



Northwestern University
1040 Passavant Pavilion
303 East Superior Street
Chicago, Illinois 60611

Editor: Gerald I. Zatuchni, M.D., M.Sc.
Managing Editor: Kelley Osborn
This publication is supported by AID/DSPE-C-0035



RESEARCH FRONTIERS IN FERTILITY REGULATION

This report is the premiere issue of a series designed to present a comprehensive review of past and current research information and experience on selected major topics in fertility regulation and contraceptive development. The aim of the series is to provide the scientific and technical community with the best possible summary of the state of the art with regard to each topic. The reports will appear approximately six times annually. As new developments occur in this rapidly changing field, the reports will be appropriately updated and reissued.

Additional copies of this publication and future issues may be obtained without charge by specific request to PARFR at the indicated address.

LONG-ACTING STEROIDAL CONTRACEPTIVE SYSTEMS

Lee R. Beck, Ph.D.
Associate Professor
Department of Obstetrics
and Gynecology
University of Alabama
Birmingham

Donald R. Cowsar, Ph.D.
Associate Director
Applied Sciences Research
Southern Research Institute
Birmingham, Alabama

Valerie Z. Pope, M.S.
Research Assistant
Department of Obstetrics
and Gynecology
University of Alabama
Birmingham

The need for effective, safe, and easy-to-use contraceptives has not diminished with the advent of the pill and the intrauterine device (IUD). On the contrary, concern regarding the safety of conventional contraceptives continues to stimulate new research. One approach to the improvement of the effectiveness, safety, and acceptability of steroidal contraceptives is to develop long-acting preparations and methods of delivery which provide programmed medication. This rationale is based on the knowledge that side effects of steroid hormones are dose-dependent. Accordingly, the degree of risk changes in proportion to the dose. The preferred dose is one that evokes contraception with minimal risk.

The therapeutic concept of minimal intervention and how it can be achieved through the programmed delivery of steroidal contraceptives is illustrated in Figure 1. The curves compare blood levels of a hypothetical steroid following either conventional or improved delivery. In this example, conventional delivery is by the oral route, and improved delivery is achieved by the use of an injectable formulation which provides programmed release. The blood level of the drug necessary for contraception is represented by a narrow zone. Values in excess of the optimal therapeutic dose constitute overmedication with a greater potential for dose-dependent side effects, whereas values below the effective range

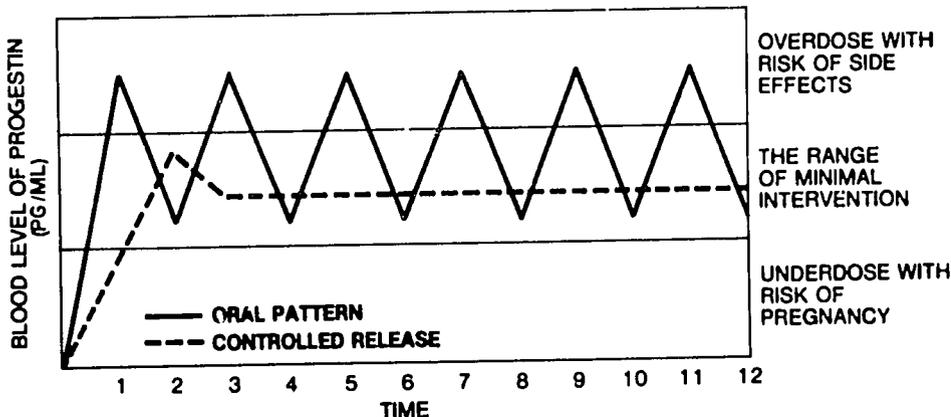


Figure 1. Blood levels of a hypothetical steroid following either oral administration or programmed delivery.

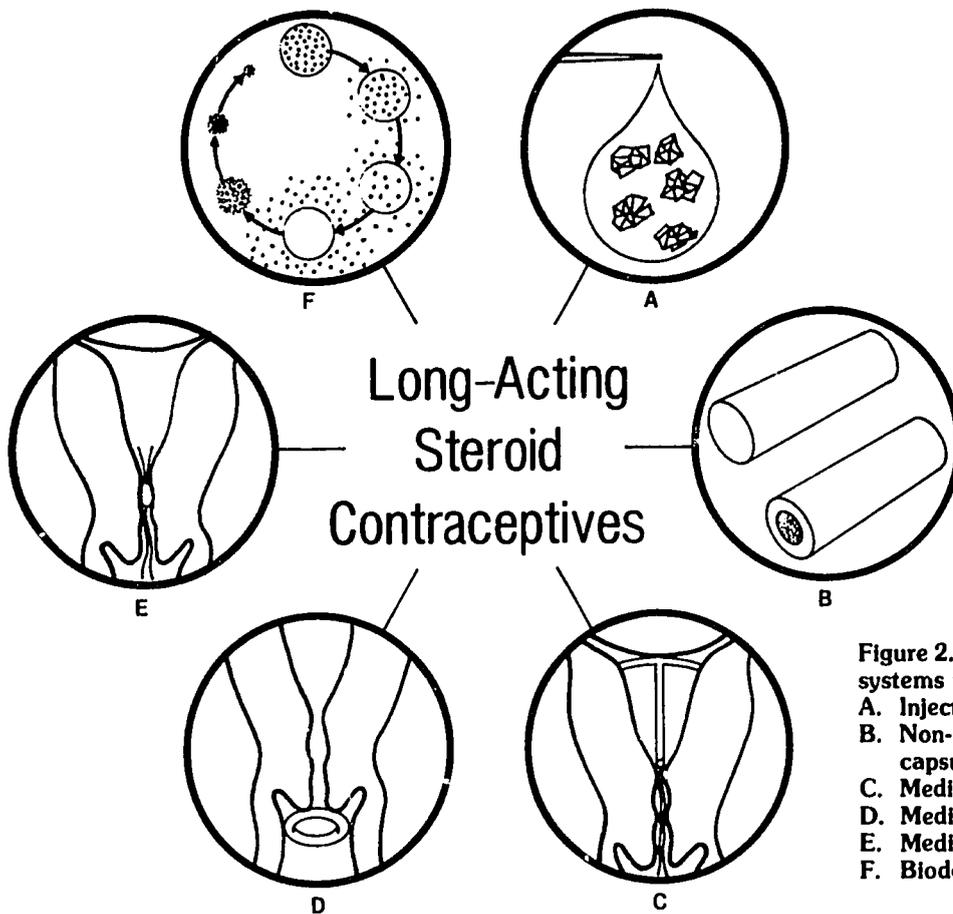


Figure 2. Long-acting steroidal contraceptive systems under current development.
A. Injectable depot formulations.
B. Non-biodegradable subdermal implants, capsules, and rods.
C. Medicated intrauterine devices.
D. Medicated intravaginal devices.
E. Medicated intracervical devices.
F. Biodegradable systems.

can result in contraceptive failure. Minimal intervention fertility control occurs when the steroid is maintained within the therapeutically effective dose range. Oral administration results in immediate high blood levels that decrease with time, and repetitive doses must be given at frequent intervals to keep the blood levels within the effective zone. This results in blood steroid fluctuations, the peaks of which exceed what is necessary for contraception.

The injectable controlled-release system meters the steroid into the blood at a controlled rate designed to maintain the blood level in the desired range, thereby achieving minimal intervention fertility control. Controlled release allows for reduction in the total amount of steroid administered over a prolonged period of time, and reduces the chance of human error by eliminating the need for repetitive self-administration.

The methods which have been employed to achieve minimal intervention fertility control using steroidal hormones are illustrated in Figure 2. These include: steroidal preparations selected for slow release and injectability (A); subdermal implants in the form of Silastic capsules and rods containing progestogens which diffuse slowly from the implant (B); medicated devices which release steroids locally in the uterus, vagina, and cervix

(C,D,E); and biodegradable systems that release steroids by diffusion and/or erosion of a biodegradable polymer (F).

Two different approaches have been used in the attempt to achieve minimal intervention fertility control, the pharmacologic approach, and the systems engineering approach. The pharmacologic approach, represented by injectable steroids, involves the use of compounds having intrinsic chemical properties which provide slow release. These include solubility of the drug in the body fluid at the injection site, affinity for receptors in the body fluid and tissues, and rate of metabolism and excretion from the body. The systems engineering approach utilizes inert drug carriers to control the rate of drug release. Although the degree of control may vary from one carrier to the next, the systems approach can be distinguished from the pharmacologic approach on the basis of a controlled-release mechanism which is neither a body nor a drug component.

Long-acting steroidal contraceptive systems can be further classified as those designed for systemic or local delivery. The implant and injectable systems, as well as medicated intravaginal devices, are designed for systemic delivery; whereas medicated intrauterine and intracervical devices are intended for local delivery. The

rationale for local delivery is to reduce the steroid load even further by direct delivery to the target organ.

The effort to develop new long-acting steroidal formulations and improved delivery systems for existing compounds represents a goal-oriented task which includes both applied and basic research of a diverse nature. The purpose of this communication is to review the state of the art relating to long-acting steroidal contraceptive systems, with emphasis on systems that have the potential to provide minimal intervention fertility control.



Injectable Depot Formulations

The first contraceptive steroids were synthetic progestogens which had to be administered at frequent intervals because they had short biologic half-lives. The synthesis and screening of new compounds led to the discovery of structures having longer durations of action which made administration by injection an attractive alternative to oral contraception.

Two progestogens, medroxyprogesterone acetate (MPA) and norethindrone enanthate (NET-EN) emerged from this early work as promising injectable formulations. Upjohn Pharmaceuticals developed MPA under the trade name Depo-Provera, and Schering AG developed NET-EN under the trade name Noristat. Other progestogens which have been administered by injection include hydroxyprogesterone caproate (Delalutin, Squibb), dihydroxyprogesterone acetophenide (Dela-droxate, Squibb), levonorgestrel undecylate (Schering AG), and levonorgestrel nonanoate (Schering AG).

Medroxyprogesterone acetate (MPA) was first used for treatment of habitual abortion and endometriosis. A single dose (1g to 4 g) of MPA for the treatment of premature labor was found to protect against pregnancy for 12 to 24 months (29). On the basis of this lead, smaller doses have been evaluated for use as long-acting injectable contraceptives. A dose of 150 mg every 90 days resulted in a pregnancy rate of 0 to 1.2 per 100 woman years following 77,000 woman years of use (11). Wilson et al. (150) suggested increasing the dose to 200 mg to protect patients who fail to return to the clinic or physician's office on schedule for repeat injections, and doses of up to 1,000 mg have been investigated with and without supplemental estrogen (158). Increasing the dose to 400 mg extends the duration of effect to six months (28, 113); however, this dose causes an increase in bleeding abnormalities.

Different treatment interval combination formulations have been investigated: 25 mg of MPA in combination with 5 mg estradiol cypionate every 30 days resulted in zero pregnancies following 623 injections in 104 women (28); and 50 mg MPA plus 10 mg estradiol every 35 days resulted in zero pregnancies in 1155 months of use in 90 women (121).

A comparison of 6- and 3-month treatment regimens, using 300 and 150 mg MPA respectively, revealed 40% drug-related dropouts in the 6-month group, compared to only 25% in the 3-month group (47). Rall (111) compared 6- and 3-month treatment intervals (450 and 150 mg MPA respectively) and found a greater pregnancy rate with the longer-acting system (0.4943 vs. 0.1069) during 220,530 woman months of use.

The major side effect of MPA is irregular bleeding. Bleeding often increases at first, but decreases with repeated injections, leading to amenorrhea. In one study, 35% of 272 women became amenorrheic during therapy (79), and another study reports a 50% incidence of amenorrhea after 3 years of continuous therapy (103). Side effects other than bleeding irregularities which have been reported include decreases in libido, weight changes, edema, and headaches (29).

Blood levels following injection of 150 mg of MPA reached a mean value of 3.57 ng/ml by one week post-treatment and declined to 0.6 ng/ml by 12 weeks (72). Blood levels in the 10 to 25 ng/ml range at 5-20 days post-treatment decreased to 5 to 10 ng/ml by 30 days post-treatment, followed by a more gradual decline through day 260 (72, 73). The minimum blood level of MPA necessary to inhibit ovulation is 0.5-1 ng/ml.

Delay in return to fertility may be a side effect of MPA treatment. Conception or return to normal menses occurred following discontinuation of treatment (250 mg MPA every 3 months) within four to seven months in 183 women following 38,599 months of use (78). In another study, 50% of the women desiring pregnancy conceived at 7 to 12 months post-treatment (79). McDaniel (88) reported an 80% pregnancy rate within 12 months. McDaniel and Pardthaisong (89) claim that return to fertility following MPA contraception is no different from that with the pill, IUD, or no contraception at all.

Norethindrone enanthate (NET-EN) was first synthesized by German scientists in 1953. Schering AG began clinical trials using NET-EN in 1957 as an injectable contraceptive. Although the drug was originally tested in a 200 mg dose given every 3 months (157), unacceptable pregnancy rates in the early clinical trials necessitated a change in the treatment interval (12, 21, 157).

Complete inhibition of fertility resulted during 1,606 woman months of use by 295 women receiving 200 mg every 8 weeks for 24 weeks with subsequent injections at 12-week intervals (52). In another study, 50 or 70 mg NET-EN in combination with 5 or 10 mg of estradiol undecylate, given once a month, resulted in zero pregnancies in 80 women following 823 treatment cycles (76); the continuation rate was 83.8% at 12 months, with only 5% drug-related dropouts.

Following intramuscular administration of 200 mg of

NET-EN, serum NET concentrations ranged from 4 to 23 ng/ml during the first 20 days, and were undetectable by 46 to 110 days post-treatment (63, 145).

Peak serum NET levels in the 8 to 23 ng/ml range occurred between day 4 and day 15 in women treated with 200 or 300 mg NET-EN (54). Serum levels remained high for the first 20 days, decreased precipitously to approximately 2 to 4 ng/ml, and then declined gradually. The minimal effective blood level of NET necessary to inhibit ovulation is approximately 1 ng/ml.

Ovulation can occur within 60 days post-treatment with 200 mg NET-EN (50, 63, 145), and the duration of ovulation inhibition varies between 45 and 150 days (38, 54, 76).

