large doses, and its freedom from side effects have been established by prolonged clinical use; the same might apply to other antigonadotrophic compounds. In rabbits, 19-nor-progesterone (Figure 1) was shown to be a more active progestational substance than progesterone (191) and to be more effective in depressing the luteinizing capacity of the guinea pig pituitary (96). Two other progesterone derivatives, 11-keto-progesterone and 11β-hydroxyprogesterone, possessed greater antiluteinizing action than would be anticipated from their small progestational activity (96). These observations suggested the possibility of developing compounds able to depress selectively the gonadotrophic activity of the pituitary and so interfere with fertility. A considerable number of steroids have been screened, in the rabbit, by the ability of a single subcutaneous injection of the compound under test (10 mg per animal) to inhibit ovulation (126). Two of 45 compounds tested were considerably more potent than progesterone *viz.*, 17α-ethinyl-19-nor-testosterone (I) and 17α-ethinyl-5(10)-oestraen-17β-ol-3-one (II). They were active by mouth and also effective in the rat. Two other norsteroids, 17α-ethyl-19-nor-testosterone (III) and 17α-methyl-19-nor-testosterone (IV), were also selected for further examination (127, 128). In addition to antifertility activity other progestational effects were assessed, including uterotrophic power and deciduomagenic capacity, while possible results on the development of the ovulated ova were studied. By mouth, compound II (norethynodrel) was a potent progestational substance although its activity was apparently much reduced by injection, for reasons unknown (42); in addition it possessed oestrogenic activity. It did not interfere with the ovarian response to injected gonadotrophin.

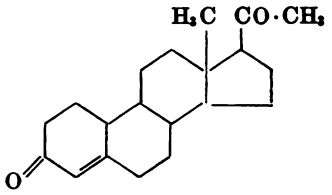
This substance (II) inhibited fertility for 70 days in female rats in doses of 2 mg on alternate days, although matings occurred and the females showed fairly regular vaginal cycles. When treatment was discontinued a sterile period averaging 26 days followed. Inhibition of ovulation appeared to be the antifertility mechanism in some animals, whereas in others ovulation had occurred but the ova had not been fertilized. Mainly fertilized ova were recovered from female rabbits pretreated with compound II (3 days) and then mated with fertile bucks; other treated and mated females produced no offspring (126). Another potent progestational substance, 17α-(2-methallyl)-19-nortestosterone inhibited ovu-

lation in rabbits as did progesterone (49a), but both substances reduced the number of females accepting males.

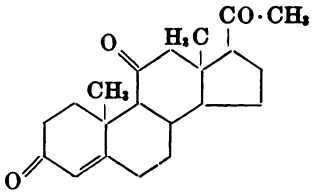
Compounds I–III were also studied in a number of sterile human subjects with normal menstrual rhythm (135, 136). The results indicated that all 3 substances inhibited corpus luteum development and presumably ovulation, in doses ranging from 5 to 50 mg, from the 5th to 25th days of 3 successive cycles. No troublesome



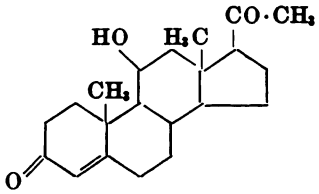
Progesterone



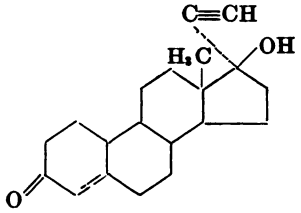
19-Norprogesterone



11-Ketoprogesterone

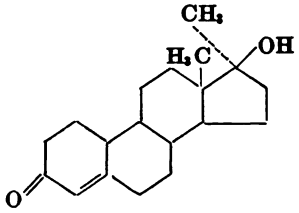


11β-Hydroxyprogesterone



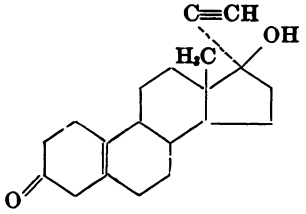
(I)

17α-Ethinyl-19-nor-testosterone (Norlutin)



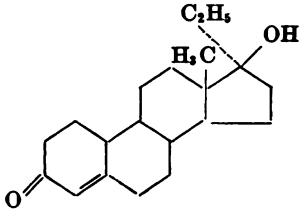
(IV)

17α-Methyl-19-nor-testosterone



(II)

17α-Ethinyl-5(10)-oestraen-17β-ol-3-one (Norethynodrel)



(III)

17α-Ethyl-19-nor-testosterone (Nilevar)

FIG 1. Progestational steroids.

effects on the incidence or duration of menstruation were reported but the possibility of damage to primordial follicles was admitted from examination of the ovaries of 7 patients. In 221 mothers norethynodrel gave complete protection against further pregnancy when taken by mouth daily for 20 days each month. During this treatment the available drug contained a small amount of a contaminant oestrogen—ethinyloestradiol-3-methyl ether. Side actions were encountered—dizziness, nausea and headache—too many apparently for it to be generally acceptable (133a). The pure substance, however, inhibited ovulation just as effectively (129a). Addition of oestrogen diminished the incidence of intermenstrual bleeding during treatment but increased other side effects. Pharmacological and clinical studies with nor-steroids have demonstrated their therapeutic usefulness in replacement therapy for progesterone and as ovulation inhibitors. The latter is not only a potential antifertility property but valuable in the relief of dysmenorrhoea (49b). The freedom from undesirable side effects enjoyed by progesterone is not, so far, a property of other synthetic progestational compounds. The fact that orally active antifertility substances of this type would necessarily be used frequently for prolonged periods, emphasizes the need for extended studies in different animal species in order to provide reassurance that the risks involved are minimal. Nevertheless, the development of compounds capable of selectively inhibiting ovulation in women without unduly disturbing the normal menstrual rhythm is a notable achievement.

