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DEVELOPMENTS IN FERTILITY CONTROL

Report of a WHO Scientific Group

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FERTILITY CONTROL

Geneva, 11-15 November 1968

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DEVELOPMENTS IN FERTILITY CONTROL

Report of a WHO Scientific Group

A WHO Scientific Group on Developments in Fertility Control met in Geneva from 11 to 15 November 1968. The meeting was opened by Dr A. M.-M. Payne, Assistant Director-General, who welcomed the members on behalf of the Director-General.

1. INTRODUCTION

On numerous occasions WHO Scientific Groups, the Advisory Committee for Medical Research, and World Health Assemblies have recommended that WHO periodically evaluate advances in fertility regulation. The purpose of this meeting was to review certain developments in present approaches to fertility control, excluding the use of oral gestogens,¹ hormonal steroids,² and periodic abstinence.³ Discussion of intrauterine devices⁴ was limited to their use as vehicles for introducing certain chemical agents into the female genital tract. The Group was aided in its work by the reports of several previous WHO Scientific Groups.⁵

Since some of the studies of new approaches now being carried out are still at the stage of animal testing, and since all future developments will necessarily have to undergo such testing, an important task for the Scientific Group was to suggest guidelines for the clinical application of knowledge derived from animal studies.

2. MALE FERTILITY AND ITS CONTROL

An agent that could safely and effectively inhibit fertility in the male, without risk of interfering with spermatogenesis and libido, would find practical application in fertility regulation. The anatomical basis of sper-

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 326.

² See *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 386.

³ See *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 360.

⁴ See *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 332; 1968, No. 397.

⁵ *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 280; 1965, Nos. 303, 304, 313; 1966, Nos. 333, 334; 1967, Nos. 341, 364; 1968, No. 403.

matogenesis and the temporal relationships of its different stages are well-documented, both in lower animals and in man. However, the essential cellular metabolic processes and the hormonal interactions leading to maturation of the spermatozoa are not understood to the same extent.

2.1 Endocrine processes

Spermatogenesis and functional maturation are under the influence of the pituitary gonadotrophins. Evidence is now available that both follicle-stimulating hormone (FSH) and luteinizing hormone (LH, sometimes called interstitial cell stimulating hormone, ICSH) are required for the development and maintenance of testicular function in man. It has been assumed that the male gonadotrophin pattern, in contrast to that of the female, is non-cyclic. However, studies in male rats suggest the existence of a circadian rhythm, probably under the influence of the pineal gland. Reported diurnal variations in plasma testosterone levels in man indirectly support the possibility of a circadian gonadotrophin cycle. It is not known whether these changes affect the development and function of spermatozoa.

Progestogens, androgens, and steroidal and nonsteroidal oestrogens are capable of blocking gonadotrophin secretion in both males and females, but there is some evidence that the mechanisms differ in the two sexes. These agents are capable of blocking spermatogenesis also; however, male libido is adversely affected by both oestrogens and progestogens. The male accessory sex glands are also regulated by this gonadotrophin-gonadal relationship.

From the standpoints both of effectiveness and of reduction of undesirable side-effects, the most desirable way in which to inhibit male fertility would be action on mature sperm prior to ejaculation.

2.2 Cellular changes and functional activity

Suppression of spermatogenesis can be recognized histologically. Infertility can also occur without histological changes and with the sperm remaining fully motile. This is designated functional sterility. Both types of action, accompanied either by cell depletion or functional change, without interference with libido, have been produced by nonsteroidal antifertility agents.

2.3 Accessory reproductive organs

The role of the accessory reproductive organs in male fertility is not fully understood. Although spermatozoa washed free from seminal plasma remain capable of fertilization, the rate of fertilization is much higher in the presence of the secretions of the accessory reproductive organs. The mechanism whereby this is achieved is not yet known.

STERILITY IN THE MALE RAT AFTER TREATMENT WITH ALKYLATING AGENTS *

| | Compound | Dose ^a | Time after treatment (weeks) | | | | | | | | | | | | | | | | | |
|---|-----------------------------|-------------------|------------------------------|---|---|---|---|---|---|---|---|----|--|--|--|--|--|--|--|--|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | | | | | |
| 1 | tretamine | 1 | | | | | | | | | | | | | | | | | | |
| 2 | isopropylmethanesulfonate | 100 | | | | | | | | | | | | | | | | | | |
| 3 | methylenedimethanesulfonate | 15 | | | | | | | | | | | | | | | | | | |
| 4 | ethylenedimethanesulfonate | 100 | | | | | | | | | | | | | | | | | | |
| 5 | propylenedimethanesulfonate | 50 | | | | | | | | | | | | | | | | | | |
| 6 | butylenedimethanesulfonate | 10 | | | | | | | | | | | | | | | | | | |

| Cell stage affected | Epididymal sperm | Spermatids | Spermatocytes | Spermatogonia |
|---------------------|------------------|------------|---------------|---------------|
| | | | | |

* The heavy black lines indicate the approximate duration of sterility (e.g., the effect of tretamine lasts from the end of the 1st week after administration to the end of the 5th week after administration).

^a In mg per kg of body weight, administered intraperitoneally.

2.4 Approaches to fertility control

Research in fertility control in the male has been carried out largely in laboratory animals, using both chemical and immunological approaches, as outlined in the following sections. None of the chemical agents described below is suitable for use in man, owing to known or potential toxicity. Similarly, immunological processes present hazards when used in man, and they suffer from a lack of specificity. Consequently, no systemic method of fertility control in man is available at present.

2.4.1 Chemical approaches

Alkylating agents and related compounds

The main categories of chemicals exhibiting selective interference with spermatogenesis are (1) derivatives of the cyclic base aziridine (ethyleneimine) and (2) esters of methanesulfonic acid.

(1) *Aziridines*. In general, the effects of different aziridines vary quantitatively rather than qualitatively. Low doses produce sterility in rats and mice, resulting from an action on the spermatid stage of development. Higher doses produce a biphasic infertility pattern, including an antispermatogonial effect associated with aspermia. When administered orally, these compounds show reduced activity, or they may be inactive.¹ Other compounds² can selectively destroy the seminiferous epithelium of the mouse. The remaining structural elements of the reproductive system, and sexual behaviour, appear to be unaffected.

(2) *Methanesulfonic acid esters*. The action of the straight-chain monoesters of methanesulfonic acid (e.g., methylmethanesulfonate) is confined to spermiogenic cells, whereas the isopropyl ester affects all premeiotic cells (see the accompanying figure). These esters, when given orally at low dose levels, will induce and maintain sterility in rats.

Diesters having the general formula $\text{CH}_3\text{SO}_2\text{O}(\text{CH}_2)_n\text{OSO}_2\text{CH}_3$, where $n = 1-4$ (compounds 4-7 in the figure) have a wide range of antifertility action in the rat, as shown in the figure. Their action is cumulative and they are effective when given orally.

(3) *Related compounds*. Hexamethylphosphoric triamide, an insect chemosterilant, also has an antispermatogenic action in rats, mice, and rabbits. Relatively high doses are required and the action is cumulative.

¹ Compounds that produce these effects include: (1) tretamine [proposed international nonproprietary name (INN) for 2,4,6-tris(1-azaridiny)-s-triazine]; (2) thiotepa [proposed INN for tris(1-azaridiny)phosphine sulfide]; (3) 1,1'-carbonylbisaziridine; (4) 1,1'-sulfinylbisaziridine.

² E.g., 1-aziridinecarboxamide, ethyl 1-aziridinecarboxylate.

The effect on spermatogenesis, with the resulting aspermia, may be prolonged. Trimethylphosphate has been tested in the rat and found to induce a functional type of sterility similar to that caused by methylmethanesulfonate.