The current consensus regarding the mechanisms of contraceptive action is that during the first 4 to 6 weeks NET-EN works through ovulation inhibition, and during the second 4 to 6 weeks through disruption of normal pituitary ovarian regulatory mechanisms, resulting in reduced or abolished LH/FSH surges and/or insufficient luteal function (26)

It has been known since the 1930s that esterification of hydroxylated steroids results in a prolonged effect and augmented biological activity. This knowledge led to the manufacture of many steroids that have potential for use as injectable contraceptives. In the first clinical trial using an injectable contraceptive, a zero pregnancy rate was reported using 500 mg of hydroxyprogesterone caproate every 4 weeks (126). After six injections, 10 mg estradiol valerate were added to control intermenstrual bleeding.

The successor of this compound, dihydroxyprogesterone acetophenide (150 mg) and estradiol enanthate (10 mg), has been effectively employed as a monthly injectable contraceptive (Deladroxate, Squibb) without a single reported pregnancy in over 15,000 woman months of use (11). Several dose combinations have been evaluated in preliminary trials, with the deladroxate dose being the most acceptable (77). This regimen, although effective, has the disadvantages of causing bleeding irregularities and requiring a strict injection

schedule (60). Return to spontaneous ovulation usually occurs within 4 to 6 months following treatment with deladroxate (60, 77, 112, 131).

Levonorgestrel nonanoate and levonorgestrel undecylate represent the newest of the injectable progestogens. Both compounds exhibit satisfactory *in vivo* release profiles in animals, and the rate of clearance from the blood has been shown to be inversely proportional to fatty acid chain length (66). Preliminary studies in humans indicated that the fatty acid moieties do not undergo hydrolysis *in vivo*, restricting the bioavailability of the drug (128). Peak blood levels of only 4 to 700 pg/ml were achieved after treatment using 100 mg of levonorgestrel nonanoate. Levels then dropped to 50 to 150 pg/ml and remained there for 130 days or more. Glycolic acid has been used to link the steroid to the fatty acid in an attempt to improve the cleavability, hence bioavailability, of these long chain fatty acid esters (67). This significantly improves the *in vivo* release profiles.

In summary, the injectable progestogens are effective, convenient, and easy to administer; they do not inhibit lactation, they free the patient from having to remember to take medication on a daily basis, and they are free from side effects of estrogen. The disadvantages are as follows: treatment cannot be immediately reversed; there is a greater incidence of irregular bleeding; there is possible delay in return to fertility; self-administration is impossible; and there is a limited choice of compounds available for use.

The most significant shortcoming of the injectable progestogens is the poor linear release profile. Figure 3 shows a typical blood profile following treatment by intramuscular injection with NET-EN. The initial blood levels of NET far exceed that which is necessary for effective contraception. There is a gradual decline during the first few weeks post-treatment before NET passes through the zone of minimal therapeutic intervention. Slow release in this instance does not satisfy the concept of minimal intervention fertility control. Similar blood profiles have been reported following treatment with MPA.

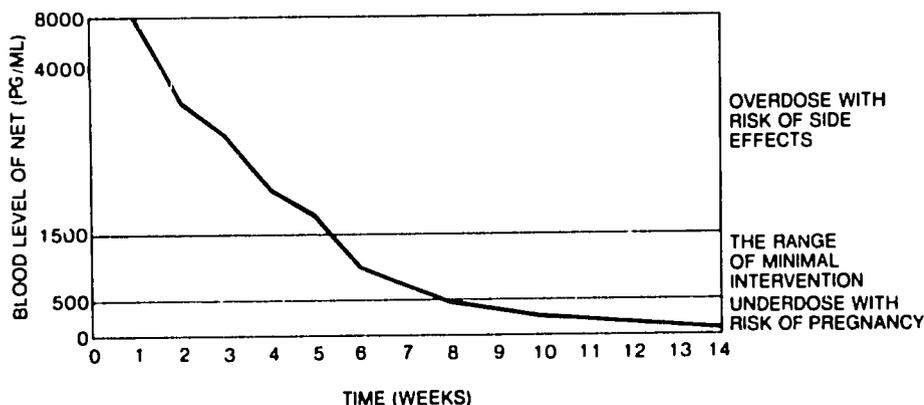


Figure 3. Plasma levels of norethisterone in a subject treated by intramuscular injection with 200 mg of norethisterone enanthate. (Source: Howard G, Warren RJ, Fotherby K: Plasma levels of norethisterone in women receiving norethisterone enanthate intramuscularly. Contraception 12:45, 1975)

Although there is little doubt that the approach is extremely attractive, the role of the injectable progestogens remains uncertain. At present, the choice is limited to only a few compounds, and although the search is ongoing, new compounds having more acceptable release profiles are not yet available.



Subdermal Implants

The evolution of contraceptive implants which utilize an inert carrier to control the rate and duration of drug release began in 1964, with the discovery that silicone rubber can be used as a carrier for prolonged drug therapy (49). The passage of steroids through silicone was first described in 1966 (44), and Segal and Croxatto (123) were the first to suggest the use of silicone rubber implants for the delivery of contraceptive steroids.

Subdermal Silastic implants impregnated with megestrol acetate (MA) were shown to be effective in preventing pregnancy in early clinical trials (30, 31, 32, 33, 132). The original aim for the implant was to administer a daily dose of progestin sufficient to inhibit fertility without interference with normal ovarian function. High pregnancy rates with single implants (31) prompted the use of multiple devices in order to achieve higher blood levels of the progestin and longer durations of release (30, 32). Four capsules provided 9 to 10 months' protection; five capsules provided protection for 12 to 15 months; and six capsules, 18 months. The Pearl Index, when five capsules were used, was 3.2, and 0.8 when six capsules were used, suggesting more effective contraception with higher doses. Intermenstrual bleeding or spotting occurred in 6% of the cycles; hypermenorrhea in 7.8% of the cycles; and the incidence of amenorrhea was only 3.6%.

In spite of promising early results, work on the implant came to an abrupt halt when investigators reported that MPA induced the formation of breast nodules in beagle dogs (45). This began a wave of studies to find an acceptable substitute for MPA (2, 15, 24-27, 34, 36, 37, 39-41, 48, 61, 68, 69, 74, 97, 110, 133, 143, 146-149).

The contraceptive effectiveness and acceptability of Silastic subdermal implants containing either levonorgestrel or norgestrienone have been compared in a multi-national clinical trial (68, 69). Four hundred ninety-two women treated with levonorgestrel capsules (180 mg) had a cumulative 12-month pregnancy rate of 0.6% and a continuation rate of 74.6%. Four hundred ninety-eight women treated with norgestrienone capsules (180 mg) had a cumulative 12-month pregnancy rate of 3.5% and a continuation rate of 79.4%. The major side effect for both steroids was irregular bleeding patterns. The rates of termination due to bleeding irregularities were 12.3% for levonorgestrel and 4.3% for norgestrienone.

Norethindrone was found to be ineffective in preventing pregnancy with use of up to 12 implants containing 20-30 mg of NET each (15, 110). Gestrigone (R-2323), delivered by Silastic implants, is effective in preventing pregnancy. However, it has been excluded from further use as a systemic contraceptive because it has been shown to elevate transaminase levels in Chilean women (2).

The 19 norsteroid ST-1435 has a high rate of *in vivo* release from Silastic capsules (40 mg/cm/day) and provides effective contraception when used as a single implant (27). The rapid rate of release, however, limits the duration to less than one year.

Lynestrenol has *in vivo* release rates from Silastic capsules even greater than ST-1435 (i.e., 60 mg/cm/day), and studies have been discontinued because of rapid release (146).

Silastic capsules 22 mm long and containing 40 mg of norethindrone acetate, and which have an *in vivo* release rate of 128 μ g NET/day, provide protection against pregnancy for approximately 10 months (15, 61, 74, 110). The short duration of effect represents a disadvantage, compared to other longer-acting implants.

The *in vivo* release profiles are similar for all progestins: rapid decrease in rate of release for the first 50 to 100 days followed by a much slower decline rate. The plasma levels of the progestins delivered from Silastic implants vary as much as threefold between individual subjects treated with identical implants (98).

In general, it can be concluded on the basis of the clinical studies that Silastic implants containing synthetic progestogens are effective in preventing pregnancy and represent an acceptable form of contraception for some populations. The duration of effect is dependent on the rate of release of the steroid from the Silastic capsule. Accordingly, steroids having higher rates of release have correspondingly shorter durations of action, whereas steroids having lower rates of release have longer durations of action, but require multiple implants in order to deliver effective daily doses. If one balances the number of capsules necessary to give contraceptive protection against duration of release and side effects, levonorgestrel and norgestrienone appear to be the most attractive steroids for use in subdermal Silastic implants. Levonorgestrel is a more effective contraceptive. The incidence of irregular bleeding, however, is significantly greater for levonorgestrel than for norgestrienone.

The use of Silastic rods instead of capsules to obtain higher rates of levonorgestrel release reduces the incidence of breakthrough bleeding and increases amenorrhea. The estimated *in vivo* release rate from rods is three to four times greater than from capsules (148). Bleeding irregularities can be controlled to some extent by adjusting the daily dose of the progestogen.

There is also some evidence to suggest that the incidence of intermenstrual bleeding decreases with time (58). Successful use of subdermal implants on a wide scale will require effective management of bleeding problems that will arise unless more effective systems can be developed. One possibility for achieving better bleeding control which is currently under investigation is to include estradiol in the implants (68).

In conclusion, subdermal dimethylpolysiloxane implants containing potent progestogens in the form of capsules and rods have been shown to provide effective contraceptive protection without serious side effects. The advantages of Silastic subdermal implants over oral contraceptives are as follows: 1) the improved therapeutic dose; 2) continuous constant release for periods of up to one year or longer; 3) the necessity of only one administration; 4) lack of vehicle discomfort; 5) reliability of administration; 6) rapid return to ovulation upon removal; 7) easy reversibility; 8) avoidance of liver portal circulation; and 9) avoidance of intermittent single bolus stimulation of the liver.

The main shortcomings of Silastic implants are as follows: 1) because they have limited surface area, as many as six or eight devices are required to provide effective blood levels of the contraceptive drug; 2) since silicone rubber is not resorbed by the tissue, the spent devices must be removed surgically; 3) variation in solubility of different synthetic steroids limits the choice of progestins that can be delivered by this route; and 4) significant variation occurs in the blood levels of individual subjects treated with identical devices.

New biodegradable implants and injectable particulate systems which offer better control of drug release and ease of administration are being designed with the aim of circumventing the objectionable features of Silastic subdermal implants, while maintaining the attractive features.



Medicated Intrauterine Systems

Medicated intrauterine systems represent a major advance toward achieving minimal intervention fertility control. The medicated IUD represents the first steroidal contraceptive system to focus on local, rather than systemic, delivery. The rationale for intrauterine steroids is based on the principle that progestogens delivered directly to the uterine lumen in low doses will act directly on the uterine mucosa, inducing changes in the endometrium which prevent implantation. Supposedly, this can occur without influencing normal ovarian function and/or causing other systemic effects.

This concept grew out of the earlier work on subdermal implants and the related development of controlled release delivery systems. The first medicated intrauterine

systems were modeled after subdermal implants. Doyle and Clewe (42) were the first to test a steroid-releasing intrauterine device in animals.

The first clinical trial using a progesterone-releasing IUD was reported by Scommegna et al. (91). Silastic capsules containing 30 mg of progesterone were placed in the uterus. The effects of progesterone released from the capsules on the endometrium were evaluated histologically. The rate of progesterone release was approximately 300 $\mu\text{g}/\text{day}$. The effects were dramatic; within 18 hr histologic changes consistent with progestational stimulation of the endometrium were apparent.

In order to improve retention, the progesterone capsules were fitted to conventional IUDs (Tatum-T) by substituting the medicated capsule for the lower part of the vertical arm of the T. The modified T had an *in vivo* rate of progesterone release of 125 $\mu\text{g}/\text{day}$. This device was used to demonstrate anti-fertility effects of intrauterine progesterone in women (19). The early handmade medicated IUDs were crude fabrications subject to significant variations of both rate and duration of drug release. The primary emphasis of the early clinical studies was to demonstrate utility, and crude systems were adequate for this purpose.

Alza Pharmaceutical Company was the first to launch a comprehensive program to develop a long-acting steroidal intrauterine contraceptive system. This led to the development of the Progestasert, a T-shaped IUD, which delivers 65 μg of progesterone per day for 365 days. The Progestasert represents a major advance toward minimal intervention fertility control. Up until this time, all controlled-release devices had high initial rates of release that decreased in linear fashion with time, whereas the Progestasert provides a constant rate of release for the life of the unit. In order to achieve this degree of control, advanced technology in polymer chemistry was focused on the problem of developing a polymeric membrane capable of providing a constant zero order rate of progesterone release with a high degree of precision and constancy. Several prototype systems having different rates and durations of release were developed and tested in animals (99). The system selected for human trial was a T-shaped device having a 36 mm vertical stem and a 32 mm transverse arm. The transverse arm contained 32 mg of crystalline progesterone in medical grade silicone oil and barium sulfate. The polymeric diffusional rate-limiting membrane consisted of a polymer of ethylene vinyl acetate containing titanium dioxide. The rate of progesterone release from the device was 65 $\mu\text{g}/\text{day}$ for a duration of 12 to 18 months.

Many studies have evaluated contraceptive effectiveness, acceptability, side effects, and mechanism of contraceptive action of Progestasert (17, 18, 43, 46, 51, 55-59, 64, 65, 83-87, 95, 99, 100, 105, 106, 114, 119,

120, 127, 129, 135, 144, 155, 156). Observations made in 1,320 women over 9,660 woman months of use revealed a pregnancy rate of 1.9 ± 0.4 per 100 users (117). Expulsion was 4.7 ± 0.6 and removal for pain and bleeding was 6.0 ± 0.7 . These statistics compare favorably with the most widely used inert and copper devices. The study confirms the earlier finding that Progestasert users experience less menstrual blood flow and dysmenorrhea than do conventional IUD users, but have more intermenstrual spotting. This study does not support the claim by Snowden (127) that Progestasert users experience a higher incidence of ectopic pregnancies. Less menstrual blood flow and dysmenorrhea appear to be the only advantages of the Progestasert over the conventional non-medicated IUD. The health benefit of decreased blood loss in anemic women is significant, however.

Intrauterine progesterone at dose levels insufficient to inhibit ovulation has been shown to have a wide spectrum of effects, including the induction of morphologic changes in the endometrium (2, 55, 58, 59, 68, 75, 84, 87, 101) and oviduct (56, 129); alteration of sperm capacitation properties of uterine fluid (65); alterations in prostaglandin concentrations of endometrium and menstrual blood (119, 120, 156); changes in biochemical, enzymatic, and trace elements in the endometrium (58, 59); alteration of the ovarian hormone levels (18); reduction in the menstrual blood loss (51, 144, 156); and inhibitory effects on embryo development and transport (65). The exact mechanism and/or combination of mechanisms which inhibit fertility is not known. The most likely explanation is that intrauterine progesterone blocks implantation by direct action on the endometrium.