Certain other substances and materials have been claimed to produce antifertility effects by interference with ovulation. Thus $\alpha\alpha$-dimethyl-β-(2-naphthyl)-valeric acid and $\alpha\alpha$-dimethyl-β[2(5,6,7,8-tetrahydro)naphthyl]valeric acid were reported to show definite antifertility activity in the rat (126); both compounds are weak oestrogens, which might explain their effects on fertility. The depressant action of *p*-hydroxypropiophenone on pituitary secretion of gonadotrophin has been carefully re-examined (66) with the conclusion that its activity is no greater than expected from its slight oestrogenic properties. Cirantin, a pyrone isolated from the peel of *Citrus aurantium*, for which antifertility activity was claimed, did not inhibit ovulation, prevent fertilization or interfere with implantation in rabbits (126).

Antigonadotrophic materials. Certain plants contain materials able to modify the action of pituitary gonadotrophins. An alcoholic extract of the American desert plant *Lithospermum ruderale*, when mixed with the diet of breeding female mice, induced irregularities in, or suppressed the oestrous cycle and caused reduced fertility (34). Extensive attempts have since been made to determine the mode of action and isolate the active constituent but the problems involved seem complex and the present state of the matter is unsatisfactory. Lithospermum extracts contain tannins and one view (167) is that the effects in mice and rats are largely if not entirely due to the nonspecific toxicity of these substances. An attempt to concentrate the active material from the plant proved unsuccessful (81). Results in mice depending on feeding Lithospermum have been open to question because of possible interference with nutrition due to the high percentage required. However, most investigations support the view that some orally

active material is present in Lithospermum which causes a reduction of reproductive capacity. In the rat aqueous extracts were shown to be much more active when injected than when given by the oral route (114). Such treatment inhibited the secretion of oestrogens and androgens with resultant atrophy of the secondary sex organs, but the direct effect of the gonadal hormones was not affected. The action of various gonadotrophic hormone preparations, injected separately from the Lithosperm extract, was inhibited in both normal and hypophysectomized rats. Lithospermum extracts could also inactivate certain pituitary fractions *in vitro, viz.;* gonadotrophin, prolactin (luteotrophin) and thyrotrophic hormone; the effect of PMS gonadotrophin was prevented although chorionic gonadotrophin was relatively unaffected. Other pituitary hormones and hormones from other sources (except adrenaline) were affected slightly or not at all. There were indications that *in vitro* and *in vivo* activities were due to different materials (57). Extracts from other species of Lithospermum have been obtained which inactivated PMS gonadotrophin when incubated with it *in vitro*, and some activity was demonstrated in *Borago officinalis* and *Rubus idaeus* (57, 58). An aqueous extract of *Lithospermum officinale* has also been used to induce a reversible inhibition of oestrus in mice (201). The activity of this material has been further examined and the inactivation of gonadotrophins found to occur after they have left the pituitary (80). The administration of extracts to patients with hypersecretory activity of the gland is mentioned and recommended. These authors report that in spite of intensive efforts, the active material was not isolated nor was any information obtained about its nature.

Because of the suggestion that Lithospermum activity might be due to tannins, and the claim that the antifertility agent present in *Pisum sativum* was *m*-xylohydroquinone (2,6-dimethylhydroquinone) (147), this compound and a number of related substances were tested for Lithospermum-like activity (113). As little as 10 μg of *m*-xylohydroquinone slowly inactivated 100 units of PMS gonadotrophin *in vitro*, while 10 mg daily subcutaneously to adult rats abolished the oestrous cycle—not without considerable loss of weight. None of the other compounds examined was as active as *m*-xylohydroquinone, and a number of aromatic carboxylic acids and polyhydric phenols were ineffective. Other workers found that a variety of quinones and polyhydroxyaromatic compounds inactivated gonadotrophins *in vitro* (138). Only 2,6-dimethylhydroquinone and hydroquinone affected reproductive processes as indicated by changes in the rat oestrous cycle, and neither was active orally; continued treatment led to a refractory state, with resumption of the oestrous cycle. According to a recent report (134a) trimethylquinone and 2-hydroxy-3-(3-methyl-2-butenyl)naphthoquinone (Lapachol) depressed the ability of the immature rat ovary to respond to PMS gonadotrophin; they did not inhibit the action of chorionic gonadotrophin or crude extracts of the rat pituitary gland.

The effects of feeding several other plants upon the fertility of laboratory animals have recently been reported (45, 46, 47). *Polygonum hydropiper*, administered as dry powdered whole plant, temporarily impaired the fertility of male and female mice and produced sterility in female guinea pigs. No evidence of

oestrogenic or androgenic activity was obtained, and it seemed likely that this material interfered with the gonadotrophic function of the pituitary. The seeds of the legume, *Psoralea corylifolia*, were oestrogenic when included in the diet of adult female mice. Both spayed and intact females exhibited intense vaginal cornification during treatment and the effect was reversible. The fertility of male mice was not impaired by treatment for a prolonged period (46). Studies concerned with the activity of the pea plant, *Pisum sativum*, are discussed later. A list has been compiled of more than 100 plants reported in the folklore of the world to affect human reproduction (37), but it seems that interest in the field of plant antigonadotrophins and the like has declined during the past few years. Until really active materials are separated in characterizable form, it seems that little progress will be made in this particular sphere.

Drugs directly affecting the ovary. Compared with spermatogenic cells, developing ova within the ovary are much more readily destroyed by irradiation than with the production of sterility (141). Furthermore, unlike the testis, the ovary has little or no recuperative powers. It is interesting that after small sterilizing doses of radiation, sterility does not develop immediately but only after an interval of time during which animals remain fertile—suggesting a differential sensitivity of the maturing follicles. The reason for the susceptibility of ovocytes to radiation is unknown. Gross damage to the ovaries can easily be recognized histologically, but minimal structural changes may nevertheless be associated with changes in fertility; these are not so readily explored systematically in the female. Thus technical difficulties contribute to the lack of information on drug damage to the female gonad. In contrast to its radiosensitivity very few drugs appear to affect the ovary directly. Myleran, as mentioned earlier, produced a specific interference with, or destruction of, spermatogonia in the male rat. Female rats given comparable treatment with this drug (2 doses of 2 mg/180 g animal, separated by an interval of 2 weeks) showed no histological damage to the ovaries 2 to 4 weeks later. Three similar doses within 4 weeks caused a disappearance of ovocytes; at 60 days a few follicles and corpora lutea remained but after 80 days none was present (13). A single dose of Myleran (2 mg/animal) to pregnant rats 5 to 7 days before parturition caused complete sterility in both male and female offspring delivered at term (14). Evidently early germinal cells in both male and female are susceptible to damage by this compound; how far the specific damage produced in the male has its counterpart in the female remains for future investigation. It is not proven that the action is, in fact, a direct one on the germinal cells. Additional work with drugs affecting male germinal cells may further knowledge of the homologies between the various male and female reproductive cells.