Bis(dichloroacetyl) diamines

The bis(dichloroacetyl) diamines that have been tested have been found to cause similar effects in rats, mice, guinea-pigs, dogs, and monkeys ; however, the effective dose per unit body weight varies considerably according to the species. These substances suppress spermatogonia, spermatocytes, and spermatids without affecting gonadotrophin secretion. Two of these compounds, WIN 13 099¹ and WIN 18 446,² have been tested in man. Courses of treatment caused a gradual fall in sperm counts to a very low level in 8–10 weeks. Their slow action militates against any practical application. Furthermore, they provoke side-effects similar to those caused by disulfiram.

Aromatic nitro compounds

Nitrofural³ and two related compounds⁴ produce an antispermatogenic effect at the primary spermatocyte stage in the rat, while sexual activity is maintained. Nitrofural also acts on spermatogonia.

Nitrothiazole and nitropyrrole derivatives have been shown to have a similar action. A dinitropyrrole derivative⁵ has been found to be the most effective of the aromatic nitro compounds that have been tested. One treatment of 500 mg per kg of body weight every 4 weeks is reported to maintain sterility in the rat.

Procarbazine

Procarbazine⁶ is a hydrazine derivative that has tumour-inhibiting properties and causes depression of spermatogenesis. In rats, high doses produce an irreversible sterility from the second week of treatment. Lower doses are followed by a temporary sterility about 11 weeks after treatment, a delay that is longer than the duration of spermatogenesis in the rat.

Antimetabolites

Limited studies of a number of antimetabolites (6-azaguanine, 6-azauridine, 6-azacytidine, 5-iododeoxyuridine, and 6-thioguanine) have shown them to induce subfertility but not sterility in experimental animals.

¹ *N, N'*-(*p*-phenylenedimethylene)bis(2,2-dichloro-*N*-ethylacetamide).

² *N, N'*-octamethylenebis(2,2-dichloro-*N*-ethylacetamide).

³ Proposed INN for 5-nitro-2-furaldehydesemicarbazone.

⁴ Nidroxyzone [proposed INN for 5-nitro-2-furaldehyde-2-(2-hydroxyethyl)semicarbazone] and nitrofurantoin [proposed INN for 1-(5-nitrofurfurylideneamino)hydantoin].

⁵ *N, N*-diethyl-2,5-dinitropyrrole-1-acetamide.

⁶ Proposed INN for *N*-isopropyl- α -(2-methylhydrazino)-*p*-toluamide.

Trace elements

The essential trace element associated with male reproduction is zinc. Zinc deficiency in experimental animals normally results in degeneration and atrophy of the testes and hypoplasia of the coagulating glands and seminal vesicles. All these changes, except testicular atrophy, can be reversed by treatment with zinc.

The gonads of the nonscrotal animals are insensitive to metals given by the parenteral route, in contrast to those of scrotal animals, which are highly susceptible.

Of the elements that have been tested, cadmium — administered by the injection of cadmium salts — produces the greatest effect. At different stages of ontogenic development, the male gonad varies in sensitivity to the toxic action of cadmium salts. It has been postulated that a specific zinc-dependent metallic enzyme appears when spermatids are transformed into spermatozoa. This would explain the sensitivity of the testes to cadmium, and the protection conferred by zinc and/or selenium.

Various trace elements have been tested for effects on reproduction and several of them produce reversible changes in testicular tissue. Copper salts, when given subcutaneously to the rat, arrest spermatogenesis and cause almost complete degeneration of the epididymal spermatozoa, and some necrosis in the head of the epididymis. Changes in the epididymis are the most constant finding with copper; however, the changes are reversible, in contrast to those resulting from equimolar doses of cadmium salts. Copper is a normal constituent of seminal plasma and spermatozoa in several species. However, copper salts, even in small amounts, exert a marked toxic effect on ejaculated sperm. This inhibition of sperm motility can be counteracted with cysteine or glutathione. The toxicity of chelating agents and metals to the sperm of the ram, bull, rabbit, and human has been investigated, and it has been found that copper or zinc combined with some chelating agents has a greater toxicity than the metal ions alone.

Agents of plant origin

Several plants or their crude extracts have been shown to exert anti-gonadotrophic effects in male rodents, but whether they actually disturb spermatogenesis is not known. Several phytoproducts (colchicine and its derivatives, podophyllotoxin and its derivatives, reserpine, psoralen, and villalstonine) have been reported to interrupt spermatogenesis and/or reduce fertility in animals. Many of these findings have not been confirmed.

It has been claimed that 2,6-dimethylhydroquinone isolated from the oil of the field pea (*Pisum sativum*) causes a reduction of fertility in man through spermatogenic arrest. This effect is said to be reversible. Similar results in rats have been reported but not confirmed. The effect of the whole plant on spermatogenesis and fertility in rats, rather than that of the putative active principle alone, should be investigated.

2.4.2 Immunological approaches

In animals, experimental induction of antispermatogenic antibodies results in the suppression of spermatogenesis, accompanied by the development of circulating and cell-bound antibodies. Animals can be sensitized by injecting testicular components, whole semen, or sperm cells, together with adjuvants. The immune serum thus obtained can immobilize and agglutinate homologous spermatozoa *in vitro*. This immunological response lasts for several months, following which normal spermatogenesis may return. It seems that cell-bound antibodies participate to a greater extent than do circulating antibodies in the *in vivo* immunological response. However, more precise knowledge of the role of both types of antibody is still needed. Recent experiments have demonstrated that man is susceptible to immunization against his own testicular tissue when it is administered with an adjuvant. Germinal cells were destroyed without apparent impairment of Leydig cells, and circulating and cell-bound antibodies resembling those encountered in sensitized animals were found. No tests of fertility after immunization have been reported to date.

The usefulness of immunological procedures for the control of fertility in man will depend on the isolation and purification of chemically defined specific antigens and improved adjuvants.

3. FEMALE FERTILITY AND ITS CONTROL

In the female, more steps in the reproductive process appear to be susceptible to regulation than in the male. In addition to suppression of ovulation, possible methods of fertility regulation include interference with (1) sperm and ovum transport, (2) fertilization, (3) implantation, and (4) embryonic development before or after implantation.

3.1 Ovulation

In normally menstruating women, a number of follicles reach a considerably advanced stage of development in each cycle. Only one of these matures completely into a Graafian follicle; the others degenerate rapidly and become atretic. The number of female germ cells attains its highest level (approximately 6 million oocytes) at about the sixth month of gestation. Henceforth, no mitotic activity occurs, but there is a continuous loss of oocytes; indeed, during infancy, more than 90% of the follicles disappear by degeneration. The factors that regulate atresia are not well understood. Were it possible to induce mass atresia of ova by pharmacological or immunological means, this would offer another approach to fertility control.

In several species, including man, follicle maturation is controlled by the follicle-stimulating hormone (FSH) and ovulation and corpus luteum formation by the luteinizing hormone (LH). The secretion of these gonadotrophins is regulated by separate hypothalamic factors, the FSH-releasing factor and LH-releasing factors. The secretion of the releasing factors is controlled partly by the central nervous system and partly by circulating gonadal hormones. In addition, pituitary gonadotrophins may exert a feedback effect via hypothalamic centres and thus modify their own secretion rate. It is also possible that the hypothalamus produces inhibitory substances to suppress the release of gonadotrophins. The isolation of these active principles might lead to new developments in fertility control.

The normal regulation of the human hypothalamo-hypophysial system is poorly understood. Present concepts are based almost exclusively on studies conducted with synthetic contraceptive steroids. The regulatory effect of natural oestrogen (17- β -estradiol) and progestogen (progesterone) remains to be established.