The need to replace the Progestasert annually is considered a disadvantage, and this has motivated the use of more potent synthetic progestins as the active principle (82, 101). New intrauterine devices that release progesterone for three years are being tested by the World Health Organization (WHO), and plans have been made to develop and test devices that release levonorgestrel at the rate of $2 \mu\text{g}/\text{day}$. Although the preliminary findings look promising, no large-scale clinical studies have been reported as yet. Theoretically, these devices will have effective life spans of 5 years or longer.

In spite of the initial promise, the medicated IUDs have not been widely accepted, and for good reason. The design of the device offers no advantage over conventional IUDs with regard to ease of insertion, expulsion, intermenstrual bleeding, pain, and uterine perforations. If the medication is taken away, what remains is a conventional IUD with all of its inherent problems. The original intent for the medicated intrauterine system was to rely on the medication for contraceptive effect, thereby affording greater flexibility in design of the device itself. Unfortunately, there has been little effort to

design smaller, less troublesome IUDs for use as intrauterine drug-delivery systems. If intrauterine progestin alone is enough to inhibit fertility, without synergistic effects of the device, then it should be possible to design devices of novel form free of those side effects which characterize conventional IUDs.

In a research program sponsored by the Program for Applied Research on Fertility Regulation (PARFR), Beck and Lewis (9) are investigating the use of fibrous polymers for delivery of contraceptive steroids to the female reproductive tract. Methods have been developed for spinning hollow fibers containing progestogens. Constant release rates of steroids from medicated fibers have been demonstrated both *in vitro* and *in vivo*, and primate studies are underway to evaluate the utility of medicated fibers for intrauterine delivery of progestogens. Fibers ranging in diameter from 0.02 to 0.05 cm, containing up to 50% by weight progesterone, have been produced using various polymers including polyethylene, polypropylene, and nylon 6.

Small rings made by looping a continuous length of fiber have been shown to be highly effective for the intrauterine delivery of progesterone in the baboon. Novel configurations of medicated fibers are being considered for intrauterine, intracervical, intravaginal, and intrascrotal delivery of drugs. Fibers have the advantage that they can be produced at extremely low cost and can be arranged into almost any configuration.

Another novel intrauterine system consists of biodegradable microspheres that provide controlled release of progestogens (92). The unique feature of this approach is that the system can be self-administered. Medicated microspheres placed in the vagina migrate spontaneously across the cervix and release the progestogen locally in the cervix and uterus. Studies are currently underway to evaluate the contraceptive potential of this system in primates.



Medicated Intravaginal Systems

The history of medicated intravaginal devices (MIVD) spans a mere decade, and there has been little change in the design of the original device, as introduced by Mishell in 1970 (95). The rationale for the MIVD is based on the knowledge that steroids rapidly penetrate the vaginal mucosa, and that foreign bodies of considerable size can be left in the vagina for long periods without discomfort. The possibility of intravaginal contraception became apparent with the discovery that Silastic rubber can be used to achieve controlled-release of steroids for extended periods of time.

The early devices consisted of cylindrical elastomers of polysiloxane molded in the form of a ring. The polymer was impregnated with the steroid. Over the years, a

variety of different steroids have been administered using intravaginal rings. Few attempts have been made to change the original design of the device. Exceptions are the use of polydimethylsiloxane in lieu of polysiloxane, and multiple layers of polymer instead of solid extrusions. Multi-layered rings in which the outer layer is drug-free provide better control of drug release (19, 20, 91). Early clinical trials using MIVDs containing medroxyprogesterone acetate established contraceptive effectiveness (5, 95, 140). Work on the MPA systems was discontinued, however, when it was shown that this steroid induces breast nodules in beagle dogs.

A variety of different progestins and rates of release of the same progestins have been tested clinically. Progesterone had no effect on ovarian function when delivered by intravaginal rings at rates as high as 4,040 $\mu\text{g}/\text{day}$ (19, 20, 140). Medroxyprogesterone acetate was effective in inhibiting ovulation in 8 out of 8 cycles when delivered at a rate of 520 to 1,280 $\mu\text{g}/\text{day}$ (95), and 136 out of 138 cycles when delivered at a rate of 860 to 1,290 $\mu\text{g}/\text{day}$ (92). Norethindrone was shown to inhibit ovulation in 4 out of 20 cycles when delivered at the rate of 50 $\mu\text{g}/\text{day}$ (81), 9 out of 22 cycles when delivered at a rate of 200 $\mu\text{g}/\text{day}$ (81), 6 out of 7 cycles at 850 $\mu\text{g}/\text{day}$ (91), and 4 out of 4 cycles at 15,030 $\mu\text{g}/\text{day}$ (91). Levonorgestrel was shown to be effective in inhibiting ovulation in 5 out of 5 cycles at 250 to 310 $\mu\text{g}/\text{day}$ (140), 58 out of 60 cycles at 279 $\mu\text{g}/\text{day}$ (94), 5 out of 6 cycles at 380 $\mu\text{g}/\text{day}$ (91), 4 out of 4 cycles at 550 $\mu\text{g}/\text{day}$ (91), and 4 out of 4 cycles at 740 $\mu\text{g}/\text{day}$ (91). RT-2323 was effective in inhibiting ovulation when delivered at a rate of 330 to 3,710 $\mu\text{g}/\text{day}$ (1, 93).

Mishell (91) concluded, on the basis of comparative clinical studies using medroxyprogesterone acetate, norethindrone, and levonorgestrel rings, that none of the progestins is clinically acceptable, owing to the high incidence of breakthrough bleeding and lack of withdrawal bleeding. For example, devices releasing 279.8 $\mu\text{g}/\text{day}$ of levonorgestrel inhibited ovulation in 61 out of 62 cycles (94). Bleeding and spotting occurred in 33%

of the cycles, however, and on 6.75% of the treatment days. Lack of withdrawal bleeding occurred in 5% of the cycles.

The use of estrogen in combination with progestin is being investigated as a means to achieve better control of bleeding (93, 137, 138). Combination devices which delivered 200-300 $\mu\text{g}/\text{day}$ of norgestrel and 175-245 $\mu\text{g}/\text{day}$ of estradiol have been shown to suppress ovulation in 100% of the cycles studied (93). Rings inserted for three weeks and removed for one give excellent bleeding control during the treatment intervals, with regular withdrawal bleeding following removal of the ring. The rings have been well tolerated by all subjects and their cohorts. The combination device has been shown to be highly effective and more acceptable from the standpoint of bleeding control than the progestogen alone devices.

The use of estrogen presents a problem because the long-term effects of estrogen delivered directly to the cervical and vaginal mucosa for extended periods of time are not known. The potential for estrogen accelerated transition of cervical dysplasia represents a major risk factor associated with the combination MIVDs.

The major advantage of the MIVD over other types of controlled release steroidal contraceptive systems is that the treatment can be self-administered and is reversible at will. Although self-administration may be an advantage in populations where motivation and compliance are good, it may be a distinct disadvantage in populations with poor motivation. Sanitation and proper care of the device may also present problems. Better design of the system may help to improve acceptability. For example, systems designed for disposal after a single insertion would eliminate sanitation concerns. Biodegradable systems might help to improve acceptability because they can be left in place, thereby eliminating responsibility of the user to remove and care for the device. Another advantage of the medicated intravaginal ring is the capability of providing minimal intervention dosing, as illustrated in Figure 4.

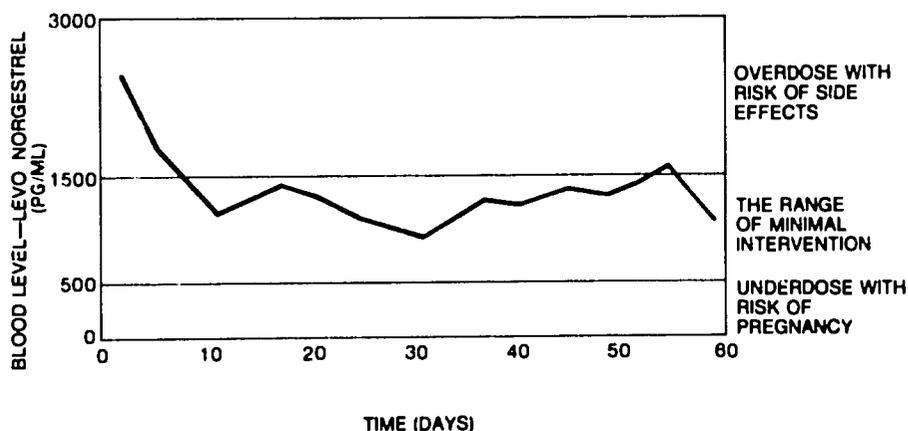


Figure 4. Serum levels of levonorgestrel in a woman bearing a levonorgestrel-medicated intravaginal ring. (Source: Toivonen J, Lahteenmaki P, Luukkainen T: The use of the contraceptive vaginal ring governed by the pattern of individual uterine bleeding. Contraception 19:401, 1979)



Medicated Intracervical Systems

The medicated intracervical device (MICD) is based on the concept that continuous local release of drugs within the cervical lumen will cause infertility. The mechanism of action is dependent upon the type of drug used. Pharmacologic agents under investigation for delivery by intracervical systems include progestogens and spermicidal compounds. The use of progestogens is expected to inhibit sperm penetration through the cervix by inducing changes in cervical mucus due to the direct action of progestogens on cervical secretory cells. Local delivery affords a method for inducing changes in the cervical mucus without concern for systemic effects that follow both oral and parenteral administration (96).

The local delivery to the cervix requires the use of a device designed for retention in the cervix as well as for controlled release. Cohen et al. (23) used plastic tubing containing progesterone sutured into the rabbit cervix to demonstrate the local effects of progesterone on normal preovulatory cervical mucorrhea. Glass and Morris (53) used silicone extrusions impregnated with chlormadinone acetate to demonstrate the effects of intracervical progestogen on fertilization, implantation, and sperm transport.

Preliminary studies in women using Silastic extrusions containing chlormadinone acetate and designed for insertion into the cervix showed effects on cervical mucus with estimated release rates of 0.15 mg/day. Cohen et al. (23) were able to induce changes in human cervical mucus by inserting a Silastic capsule containing progesterone into the cervix. Although these early studies establish the feasibility of inducing alterations in cervical mucus by direct delivery of progestogens, the devices were poorly designed and not well retained in the cervix.

Moghissi et al. (96), undertook a comprehensive program of research to design and test intracervical devices. After evaluation of several different designs, a prototype system was selected for more comprehensive study. The device consists of a polypropylene spine supporting a cylinder of silicone rubber. The cylinder serves as the drug reservoir. Branched arms at the end of the spine hold the device in place. An inserter is used to position the device in the cervix. Different designs of the arm configuration have been tested as part of multi-center trials conducted by the World Health Organization (WHO) (70). On the basis of these trials, systems have been selected for further clinical studies.

Intracervical devices that release the spermicide quinine are also being tested by WHO. The quinine-releasing device is similar in design to the steroid-releasing device. Phase I multi-center clinical trials of devices releasing 20 $\mu\text{g}/\text{day}$ of quinine sulfate showed inhibition of sperm migration in 80% of the post-coital tests. No local or systemic side effects were recorded (70).

Medicated intracervical devices have yet to be tested for contraceptive effectiveness. Assuming the MICD proves to be effective, there still remains the question of acceptability. There should be no problem in producing devices which provide controlled release for long periods of time. Moreover, the MICD is well suited for local delivery. Therefore, systemic side effects should not be a problem. Another advantage is that treatment is reversible. A potential objection to MICDs is that the system requires careful placement in the cervix by trained personnel. The possibility that the device might function as a conduit for infectious microorganisms from the vagina to the uterus requires further investigation. Finally, a major design problem still exists with regard to developing an intracervical device that remains in place and is not expelled.



Biodegradable Systems

The use of biodegradable polymers for the programmed delivery of steroid hormones represents a major advance in contraceptive technology, the full impact of which is yet to be realized. On the surface there is the obvious advantage that biodegradable systems comprising drug polymer combinations eliminate the need for device removal following use. An underlying and more significant consideration is that the use of polymers that biodegrade allows for greater flexibility in system design (3).

From the work on non-biodegradable subdermal implants, it is clear that most progestogens cannot be effectively administered using polymeric implants because the rate of release of the steroid is not sufficient to maintain the blood level in a therapeutically effective range. Although increasing the number of implants increases the total daily dose, this has not proved to be a practical solution because it is difficult to implant multiple large devices. An alternative to increasing the number of implants is to increase the surface-to-volume ratio using multiple small particles (mini implants). Theoretically, the size of the implant can be reduced to the point at which the rate of steroid release will be sufficient to maintain an effective blood level, providing a sufficient number of particles are administered. Moreover, by reducing the size of the particles to 200 μm or less, the system can be injected as a suspension, thereby providing an improved method of administration. The rate of release can be adjusted to accommodate the potency of the steroid by selection of particle size. Another potential advantage of the small particles is that the daily dose can be adjusted by changing the quantity of particles injected. This is not possible with large implants. The problem with this approach is that small polymer drug particles, unlike large implants, cannot be removed following use. Accordingly, development of small particle drug-delivery systems depends on the use of polymers which biode-

grade in body tissues and the technology for incorporating steroid hormones in small polymeric particles.

Polymers with hydrolytically unstable chemical structures and those readily broken down by hydrolytic enzymes are potentially useful as biodegradable drug carriers in the form of implants and/or small particles (4, 62). By design, the degradation products of these polymers pass directly into the systemic circulation along with the drugs they release. Consequently, the biodegradable polymeric carriers require both careful selection and extensive toxicologic evaluation to ensure their safety before they can be tested clinically. To increase the likelihood that the large amount of time, effort, and expense required to develop a technically reliable delivery system will not be wasted due to unanticipated toxicity of the biodegradable polymer, the earliest contraceptive formulations have been based on clinically proven drugs and polymeric forms of natural metabolites, e.g., lactic acid and various amino acids. Newer systems are being developed, however, in which non-biologic chemicals have been used for synthesis of new, highly reliable drug-releasing polymers. If these new biodegradable polymers prove to be toxicologically safe, a broad spectrum of new contraceptive formulary will soon be available for clinical trials.