Female rats treated with relatively large doses of 6-mercaptopurine (3 doses of 30 mg/kg) prior to mating, produced litters with a significant increase in the number of stunted and resorbed foetuses (178). This might be due to incorporation of the drug in the developing ovum, but the effect was small. In general, of a number of antimetabolites studied, there was little evidence of direct damage to the ovary. Administration of triethylenemelamine (TEM) to female rats in

doses producing marked antifertility effects in males did not impair their subsequent fertility (75).

VI. CHEMICAL INTERFERENCE WITH GESTATION

Before implantation. Assuming that pregnancy supervenes, the events following ovulation are briefly as follows. In the ovary, the site of each ruptured follicle becomes rapidly occupied by cells which form the corpus luteum (producing progesterone). The shed ovum or ova pass into the oviducts and undergo the final maturation division, the last occurring shortly after fertilization, in the upper reaches of the genital pathway. In mammalian fertilization, spermatozoa await the arrival of ova in the oviducts. During this time the sperm are also believed to undergo an enhancement of fertilizing power ("capacitation") involving unknown biochemical mechanisms (27). Fertilization marks the onset of gestation. No substances are known directly to interfere with the fertility of the ovulated ovum or with the fertilization process *in vivo*—the question of hyaluronidase inhibitors has already been considered. Important factors here are the timing of drug administration in relation to ovulation and the problem of obtaining adequate concentration of drugs in the tubal fluid forming the environment of the shed ovum. The effects of gonadal hormones on the ovulated ovum and the early fertilized ovum referred to below are probably due to alterations in the delicately balanced hormone conditioning of the tubes and uterine mucosa, rather than to a direct effect on the ovum. In any case differentiation between these actions is technically very difficult.

After fertilization, the ovum moves along the genital pathway developing as it proceeds by successive divisions into a minute hollow sphere of cells—the blastocyst. It is believed that the rate of passage along the tubes is critical, as is the composition of the tubal and uterine fluid for the nourishment of the embryo. Proportionately, this initial stage of gestation lasts for a considerable time in some species. Thus in the rat, implantation does not occur until the seventh day—which represents one-third of the entire period of pregnancy. Although direct chemical attack in these early days is difficult to achieve, it had been known for many years that sex hormones are able to produce interesting effects on this phase. According to experimental conditions it is possible to hasten or hinder the transport of ova to the uterus after ovulation. Thus injections of oestradiol caused acceleration of the rate of passage of fertilized ova through the oviducts of mice (20) and rabbits (199) with resultant infertility; the ova degenerated in the uterus, apparently from lack of the correct sustaining fluid. However, daily injection into pregnant mice and rabbits of small doses of oestrogen, commencing from the day of impregnation, caused retention of most ova in the oviducts, possibly due to closure of the tubo-uterine junction—the so-called "tube-lock." Development proceeded normally for a time but the ova then degenerated and pregnancy failed (19, 21, 129, 198). Those gaining entry to the uterus also showed signs of degeneration by the fourth day (19). Testosterone propionate in mice produced the same result (18). Small oral doses of ethinyl-oestradiol or diethyl-stilboestrol prevented implantation of the blastocyst in the

rabbit if given soon after ovulation, or terminated early pregnancy (122). The administration to female rats of oestriol—one of the less active metabolites of oestradiol-17β—for 6 days from the day of mating reduced the live births to 33% of the controls. Used before mating the litter size was reduced to 60% of the controls (196). Evidence suggested that oestriol interfered with conditioning of the endometrium. Injections of oestrone in rabbits on days 3 and 4 after copulation could completely prevent implantation (129).

The importance of the correct environment during the early days of gestation is indicated by recent experiments involving the transfer of fertilized mouse eggs to other females in a post-coital state (98). Ova transferred 3½ days after copulation to other females 2½ days post coital gave a high proportion of successful pregnancies; 2½-day ova into 3½-day conditioned females produced a low yield of pregnancies. The progression of fertilized ova along the tubes appears to be carefully organized so that the site of implantation is reached at a particular time. In rabbits, fertile ova were not transported through the oviducts of oestrous or pseudopregnant rabbits (although cleavage continued normally) except following a recent ovulation (3). On the other hand, also in rabbits, the transfer of embryos from the oviducts of luteal phase animals into the oviducts of other luteal phase animals, gave results just as good as when embryos were introduced into the oviducts of prepared follicular phase animals, *i.e.*, mated to vasectomized males 1 day previously. Furthermore, embryos from follicular phase females survived equally well when transplanted into either follicular phase or luteal phase animals (11).

Recent experiments in the rat (30) have demonstrated that injection of progesterone (4 mg/day, s.c.) delayed nidation (implantation) until such time as 1 μg of oestrone was given in addition to the progesterone. It was claimed that the embryos were maintained in the uterus in a viable, resting condition during progesterone treatment. Blastocysts recovered up to 28 days after mating showed little advance in development with time. Delayed implantation was not consistently observed in rats ovariectomized 4 days after mating and treated with progesterone; with ovariectomy on day 3 and a delay of 8 days before commencing hormone therapy, nidation did not occur.