Under the influence of gonadotrophins (predominantly FSH), the maturing follicle secretes increasing amounts of oestrogenic hormone. Following ovulation, the ruptured follicle is converted into a corpus luteum that secretes both oestrogen and progestogen. The hypophysial control of the endocrine function of the corpus luteum in the human species and the factors (e.g., luteolysin) that induce regression of the corpus luteum are incompletely understood. It is believed, however, that the steroidogenic function of the corpus luteum is one of the steps of the reproductive processes that are susceptible to regulation.

3.2 Capacitation, fertilization, and tubal passage

Capacitation is the induction of certain physiological changes in ejaculated spermatozoa in the female genital tract that enable them to penetrate the zona pellucida of the egg. The time required for this process varies from one species to another. It is not known whether capacitation occurs in man.

Fertilization in animals is defined as a series of events in which two cells—the ovum and the sperm, whose nuclei have previously undergone meiotic division—unite to form a single cell, the zygote. From the biological point of view, fertilization is a complex process that starts with the penetration of sperm into the cytoplasm of the egg and includes the subsequent formation, development, and syngamy of the male and female pronuclei until the union of maternal and paternal genetic material is complete.

However, there are many events that are essential preliminaries to fertilization, especially in animals in which fertilization occurs internally. Such events include normal gametogenesis, ovulation, maturation of eggs

after ovulation, correct time of mating, ejaculation, transport and capacitation of sperm, and the meeting of sperm and eggs at the right time and in the right location. It is clear that disturbance of any of these preliminaries will ultimately disturb fertilization.

The normal development of fertilized eggs, the proper timing of egg transport from the tube to the uterus, and the development of eggs are all of critical importance for implantation and future development of embryos. However, the fact that ovum implantation may occur in women following surgical transplantation of the ovary into the uterus after bilateral salpingectomy suggests the necessity for further investigation to establish the significance of tubal factors in women.

Fertilization is inhibited during pseudopregnancy in certain species, a phenomenon that is attributed to a disturbance of sperm transport. Recent experiments have shown that the administration of progesterone or progestational compounds before ovulation disturbs sperm transport as well as sperm capacitation, thus inhibiting fertilization to a certain degree. The accelerated egg transport that follows such treatment also causes some disturbance of fertilization.

The reaction that occurs at fertilization between certain components of eggs and of sperm, such as fertilizin and antifertilizin, has been long considered to resemble the antigen-antibody reaction in immunology. The evidence of the existence of such a mechanism in mammals is as yet incomplete.

3.3 Blastocyst formation and implantation

The importance of synchronization between the developmental stage of the eggs and that of uterine endometrium for implantation and embryonic growth, as influenced by progesterone secretion from the corpora lutea, has been demonstrated in many species by egg-transfer techniques. Recent experiments indicate that pretreatment of rabbits with progesterone not only inhibits fertilization and disturbs the normal transport of eggs from the tube to the uterus but also causes degeneration of the eggs *in utero*. Ovariectomy in mice on the second and third days after mating is not invariably followed by the failure of implantation. Studies of oestrogen production in the rat and mouse, using histological and uridine-uptake techniques, indicate that it reaches a peak on the third day, subsequently remaining at the same level in the rat and declining in the mouse.

It should be noted that various oestrogens and anti-oestrogens are effective as antifertility agents in the rabbit when given postcoitally, although it is not believed that oestrogen is necessary for implantation in that species.

In the rodent and in man, it has not as yet been possible to separate with certainty oestrogenicity and anti-implantation effects. Although oestrogens in doses greatly exceeding physiological levels will interfere with

pregnancy in rodents by causing degeneration of the maternal portion of the placenta, they do not appear to interfere with an implanted pregnancy in infrahuman primates or man.

3.4 Early embryonic development

Agents that interfere with early embryonic development after implantation may act either directly on the foetus or indirectly on the implantation site or the placenta.

3.5 Chemical approaches to fertility control

3.5.1 Agents influencing ovulation

The available information suggests that the neural stimulus for ovulation originates in one or more centres outside the hypothalamus. Very little is known about the location of such extrahypothalamic centres or about the nerve fibres that transmit the stimulus for ovulation to hypothalamic nuclei. Experiments in the rat have indicated that these fibres enter the hypothalamus from its frontal part. Anatomical and biochemical evidence suggests that there are cholinergic as well as adrenergic fibres, impinging on the hypothalamic nuclei, that play a role in initiating the synthesis of the gonadotrophin releasing factors.

Nonsteroidal agents that inhibit ovulation by acting on the central nervous system operate either by suppressing the neural stimulus for ovulation at its origin or by inhibiting its transmission to the hypothalamus. The mode of action of these drugs thus differs from that of the anti-ovulatory steroids, which have been shown to reduce the synthesis and release of gonadotrophin releasing factors at the hypothalamic level.

The agents discussed in this section do not appear to be useful for clinical application because of their behavioural effects.

(1) *CNS depressants*. A list of CNS depressants that have been studied in animals as possible inhibitors of ovulation is given below :¹

| <i>Drug</i> | <i>Species</i> | <i>Drug</i> | <i>Species</i> |
|---------------|--------------------|------------------------|----------------|
| diethyl ether | rat | diallylbarbituric acid | rat |
| ethanol | rat, rabbit | barbital | hen |
| morphine | human, rat, rabbit | probarbital | hen |
| phenobarbital | rat | diphenylhydantoin | rat |
| pentobarbital | rat, rabbit | chlorpromazine | human, rat |
| amobarbital | rat | meprobamate | rat |

¹ For references to the original reports, see Gold, E. M. & Ganong, W. F. (1967), in : Ganong, W. F. & Martini, L. (ed.) *Neuroendocrinology*, vol. 2, p. 377, New York, Academic Press.

These compounds inhibit the release of gonadotrophins and prevent ovulation, in both spontaneous and "reflex" ovulators (i.e., animals that ovulate only after coitus and other sexual stimuli). In species that ovulate spontaneously, CNS depressants must be given before the so-called "critical period"—i.e., before the neurogenic stimulus for ovulation has reached the hypothalamus. Several of these compounds block the ovulatory response induced in immature animals by pregnant mare serum (PMS) gonadotrophin; a critical period after which the drugs become ineffective has also been described in this case. A few data obtained in animal experiments indicate that the inhibition of ovulation induced by these agents may be overcome by sexual stimuli. Only morphine and chlorpromazine have been found to be effective in women.

(2) *Cholinergic blocking agents.* The anticholinergic drugs listed below¹ are highly effective in blocking ovulation and in preventing the cyclic release of gonadotrophins in both spontaneous and "reflex" ovulators:

| <i>Drug</i> | <i>Species</i> |
|----------------------|-----------------------|
| atropine | rat, rabbit, hen, cow |
| methantheline | rat, rabbit |
| tridihexethyl iodide | rat |

These agents probably prevent the transmission of stimuli in the cholinergic pathways impinging on the hypothalamus.

(3) *Adrenergic blocking agents.* The following adrenergic blocking agents have been shown to inhibit ovulation in both spontaneous and "reflex" ovulators when given before the critical period:¹

| <i>Drug</i> | <i>Species</i> |
|-------------------------|------------------|
| dibenamine ² | rat, rabbit, hen |
| phenoxybenzamine | rat |
| SKF-501 ³ | rat, rabbit, hen |

All anti-adrenergic agents studied so far belong to the "alpha" type. It is important to know whether drugs that can specifically counteract the effects of adrenergic mediators on "beta" receptors (e.g., propranol and its derivatives) can also inhibit the release of gonadotrophin.

(4) *Depletors of brain catecholamines and inhibitors of catecholamine synthesis.* Reserpine and other drugs that deplete brain stores of monoamines (norepinephrine and serotonin) have been shown to suppress the secretion of FSH and LH and to block ovulation in mammals, including man. That norepinephrine is the neurotransmitter involved in the rat has

¹ See footnote, p. 14.