Lactic acid, $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{H}$, a common intermediate or end-product of carbohydrate metabolism, has two optical isomers, as it contains an asymmetric carbon atom. Polylactic acid (PLA), therefore, exists either as a

highly crystalline stereoregular polymer, L(-)-PLA or D(+)-PLA, or a highly amorphous racemic polymer, D,L-PLA. Although the appearance and mechanical properties of these PLA thermoplastics are similar to the common commercial polyesters (e.g., Dacron), because they are totally aliphatic, they decompose when exposed continuously to water (116), e.g., tissue fluids. Although physical-chemical parameters such as polymer molecular weight, acid content, and degree of crystallinity and implant size all affect the rate of resorption of PLA, as a general rule, D,L-PLA lasts from 6 to 12 months when implanted in soft or hard tissues, and L(-)-PLA or D(+)-PLA is totally resorbed within from 12 to 30 months post-implantation (6, 16, 90, 116). Copolymerization of lactic acid with glycolic acid accelerates the resorption rate of the implant.

Since the biodegradation byproduct of PLA is lactic acid, a non-toxic biochemical, PLA was the first biodegradable polymer investigated for use as a long-acting drug carrier for contraceptive steroids (71), and it continues to be the polymer of choice of many investigators for the delivery of steroids and a variety of other drugs (7, 8, 10, 102, 107, 108, 134, 152-154). The earliest contraceptive doses based on PLA were simple drug-polymer mixtures.

Rods and films were implanted surgically, or the composites were ground into powders for injection. While these systems prolonged the release of the drug, they often lacked the precision required for long-term controlled drug release.

In a program sponsored by PARFR, Dynatech Research Corporation has developed a biodegradable implant which consists of a copolymer containing 90% lactide and 10% glycolide, blended with 50% by weight levonorgestrel. The mixture is extruded into rods designed for implantation under the skin (151). Following implantation, levonorgestrel is released at a constant rate by diffusion and bioerosion of the polymer. The prototype system has a duration of levonorgestrel release *in vivo* of approximately 8 years. The low rate of release requires the use of multiple implants to achieve adequate daily doses. Work is currently underway to increase the rate of release and shorten the duration of action.

In another program funded by PARFR, investigators at the University of Alabama in Birmingham and Southern Research Institute have developed a new long-acting, injectable contraceptive which provides continuous controlled release of the steroid norethindrone (NET) for a precise period of 6 months following a single intramuscular injection (8). The prototype system consists of microcapsules made of the biodegradable polymer D,L-PLA, in which micronized crystals of NET are homogeneously dispersed. Following intramuscular injection, NET is slowly released from the microcapsules at

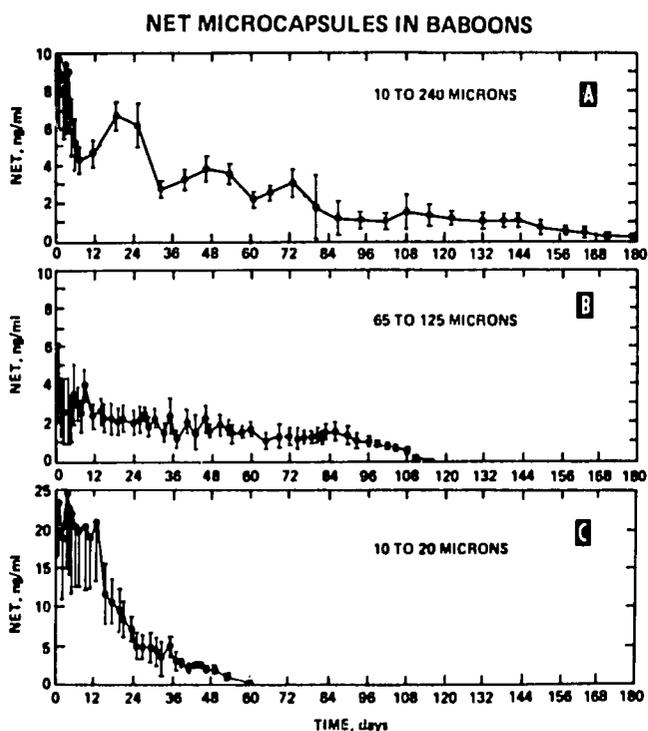


Figure 5. Comparison of the mean serum levels of NET in three different groups of baboons treated by intramuscular injections of microspheres containing norethisterone.

a constant rate by diffusion of the steroid from the polymer matrix. Three different doses of a standard preparation of microcapsules have been tested in normally cycling female baboons (4 to 5 baboons/group). Following injection of either 300, 200, or 100 mg of microcapsules containing 75, 50, or 25 mg of NET, blood samples were collected at selected intervals and analyzed for NET, estrogen, and progesterone by radioimmunoassay. All three doses provided continuous NET release for 6 months following injection. The NET serum profiles for the different doses are parallel, and ovulation was inhibited in all baboons for 6 months following treatment. By adjusting the size of the microspheres and the composition of the polymer, a system having 90 days' duration of release has been developed (10). Clinical studies of the 90-day injectable system are scheduled for mid-1980. The technology is now available to produce injectable microspheres having almost any desirable rate and duration of release of different steroids (7) (Figure 5).

The use of small particles allows for precise manipulation of the blood levels of the drug. An increase or decrease in the quantity of particles injected results in corresponding changes in the blood levels of the steroid (7, 10).

The injectable microsphere system provides a number of unique advantages:

- 1) The microspheres can be administered by injection;
- 2) The rate and duration of drug release *in vivo* can be predetermined by selection of microsphere size and percent loading of steroid (Figure 5);
- 3) The microspheres biodegrade without causing any harmful effects at the site of injection;
- 4) Blood levels of the steroids can be regulated by dose selection of microspheres;
- 5) The system can be sterilized without sacrifice of performance;
- 6) Continuous progestational treatment utilizing the system at doses which inhibit ovulation has no carry-over effects on fertility;
- 7) The system provides the degree of control necessary to achieve minimal intervention therapy (Figure 5).

The prototype microsphere systems can easily accommodate combination steroid therapies without compromising performance. This can be done by combining microspheres containing different steroids. Various species of microspheres can be blended to achieve preparations with different combinations of drugs. For example, microspheres containing estradiol might be blended with microspheres containing norethindrone to achieve a combination injectable contraceptive. The daily rate and duration of release of each steroid can be adjusted independently of the other by changing size, percent drug loading, and dose of the different micro-

sphere species. This flexibility allows for the design of systems which deliver the optimum quantity of a steroid and/or combination of steroids to the target organs.

The microspheres are well suited for placement directly within target tissues to achieve local drug delivery. The use of medicated biodegradable microspheres for intravaginal, intracervical, and intrauterine delivery of drugs is currently under study. Microspheres might also be adapted for use as a controlled release matrix for contraceptive inhalants and nasal sprays. The local release of drugs directly at the level of the target organ by strategically placed microspheres represents the ultimate in minimum intervention. Moreover, biodegradation of the delivery system eliminates the problem of device removal.

In a study funded by the National Institutes of Health, Pitt et al. (23, 102, 107) have shown that poly(ϵ -caprolactone), an aliphatic polyester similar to polylactic acid, is an excellent biodegradable drug carrier for contraceptive steroids. Polycaprolactone can be extruded as small-bore tubing which, in turn, can be filled with steroids to produce implants analogous to earlier ones made of silicone rubber. When the device is implanted with a trocar, the steroid is released by membrane diffusion at a constant rate for 6 or 12 months. Since the polymer is resorbed after the drug is released, the implants do not require surgical removal unless reversal of the treatment is desired. Extensive toxicologic evaluations of the polycaprolactone implants are currently underway.

Synthetic polypeptides have also been extensively investigated as biodegradable polymeric carriers for contraceptive steroids. In one successful approach, Sidman et al. have synthesized copolymers of glutamic acid and ethyl glutamate (Glu-EGlu) and have used these plastics to fabricate both implantable drug-filled tubes (similar to the polycaprolactone devices described above) and matrix rods (124, 125). Glu-EGlu devices which release steroids by diffusion at nearly constant rates for periods ranging from one month to one year have been reported. In an alternative approach, Petersen et al. (104) have bonded steroids covalently to a derivative of polyglutamic acid which has either a hydroxyethylamide or a hydroxypropylamide pendant group to achieve separation of the drug from the polypeptide backbone (22). These polymeric prodrugs of norethindrone undergo retrograde cleavage of the labile linkages to slowly release the progestin at a nearly constant rate. Preliminary studies in rats indicate that these formulations may have a duration of action of one year or more.

Except for the polymeric prodrug system and the Dynatech implant, all of the biodegradable systems discussed so far rely principally on membrane-moderated diffusion as the mechanism to control the rate and duration of drug release. The Alza Corporation is de-

veloping a new family of biodegradable polymers that undergo a time-predictable hydrolytic erosion process when they are placed in contact with living tissue (14, 117). These materials, called Chronomer, are poly(orthoesters), and they are described as hydrophobic, tractable, biocompatible, and non-toxic. The current prototype for a bioerodible implant for contraception is comprised of 20% micronized norethindrone and 10% stabilizing buffer suspended in a matrix of Chronomer. The mixture is fabricated as a rod with rounded ends 14 mm long and 3 mm in diameter. When the rods are implanted, the orthoester linkages on the surface hydrolyze, and the implant erodes to release entrapped drug. Nearly constant plasma levels of the drug can be maintained for up to 6 months with the Chronomer system. Extensive toxicologic studies are now underway, and clinical trials are expected to begin in 1980.

A very promising feature of biodegradable systems is their possible utilization to achieve "programmed" release. In the case of female fertility regulation, constant plasma levels of a steroid hormone do not allow maintenance of a regular menstrual pattern in all women. For this reason, pilot investigations are exploring the feasibility of constructing devices allowing pre-programmed release profiles, in which the active agent is made available only during specific periods (13).

Biodegradable implants and small particulate systems are at an early stage of development. There is ample evidence to suggest, however, that biodegradable systems satisfy most of the requirements for safe and effective contraception. The systems under development allow for selection of the most appropriate steroids, based on clinical effectiveness and safety; provide minimal intervention dosing capabilities; and minimize the degree of patient compliance by building extended duration of delivery into the dosage form.

The development of more sophisticated systems lies well within the realm of current technical capabilities, and the future of biodegradable systems for fertility control looks promising.

CONCLUSIONS

One of the major problems with use of steroidal contraception in developing countries is the need to take a pill on a daily basis. Even where motivation for daily use of contraceptives is good, compliance is often poor, owing to inadequate education or understanding. Another limitation is the relatively great expense of the drugs. Hence, there is a great need to develop steroidal contraception that can be administered on a long-acting basis.

Several injectable steroidal contraceptives are presently being used in developing countries, but although these methods eliminate the need for daily motivation to take a pill, they have not been widely accepted because of problems with irregular menstrual bleeding and amenorrhea, and with related psychosexual and religious considerations.

Accordingly, it is hoped that the principles outlined in this communication that emphasize a constant low blood level of steroid will result in adequate fertility control without the present bleeding problems. If this can be accomplished, we will have come a long way toward providing adequate and acceptable fertility control for women in developing countries and in developed countries as well.

The work to develop long-acting contraceptive systems follows a trend toward the application of more sophisticated technology and wider diversity of technological expertise. The new systems being developed require input from biomaterial specialists, life scientists, and clinicians. The multidisciplinary approach to research requires centralization of expertise and a broad funding base in order to be effective. The current philosophy of government funding is leading in the opposite direction. The technical expertise required to develop highly sophisticated long-acting steroidal contraceptive systems is available. The challenge which lies ahead is to focus the full spectrum of this expertise on the problem. This review is intended to describe where we have been so that we can better plan where we should be going.