These different experiments illustrate the influence oestrogens and progesterone can exert on the movements and viability of the ovum and the species variability. In the physiological state the hormone balance ensures the correct rate of passage, conditions the uterus to receive the ovum, and facilitates implantation. Possible approaches for the development of antifertility compounds are evident. Experiments planned to test the ability of substances to interfere with fertility in the female must pay due regard to the time factors and probable species variations. An adequate knowledge of the underlying physiological events in the species under investigation is essential.

A recent development is the intriguing discovery that antifertility effects in female rats can be produced during the first 4 days post coitus by oral administration of a triphenyl ethanol derivative, 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-*p*-anisylethanol, MER-25 (152). This substance was not only effec-

tive by mouth but its action was remarkably selective and influenced fertilized ova during their sojourn within the oviducts. Administered during this oviducal phase it was entirely successful in preventing implantation of fertilized ova. Treatment applied during 1 day only could also be completely effective; it was ineffective if delayed until day 4 of gestation (counting the day of insemination as day 0). Developing, fertilized ova recovered from the oviducts of treated animals showed cell abnormalities and degenerative changes; from the third day onwards no ova were recovered. In control animals at this time segmenting ova could be found approaching the utero-tubal junction. The action of this substance is thought to be a cytotoxic process although it is not yet established whether this is direct on the fertilized ovum, or due to interference with an essential function provided via the maternal duct system. Treated females (*e.g.*, 25 mg/kg daily for 3 days) appeared to be pseudopregnant for 15 days before resuming a normal oestrous cycle. The subsequent fertility of treated females and of their offspring seemed unimpaired. A mode of action involving interference with corpora lutea or the development of decidual tissue in the uterus has been excluded. The authors point out that the possibility remained that the antifertility effect of this substance is connected with its known anti-oestrogenic activity (87). A recent account of the pharmacological effects of MER-25 (87a) suggests that its anti-oestrogenic activity is due to competition at the target organ, since it is active in ovariectomized animals receiving the natural hormones or synthetic analogues. It is only a feeble antigonadotrophin. Administered to lactating rats, infertile females but fertile male offspring resulted suggesting adverse effects on the germinal epithelium of immature females.

Interference with implantation. This is a vital stage of the reproductive process, during which the minute blastocyst develops a trophoblast and becomes located in a particular part of the uterus. Changes are then initiated resulting in the formation of the placenta—an organ derived in part from the trophoblast and also from the endometrium of the uterus. The corpus luteum (secreting progesterone) and later the formed placenta (which produces both progesterone and gonadotrophin) contribute to the suppression of ovulation for the duration of gestation. Little is known concerning the physiological mechanisms involved in the implantation process. The presence of the blastocyst provokes a reaction in the nearby endometrium with the formation of decidual tissue, which represents the initial stages of the maternal part of the placenta. The role of progesterone in the conditioning of the uterus has been mentioned and the presence of this hormone is, of course, essential for the maintenance of pregnancy. The continued presence of oestrogen antagonizes the progestational phases of endometrial development and prevents implantation of the blastocyst. If implantation has occurred, oestrogen can still terminate the pregnancy, as observed many years ago (120, 122). The development of antiprogestational substances competing directly with the natural hormone and thereby terminating pregnancy in its early stages, is an attractive possibility. Analogues of progesterone may be found, which lack progesterone-like action but can directly antagonize the effects of this hormone. Administration of pregnandione and pregnan-3α-20α-diol in various

proportions was found to exert a quantitative restriction on progesterone-induced development of decidual tissue in the rat uterus, which appeared to be of a competitive nature (68). Given to female rats for 7 days before mating, however, these steroids merely tended to reduce the litter sizes (196).

Much interest was aroused by a series of publications centering around the claim that 2,6-dimethylhydroquinone (*m*-xylohydroquinone) was an effective antifertility substance in rats, due to its antiprogestational activity. A natural source of this substance is an oil extracted from the field pea (*Pisum sativum*) (146). Ingestion of peas or injection of the oil was reputed to cause sterility in rats; in women the oil caused postponement of menstruation and infertility (144, 146, 147). Work with the hydroquinone has been vigorously pursued, including extensive clinical trials (145, 148, 149). In spite of the results claimed, independent workers have failed to confirm that the xylohydroquinone has any effect upon the fertility of rats. Thus, administered to mature female rats under strictly controlled conditions before mating or during the first trimester of gestation it "failed to delay mating, prevent implantation or to affect a single foetus or litter" (177). At the same time there are other reports that this chemical possesses antifertility activity. Although no effect was observed in rabbits, activity was demonstrated in rats following injection or oral administration (126). A single dose of the hydroquinone (1 mg) administered to breeding mice was also reported to cause abortion, resorption or still-birth, but the evidence indicated that the drug did not prevent nidation (8a).

In the face of conflicting evidence from reputable sources, it is difficult to assess the validity of data. At least it would appear that the activity of *m*-xylohydroquinone is undramatic and somewhat difficult to demonstrate. There seems to be no published work confirming the activity of the field pea or the oil derived from it.

Inhibition of the formation of decidual tissue in the uterus is a convenient test for the ability of a substance to interfere with implantation. Injury to the uterus at a particular time in the oestrous cycle has long been known to result in the transient growth of a cell mass of maternal (decidual) tissue. This growth (a deciduoma) is analogous to the maternal part of the early placenta. Its development necessitates an initial conditioning of the uterus with oestrogen and progesterone, which may be achieved by inducing pseudopregnancy (electrical stimulation of the cervix or mating with vasectomized males). This is followed by application of a nonspecific stimulus, mechanical or chemical, to the uterine lining which can be regarded as an artifical counterpart to the reaction ordinarily provoked by the presence of the blastocyst. In practice, the conditioned uterine horns are traumatized on the fourth day, the substance under test being applied topically to one horn of the uterus at the same time. Alternatively, one horn only can be traumatized, the other serving as a control. The results are assessed by comparing the two horns four days later. The method presents a valuable screening test for detecting potential antifertility action. In this way, inhibition of deciduomata formation in the rat occurred with oestrogens (195), ACTH, cortisone, DOCA, testosterone and pregnandiol (67). Although the most active

of this group, pregnandiol was not impressive as an antifertility agent (196) but this might be related to the timing of treatment.