² *N*-(2-chloroethyl)dibenzylamine.

³ *N*-(2-chloroethyl)-*N*-ethyl-9-fluoreneamine hydrochloride.

been demonstrated by using specific depletors (e.g., syrosingopine and tetrabenazine) and specific inhibitors (e.g., α -methyltyrosine) of catecholamine synthesis. There is some indication that hypothalamic serotonin might play some role in other rodents.

(5) *Monamine oxidase (MAO) inhibitors*. The MAO inhibitor pargyline blocks spontaneous ovulation in the rat if given prior to the critical period. Experiments with pargyline in combination with monoamine precursors have shown that the effect of pargyline on ovulation is not due to an increase of hypothalamic stores of monoamines.

(6) *Serotonin blocking agents*. Serotonin antagonists (e.g., lysergic acid diethylamide and methysergide) cause a consistent and significant reduction in the incidence of ovulation induced by PMS in mice and rats, but are less effective in spontaneous ovulators; this suggests that in these species serotonin does not play a physiological role in ovulation and indicates that not all test systems used so far for studying inhibitors of ovulation are fully adequate.

3.5.2 *Antigonadotrophic substances*

Metallibure¹ has been reported to be an effective inhibitor of pituitary gonadotrophin in various species of rodent and monkey and in women. In post-menopausal subjects, normally-menstruating women, and men with prostatic carcinoma, urinary gonadotrophin levels were reported to be considerably reduced. In normally-menstruating women, ovulation was inhibited. However, since this compound has been reported to cause side-effects, it is unlikely that it will be tested clinically.

Several plants or their crude extracts have been reported to possess antigonadotrophic properties and to disturb the oestrous cycle in rodents. Oestrogenic substances of plant origin (isoflavones, isoflavenes, isoflavonones, steroids, and stilbenes) and other phytoproducts (antimitotic compounds, alkaloids, hormones, antibiotics, amino acids, and other compounds) have been found to exert one or the other type of effect on reproductive processes in female rodents. At present there is no prospect that any of these substances will find clinical application as a contraceptive.

3.5.3 *Agents interfering with fertilization*

(1) *Hyaluronidase inhibitors*. It has been considered that the presence of the enzyme hyaluronidase in spermatozoa is necessary for passage of the spermatozoa through the cumulus oophorus, corona radiata, and zona pellucida of the egg. It has been reported that the treatment of rabbit

¹ Proposed international nonproprietary name for: 1-methyl-6-(1-methylallyl)-2,5-dithiobiurea.

sperm with nitrated hyaluronic acid (a hyaluronidase inhibitor), or the deposition of this acid in the vagina before mating, has the effect of inhibiting fertilization *in vivo*. Phosphorylated hesperidin, another hyaluronidase inhibitor, has also been reported to act as an antifertility agent in the rat, mouse, and human. However, no effect on fertilization and no other antifertility effect could be demonstrated when phosphorylated hesperidin was deposited in the tubes of rabbits, added to rabbit sperm intended for insemination, injected intraperitoneally into rats, or fed to rats. Other hyaluronidase inhibitors tested include trigentic acid and a polymer of 2,5-dihydroxybenzenesulfonic acid which inhibited fertilization when mixed with rabbit semen before insemination. Ammonium aurine tricarboxylate, when administered to male rats, was also reported to prevent sperm from penetrating cervical mucus or the egg membranes. However, the latter effect could not be reproduced in the mouse. The antifertility effect of polyphloretin phosphate in rats has also been reported, but whether it inhibits sperm penetration or affects other reproductive processes is not known.

(2) *Trace elements.* Cadmium interferes with the trophic effect of oestrogen on the uterus of ovariectomized rats. The topical application of cadmium in one of the horns of the rat uterus prevents implantation in both horns for 1 month, without affecting ovulation.

In experimental animals, copper deficiency is associated with a great variety of disorders and with impaired reproductive performance (foetal death, abnormalities in the oestrous cycle, etc.). The intravenous injection of copper salts in toxic or near-toxic doses induces an LH discharge, and thereby ovulation, in the rabbit. In the rat, the electrolytic deposition of copper in the pre-optic region of the hypothalamus induces ovulation. The physiological mechanisms leading to ovulation are activated by copper and other metallic ions through the hypothalamic-hypophysial system.

Endometrial effects : copper or zinc wires placed in the lower portion of one uterine horn of the rabbit produce a decrease in the number of implantations in that horn, but no significant alteration in the other horn. Silver, tin, and magnesium do not have this effect. Some of these metals produce a change in the ratio of the glandular to the stromal portions of the endometrium—e.g., copper and zinc induce a proliferation of mucosal stroma and silver induces glandular proliferation.

The effects of metallic copper on women of proved fertility have been studied. One group of women used a new type of plastic intrauterine device to which was attached a copper wire with a surface of 60 mm²; a second group of women, using the same device without copper, served as controls. The contraceptive action demonstrated after 9 months of use is indicated in the accompanying table. The histological appearance of the endometrium was normal, but the specific site of action of the copper (i.e., sperm, endo-

NET CUMULATIVE RATES OF EVENTS AND CLOSURES IN 9 MONTHS
OF USE OF SELECTED INTRAUTERINE DEVICES *

| Events and closures | Rates per 100 users of: | | |
|----------------------------------|-------------------------|----------------------|-----------------|
| | T device | T device with copper | Lippes loop "D" |
| Events | | | |
| Pregnancies | 12.8 | 2.54 | 2.0 |
| Expulsions | | | |
| First | 5.2 | 5.20 | 8.8 |
| Subsequent | 0.27 | 0.48 | 2.4 |
| Removals | | | |
| Medical reasons | 1.9 | 3.08 | 12.8 |
| Planned pregnancies | 1.2 | 0.96 | 0.6 |
| Other reasons (personal) | 0.95 | 1.68 | 1.7 |
| Closures | | | |
| Pregnancies | 12.8 | 2.50 | 1.9 |
| Expulsions | | | |
| First | 2.3 | 1.26 | 2.6 |
| Subsequent | 0.27 | 0.48 | 1.4 |
| Removals | | | |
| Medical reasons | 1.92 | 2.18 | 10.8 |
| Planned pregnancies | 1.63 | 1.76 | 0.4 |
| Other reasons (personal) | 0.95 | 1.68 | 1.5 |
| Total closures | 19.87 | 9.86 | 18.6 |
| Continuation rate | 80.13 | 90.14 | 81.4 |
| Total woman months of use | 5 989 | 2 558 | 55 698 |

* Zipper, J., Tatum, H. J., Pastene, L. & Medel, M., unpublished data.

cervical secretion, endometrium, tubes, or blastocysts) has not yet been elucidated.

(3) *Temperature*. A departure from normal temperatures may interfere with fertilization, but it is unlikely that such treatment will have practical application to fertility control in man.

3.5.4 *Agents interfering with implantation*

(1) *Ergot alkaloids*. The anti-implantation effect of ergot alkaloids, particularly ergocornine, has been extensively investigated in rats. Implantation is inhibited through interference with luteal function, leading to the suppression of decidua formation. Ergocornine is effective in mice but is

ineffective in rabbits and guinea-pigs. In limited studies in women, it was claimed that postcoital administration prevented pregnancy. However, the compound is toxic and there is little prospect of its use as a clinical contraceptive.

(2) *Monaminoxidase inhibitors*. It has been reported that a large number of hydrazine derivatives, all of which are monoamine oxidase inhibitors, interrupt pregnancy in rats, mice, and rabbits when given after coitus. It is believed that these compounds act by reducing uterine sensitivity to progesterone and not by virtue of their monoaminoxidase-inhibiting property.