REFERENCES

1. Akinla O, Lahteenmaki P, Jackanicz TM: Intravaginal contraception with the synthetic progestin, R-2323. *Contraception* 14:671, 1976.
2. Alvarez F, Robertson DM, Montes de Oca V, Sivin I, Brache V, Faundes A: Comparative clinical trial of the progestins R-2323 and levonorgestrel administered by subdermal implant. *Contraception* 18:151, 1978.
3. Baker RW, Lonsdale HK: Controlled release—mechanisms and rates. In Tanquary AC, Lacey RE (eds): *Controlled release of biologically active agents. Advances in Experimental Medicine and Biology*. New York, Plenum Press, Vol 47, 1974, pp 15-72.
4. Baker RW, Lonsdale HK: Erodible controlled release systems. Division of Organic Coating and Plastics Chemistry, 171st American Chemical Society Meeting. New York, 1976, pp 235-242.
5. Beck LR, Cowsar DR: Personal communication.
6. Beck LR, Cowsar DR, Lewis DH: Systemic and local delivery of contraceptive steroids using biodegradable microcapsules. In Hafez ESE, Van Os WAA (eds): *Polymeric Delivery Systems. Proc Internat Symposium on Medicated IUDs and Polymeric Delivery Systems*. Amsterdam, Holland, June 27-30, 1979.
7. Beck LR, Cowsar DR, Lewis DH, Cosgrove RJ, Riddle CT, Lowry SL, Epperly T: A new long-acting injectable microcapsule system for the administration of progesterone. *Fertil Steril* 31:545, 1979.
8. Beck LR, Cowsar DR, Lewis DH, Gibson JW, Flowers CE: New long-acting injectable microcapsule contraceptive system. *Am J Obstet Gynecol* 135:419, 1979.
9. Beck LR, Lewis DH: Personal communication.
10. Beck LR, Pope VZ, Cowsar DR, Lewis DH, Tice TR: Evaluation of a new three-month injectable contraceptive microsphere system in primates (baboon). *J Contracep Del Systems* 1:79-86, 1980.
11. Benagiano G: Long-acting systemic contraceptives. WHO Symposium on Advances in Fertility Regulation. Copenhagen, Bogtrykkeriet Forum, 1976, pp 323-360.
12. Benagiano G, Diczfalusy E: Multinational comparative clinical evaluation of the long injectable contraceptive steroids: norethindrone oenanthate and medroxyprogesterone acetate. I. Use-effectiveness. *Contraception* 15:513, 1977.
13. Benagiano G, Gabelnick HL: Biodegradable systems for the sustained release of fertility-regulating agents. *J Steroid Biochem* 11:449, 1979.
14. Benagiano G, Schmitt E, Wise D, Goodman M: Sustained release hormonal preparations for the delivery of fertility regulating agents. *J Polymer Sci (In press)*, 1979.
15. Bhatmagar S, Srivastava VK, Takkar D, Chandra VL, Hingorani V, Laumas KK: Long-term contraception of steroid-releasing implants. II. A preliminary report on long-term contraception by a single Silastic implant containing norethindrone acetate (ENTA) in women. *Contraception* 11:505, 1975.
16. Brady JM, Cutright DE, Miller RA, Battistone GC, Hunsuck EE: Resorption rate, route of elimination, and ultrastructure of the implant site of polylactic acid in the abdominal wall of the rat. *J Biomed Materials Research* 7:155, 1973.
17. Brenner PF, Cooper DL, Mishell D: Clinical study of a progesterone-releasing intrauterine contraceptive device. *Am J Obstet Gynecol* 121:704, 1975.
18. Bryant-Greenwood GD, Greenwood FC, Hale RW, Morshige WK: Hormonal evaluation of the intrauterine progesterone contraceptive system. *J Clin Endocrinol* 44:721, 1977.
19. Burton FG, Skiens WE, Duncan GW: Low-level, progestogen-releasing vaginal contraceptive devices. *Contraception* 19:507, 1979.
20. Burton FG, Skiens WE, Gordon NR, Veal JT, Kalkwarf DR, Duncan GW: Fabrication and testing of vaginal contraceptive devices designed for release of pre-specified dose levels of steroids. *Contraception* 17:221, 1978.
21. Chinnatamby S: A comparison of the long-acting contraceptive agents, norethindrone oenanthate and medroxyprogesterone acetate. *Aust NZ J Obstet Gynaecol* 11:233, 1971.
22. Choi NS, Heller J: Erodible agent releasing device comprising poly(orthoesters) and poly(orthocarbonates). U.S. Patent 4,138,344, Feb 6, 1979, Assignee: Alza Corporation.
23. Cohen MR, Pandya GN, Scommegna A: The effects of an intracervical steroid-releasing device on the cervical mucus. *Fertil Steril* 21:715, 1970.
24. Coutinho EM: Clinical experience with implant contraception. *Contraception* 18:411, 1978.
25. Coutinho EM, Da Silva AR: One year contraception with norgestrienone subdermal Silastic implants. *J Fertil Steril* 25:170, 1974.
26. Coutinho EM, Da Silva AR, Carreira CM, Chaves MC, Adeodato-Filho J: Contraceptive effectiveness of Silastic implants containing the progestin R-2323. *Contraception* 11:625, 1975.
27. Coutinho EM, Da Silva AR, Kraft HG: Fertility control with subdermal capsules containing a new progestin (ST-1435). *Int J Fertil* 21:103, 1976.
28. Coutinho EM, de Souza JC: Conception control by monthly injections of medroxyprogesterone suspension and long-acting oestrogen. *J Reprod Fertil* 15:209, 1968.
29. Coutinho EM, de Souza JC, Csapo AI: Reversible sterility induced by medroxyprogesterone injections. *Fertil Steril* 17:261, 1966.
30. Coutinho EM, Mattos CER, Sant'Anna ARS, et al.: Long-term contraception by subcutaneous Silastic R capsules containing megestrol acetate. *Contraception* 2:313, 1970.
31. Croxatto H, Diaz S, Atria P, et al.: Contraceptive action of megestrol acetate implants in women. *Contraception* 4:155, 1971.
32. Croxatto H, Diaz S, Rosati S, et al.: Adnexal complications in women under treatment with progestagen implants. *Contraception* 12:629, 1975.
33. Croxatto H, Diaz S, Vera R, Etchart M, Atria P: Fertility control in women with a progestagen released in microquantities from subcutaneous capsules. *Am J Obstet Gynecol* 105:1135, 1969.
34. Croxatto H, Diaz S, Quinteros E, Simonette L, Kaplan E, Rencoret R, Leixeland P, Martinez C: Clinical assessment of subdermal implants of megestrol acetate, d-norgestrel and norethindrone as a long-term contraceptive in women. *Contraception* 12:615, 1975.
35. Dallenbach-Hellweg G, Sievers S: Die histologische reaction des endometrium auf lokal applizierte gestagene virchows arch. *A Path Anat Histol* 368:289, 1975.
36. Da Silva AR, Coutinho EM: Long-acting subdermal implants containing norgestrienone for one or two years contraception. *J Fertil Steril* 28:379, 1977.
37. Da Silva AR, Coutinho EM: Eighteen months contraception following subdermal insertion of Silastic capsules containing norgestrienone. *Int J Fertil* 23:185, 1978.
38. Dhall K, Dash RJ, Chadha U, Rastogi GK, Devi PK: Effect of three injections of norethisterone oenanthate on ovarian function. *Indian J Med Res* 69:93, 1979.
39. Diaz S, Pavez M, Quinteros E, Diaz J, Robertson DN, Croxatto HB: Clinical trial with subdermal implants containing norgestrienone. *Contraception* 18:429, 1978.
40. Diaz S, Pavez M, Quinteros E, Robertson DN, Croxatto HB: Clinical trial with subdermal implants of the progestin R-2323. *Contraception* 16:155, 1977.
41. Diaz S, Pavez M, Robertson DN, Croxatto HB: A three-year clinical trial with levonorgestrel Silastic implants. *Contraception* 19:557, 1979.
42. Doyle LL, Clewe TH: Preliminary studies on the effect of hormone-releasing intrauterine devices. *Am J Obstet Gynecol* 101:564, 1968.

43. Doyle L, Clewe T, Chandler G: Hormone-releasing Silastic intrauterine devices: effect of provera-releasing intrauterine devices in two species of primates. *Fertil Steril* 26:649, 1975.
44. Dziuk PJ, Cook B: Passage of steroids through silicone rubber. *Endocrinology* 78:208, 1966.
45. Editorial: Further doubts about oral contraceptives. *Br Med J* 5691:252, 1970.
46. Editorial: Which IUD? *Lancet* 1:1239-1240, 1976.
47. El Mahgoub S, Karim M, Ammar R: Long-term use of depot medroxyprogesterone acetate as a contraceptive. *Acta Obstet Gynecol Scand* 51:251, 1972.
48. Faundes A, de Mejias VB, Leon P, Robertson D, Alvarez F: First year clinical experience with six levonorgestrel rods as subdermal contraception. *Contraception* 20:167, 1979.
49. Folkman J, Long DM: The rise of silicone rubber as a carrier for prolonged drug therapy. *J Surg Res* 4:139, 1964.
50. Fotherby K, Howard G, Shrimanker K, Elder M, Bye PG: Occurrence of ovulation in women receiving the injectable contraceptive norethisterone oenanthate. *Contraception* 18:535, 1978.
51. Gallegos A, Aznar R, Merino G, Guizer E: Intrauterine devices and menstrual blood loss. A comparative study of eight devices during the first six months of use. *Contraception* 17:153, 1978.
52. Giwa-Osagie OF, Savage J, Newton JR, Osato F: Norethisterone oenanthate as an injectable contraceptive: use of a modified dose schedule. *Br Med J* 1:1660, 1978.
53. Glass RH, Morris JM: Antifertility effects of an intracervical progestational device. *Biol Reprod* 7:160, 1972.
54. Goebelsmann U, Stanczyk FZ, Brenner PF, Goebelsmann AE, Gentschein EK, Mishell DR: Serum norethindrone (NET) concentrations following intramuscular NET enanthate injection. Effect upon serum LH, FSH, estradiol and progesterone. *Contraception* 19:283, 1979.
55. Gonzalez A, Aznar R, Revilla MC: Abstract: Ultrastructural changes in endometrium of women exposed to progesterone-releasing intrauterine contraceptive devices for more than 5 years. In Hafez ESE, van Os W (eds): *Medicated IUDs and Polymeric Delivery Systems: International Symposium*. Amsterdam, Holland, June 27-30, 1979.
56. Haefeli M, Zodrow W, Spornitz UM, Ludwig KS, Wetwillor A: Abstract: The progestin-releasing IUD. In Hafez ESE, van Os W (eds): *Medicated IUDs and Polymeric Delivery Systems: International Symposium*. Amsterdam, Holland. June 27-30, 1979.
57. Hagenfeldt K: The modes of action of medicated intrauterine devices. *J Reprod Fertil (Suppl 25)*: 117-132, 1976.
58. Hagenfeldt K, Landgren B-M: Contraception by intrauterine release of progesterone - effects on endometrial trace elements, enzymes and steroids. *J Steroid Biochem* 6:895, 1975.
59. Hagenfeldt K, Landgren B-M, Edstrom K, Johannisson E: Biochemical and morphological changes in the human endometrium induced by the Progestasert device. *Contraception* 16:183, 1977.
60. Herzog GM, Soule SD: Control of fertility by monthly injection of estrogen-progestagen. *Obstet Gynecol* 32:111, 1968.
61. Hillier SG, Jha P, Griffiths K, Laumas KR: Long-term contraception by steroid-releasing implants. VI. Serum concentration of norethindrone levels in women bearing a single Silastic implant-releasing norethindrone acetate. *Contraception* 15:473, 1977.
62. Hopfenberg HB: Controlled release from erodible slabs, cylinders, and spheres. In Paul DR, Harris FW (eds): *Controlled Release Polymeric Formulations*. ACS Symposium Series 33. Washington, DC, American Chemical Society, 1976, pp 26-32.
63. Howard G, Warren RJ, Fotherby K: Plasma levels of norethisterone in women receiving norethisterone oenanthate intramuscularly. *Contraception* 12:45, 1975.
64. Hudson R, Hemphill P, Tillson SA: Preclinical evaluation of intrauterine progesterone as a contraceptive. I. Local contraceptive effects and their reversal. *Contraception* 17:465, 1978.
65. Hudson R, Pharriss BB, Tillson S, Reno F: Preclinical evaluation of intrauterine progesterone as a contraceptive agent. III. Embryology and toxicology. *Contraception* 17:489, 1978.
66. Humpel M, Kuhne G, Schulze PE, Speck U: Injectable depot contraceptives on d-norgestrel basis. I. Pharmacokinetic studies in dog and baboons. *Contraception* 15:401, 1977.
67. Humpel M, Schulze PE, Speck U: A new principle of injectable depot contraceptives. I. Drug selection and studies in monkeys. *Contraception* 19:411, 1979.
68. International Committee for Contraception Research of the Population Council: Contraception with long-acting subdermal implants. I. An effective and acceptable modality in international clinical trials. *Contraception* 18:315, 1978.
69. International Committee for Contraception Research of the Population Council: Contraception with long-acting subdermal implants. II. Measured and perceived effects in international clinical trials. *Contraception* 18:335, 1978.
70. Intracervical devices. In: *Special Programme of Research, Development, and Research Training in Human Reproduction*. Seventh Annual Report. Geneva, World Health Organization, 1978, p 79.
71. Jackanicz TM, Nash HA, Wise DL, Gregory JB: Polylactic acid as a biodegradable carrier for contraceptive steroids. *Contraception* 8:227, 1973.
72. Jeppsson S, Johannisson EDB: Medroxyprogesterone acetate, estradiol, FSH and LH in peripheral blood after intramuscular administration of depo-provera to women. *Contraception* 14:461, 1976.
73. Jeppsson S, Johannisson ED, Ljungberg O, Sjoberg NO: Endometrial histology and circulating levels of medroxyprogesterone acetate (MPA), estradiol, FSH and LH in women with MPA induced amenorrhoea compared with women with secondary amenorrhoea. *Acta Obstet Gynecol Scand* 56:43, 1977.
74. Jeyaseelan S, Takkar D, Bhuyan UN, Laumas KR, Hingorani V: Local tissue response to Silastic implant containing norethindrone acetate in women. *Contraception* 15:39, 1977.
75. Jones R, Cohen M, Bell J: Local progestational effect of norgestrel in an intrauterine device. *Contraception* 8:439, 1973.
76. Karim M, el Mahgoub S: Conception control by cyclic injections of norethisterone enanthate and estradiol undecylate. *Am J Obstet Gynecol* 11:740, 1971.
77. Keifer WS, Lee AF, Scott JC: A clinical evaluation of a monthly injection for conception control. *Am J Obstet Gynecol* 107:400, 1970.
78. Kirton KT, Cornette JC: Return of ovulatory cyclicity following an intramuscular injection of medroxyprogesterone acetate (Provera). *Contraception* 10:39, 1974.
79. Kora S, Virkar K: Incidence of pregnancy, changes in menstrual pattern, and recovery of endometrial function after discontinuation of medroxyprogesterone acetate therapy. *Fertil Steril* 26:121, 1975.
80. Landgren BM, Diczfalusy E: Pharmacodynamic effects of the norethisterone minipill. Swedish Med Res Council, Reproductive Endocrinol Res Unit and Dept Ob/Gyn. Karolinska Sjukhuset, 404 ol Stockholm 60, Sweden.
81. Landgren BM, Johannisson E, Masiaroni B, Diczfalusy E: Pharmacokinetic and pharmacodynamic effects of small doses of norethisterone released from vaginal rings continuously during 90 days. *Contraception* 19:253, 1979.
82. Luukkainen T, Nilsson CG: Sustained intrauterine release of d-norgestrel. *Contraception* 18:451, 1978.
83. Martinez-Manautou J: Contraception by intrauterine release of progesterone:

- clinical results. *J Steroid Biochem* 6:889, 1975.
84. Martinez-Manautou J, Aznar R, Maqueo M, Pharriss BB: Uterine therapeutic system for long-term contraception. II. Clinical correlates. *Fertil Steril* 25:922, 1974.
85. Martinez-Manautou J, Aznar R, Rosado A, Maqueo M: Clinical experience with the intrauterine progesterone-releasing system. Proceedings of the Third International Conference on Intrauterine Contraception, Cairo, December 1974.
86. Martinez-Manautou J, Aznar T, Rosado A, Maqueo M: Clinical experience with intrauterine progesterone releasing systems. In Hefnawi F, Segal SJ (eds): *Analysis of Intrauterine Contraception*. Amsterdam, North Holland, 1975, pp 173-184.
87. Martinez-Manautou J, Maqueo M, Aznar R, Pharriss BB, Zaffaroni A: Endometrial morphology in women exposed to uterine systems releasing progesterone. *Am J Obstet Gynecol* 121:175, 1975.
88. McDaniel EB: Return of fertility following discontinuation of three-month contraceptive injections of DMPA plus routine oral estrogen supplement: a preliminary report. *Fertil Steril* 22:802, 1971.
89. McDaniel EB, Pardthaisong T: Return of fertility after depo-medroxyprogesterone acetate DMPS contraceptive injections. *J Steroid Biochem* 9:861, 1978.
90. Miller RA, Brady JM, Cutright DE: Degradation rates of oral resorbable implants (polylactates and polyglycolates) — rate modification with changes in PLA/PGA copolymer ratios. *J Biomed Materials Research* 11:711, 1977.
91. Mishell DR Jr, Lumkin M: Initial clinical studies of intravaginal rings containing norethindrone and norgestrel. *Contraception* 12:253, 1975.
92. Mishell DR Jr, Lumkin ME, Stone S: Inhibition of ovulation with cyclic use of a progesterone impregnated intravaginal device. *Am J Obstet Gynecol* 113:927, 1972.
93. Mishell DR Jr, Moore DE, Roy S, Brenner PF, Page MA: Clinical performances and endocrine profiles with contraceptive vaginal rings containing a combination of estradiol and d-Norgestrel. *Am J Obstet Gynecol* 130:55, 1978.
94. Mishell DR Jr, Roy S, Moore DE, Brenner PF, Page MA, Gentschein E, Fisk PD: Clinical performances and endocrine profiles with contraceptive vaginal rings containing d-Norgestrel. *Contraception* 16:625, 1977.
95. Mishell DR Jr, Talas M, Parlow AF, Moyer DE: Contraception by means of a Silastic vaginal ring impregnated with medroxyprogesterone acetate. *Am J Obstet Gynecol* 107:100, 1970.
96. Moghissi KS, Burton FG, Skiens WE, Leininger RI, Sikov MR, Duncan GW, Smith LG: An intracervical contraceptive device. In Gabelnick HL (ed): *Proceedings: Drug Delivery Systems*. DHEW Publication No (NIH) 77-1238, 1977, p 79.
97. Moore DE, Roy S, Stanczyk FZ, Mishell DR: Bleeding and serum d-norgestrel, estradiol and progesterone patterns in women using d-norgestrel subdermal polysiloxane capsules for contraception. *Contraception* 17:315, 1978.
98. Nash HA, Robertson DN, Jackanicz TM: Release of contraceptive steroids from sustained release dosage forms and resulting plasma levels. *Contraception* 18:395, 1978.
99. Newton J, McEwan J: Hormone releasing IUDs — a proper perspective view. *Fertil Steril* 1:35-36, 1977.
100. Newton J, Szontagh F, Lebech P, Row P: A collaborative study of the progesterone intrauterine device (Progestasert). *Contraception* 19:575, 1979.
101. Nilsson CG, Luukkainen T, Arko H: Endometrial morphology of women using a d-norgestrel-releasing IUD. *Fertil Steril* 29:397, 1978.
102. Nuwayser ES, Williams DL, Meier PM, Wu TC, Merchant SZ, Kerrigan JK: A microcapsule system for drug delivery. In Gabelnick HL (ed): *Proceedings: Drug Delivery Systems*. DHEW Publication No (NIH) 77-1238, 1977, pp 193-251.
103. Parveen L, Chowdhury AO, Chowdhury Z: Injectable contraception (medroxyprogesterone acetate) in rural Bangladesh. *Lancet* 2:946, 1977.
104. Peterson RV, Anderson JM, Anderson CG, Fang SM, Feijen J, Gregonis DE, Mitra S, Kim SW: Controlled release of progestins from poly (α -amino acid) carrier. Proceed 6th International Symposium on Controlled Release of Bioactive Materials, New Orleans, Louisiana, August 1979, pp 1-21 - 1-27.
105. Pharriss BB, Erickson R, Bashaw J, Hoff S, Place VA, Zaffaroni A: Progestasert: An uterine therapeutic system for long-term contraception. I. Philosophy and clinical efficacy. *Fertil Steril* 25:915, 1974.
106. Pharriss BB, Place VA, Senaelbeck L, Schmitt E: Steroid delivery systems for contraception. *J Reprod Med* 17:91, 1976.
107. Pitt CG, Christensen D, Jeffcoat AR, Kimmel GL, Schindler A, Wall ME, Sweidinger RA: Biodegradable polymers and sustained delivery of contraceptive drugs. In Gabelnick HL (ed): *Proceedings: Drug Delivery Systems*. DHEW Publication No (NIH) 77:1238, 1977, pp 141-192.
108. Pitt CG, Jeffcoat AR, Zweidinger RA, Schindler A: Sustained drug delivery systems I. The permeability of poly (E-caprolactone), poly (D,L-lactic acid), and their copolymers. *J Biomed Materials Research* 13:497, 1979.
109. Place VA, Pharriss BB: Progress in development of Progestasert 65 progesterone therapeutic system for contraception. *J Reprod Med* 13:66, 1974.
110. Rahman SA, Jha P, Jain AK, Laumas V, Kumar D, Laumas KR: Effect of norethindrone acetate-releasing single implant on hypothalamo-hypophyseal-gonadal axis in women. Symposium on General and Comparative Endocrinology. Abstract p 77, 1976.
111. Rall HJ, van Niekerk WA, Engelbrecht BH, van Schalkwyk DJ: Comparative contraceptive experience with three-month and six-month medroxyprogesterone acetate regimens. *J Reprod Med* 18:55, 1977.
112. Reifenstein EC, Pratt TE, Hartzell KA, Shafer WB: Artificial menstrual cycles induced in ovulating women by monthly injection of progestagen-estrogen. *Fertil Steril* 16:652, 1965.
113. Ringrose CA: Effects of medroxyprogesterone acetate employed as a long-term injectable contraceptive. *J Reprod Med* 8:75, 1972.
114. Rosado A, Hicks JJ, Aznar R, Mercado E: Intrauterine contraception with the progesterone I device - interference with the metabolic activity and capacitation of spermatozoa. *Contraception* 9:39, 1974.
115. Saxena BN, Shrimanker K, Fotherby K: Radioimmunoassay of serum norethisterone oenanthatate levels in women after intramuscular administration. *J Steroid Biochem* 8:1117, 1977.
116. Schindler A, Jeffcoat R, Kimmel GL, Pitt CG, Wall ME, Sweidinger R: Biodegradable polymers for sustained drug delivery. In Pearce EM, Schaeffgen JR (eds): *Contemporary Topics in Polymer Science*, Vol 2. New York, Plenum Publishing Corp, 1977, pp 251-289.
117. Schmitt EE: Pharmaceutical composition of poly(orthoester) co- and homopolymers and poly(orthocarbonate) co- and homopolymers having carbonyloxy functionality with drug. U.S. Patent 4,155,922, May 22, 1979, Assignee: Alza Corporation.
118. Scommegna A, Avila T, Luna M, Rao R, Dmowski WP: Fertility control by intrauterine release of progesterone. *Am J Obstet Gynecol* 43:769, 1974.
119. Scommegna A, Ileakis J, Rao R, Dmowski P, Rezai P, Auletta F: Endometrial prostaglandin F content in women wearing nonmedicated or progestin-releasing intrauterine devices. *Fertil Steril* 29:500, 1978.
120. Scommegna A, Ileakis J, Sotrel G, Auletta FJ: The effect of intrauterine progesterone treatment on the endometrial

- prostaglandin F content in the rabbit. *Fertil Steril* 28:581, 1977.
121. Scommegna A, Lee AW, Borushek S: Evaluation of an injectable progestin-estrogen as a contraceptive. *Am J Obstet Gynecol* 107:1147, 1970.
122. Scommegna A, Pandya GN, Christ M, Lee AW, Cohen MR: Intrauterine administration of progesterone by a slow releasing device. *J Fertil Steril* 21:201, 1970.
123. Segal SJ, Croxatto HB: Single administration of hormones for long-term control of reproductive function. Presentation at the XXIII Meeting of American Fertility Society, April 14-16, 1967, Washington, DC.
124. Sidman KR, Schwoppe AD, Steber WD, Rudolph SE, Poulin SB: Use of synthetic polypeptides for biodegradable drug delivery systems. *Polymer Preprints* 20:27, 1979.
125. Sidman K, Steber WD, Burg AW: A biodegradable drug delivery system. In Gabelnick HL (ed): *Proceedings: Drug Delivery Systems*. DHEW Publication No (NIH) 77-1238, 1977, p 120.
126. Siegel I: Conception control by long-acting progestagens—preliminary report. *Obstet Gynecol* 21:666, 1963.
127. Snowden R: The Progestasert and ectopic pregnancy. *Br Med J* 2:1600, 1977.
128. Spona J, Weiner E, Nieuweboer B, Humpel M, Schneider W, Johansson ED: Injectable depot contraceptives on d-norgestrel basis. II. Clinical pharmacokinetic studies with d-norgestrel undecylate in women. *Contraception* 15:413, 1977.
129. Spornitz UM, Ludwig KS, Mall M: Abstract: Effect of progesterone containing IUD on the morphology of the endometrium and oviduct. In Hafez ESE, van Os W (eds): *Medicated IUDs and Polymeric Delivery Systems: International Symposium*. Amsterdam, Holland, June 27-30, 1979.
130. Stanczyk FZ, Hiroi M, Goebelsmann U, Brenner PF, Lumkin ME, Mishell DR Jr: Radioimmunoassay of serum d-Norgestrel in women following oral and intravaginal administration. *Contraception* 12:279, 1975.
131. Taymor ML, Planck S, El Yahia C: Ovulation inhibition with a long-acting parenteral progestagen-estrogen combination. *Fertil Steril* 15:653, 1964.
132. Tejuja S: Use of subcutaneous Silastic capsules for long-term steroid contraception. *Am J Obstet Gynecol* 197:954, 1970.
133. Tejuja S, Malhotra U, Bhinder G: A preliminary report on the contraceptive use of subdermal implants containing norethindrone. *Contraception* 10:361, 1974.
134. Thies C: Characterization of microcapsules containing naltrexone or naltrexone pamoate. In Paul DR, Harris FW (eds): *Controlled Release Polymeric Formulations*. ACS Symposium Series 33. Washington DC, American Chemical Society, 1976, pp 190-194.
135. Tillson SA, Marian M, Hudson R, Wong P, Pharriss B, Aznar R, Martinez-Manautou J: The effect of intrauterine progesterone on the hypothalamic-hypophyseal-ovarian axis in humans. *Contraception* 11:179, 1975.
136. Toivonen J, Lahteenmaki P, Luukkainen T: Pituitary and gonadal function during the use of norgestrel-estradiol vaginal rings. *Contraception* 18:201, 1978.
137. Toivonen J, Lahteenmaki P, Luukkainen T: The use of the contraceptive vaginal ring governed by the pattern of individual uterine bleeding. *Contraception* 19:401, 1979.
138. Toivonen J, Lahteenmaki P, Luukkainen T: Bleeding and ovulation control with use of a small contraceptive vaginal ring releasing levonorgestrel and estradiol. *Contraception* 20:11, 1979.
139. Victor A, Edqvist LE, Lindberg P, Elamsson K, Johansson EDB: Peripheral plasma levels of d-Norgestrel in women after oral administration of d-Norgestrel and when using intravaginal rings impregnated with d-Norgestrel. *Contraception* 12:261, 1975.
140. Victor A, Johansson EDB: Pharmacokinetic observations on medroxyprogesterone acetate administered orally; and intravaginally. *Contraception* 14:319, 1976.
141. Viinikka L, Victor A, Janne O, Raynaud JP: The plasma concentration of a synthetic progestin R-2323 released from polysilastic vaginal rings. *Contraception* 12:309, 1975.
142. Wadsworth PF, Heywood R, Allen DG, Sortwell RJ, Walton RM: Treatment of rhesus monkeys (*Macaca mulatta*) with intrauterine devices loaded with levonorgestrel. *Contraception* 20:177, 1979.
143. Waise J, Marker L, Holma P, Varilainen E, Osler M, Pyorala T, Johansson E, Luukkainen T: Long-term contraception with norethindrone subcutaneous capsules. *Ann Clin Res* 8:93, 1976.
144. Wan LS, Ying-Chih H, Manik G, Bradley B: Effects of the Progestasert on the menstrual pattern, ovarian steroids and endometrium. *Contraception* 16:417, 1977.
145. Weiner E, Johansson EDB: Plasma levels of norethindrone after i.m. injection of 200 ng norethindrone enanthate. *Contraception* 11:419, 1975.
146. Weiner E, Johansson EDB: Effects on the ovarian function of subcutaneous implants containing lynestrenol. Presented at XVIII Nordiska Gynekologkongressen, Uppsala, June 4-8, 1974. Abstr No 92. *Acta Obstet Gynecol Scand Suppl* 47:50, 1976.
147. Weiner E, Johansson EDB: Plasma levels of d-norgestrel, estradiol and progesterone during treatment with Silastic implants containing d-norgestrel. *Contraception* 14:81, 1976.
148. Weiner E, Johansson EDB: Contraception with d-norgestrel Silastic rods: Plasma levels of d-norgestrel and influence on the ovarian function. *Contraception* 14:551, 1976.
149. Weiner E, Johansson EDB, Wide L: Inhibition of the positive feedback of estradiol during treatment with subcutaneous implants of d-norgestrel. *Contraception* 13:287, 1976.
150. Wilson E: Use of long-acting depot progestagen in domiciliary family planning. *Br Med J* 2:1435, 1976.
151. Wise DL, Gresser JD, Beck LR, Howes JF: Kinetics of release: drug diffusion from and erosion of polymer matrices. In Hafez ESE, van Os WAA (eds): *Polymeric Delivery Systems. Proc Internat Symposium on Medicated IUDs and Polymeric Delivery Systems*. Amsterdam, Holland, June 27-30, 1979.
152. Wise DL, McCormick GJ, Willet GP, Anderson LC, Howes JF: Sustained release of sulphadiazine. *J Pharm Pharmacol* 30:686, 1978.
153. Yolles S, Eldridge J, Leafe TD, Woodland JHR, Blake DR, Meyer FJ: Long-acting delivery systems for narcotic antagonists. In Tanquary AC, Lacey RE (eds): *Controlled Release of Biologically Active Agents. Advances in Experimental Medicine and Biology*. New York, Plenum Press, Vol 47, 1974, pp 177-193.
154. Yolles S, Leafe TD, Woodland JHR: Long-acting delivery systems for narcotic antagonists. II. Release rates of naltrexone from poly(lactic acid) composites. *J Pharm Sci* 64:348, 1975.
155. Zador G, Nilsson BA, Nilsson B, Sjoberg NO, Westrom L, Wiese J: Clinical experience with uterine progesterone system (Progestasert). *Contraception* 13:559, 1976.
156. Zahradnik H, Kraut E, Flecken A, Breckwoldt M: Local effect of progesterone on prostaglandin F_{2α} levels in menstrual blood. *Acta Endocrinol (Suppl)* 208) 84:93, 1977.
157. Zaňartu J, Navarro C: Fertility inhibition by an injectable progestagen acting for three months. *Obstet Gynecol* 31:627, 1968.
158. Zaňartu J, Rice-Wray E, Goldzieher JW: Fertility control with long-acting injectable steroids. A preliminary report. *Obstet Gynecol* 28:513, 1966.