Of particular interest was the demonstration that a single injection of ergotoxine (0.175 mg/rat, s.c.) any time between fertilization and implantation, prevented deciduoma formation in the rat (158). The action could be reversed by the administration of progesterone (1.0 mg) at the time of traumatization. Ergotoxine (1 to 2 mg/rat) also rapidly terminated pseudopregnancy and early pregnancy (156), its action extending from the day preceding insemination until the eighth day. The action was not mediated via adrenocortical stimulation, since the full effect was retained after adrenalectomy (161). Injection of prolactin antagonized the ergotoxine action, because of stimulation of the ovary and liberation of additional progesterone, indicating the integrity of ovarian function (155). Histologically there appeared to be neither stimulation of follicular development nor depressed luteinization due to the ergotoxine. A local oxytocic effect on the uterus was discounted. Other oxytocics (pitocin and ergometrine) suppressed decidual growth when applied *locally* to the uterus but were inactive systemically. The conclusion was that the effect of ergotoxine was probably independent of its better known pharmacological properties; in some way it appears to disturb the hormone balance via the hypophysis, and not by direct interference with progesterone or gonadotrophins (157). The ergotoxine alkaloids—ergocryptin, ergocornine and ergocristine—were all active, in descending order of magnitude. Butazolidine (3,5-dioxo-1,2-diphenyl-4-*n*-butylpyrazolidin) is also said to be capable of prematurely terminating pseudopregnancy and inhibiting deciduoma formation in the rat (139), possibly via a direct action on the central nervous system. Apart from this unconfirmed report the systemic activity of the ergotoxine alkaloids remains unique.

The idea that a common intermediary process, perhaps histamine release, might function in the response of the endometrium to the blastocyst and various forms of trauma has been explored. It has been stated that as little as 1 to 10 μg of histamine introduced into the uterine lumen will stimulate the growth of decidual tissue in the conditioned uterus, even in the absence of trauma (163). A number of antihistamines (*e.g.*, diphenhydramine or pyrathiazine), applied locally to the endometrium of one uterine horn at the time of traumatization, inhibited decidual growth (159), and the effect was not antagonized by progesterone. The traumatized but untreated contralateral horn always produced a large deciduoma, the mechanism of antihistamine action thus appearing to be directly at the cellular level. Negative results were obtained with local anaesthetics (dibucaine tetracaine and chlorbutol) and pressor substances (Pitressin and serotonin), suggesting that ancillary pharmacological properties of antihistamines were not involved in their uterine effect (160). Adrenaline was active although at a high dose (0.38 mg). None of these substances (including antihistamines) were effective by the parenteral route. The activity of adrenaline raised the possibility that antihistamines were effective by a reinforcement of the pressor action of adrenaline (89). However, pyrathiazine showed no such pressor enhancement although the most effective inhibitor of the decidual cell response.

Further investigations may elucidate the mechanism of the ergotoxine effect and lead to the development of orally active antifertility substances of this type. An extension of this work to species other than the rat would also be of interest. The production of antifertility substances with the antihistamine type of activity is possible but considerable increase in potency would be necessary as judged by the required topical concentrations. The available data on this interesting work has been recently collected (163).

Destruction of the developing embryo. The possibility of selective destruction of the implanted embryo by the direct action of substances administered to the mother, has been investigated by several groups of investigators over the past few years. It is not surprising that the compounds used exhibit maternal toxicity; the question is whether a sufficiently large differential effect can be achieved to permit chemically induced abortion with negligible maternal risk. Compounds may be developed which are safe for occasional use under proper medical supervision; their continued indiscriminate use raises serious problems of chronic toxic effects which will not easily be discounted. Direct destruction of the embryo may be achieved in the pre-implantation phase, at the time of implantation or during the subsequent development of the embryo. In the latter instance there is the complication of the placenta, although this structure could provide an additional point of attack. So far, however, no substances are known which affect this organ selectively. Destruction of the embryo in rats and mice is usually followed by resorption except in the later days of gestation. The periods of pre-implantation and implantation present the difficulty of attaining an adequate concentration of drug in the tubal fluid and the problem of differentiating between a direct action on the embryonal cells and an indirect effect mediated by hormonal imbalance. In testing compounds for abortifacient activity, it is usual to examine the susceptibility of the embryo at selected times during gestation, representing the 3 phases referred to above, and to determine the minimum dose producing 100% destruction of litters. In addition, an assessment of the damage produced in maternal tissues, *e.g.*, the haematopoietic system, should be made. Evidence of permanent effects on the maternal reproductive tissues can be sought by subsequent fertility tests on treated animals and on their offspring.

So far, work has been concerned with the effects of various antimetabolites and other cytotoxic agents which interfere with cell division. Most of these substances have already been tested for antitumour activity and a few are useful in the palliative treatment of certain forms of human malignant disease. The results of tests of abortifacient activity have been disappointing in regard to potential usefulness in human subjects, although presenting interesting problems for further investigation, *e.g.*, the ability of occasional foetuses to withstand doses of antimetabolites lethal to other members of the same litter, the resistance of the placenta to these substances and the varying susceptibility of embryos according to their gestation age.

a) *Antimetabolites.* Inclusion in the diet of the vitamin analogue, desoxypyridoxine (2, 4-dimethyl-3-hydroxy-5-hydroxymethylpyridine), produced early suppression of oestrus cycles in rats, although other signs of toxicity developed (107a).

Feeding the drug for 22 days prior to breeding was necessary to cause complete failure to produce litters.