(3) *2,6-dimethylhydroquinone*. There has been a single report that 2,6-dimethylhydroquinone causes a reduction of fertility in women when administered orally in doses of 300–500 mg on the 16th and 21st days of the menstrual cycle, but this has not been confirmed.

(4) *Oestrogens and related compounds*. In laboratory and most domestic animals, oestrogens prevent the development of the blastocyst and prevent implantation or terminate pregnancy at practically all stages. An oestrogen-sensitive centre in the hypothalamus presumably affects gonadotrophin synthesis and/or release. Although relatively low doses of cyclic oestrogens may cause the release of LH, persistently higher levels prevent ovulation and inhibit follicular growth. When given to females shortly after mating, oestrogens disturb tubal transport. This may result either in the retention of ova in the Fallopian tubes or the accelerated passage of ova through the tubes. When given later, oestrogens prevent the implantation of ova even if they have arrived in the uterus within the normal time. As pregnancy progresses, higher doses will cause resorption of the established embryo and abortion of the foetus. However, there is a considerable body of evidence that, quite apart from the priming effect of oestrogen on the uterus (which it prepares for the action of progesterone), additional oestrogen is needed in at least some rodent species to give full uterine sensitivity and thus to permit normal implantation.

Accumulating evidence indicates that anti-oestrogens are capable of preventing normal tubal transport and implantation in rodents. It has been questioned whether the antifertility action of some extensively studied synthetic compounds depends on their anti-oestrogenic properties. It seems certain that dimethylstilbestrol (DMS), very probable that nafoxidine¹ hydrochloride, and possible that MER-25² act because of their oestrogenic properties. It also seems likely that most anti-oestrogens so far described have antifertility effects because they are weak oestrogens or pro-oestrogens.

¹ Proposed INN for 1-{2-[p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]ethyl}pyrrolidine.

² 1-{p-[2-(diethylamino)ethoxy]phenyl}-2-(p-methoxyphenyl)-1-phenylethanol.

However, there remain such compounds as U-11555A¹ and MRL-37,² which do not seem to be sufficiently oestrogenic to account for their antifertility potencies (although MRL-37 closely resembles MER-25). Other compounds, such as *meso*-DMA³ and *erythro*-MEA,⁴ although quite potent as oestrogens, have much higher antifertility potencies than are reasonably explicable on the basis of an oestrogenic action. Anti-oestrogenic activity, or an activity in some way dissociated from typical oestrogenicity, could explain the action of each compound investigated to date. Since compounds such as DMS and DMA do not act as anti-oestrogens when administered by the intravaginal route, their antifertility effects when administered by subcutaneous injection should be ascribed to anti-oestrogenicity only with caution. It remains possible that the antifertility action of the types of compound under discussion is due to oestrogenic activity that is not detected by conventional tests. For example, DMS and certain related compounds show high potency when tested in assays of the uptake of tritiated uridine into uterine RNA in the mouse, a response typical of oestrogens.

It has been assumed that certain substances used in fertility control, particularly MER-25, exert a zygotoxic action. Recent work has demonstrated that this is not so with effective doses in rodents and rabbits. Ovum transfer from treated or control donors to treated or control hosts has shown that the uterus and not the zygote is affected when MER-25, DMS, nafoxidine hydrochloride, U-11555A, ORF 3858,⁵ and MRL-37 are used. Similar results were obtained when estradiol and ethinylestradiol were given during the first 3 days after mating, indicating that although this oestrogen, like others, may cause failure of nidation through a disturbance of tubal transport, the uterus is also affected and is not able to accept the zygote at the normal time of implantation.

Recent observations suggest that oestrogens will prevent implantation of the ovum when administered post-coitally in large doses to rhesus monkeys or to women. In the monkey and man, in contrast to lower species, oestrogens apparently have no effect once implantation has occurred. Since the time required for implantation is approximately 6 days, the duration of administration in women has usually been 4-6 days, depending to some extent on how long after coitus the patient presented herself. Limited clinical success has been obtained with daily doses of 50 mg of stilbestrol; doses of 3-10 mg per day have been shown to be completely ineffective. Laboratory studies suggest that if the compound is administered over a shorter period, high doses may be required. One of the limitations of this

¹ 2-[*p*-(6-methoxy-2-phenylinden-3-yl)phenoxy]triethylamine.

² 2-[*p*-(*p*-methoxy- α -phenylphenethyl)phenoxy]triethylamine.

³ (\pm)-4,4'-(1,2-dimethylethylene)diphenol.

⁴ *erythro*-4,4'-(1-ethyl-2-methylethylene)diphenol.

⁵ 5-ethyl-6-methyl-4-phenyl-3-cyclohexene-1-carboxylic acid.

approach at present is the difficulty of determining the time of ovulation. It is obvious that high doses of oestrogen cannot be given throughout the cycle without suppressing ovulation and encountering many more side-effects than occur with the anti-ovulatory compounds now in use.

The most consistent findings following oestrogen administration during this period are the lowering of the basal body temperature to the pre-ovulatory level and a "retarded" endometrium with persistence of basal vacuoles even up to the time of menstruation. Stromal oedema is also seen. Nausea has been the principal side-effect noted.

Apart from a large number of steroid derivatives, which usually show a broad range of undesirable biological activity, two main types of compound have been intensively investigated to date. They are (a) stilbene and dibenzyl derivatives closely related to the potent synthetic oestrogens diethylstilbestrol and hexestrol and (b) basic ethers of some phenolic triarylethylene, triarylethane, or triarylethanol derivatives, and their ring-fused analogues. All the stilbestrol derivatives among type (a) compounds seem to owe their activity to oestrogenic properties. The dibenzyl derivatives show varying degrees of oestrogenic and antifertility activity, which makes them of greater interest (cf. *erythro*-MEA). Type (b) compounds, which show varying oestrogenic, anti-oestrogenic, and antifertility activities, include some 2,3-diaryllindenes, such as U-11555A, and several 1,2-diaryl-3,4-dihydronaphthalenes, of which nafoxidine shows the greatest activity. The indene derivative U-11555A suffers the disadvantage of chemical instability, and causes photosensitivity in some species.

Various heterocyclic analogues of U-11555A, nafoxidine, and similar compounds have shown weak antifertility activity. Several of a series of 18 derivatives of 2,3-diphenylindole were found to be effective; 5-fluoro-3-phenyl-2- $\{p$ -[2-(1-pyrrolidinyl)ethoxy]phenyl}indole and 3-phenyl-2- $\{p$ -[2-(1-pyrrolidinyl)ethoxy]phenyl}indole prevented implantation in the rat when administered in daily doses of 10 mg per kg of body weight on days 1-5 after coitus. Many related compounds have been described.

On the basis of results obtained in rats, it appears that U-10293,¹ a diazocine compound, shows promise. It is oestrogenic, antigonadotrophic, nontoxic, and nonteratogenic.

Another compound, U-11634,² prevents implantation in rats when administered orally or subcutaneously at a level of 2.5 mg per kg of body weight for 7 days from pro-oestrus. A single oral dose of 10 mg per animal on days 3-6 after coitus is equally effective. The compound is devoid of hormonal properties and is not teratogenic. However, it is toxic and there is little prospect of its use as a contraceptive.

¹ 2,8-dichloro-6,12-diphenyldibenzo(*b,f*) (1,5) diazocine.

² 5- $\{[(\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)oxy]methyl}-2-oxazolidinethione.

The dibenzyl derivative ORF 3858 is a promising compound that has potent postcoital antifertility action in rabbits and rhesus monkeys. Cyclofenil¹ and F6103² are unsymmetrical diphenylethylene derivatives that exhibit oestrogenic activity. They are thought to oppose the action of progesterone. The efficacy of F6103 as a postcoital contraceptive agent in man remains to be established.