Northwestern University
1040 Passavant Pavilion
303 East Superior Street
Chicago, Illinois 60611

Editor: Gerald I. Zaluchni, M.D., M.Sc.
Managing Editor: Kelley Osborn
This publication is supported by AID/DSPE-C-0035



RESEARCH FRONTIERS IN FERTILITY REGULATION

CURRENT STATUS OF RESEARCH ON HORMONAL CONTRACEPTION IN THE MALE

Emil Steinberger, M.D.

*Professor and Chairman
Department of Reproductive Medicine and Biology
University of Texas Medical School
Houston, Texas*

Although much has been written about the need for a chemical contraceptive for the male, not a single systemic male contraceptive has been developed to the stage of serious clinical testing. Yet efforts to develop a male contraceptive antecede similar efforts to develop a female contraceptive.

In the mid-1950s, potent synthetic oral progestins were developed and a few years later, estrogen-progestin preparations were shown to be highly effective oral contraceptives. These agents were rapidly approved by regulatory agencies and became available for general use by millions of women. Work toward the development of a systemic male contraceptive began in 1950, in the laboratory of W.O. Nelson (13). His theoretic approach, however, was entirely different from that utilized in the female. In the female, focus was on the physiologic mechanisms providing negative feedback regulation of the pituitary gonadotropins by gonadal steroids (9). In the male, the initial approach focused upon interruption of spermatogenesis by chemical means. Work was hindered, however, by the limited knowledge of male reproductive tract physiology, and during the subsequent 30 years, the development of a male contraceptive did not advance beyond the confines of research laboratories. The necessary basic knowledge has not yet been acquired, and a hormonal approach similar to that utilized in the female has not been pursued with appropriate vigor.

BASIC APPROACHES

The available physiologic data suggest four approaches to curtailment of fertility in the male.

1) Interference with spermatogenesis.

Producing an azoospermic state undoubtedly would be the most effective means of male contraception; lack of spermatozoa in the semen is definitely associated with sterility. Sperm production might be blocked by specific antispermatogenic cytotoxic agents or other substances that interfere with the spermatogenic process, or by hormonal interference with germinal epithelium maturation, e.g., blockage of pituitary gonadotropins. The latter could be achieved by suppression of gonadotropin production with gonadal steroids; suppression of FSH production by application of the negative feedback mechanism with an inhibin-like substance; suppression of gonadotropin production by interference with GnRH at the pituitary gland level; or by a direct chemical action on the biosynthetic processes involved in formation of gonadotropins in the pituitary gland.

2) Interference with the mechanisms responsible for sperm motility

Under normal physiologic conditions, spermatozoal motility is necessary for fertilization to occur. The biochemical mechanisms responsible for motility are complex and amenable to chemical regulation. Although no specific agents have as yet been found which can interfere with the development of motility in the germinal cells, a number of clinical conditions have been described in which men showing perfectly normal spermatogenesis produce immotile spermatozoa. This defect may be secondary to a biochemical abnormality in the seminiferous epithelium. Thus, it is possible that a similar but reversible biochemical defect in the seminiferous epithelium could be induced by chemical means, and immotile spermatozoa would be produced.

3) Interference with epididymal sperm maturation.

Spermatozoa acquire fertilizing capacity as they traverse the epididymis. This capacity is apparently related to the action of testosterone in the epididymis. An agent capable of interfering with this process could serve as a contraceptive. In studies utilizing the antiandrogen cyproterone acetate, Prasad and colleagues observed loss of motility, viability, and fertilizing capacity of epididymal spermatozoa in the rat (19). However, the large doses of cyproterone acetate required to produce this condition also affected other androgen target tissues, negating the effectiveness of this approach (18). More potent androgens and drug delivery systems which would preferentially expose the epididymis to the antiandrogen activity would be necessary for this to be a feasible approach to male contraception.

4) Interference with the formation or activity of acrosomal enzymes

Various proteolytic and mucolytic enzymes are packaged in the acrosome. These enzymes are apparently essential if the spermatozoa are to traverse the female reproductive tract and the cellular envelopes surrounding the ovum, and to penetrate the ovum. Interference with the synthesis or function of these enzymes might render the spermatozoon incapable of fertilizing the ovum.

Understanding the molecular mechanisms responsible for hormonal control of spermatogenesis and Sertoli cell function has opened up a number of other avenues for interference with sperm production (39). As knowledge of testicular physiology increases, more points of potential attack are likely to be discovered.

MAJOR RESEARCH EFFORTS

Research efforts toward development of a male contraceptive have taken two major directions. 1) Initial studies were focused on the development of chemical agents with a direct action on the seminiferous epithelium. 2) More recent research has been directed toward interfering with spermatogenesis by hormonal means. The following discussion considers these two major approaches.

Antispermatozoic Agents

The work on antispermatozoic agents was stimulated by observations made in the 1940s that certain cytotoxic agents, e.g., nitrogen mustards, arrest spermatogenesis at dose levels below those having systemic toxicity (5). Although these compounds could not be considered as potential contraceptives, primarily because of their alkylating, mutagenic, teratogenic, and carcinogenic properties, other agents capable of direct interference with

the spermatogenic process, but devoid of these undesirable characteristics, might be developed.

The first chemical compounds tested specifically for their potential application as contraceptives were the nitrofurans, compounds used to treat urinary tract infections in men (13, 14, 15). Several nitrofurans were found to arrest spermatogenesis in the rat at a dose level that was free of systemic effects. The spermatogenic arrest was reversible upon cessation of treatment. The offspring of animals which recovered fertility showed no abnormalities for up to F-3 generations (15). A limited trial in humans utilizing one of the nitrofurans, however, showed an unsatisfactory therapeutic index (12).

Subsequently, the thiophens, a class of compounds similar to nitrofurans in their chemical structure, were tested and shown to possess antispermatozoic activity (24). This research led to the discovery of a number of other chemical agents capable of inducing spermatogenic arrest in the rat: the dinitropyrroles (16), the diamines (1), and recently the indol-carboxylic acids (2). The diamines, when tested in men, produced undesirable Antabuse-like side effects (4). The other agents were not clinically tested.

Although most reports in the literature are concerned with the above-mentioned compounds, a large and diverse number of other chemical agents, such as deuterium oxide, cadmium, and fluoroacetamide, were also shown to produce a specific effect on the seminiferous epithelium. Most of these studies were sporadic rather than the result of a major, organized effort directed toward the synthesis of analogues with more specific effects on the seminiferous epithelium, and the biochemical mechanisms responsible for the induction of spermatogenic arrest by these compounds were not considered. The studies were not goal oriented, properly supported, or carried out beyond the very early stages of investigation; they were usually limited to demonstration of a histologic phenomenon of spermatogenic arrest or sterility.

Recent reports from China describe a new chemical antifertility agent, gossypol, which has apparently been administered to several thousand men during the past 8 years and has proven to be highly effective and nontoxic and to have reversible effects (11). Gossypol is found primarily in cotton seed, but is also present in the root and stem of the cotton plant. The compound probably exists in several isomeric forms. It is toxic to nonruminant animals; it reduces the oxygen-carrying capacity of blood, is irritating to the gastrointestinal tract, and in large doses produces pulmonary edema, shortness of breath, and paralysis. It is used industrially as a rubber antioxidant and as a stabilizer for vinyl polymers.

According to the reports of Chinese investigators, gossypol produces its antifertility effect in men by "killing" the spermatozoa in the epididymis rather than by a

direct effect on spermatogenesis. The effective dose is 12 to 20 mg administered daily for two and one-half months, or until the infertile state is reached. Subsequently, a maintenance dose of 12.5 mg administered twice weekly maintains infertility. Fertility is reestablished within several months after discontinuation of therapy. The effectiveness is reported to be 99% and, except for a slight decline in blood potassium levels, no other adverse effects have been reported.

While these initial reports suggest that gossypol may indeed be effective in producing male infertility without systemic toxicity, more information will be required before these optimistic conclusions can be accepted. Regardless of the outcome of further studies on gossypol, these reports demonstrate the antifertility effects of another class of chemical compounds, and demonstrate the potential of this approach to male contraception.

Hormonal Agents

Despite a clear clinical demonstration some 40 years ago that administration of androgens will induce reversible azoospermia and thus infertility, it was not until the 1970s that serious investigation of a hormonal approach to male contraception began—two decades after similar studies were begun in the female.

Several assumptions, although they are unsupported by data, seem to have served as the basis for this lack of interest in hormonal contraception for the male:

- 1) In order to maintain the azoospermic state, dangerously high doses of androgens would be required.
- 2) Because natural androgens are ineffective when administered orally, a parenteral route would have to be utilized to administer the contraceptive agent; this would preclude the use of androgens. No adequate, synthetic, orally effective androgen is presently available and no strong incentive exists to develop one. This lack of incentive stands in contrast to the incentive for the development of orally active progestins, which were synthesized initially for treatment of common disorders in the female reproductive system rather than for contraceptive use.
- 3) The probable acceptance rate of a male systemic contraceptive which would interfere with testicular function is believed by some investigators to be low.

All three major classes of gonadal steroid hormones (estrogens, progestogens, and androgens) suppress production of pituitary gonadotropins and induce azoospermia, or varying degrees of oligospermia.

Estrogens. These hormones cannot be seriously considered as potential candidates for use in the male, because of their feminizing effects and other serious side effects.

Progestins. When used alone, progestins will induce oligospermia, but the degree may not be sufficient to

assure sterility and the suppression of testicular testosterone production will induce the totally unacceptable side effect of impotence.

Androgens. The data accumulated in the late 1960s and early 1970s demonstrated the pivotal role of testosterone in spermatogenesis and show that its effect requires intratesticular concentrations several times greater than concentrations in the blood. This information prompted some investigators to consider the possibility that testosterone alone might be utilized as a male contraceptive.

Steroid combinations. The use of a combination of steroids has also been considered. In this approach, one class of steroids, e.g., danazol, or one of the synthetic progestins, would be utilized to suppress the pituitary gonadotropins, while testosterone would be used to maintain libido. The testosterone might act in a synergistic fashion with progestins or danazol to induce azoospermia. Studies considering these approaches are discussed in the following section.

- 1) **Danazol-testosterone combination.** A number of studies have been conducted with the danazol-testosterone combination (6, 21). Oligospermia or azoospermia was induced and maintained with regularity. However, in many cases suppression of sperm production to 5 million/ml or less was achieved rather than azoospermia. No serious side effects have been reported.

The testosterone preparation used in most studies has been testosterone enanthate (TE) in oil; a dose of 200 mg every 3 or 4 weeks has been shown to prevent impotence.

- 2) **Progestin-testosterone combination.** A number of progestins have been investigated sporadically as potential candidates for this combination approach. Recently in a major study organized and coordinated by the International Committee for Contraception Research, several progestin-androgen preparations were tested utilizing 25 different dosage regimens. A total of 35 clinical studies were conducted in different parts of the world. Of the 189 men enrolled in the studies and treated for sufficiently long periods to observe the full effect of the steroids, 81% showed sperm suppression below 10 million. Of the various progestins, R2323 (17 α -ethinyl-17 β -hydroxy-18-methyl-4,9,11-estriene-3-one) was most effective. In 73% of men treated with R2323 and testosterone enanthate, sperm counts of less than 1 million/ml were observed.

Only minor side effects were documented with the effective regimens. The most frequently observed side effect, decline of libido during early phases of treatment, was most common in men receiving megestrol acetate and depot-medroxyprogesterone acetate. Other side effects were moderate weight gain and, in a few subjects, reversible gynecomastia.

Elevated serum transaminase levels were observed in Scandinavian and South American subjects, most commonly in men receiving R2323. Transaminase levels returned to normal in all instances after discontinuation of treatment (21).

These studies suggest the potential for the use of progestins as a male contraceptive. It would have been interesting to compare the physiologic characteristics of those men who responded to therapy to the characteristics of men receiving the same medication but who failed to respond. This information might provide an explanation for the differences in response.

TESTOSTERONE STUDIES

New knowledge in the areas of physiology and biochemistry of the testes during the past 15 years has elucidated a number of regulatory mechanisms associated with spermatogenesis. It has been shown that the intratesticular levels of testosterone are much higher than circulating levels, that the high intratesticular levels of testosterone are probably required for normal progression of spermatogenesis, and that there is an oscillatory pattern to the plasma testosterone levels. These findings suggest that the oscillatory patterns may be of importance in the feedback mechanism between the gonads and the hypothalamic-pituitary axis.

On the basis of these findings, a hypothesis was proposed, suggesting that pituitary LH production could be blocked by administration of testosterone at a dose that would result in a nonoscillatory normal level of plasma testosterone. Under these conditions, a steady state should be created, characterized by suppressed LH levels, normal plasma testosterone levels, markedly decreased endogenous testosterone production resulting in low intratesticular testosterone levels, and cessation of spermatogenesis. On the basis of a case study in 1973,

which showed the hypothesis to be viable, it was suggested that initially, administration of relatively high doses of testosterone enanthate is essential for reliable induction of azoospermia, which then could be maintained with a much lower dose producing normal circulating levels of testosterone (22, 23).

Subsequent studies with testosterone enanthate (TE) conducted in our laboratories (25, 26, 27) differ from those reported in the literature (3, 17, 30) in two major aspects. In our studies, the duration of treatment was one year, while reports in the literature deal with much shorter periods of treatment time. We employed an "induction phase" during which high doses of TE were administered for a short period (200 mg of TE twice a week for 2 weeks and then at weekly intervals for an additional 2 weeks). Several maintenance schedules were tested, varying the time interval between injections rather than varying the dose. The induction phase resulted in azoospermia or severe oligospermia (less than 0.3 million/ml) in all subjects who adhered to the injection schedule, and was found to be absolutely essential for the initial achievement and for the subsequent maintenance of azoospermia. An average of 53 days was required for the sperm count to drop to less than 1 million/ml; 69.3 days were required for azoospermia. Severe oligospermia (less than 300,000/ml) or azoospermia was observed only when the induction phase was employed and when the maintenance schedule consisted of 200 mg TE administered every 10 days.