The effects of folic acid antagonists on fertility are well known. In rats on a folic acid deficient diet, a crude preparation of folic acid antagonist given to the females as late as 9 days after breeding caused complete resorption of foetuses (107). Injection of mice or rats with small doses of the antifolic compound, 4-aminopteroylglutamic acid (aminopterin), during the first week of gestation also produced complete resorption of embryos (188, 189). Smaller doses of antifolic compound or postponement of treatment until the eleventh day after insemination produced malformations in surviving foetuses (106). Normal litters resulted when treatment commenced on day 15. According to others (103), a single dose of aminopterin (0.2 mg/kg) between days 9 and 12 of gestation was frequently lethal to the embryo; if it survived there was usually no evidence of teratogenic disturbance. The maximum susceptibility of the embryo was on the ninth day—shortly after implantation. This dose had little effect before the eighth day or after the fifteenth. The maternal LD50 was about 3 mg/kg. The effects of antifolic compounds, according to circumstances of administration, extend over the first two-thirds of pregnancy in the rat, with greatest susceptibility at or soon after implantation. Inhibition of ovulation in rodents required doses close to those producing lethal effects (107). Apparently embryonic tissues are much more sensitive to deprivation of folic acid than those of the adult. Two pyrimidine derivatives, 2,4-diamino-5(3′,4′-dichlorophenyl)-6-methylpyrimidine and 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, resembled the 4-amino-analogues of folic acid in their actions (125). Their administration to pregnant rats could not prevent implantation entirely, but led to later malformation or death (180).

As a means of producing therapeutic abortion aminopterin has been found to be too uncertain in its action. Apart from maternal toxicity, as reflected in haematopoietic changes, a complication was the independent existence of the placenta in pregnancies of more than one month duration with the likelihood of its persistence and continued growth after death of the foetus. In addition, insufficient treatment carried the risk of production of foetal abnormalities (175, 179).

o-Diazoacetyl-L-serine (Azaserine), an antibiotic isolated from a *Streptomyces* (6) shows both antitumour (173b) and abortifacient activity. Its effects on the embryo range from complete destruction and resorption to teratogenic manifestations, depending upon the dose, frequency of administration and the stage of gestation. The maximal effect occurred just after implantation (days 8 and 9), when a single intraperitoneal dose (2.5 to 5 mg/kg) resulted in complete litter destruction (103, 104, 181); insufficient doses produced malformed foetuses. Later in gestation the embryos were unaffected even by a dose 10 times that effective on day 8 (105). There was a wide margin of safety between the abortifacient dose and the single LD50 (about 100 mg/kg, i.p.) (103, 173). Offspring born after repeated abortions were unaffected and in turn produced normal litters. Azaserine is known to interfere with glutamine utilization in purine biosynthesis (60a). How far this is an adequate explanation of its lethal action on the embryo

remains to be seen; attempts to antagonize the toxicity of Azaserine by administration of amino acids (including glutamic acid) to mice were unsuccessful (173).

Another antibiotic, 6-diazo-5-oxo-L-norleucine (DON), was more potent than Azaserine. It is a diazo-ketone also isolated from a *Streptomyces*, and produces marked inhibition of a variety of experimental tumours. From implantation to midterm, 2 doses (0.5 mg/kg) caused total destruction of litters in rats. Again, the action appeared to be directly on the foetus with no adverse or cumulative maternal effect (182). Large doses of adenine sulphate, although ineffective against the toxic effects of Azaserine protected the embryo to a considerable extent against DON, supporting the view that the latter inhibited the *de novo* synthesis of purine bases. Alazopeptin, isolated from *Streptomyces griseus planus*, has recently been tested for antifertility activity (185). This substance is apparently a dipeptide, also containing a diazo-keto-amino acid (39). Its effect on pregnancy was comparable to that of 6-diazo-5-oxo-norleucine, and both substances showed a wide differential toxicity between mother and foetus on the basis of a single dose. However, these diazo-amino acids exhibit greatly enhanced toxicity when administered daily. In spite of resorption of embryos, placentas frequently survived treatment with these substances.

Oral administration to pregnant rats of 6-mercaptopurine produced foetal deaths and stunted growth in survivors (178). The most sensitive phase was the period of implantation (days 7 and 8 of gestation), when two successive daily doses (10 mg/kg each) produced resorption of 90% of embryos. Occasional foetuses survived and gross malformations were not seen; larger doses had little effect on earlier or later stages of pregnancy. There was evidence that treatment prior to mating damaged ovarian follicles. Certain other synthetic purine antagonists (2,6-diaminopurine, 6-chloropurine and thioguanine) behaved similarly to 6-mercaptopurine, although their action extended to earlier and later stages of gestation (184). Thioguanine was the most toxic compound to foetuses, 100% litter failure being induced by 2 doses (10 mg/kg, i.p.) on days 7 and 8, while surviving foetuses from insufficient treatment were stunted and malformed. It was about 10 times more toxic than 6-mercaptopurine to adult rats (125a). Many placentas persisted after antipurine treatment and there was no evidence of damage at term to the maternal bone marrow, lymphoid tissue or intestinal epithelium. The great recuperative power of these tissues in the rat should, however, be borne in mind.

A number of other purine antagonists were inactive (187), *viz.*, 2-aza-adenine, 8-aza-guanine (guanozolo), 8-aza-xanthine, 8-aza-dimethylxanthine.

b) *Cytotoxic alkylating agents.* Aliphatic nitrogen mustards, *e.g.*, mustine (HN_2), produced lethal effects on rat and mouse embryos (35, 61, 103). Smaller doses caused foetal abnormalities without noticeable maternal harm when administered to mice (35) between days 11 and 12 of gestation (1 to 2 μg/mouse, i.p.) and rats (103) between days 10 and 16 at about one-fifth of the maternal LD50 (0.5 mg/kg). The toxic effect of nitrogen mustard is less specific than that of antimetabolites, and foetal susceptibility continues when sensitivity to antimetabo-

lites has virtually disappeared (103). In rats, injection of the aromatic nitrogen mustard, Chlorambucil (2 doses, 10 mg/kg on days 7 and 8 or 11 and 12) caused resorption of 99% of the foetuses. After one such dose on day 8 the same percentage were dead at the end of pregnancy (187). A short course of TEM to rats (5 daily doses, 0.2 mg/kg) commencing on the fourth day after insemination resulted in complete failure of litter production with no evidence of foetuses in animals examined at various times after treatment (75). On days 7 and 8 of gestation 2 doses of TEM (0.5 mg/kg) caused 100% destruction of embryos in the rat (183), but susceptibility to this substance diminished rapidly after implantation; it also appeared to damage the placenta. Triethylenephosphoramide (TEPA) was 100% effective on days 7 and 8 or 11 and 12 (2 doses, 5 mg/kg, i.m.). Stunted foetuses and malformations occurred after insufficient treatment with TEM or TEPA. Most of the maternal bone marrow was temporarily destroyed by these compounds although the animals gained weight (176).