In contrast to DMS and *erythro*-MEA, most of the triarylalkane and triarylalkene derivatives listed above possess the desirable feature of activity when administered orally. However, the possibility that an ideal non-oestrogenic, potent antifertility drug might be found among them is perhaps limited by their potential carcinogenicity. Furthermore, the teratogenicity of triparanol (a close analogue of MER-25) in rats illustrates the necessity of critical evaluation of compounds of this type.

Little or no oestrogenic or antifertility activity has been found in various chlorinated stilbene derivatives and dibenzyl derivatives other than those described above, or in derivatives of angolensin,³ deoxybenzoin, indan, phenanthrene, benzene, cyclohexane, and hydrodibenzyl. However, anti-oestrogenic activity, sometimes pronounced, has often been found in intra-vaginal tests. Some chrysene derivatives have shown antifertility action, which is almost certainly correlated with oestrogenic activity.

Preliminary studies indicate that diethylstilbestrol has an antifertility effect both in rhesus monkeys and in women. ORF-3858 and compound 66/179⁴ have been tested only in rhesus monkeys, and should be investigated further. In addition, U-10293, *erythro*-MEA, CN-55945-27,⁵ and ICI 46474⁶ seem to deserve further study as anti-implantation agents for post-coital administration.

3.5.5 Agents interfering with early embryonic development

Although studies of agents interfering with early embryonic development have not yet led to the discovery of a compound likely to be of use in fertility regulation, they provide techniques for evaluating certain aspects of drug safety related to teratogenicity.

The agents that have been used in such studies can be conveniently classified as (1) metabolites, (2) metabolic analogues (antimetabolites), (3) enzyme inhibitors, (4) antimitotic compounds, (5) cytostatic compounds, (6) vitamins and vitamin analogues, (7) hormones and synthetic compounds

¹ Proposed INN for 4,4'-(cyclohexilidenemethylene)diphenol diacetate ester.

² α -(*p*-hydroxyphenyl)- α -(2-methylcyclohexylidene)-*p*-cresol diacetate.

³ 2',4'-dihydroxy-2-(*p*-methoxyphenyl)propiofenone.

⁴ 1-{2-[*p*-[2-phenylnaphtho(2,1-*b*)furan-3-yl]phenoxy}ethyl}pyrrolidine.

⁵ 1-{2-[*p*-[α -(*p*-methoxyphenyl)- β -nitrostyryl]phenoxy}ethyl}pyrrolidine citrate.

⁶ (*Z*)-2-[*p*-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine.

with hormonal activity, and (8) X-rays and other types of radiation. In evaluating the effect of these agents, the following points must be borne in mind.

Embryos of identical gestational age differ in their degree of development; this phenomenon is encountered between, as well as within, litters of polytocous animals. Such developmental variability is also a well-known phenomenon in human multiple pregnancy.

A relatively high incidence of abortive embryos (about 10%) occurs in highly fertile animals. The same is true of early human embryos. In some human abortuses and hydatidiform moles, there has been evidence of basic chromosomal defects.

There is experimental support for assuming that some embryos may abort as a result of aging gametes; postovulatory aging of eggs, in particular, appears to lead to abnormalities such as polyspermy, spontaneous activation and fragmentation, and loss of fertilizability.

Agents administered to the mother (especially those given parenterally, but also those given orally) can reach unimplanted free-lying embryos very rapidly. In some instances, the penetration of embryos by such substances probably takes place within minutes of maternal application. The extent of the damage will depend in each case on both the maternal and embryonic genotypes; consequently, accurate prediction of embryonic vulnerability to damage from a given exposure to such agents is extremely difficult.

Several agents, especially those of hormonal nature, tend to affect embryos secondarily rather than primarily—i.e., by inducing profound alterations in the uterine environment, both in the endometrium and the myometrium.

A placental barrier exists between the mother and the embryo (probably not identical with the so-called immunological barrier), which regulates quantitatively the influx of metabolites and foreign substances into embryos. In general, even if penetration of the blastocoelic space occurs, the concentration of such substances in the embryo is only a fraction of the concentration in the maternal blood stream. The mechanism is unknown, but animal experiments have given some indications that small amounts of oestrogen can destroy the regulatory power of this barrier between mother and embryo.

Morphologically distinct areas of the embryo react quite differently to a given agent. As a rule, the embryo proper (also referred to as the embryonic disc or the inner cell mass) is far more sensitive than the extra-embryonic elements (comprising the trophoblast, trophoblastic knobs, and endoderm).

3.5.6 *Agents interfering with embryonic development after implantation*

Although oestrogens and possibly other compounds will disrupt an established implantation site in rodents, no such effect has been observed

in primates. However, there are a number of cytotoxic agents with anti-trophoblastic or antizygotic activity that will destroy the developing embryo. Effective antizygotic doses of most of these compounds are highly toxic to the mother. The available evidence suggests that all are actually or potentially teratogenic when given in subeffective doses or at critical times of embryonic development, which vary for different drugs. Although women have occasionally been reported to have had normal fetuses after courses of cytotoxic agents during pregnancy, it seems safer to carry out therapeutic interruption of pregnancy in such cases, especially if the compound has been taken during the first trimester.

(1) *Antimetabolites*. In the use of antimetabolites for the interruption of gestation in women, the most extensive experience has been obtained with aminopterin. In one study, when this compound was given during the first trimester in 52 pregnancies, 18 failed to abort and at least 10 foetal malformations were reported. Similarly, malformations have been reported following the use of mercaptopurine and busulfan. A clinical study of 6-azauridine has been reported, although this compound has been found to be teratogenic in lower species.

(2) *Alkylating agents*. Pregnancy has been interrupted following the administration of nitrogen mustard, tretamine, chlorambucil, cyclophosphamide, and busulfan to women in the first trimester of pregnancy. Malformed fetuses have also been found in these studies.

(3) *Antimitotic and miscellaneous cytotoxic agents*. Colchicine, deacetylmethylcolchicine, urethane, and vinblastine have been administered to a limited number of pregnant women without demonstrable effect on the foetus, although some of these agents are effective abortifacients (or have proved to be teratogenic) in lower species.

When used for cancer chemotherapy, cytotoxic agents appear to be less dangerous to the foetus, in terms of teratogenicity, if administered during the second and third trimester of pregnancy. The toxicity, potential teratogenicity, and relative ineffectiveness of the agents now available suggests that they are not suitable for fertility control.

(4) *Intra-amniotic solutions*. The intra-amniotic instillation of hypertonic solutions of dextrose and saline can be used to interrupt pregnancy in the second trimester. Some success has been reported with the intrauterine administration of monoaminoxidase inhibitors (e.g., pargyline hydrochloride). In animals, deacetylmethylcolchicine is much more effective if administered intra-amniotically. The investigation of embryotoxic compounds of sufficient molecular size to prevent passage through the placenta might prove rewarding.

3.6 Immunological approaches to fertility control

The use of immunological procedures to control the fertility of the human female is not yet a practical proposition. However, the immunological approach with species-specific antigens is potentially useful, provided the specificity of sperm antigens can be improved so that the antibodies formed do not react with any of the antigenic determinants present in other tissues. Another prerequisite is the development of immunization procedures by which a high concentration of antispermatic antibodies can be maintained in the female genital tract.

The presence of circulating antibodies to spermatozoa has been demonstrated in laboratory animals after immunization with sperm cells, semen, or testicular tissue with the addition of adjuvant. However, this does not invariably result in temporary sterility. Furthermore, the administration of antisperm sera did not affect the reproductive capacity of the animals.

It has been found that active immunization procedures give rise to a high titre of antibodies in the circulation, but not in the vaginal and uterine fluids. On the other hand, it has been reported that intravaginal sensitization with sperm produces antibodies that can be detected in the vaginal fluid but not in the circulation. Whether such "local" antibodies can immobilize the sperm in the vagina and/or in the uterus remains to be established.

The evidence obtained thus far in clinical studies is even less conclusive. Sperm-agglutinating antibodies have been reported to be present in the serum of women with unexplained infertility. However, such antibodies have also been found—although less frequently—in the serum of fertile women. That such antibodies may play a role in infertility is suggested by the finding of a fall in their titres following abstinence or the use of a condom, associated with a correction of the infertile state in a few cases.

The finding of immobilized sperm following the intravaginal instillation of seminal antigen suggests that the induction of "local" antibodies also offers a possible means of fertility control in the human.

Animal experiments indicate that immunization with gonadotrophin preparations obtained from another species may lead to impaired fertility. In clinical studies the administration of pregnant mare's serum gonadotrophin (but not that of human hypophysial or urinary gonadotrophins) resulted in the formation of antigonadotrophins.

Animal studies with the production of antibodies against placental preparations have also been reported. The injection of such antiplacental sera into pregnant rats resulted in a high incidence of abortion. However, since the antigenic determinants of placental and kidney preparations are somewhat similar, this treatment evoked a nephrotic condition.

4. APPLICATION OF RESULTS OF ANIMAL STUDIES TO MAN

4.1 Small laboratory animals

The general recommendations for preclinical and clinical evaluation made in the reports of three WHO Scientific Groups¹ are also applicable to the testing of antifertility drugs. The difficulty of extrapolating to man the observations made in small laboratory animals is especially marked in research on antifertility agents because of the wide variation in the reproductive processes of different species. For convenience, many biological assays of antifertility drugs are performed in rats, rabbits, mice, and hamsters. There has been no systematic attempt to correlate the results of animal assays with those of assays carried out in humans, and in studies of humans there has been a failure to utilize pharmacological principles to determine the dose-response characteristics of antifertility drugs. The data that are available are limited almost entirely to the steroidal hormones. In the search for new types of antifertility drug, better guidelines are required than those available at present.

4.2 Infrahuman primates

Recently, greater emphasis has been given to the testing of antifertility drugs in infrahuman primates. Various species seem to be suitable for studies of postovulatory agents used before or during implantation or to interrupt gestation, but their degree of similarity to women is not known. Moreover, when compounds different from the natural hormones are used, the responses of different species of primate may vary as significantly as they sometimes do between the rat and the mouse. The rhesus monkey is the primate most commonly used. It should be noted that although this monkey has considerable similarity to man, it shows differences in the timing and mode of implantation; in the placental production of chorionic gonadotrophin; and in the foetal, maternal, and placental production and metabolism of oestrogen.

Studies in infrahuman primates should emphasize investigation of the basic physiology of primate reproduction and the mechanisms of drug action rather than drug screening. However, in evaluating promising post-coital and postovulatory contraceptives or agents that interrupt pregnancy at later stages, infrahuman primates may offer a useful intermediate step if testing in man involves undue risk to the mother or foetus. This may also be true of antifertility compounds for use in the male.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 341; 1967, No. 364; 1968, No. 403.

5. GUIDELINES FOR STUDIES IN MAN

5.1 Pharmacological studies

In undertaking any evaluation of a contraceptive method in man the primary concern must be the safety of the user and the well-being of any offspring that may be born following contraceptive failure. The need for laboratory control and follow-up of patients increases when little is known of the effect of a given method or agent in man. It follows that no group of patients should be so large as to make it impossible to meet this need. Furthermore, exploratory studies of methods not previously tested in man that could influence foetal development should not be contemplated unless pregnancies resulting from contraceptive failure can be interrupted. In general, methods based on the systemic administration of drugs are of more concern than those that may be considered purely local or mechanical.

5.2 Clinical trials

5.2.1 *Local methods*

It is becoming increasingly possible that antifertility agents may be placed in the uterine cavity to exert a local effect, perhaps in specially designed forms so as to provide a prolonged continuous release. Prior to clinical trial with such agents, information should be available on the amounts being released, the effects on reproductive organs in animals, possible systemic effects that might have a bearing on safety, the reaction of the peritoneum to the substance (to anticipate difficulties in the event of uterine perforation), and possible teratogenic effects.

In exploratory clinical studies with locally acting agents, the goals should be (1) to evaluate contraceptive effectiveness, the reversibility of the effect, and menstrual changes or other side-effects directly related to the method, and (2) to detect any systemic effects. The number of users should be gradually and cautiously increased. A satisfactory indication of the effectiveness of a method and of the general incidence of side-effects can be obtained from the observation of 200 patients for 1 year. Systemic effects can be detected in much smaller groups.

In the next phase of evaluation, the acceptability of the method—including its use-effectiveness and the incidence of side-effects—is determined in populations of up to 1000 patients. At this time, provision should also be made to collect prospectively data on the incidence of possible neoplastic or other abnormal effects on the tissues or cells of the uterine endometrium and cervix, and on other abnormalities of the genital tract. In order to define the risks of teratogenicity, special attention should be given to the complete examination, including chromosomal studies and a routine medical follow-up, of all children born after contraceptive failure who may have been exposed to the local effects of the agent.

5.2.2 *Systemic methods*

Chemical agents that act systemically to interfere with male or female fertility must be given particular consideration from the standpoint of safety. Certain agents or routes of administration may give rise to special problems, but in general, attention must be paid to (1) acute and subacute toxicity, (2) intermediate safety, (3) long-term and/or latent effects, and (4) teratogenicity. The data that are necessary include the LD₅₀ in several species, the reasons for death, and the pharmacodynamic action of the drug. Emphasis must be placed on periodic function tests of both endocrine and parenchymatous organs. Such function tests should be extended to healthy subjects during the second stage of the clinical trial.

Subacute toxicity studies in two species, one not a rodent, must precede the preliminary clinical trial. In such toxicity studies, the maximum dosage that will be used in the clinical trial should be given. Such tests should help to establish the maximum tolerable dose and duration of administration. It is important to use the same species to determine both primary and secondary effects. By definition, clinical studies are short-term, rarely involving more than 30 consecutive days of treatment, and they should involve individuals in whom there is no risk of pregnancy. Sufficient information on safety should be furnished by 90-day toxicity studies; complete data should be available on functional tests of endocrine organs and organs such as the liver and kidney, and on pathological changes. Clinical pharmacological studies should be carried out, using laboratory tests to study any modification in the function of one or more organs or organ systems in small numbers of carefully chosen and controlled subjects. Before clinical trials to determine the antifertility effect of any drug may be undertaken, chronic-toxicity studies in two species must also be completed. The duration of such toxicity studies must exceed the planned duration of the clinical trial.

The most critical aspect of any clinical evaluation of an antifertility agent is the first attempt to demonstrate a contraceptive effect. The clinical studies usually involve, for each given dose level, about 100–200 healthy subjects of proved fertility for periods up to 1 year. Patients should be followed up at monthly intervals to evaluate side-effects and to establish as soon as possible the onset of any pregnancies in order that exposure to the drug may be discontinued. Patients should receive appropriate clinical and laboratory tests.

Evaluation programmes involving entirely new concepts of fertility control must be undertaken with caution. This is particularly true of drugs that interfere with implantation or that have a cytotoxic effect on the embryo, but must also be seriously considered with agents that modify the development or maturation of spermatozoa but do not totally suppress spermatogenesis.