Myleran produced its maximum effect before implantation (days 4 and 5, 10 mg/kg) but, surprisingly, only 50% of the embryos were destroyed; thereafter its effectiveness diminished rapidly (187). Delayed maternal lethal effects resulted from this treatment, due to interference with haematopoiesis. Reference has already been made to the fact that a single injection of this drug to rats (2 mg/rat, i.p.) 5 to 7 days before parturition, caused complete sterility in male and female offspring (14), although earlier in pregnancy "quite large doses do not interfere with its course and the F_1 and F_2 generations are apparently healthy" (52a). A formidable bar to the practical use of alkylating agents, however satisfactory the results in experimental animals, is the possibility of carcinogenesis or transmissible mutagenic effects.

c) *Antimitotics.* N-desacetylthiocolchicine (197) and N-desacetylmethylcolchicine (Demecolcin) (142, 193) produce foetal death in rodents. The former substance (LD50 in rats by single injection, 175 mg/kg, i.p.) was 25 to 100 times less toxic than colchicine (16). Rats tolerated daily intraperitoneal doses of 3 mg/kg indefinitely and 8.5 mg/kg for 21 consecutive days.

In rats before implantation the thiocolchicine (2 doses, 7 mg/kg, i.p.) was relatively ineffective, but from the 10th day onwards destroyed all litters; a a smaller dose (2 doses, 5 mg/kg) was still effective at mid-term but not later (186). Even in the terminal phases of pregnancy foetal death could readily be achieved by colchicine derivatives, in contrast to the failure of antimetabolites. Repeated abortion with the thiocolchicine did not impair the subsequent fertility of treated females or cause abnormalities in their offspring. Some damage to maternal tissue was manifest as depression of the peripheral white cell count and prolongation of the prothrombin time.

Demecolcin (LD50, 35 mg/kg) interrupted pregnancy in rats without maternal mortality (2.5 to 7.5 mg/kg, s.c.) from the time of implantation to the last phases of gestation (186). Similar doses to rabbits, 13 to 16 days pregnant, were also effective but it was less active orally (40). In mice desacetylthiocolchicine did not interrupt gestation in doses of 30 μg daily from the sixth to twelfth days of pregnancy; Demecolcin (50 μg per mouse over the same period) interrupted

pregnancy in 100% of mothers without maternal injury (200). These rat experiments suggest a higher dose of desacetylthiocolchicine may be necessary to interfere with gestation in mice. No gross malformations were encountered among surviving foetuses in rats treated with the colchicine derivatives (186), but anatomical abnormalities occurred in mice after insufficient treatment (200). These were not transmitted by subsequent breeding, although impaired fertility was seen in some animals through 3 successive generations. Histological damage to the ovaries with many arrested mitoses in the follicles, required high doses of Demecolcin (150). In general, the colchicine derivatives killed foetuses when given any time in the latter half of pregnancy, even on the day before littering. It is unfortunate that the placenta frequently survived treatment with these compounds.

Other antimitotic substances possess a differential action on the foetus. Thus podophyllotoxin, from the mandrake or May apple root, is one-sixth as toxic as colchicine. It is more toxic to young than adult animals and interrupts pregnancy in mice, rats and rabbits (41, 187, 202). Although relatively ineffective by mouth, a single injection to mice (1.5 mg per animal), between the third and fourteenth day after insemination, terminated pregnancy (40, 200). No side effects were reported and several successive abortions appeared not to impair the subsequent fertility of the mothers, nor interfere with bone marrow function; however, the LD50 was about 3.5 mg per mouse. Two related constituents of podophyllin resin, α- and β-peltatin (79), had similar antifertility action and were less toxic than podophyllotoxin. A single dose of α-peltatin (2 mg per mouse) on day 3 of pregnancy interrupted 34 of 37 pregnancies. Two similar daily doses of either peltatin caused a disturbing number of maternal deaths, due apparently to acute diarrhoea and not to damage to liver, haematopoietic or cardiac tissues (200). Histological evidence of tissue damage has, however, been reported (79). There is clearly little margin of safety with these compounds.

D-Usnic acid, an antimitotic substance found in many lichens, usually prevented pregnancy in mice if given immediately after copulation (1.5 mg per mouse). Resistance developed with repeated administration so that pregnancy ensued although fertility was subnormal. There was no indication of transmission of effects to progeny (202).

Antimitotic activity as assessed in plants or invertebrates, or even in mammalian tissue culture, does not necessarily indicate antifertility activity in the intact mammalian organism (200). The following mitotic poisons did not prevent pregnancy in female mice: 9,10-phenanthraquinone (85), 4-dimethylaminochalcone (43), vitamin K and its synthetic analogues and 1,4-naphthoquinone (200). Stilboestrol has antimitotic properties (70). Its monobenzyl ether—a feeble oestrogen—interrupted pregnancy in most mice, when given (400 μg per animal) on the third day of gestation (200). A comparison with the result of stilboestrol administration at various stages of gestation would be interesting. Podophyllotoxin and stilboestrol have been reported to be synergistic in producing damage to experimental tumours (203); β-peltatin and stilboestrolmonobenzyl ether together showed enhanced activity in pregnant mice. Combinations of phenanthra-

quinone with β-peltatin, and thiocolchicine with β-peltatin were said to exhibit marked antagonism (200).