Although comprehensive tests for teratogenicity in lower animals are essential before any clinical studies of antifertility drugs can be undertaken, the value of such tests for predicting the effects of drugs in humans has not been established.¹

Following the selection of the dose range of an antifertility drug, larger programmes involving 500–1000 patients may be undertaken, including appropriately selected control groups, to determine more precisely the efficacy of the drug and the incidence of side-effects. A thorough medical history of each prospective subject should be taken and a complete medical examination, including Papanicolaou smears in females, should be made. Only healthy adults of proved fertility should be included.

Patients should be seen regularly by qualified medical or paramedical personnel, preferably not less frequently than every 3 months, to rule out pregnancy, to ascertain the patient's general health, and to learn of any side-effects that may have occurred. Significant deviations from the norm in individual health status or the occurrence of unexpected side-effects should be reported immediately to the medical supervisor for investigation, evaluation and disposition. It is most important that all those who continue taking the contraceptive agent and those who drop out of the programme be followed up. Efforts should be made to study the fertility of subjects who have discontinued the method, and the growth and development of their subsequent offspring. Each patient should be given a careful and complete general medical examination every year for the duration of the study and, if possible, for some years afterwards. Particular attention must be given to the physical and cytological diagnosis of cancer in hormonally responsive tissues, particularly those having a high frequency of carcinoma (e.g., breast, uterine cervix and endometrium, and prostate gland). Such diagnostic cancer surveys should be made annually together with the general medical examination.

Special laboratory studies may be indicated for all or part of the group to define more exactly metabolic or functional problems discovered during the preliminary testing in the previous stage of evaluation.

The health risk to a population from a contraceptive agent can best be determined by increasing both the duration of the studies described above and the number of subjects under observation, based upon existing safety data. A retrospective evaluation may be the most efficient if not the only way to study the risk of events whose incidence is extremely low.

It must be realized that, as experience with a given agent accumulates, its use will be extended from normal individuals to those with pathological conditions. Before this can be done there must be evidence that the agent does not aggravate the disease either acutely or chronically nor change

¹ See *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 364.

therapeutic requirements. The metabolic and functional studies noted previously should be helpful in detecting such problems.

6. RESEARCH RECOMMENDATIONS¹

6.1 Male fertility and its control

In relation to male fertility and its control, studies should be carried out in the following areas :

(1) The role of the pineal gland in the control of gonadotrophin secretion and the similarities and differences between the male and the female in neuro-endocrinological control of gonadotrophin secretion (2.1).

(2) Factors that might affect the permeability of the seminiferous tubules and modify testicular fluid balance (2.2).

(3) The functions of Sertoli cells and agents affecting them (2.2).

(4) The basic mode of action of chemical agents that act on male reproductive processes. Studies should include the determination of drug distribution and metabolism and changes in enzyme and hormone levels, particularly those occurring in spermatogenic cells, in cells associated with spermatogenesis, and in accessory reproductive organs (2.1-2.4).

(5) The passage of exogenous agents into the semen, the time they take to reach the semen, the duration of their effect in the semen, the extent to which they are bound to spermatozoa, and the relationship, if any, of all these factors to male fertility and to pregnancy wastage (2.2-2.4).

(6) The biochemistry and pharmacology of the epididymis and ductus deferens during sperm maturation and ejaculation (2.2, 2.3).

(7) The role of the accessory gland secretions in man (2.3).

(8) Exogenous factors that arrest sperm motility in the ejaculate (2.4).

(9) The development of compounds that could rapidly induce and maintain a functional type of sterility with minimal toxicity and risk of genetic damage (2.4.1).

(10) The relationship between trace elements and enzyme systems in the control of male fertility (2.4.1).

(11) Chelating agents that can specifically bind zinc in the testis (several chelating agents that bind zinc have been shown to have an affinity for, and to affect, specific tissues) (2.4.1).

(12) The use of testosterone derivatives as carriers to direct chelates and/or metals preferentially to specific target organs (2.4.1).

¹ For convenience, the recommendations appear in the order in which the subject is treated in the text. The figures in parentheses refer to sections in the text.

6.2 Female fertility and its control

In relation to female fertility and its control, studies in the following areas are recommended :

(1) The relevance of the different variables routinely used to detect and predict ovulation, and the development of new techniques (3.1).

(2) The significance and mechanism of the thermal changes in the progestational phase of the menstrual cycle (3.1).

(3) The location in the CNS of the centres from which the stimulus for ovulation originates, and of the nervous pathways through which stimuli are transferred to the hypothalamus. The development of drugs that interfere at this level to block ovulation should be attempted (3.1, 3.5.1).

(4) Factors that affect atresia of the follicles (3.1, 3.5.1).

(5) Factors that stimulate or inhibit the endocrine function of the corpus luteum (3.1, 3.5.1).

(6) The minimum life span of the corpus luteum that permits the maintenance and continuation of pregnancy in primates (3.1, 3.5.1).

(7) Gonadotrophin inhibitors from various sources (hypothalamus, thymus, plasma, urine, etc.) and possible inhibitors of releasing factors (3.1, 3.5.2).

(8) *In vitro* systems suitable for the study of fertilization (3.2).

(9) The duration of the fertilizing ability of sperm in the female genital tract (3.2).

(10) The significance of uterine and tubal motility for fertilization and implantation (3.2, 3.3).

(11) Sperm capacitation and fertilization of the egg by endocrine and chemical treatment, from both the morphological and biochemical standpoints (3.2, 3.5.3).

(12) Physicochemical changes in cervical mucus produced by progestational agents, and the influence of such changes on the fertilizing ability of spermatozoa (3.2, 3.5.3).

(13) Effects of the sex hormones on the metabolism of the endometrium and endosalpinx ; the interaction between the uterine environment and the implanting egg ; and hormonal requirements for implantation, particularly in primates (3.3, 3.5.4).

(14) Drug exchange between mother and conceptus at different stages of pregnancy (3.4, 3.5.5, 3.5.6).

(15) The correlation between duration of exposure to antifertility drugs and target organ response at different dose levels (3.5).

(16) The variation over a period of time in the effects of fertility regulating agents on metabolic processes, including enzyme induction (3.5).

(17) The details of the regulation of gonadotrophins by steroid feedback mechanisms in man (3.5, 3.5.1).

(18) The effect on ovulation of anti-adrenergic agents that block beta receptors (3.5.1).

(19) Methods for the continuous slow release of metals, alloys, and chelating agents into the female genital tract (3.5.3).

(20) The mechanisms of the emetic effect of oestrogens in relation to their chemical structure (3.5.4).

(21) Chemical agents that prevent implantation, and their mechanism of action (3.5.4).

(22) Agents that interrupt gestation, and their mechanism of action (3.5.5, 3.5.6).

(23) Specific sperm or semen antigens that do not cross-react with antigenic determinants of other tissues (3.6).

(24) The site of immunological reactions that interfere with fertility (3.6).

(25) Methods of inducing high local concentrations of specific anti-spermatic antibodies in the female genital tract (3.6).

(26) The mechanism of action of adjuvants in the potentiation of antigenicity, and the improvement of existing adjuvants for immunization (3.6).

6.3 General recommendations

Without further research on basic reproductive physiology, the rational development of new methods of fertility regulation would be hindered. Many of the above recommendations have been made with this in mind. In addition, the Group make the following general recommendations :

(1) Systematic comparative studies should be made of the similarities and differences between experimental animals and man, from the points of view of reproductive physiology and pharmacology. New techniques should be developed for the testing of antifertility compounds.

(2) Attempts should be made to study the effects of promising anti-fertility compounds in more than one species—including, if possible, primates—at an early stage of the investigation of such compounds.

(3) Studies should be undertaken to determine the formulations of, and routes of administering, fertility regulating agents that are most efficient for obtaining the required target organ response.

Annex

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