Miscellaneous. The carcinogen, methyl cholanthrene, interrupted pregnancy in mice when injected on day 1 or day 7 of gestation (*e.g.*, 0.5 to 1.0 mg per mouse) (71a). At midterm, it was comparatively ineffective. The mode of action was not established, but a direct toxic effect on the embryo seemed likely since the compound remained active in ovariectomized, pregnant mice receiving progesterone (1.5 mg/day).

Certain nitriles produce selective death of the rat foetus in the late stages of pregnancy. The seeds of certain members of the pea family (*Lathyrus hirsutus*, *L. pusillus* and *L. odoratus*) produced deformities in growing animals (Lathyrism) and embryonic death. The active principle of the sweet pea (*Lathyrus odoratus*) has been characterized as (β-L-glutamylamino)propionitrile (151). Aminoacetonitrile, β-aminopropionitrile and *bis*-(β-cyanoethyl)amine all produced effects resembling those following feeding a high percentage of pea meal (168). For example, small quantities of β-aminopropionitrile (*e.g.*, 0.1 % in the diet) were apparently harmless up to the 15th day of gestation. Administered for one further day, or if treatment commenced on the 17th day onwards uniformly lethal effects on the embryo could be produced; the nature of the toxic action is not known. The fertility of adult male or female rats was unaffected. *Tris*-(β-cyanoethyl) amine, β-dimethylaminopropionitrile and β-mercaptoethylamine were inactive. These substances hardly enter the category of potential antifertility agents, but their ability selectively to cause foetal death late in gestation is of interest.

Antifertility effects due to minute amounts of selenium in the drinking water of rats have also been described (140). Although 1.5 to 2.5 parts per million were apparently harmless over 2 generations, 7.5 ppm prevented reproduction in the female, whilst male fertility was unaffected.

Lipopolysaccharides from bacteria possess abortifacient properties (187). Expressed as the percentage of rat litters completely destroyed by the dose given, the best results were: material from *B. typhosus*, 90% (2 mg/kg on day 12), from *S. marcescens*, 100% (2 mg/kg on day 15) and *B. abortus*, 100% (30 mg/kg on day 11). Few placentas survived in these experiments.

VII. CONCLUSIONS

Compounds have been found which interfere selectively with various phases of the intricate reproductive process in both male and female. Some act directly on the developing gametes or on the embryo, while others appear to disturb the delicate hormonal balance in the female.

Control of human male fertility offers little prospect of success at the present time. In the rodent, spermatogonia, spermatocytes and early spermatids can be selectively attacked by various types of chemical agents, with the later appearance of episodes of sterility. Late spermatids and spermatozoa have not been selectively damaged so far. Interference with hormonal control seems to be an unprofitable approach in view of the lengthy period of spermatogenesis. More promising would be selective damage to the male gametes during their stay in

the epididymis since, in rodents, sperm are constantly being renewed irrespective of mating. The same might apply to man, in which case predictable periods of sterility could be produced by suitable drugs. The sperm tail is a unique structure which may well depend upon special biochemical processes for its proper function. During the epididymal phase, when sperm are inactive, selective interference could ensure that they remained non-motile after emission. In this way fertility control might be achieved with minimal risk. The application of cytotoxic substances, however, will always carry the possibility of damage to cell nuclei with the dangers of carcinogenesis and alterations in genetic material.

Interference with the reproductive process in the female offers greater prospects of control. Steroids have been developed which successfully prevent ovulation after oral administration. These substances appear to act by suppression of gonadotrophin secretion and the method necessitates treatment during the greater part of each cycle in women, while permitting a continuation of normal menstrual rhythm. Such anovulatory cycles are well recognised events in otherwise normal individuals. The risks from prolonged depression of pituitary activity and endocrine imbalance by such means cannot yet be adequately assessed. In view of the lack of toxicity of progesterone, the development of progesterone-antagonists as potential antifertility substances would be of considerable interest. All substances acting by interfering with the hormonal balance in the female possess the disadvantage of requiring timed administration during the menstrual cycle.

The pre-implantation and implantation phases of development can be successfully attacked in experimental animals; in human beings they are, relative to gestation, of very short duration so that the time of administration may well be critical. The post-implantation embryo can still be destroyed in rodents, but, generally speaking it becomes more resistant with time. Unfortunately, too, the placenta frequently survives. Available compounds are also too toxic and there is the risk that insufficient treatment at any stage of gestation will result in the production of abnormal offspring.

The chemical control of proliferating cell systems is also the problem of cancer chemotherapy. Comparative studies of substances interfering with the multiplication of normal cells in the germinal epithelium in different species, may contribute to an understanding of the complexities of the malignant process. It has been said "the real problem of cancer is to understand the processes of control, by which normal cells from the fertilized ovum to the end of life are maintained in morphological and functional conditions appropriate to the needs of the organism at the time" (22). Similar knowledge concerning the developing gametes could facilitate the rational development of antifertility substances.

Compounds interfering directly or indirectly with proliferating cell systems in the body possess potential dangers which may be very difficult to assess from studies on experimental animals, however varied these may be. The chief hazards would arise from the widespread use and abuse of antifertility compounds by the reproductive age group of the community. The toxic manifestations of the drugs used could emerge only after years of treatment and may appear in future genera-

tions as a result of damage to genetic material. There is also a real risk of carcinogenesis, permanent damage to reproductive capacity and to haematopoiesis. Even the use of direct spermicidal compounds could result in the induction of cancer. It is a vital necessity to carry out detailed and prolonged toxicological tests in a variety of species on any compound likely to reach human application. These must include tests for the hazards referred to above and extend over several generations. In this connection, adequate liaison between the pharmacologist, experimental pathologist, geneticist and appropriate workers in the cancer field is essential.

The development and study of antifertility compounds present a largely unexplored chapter in pharmacology, the impetus to which is now being provided by overpopulation. There is reason to suppose that the safe chemical control of fertility can be achieved, but in no other branch of pharmacology is the potential hazard so great from the exploitation or misuse of drugs.

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