On this schedule, the azoospermia was maintained throughout the 1-year study period. Administration of 200 mg TE every 2 weeks during the maintenance phase resulted in sperm counts of less than 1 million/ml throughout the experimental period; in 10 out of 12 of these subjects, azoospermia or a sperm count below 0.3 million/ml was maintained. Administration of TE every 3

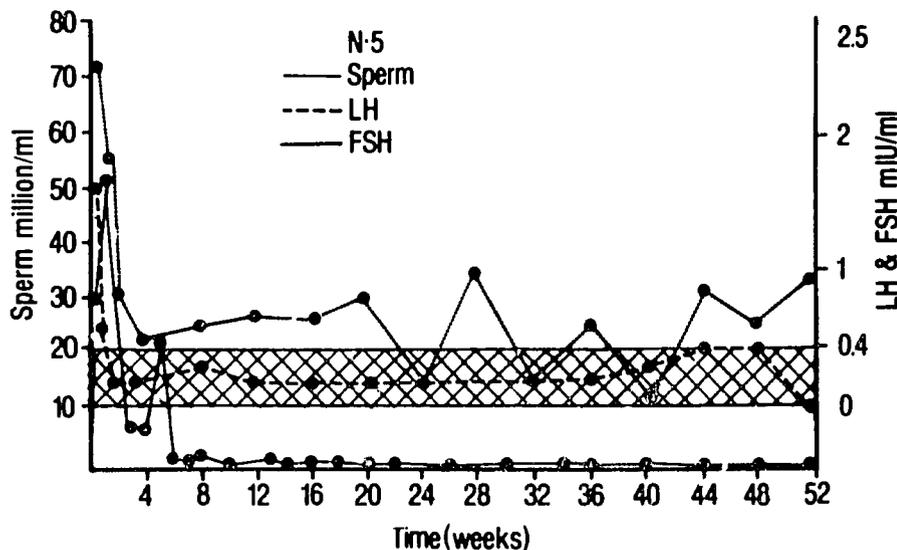


Figure 1. Sperm counts and gonadotropin levels in subjects receiving TE injections every 10 days during the maintenance phase. The shaded area represents the undetectable range for FSH and LH. (From Steinberger E, et al: *Int J Androl (Suppl)* 2:748, 1978).

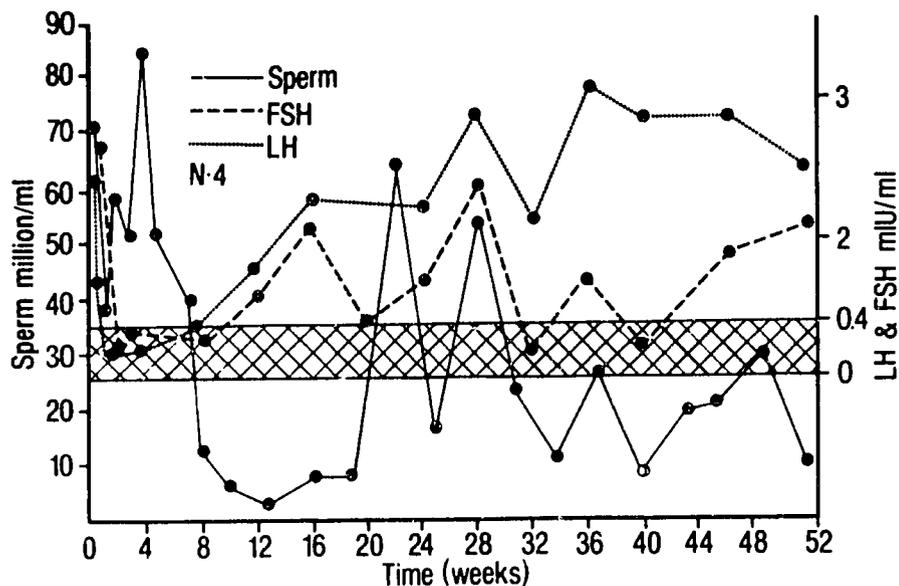


Figure 2. Sperm counts and gonadotropin levels in subjects receiving TE injections every 3 weeks during the maintenance phase. The shaded area represents the undetectable range for FSH and LH. (From Steinberger E, Smith KD: *Fertil Steril* 28:1320, 1977).

weeks resulted in sporadic sperm production during the 1-year period.

The three maintenance schedules—injections of 200 mg TE every 10 days (Group I), every 2 weeks (Group II), or every 3 weeks (Group III)—produced three totally different patterns of sperm output: maintenance of azoospermia, maintenance of sperm counts of below 1 million/ml, or serious breakthrough in sperm production (Figures 1 and 2).

Analysis of changes in the circulating hormone levels during the maintenance phase in the three experimental groups provided information concerning the probable hormonal mechanisms responsible for the differences in sperm production. Analysis of LH levels revealed that only in Group I were the plasma LH levels suppressed to undetectable levels throughout the maintenance period (Figure 1). In the other two groups, sporadic spikes in LH levels were noted at various time intervals during the maintenance period (Figure 2). Thus, the maintenance of azoospermia was associated with uninterrupted suppression of plasma LH to undetectable levels.

It appears that complete suppression of LH is absolutely essential to prevent stimulation of endogenous testosterone production, which in turn may result in sperm production. In all three groups, plasma testosterone levels returned to the pretreatment level or to a normal range for adult males after the induction phase. However, since the design of the study called for measurement of hormone levels prior to each TE injection, the data reflect blood hormone levels 10 days, 2 weeks, or 3 weeks after an injection, rather than plasma levels at shorter time intervals after the injection.

The possibility was considered that higher testosterone levels may be present shortly after the injection. To

obtain information concerning this question, plasma testosterone levels were measured daily in one of the subjects for three cycles of injections, 22 weeks after initiation of therapy. At no time interval after the injection did the plasma testosterone levels rise above the normal range.

To obtain information on the kinetics of plasma testosterone levels after a single injection of TE, a normal adult male and a castrated male were injected with 200 mg TE, and plasma testosterone levels were measured daily at 1:00 p.m. for 10 days (Figure 3). In the normal individual, the peak in plasma testosterone levels occurred within 2 days after the injection, while in the castrated individual, the peak was not reached until the 4th day. In the normal individual, the peak level was more than three times the normal preinjection baseline. In the castrated subject, the peak reached only the range of a normal adult male. The drop to the preinjection baseline was slower in the castrated male than in the normal one.

These data can be interpreted in the following fashion. In the castrated male, only the injected (exogenous) testosterone is measured, while the testosterone levels in the intact subject reflect the sum of both the endogenous and the exogenous (injected) testosterone. When the curves depicting the testosterone levels in the normal male and in the castrated male are superimposed (Figure 4), one can extrapolate the pattern of changes of endogenous testosterone production in the normal male at the various time intervals after a single testosterone enanthate injection, if an assumption is made that the metabolic and clearance rates are similar in both subjects.

During the first 2 days after the injection, the elevated plasma testosterone levels in the normal subject reflect the sum of testosterone production by the testes and the

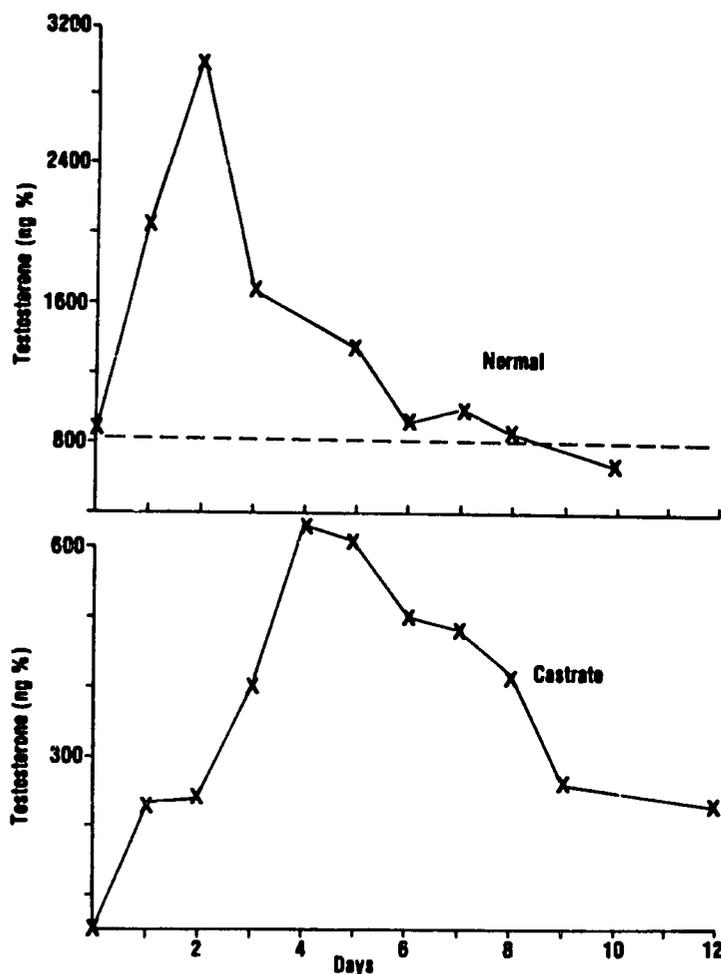


Figure 3. Plasma testosterone levels measured at daily intervals in a normal adult male and a castrated male after a single injection of 200 mg TE.

amounts of testosterone released from the injection site. These elevated testosterone levels suppress LH through a feedback mechanism. The resulting suppression of LH produces a rapid drop in endogenous testosterone. Progressively greater fractions of circulating testosterone are derived from the injection site and less is produced by the testes. By the 9th or 10th day after the injection, the plasma testosterone level drops below the preinjection baseline, and most of the testosterone in circulation is probably derived from the injection site. This change in plasma levels (probably in conjunction with changes in the oscillatory pattern, discussed below) results in restoration of LH production. Gradually, as the levels derived from the administered testosterone decline, the endogenous testosterone production by the testes increases, plasma levels return to normal, and feedback homeostasis is restored.

An interesting aspect of these data is the observation that the plasma testosterone decay curve crosses the preinjection baseline 9 days after injection and drops below the baseline on the 10th day. In our experimental subjects, a 10-day injection interval resulted in continuous suppression of plasma LH, and maintenance of

azoospermia. Thus, this empirically derived schedule coincides with the experimental data, and suggests that maintenance of azoospermia depends on the presence of a testosterone-negative feedback on LH production resulting in suppression of testicular androgen production. The suppression of testosterone production results in diminished intratesticular testosterone levels and cessation of spermatogenesis (10). The data also suggest that a delay in TE injections beyond 10 days will result in reactivation of LH secretions secondary to a further drop in circulating testosterone levels, followed by stimulation of testicular testosterone production, an increase in intratesticular testosterone levels, activation of the spermatogenic process, and reestablishment of sperm production.

A temporal analysis of the relationship between the testosterone injection patterns, plasma hormone levels, and sperm production in subjects who showed breakthrough sperm production seems to support these conclusions. For example, in one of the subjects an induction phase of approximately 7 weeks was associated with elevation of plasma testosterone levels, suppression of plasma LH levels to an undetectable range, and

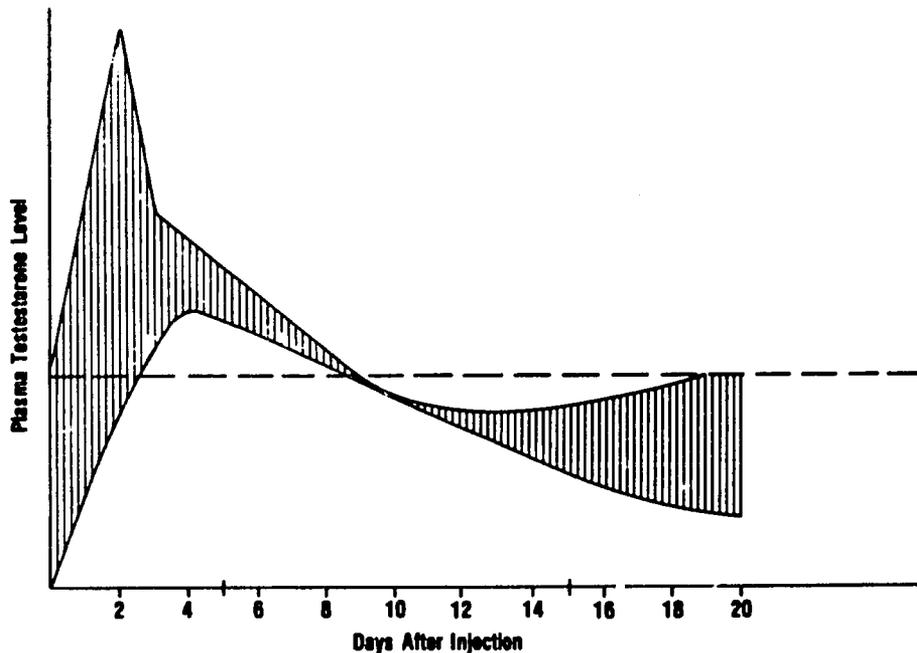


Figure 4. Hypothetical relationship between testosterone levels derived from endogenous and exogenous sources after a single injection of TE in a normal male. The upper line is derived from the data obtained in the normal subject of Figure 3. The lower line is constructed from the data obtained in the castrated male of Figure 3. It is proposed that the shaded area between the two lines represents the contribution of the endogenous testicular testosterone production to the total level in circulation.

suppression of sperm production to azoospermia (Figure 5). A single missed injection resulted in a 26-day interval between injections and was followed by an elevation of LH levels, which in turn was followed in time by an elevation in testosterone levels (despite decreased frequency of TE injections, suggesting resumption of testicular testosterone production) and by the appearance of a substantial number of spermatozoa in the ejaculate several weeks after these hormonal changes.

From the data reported here, and from data reported by other investigators, it is impossible to conclude whether LH synthesis and release is regulated by the absolute integrated levels of circulating testosterone or by other factors, such as the character of plasma testosterone oscillations (amplitude or frequency). If LH is regulated

by the integrated mean level of testosterone in the blood, then the regulatory effect must occur at a relatively narrow range close to the average plasma testosterone levels. On the other hand, it is possible that LH levels are regulated by the oscillations of testosterone levels, either the amplitude of the oscillations, their frequency, or both.

To investigate this possibility, we studied the oscillatory pattern of plasma testosterone levels in a normal subject receiving TE. The measurements were obtained every 2 minutes for 1 hour prior to injection of 200 mg TE (Figure 6) and 3, 7, and 10 days after the injection (Figures 7, 8 and 9). The results obtained during the preinjection period revealed an oscillatory pattern with a mean plasma testosterone level of 829 ± 50 ng% and a coefficient of variation of 30.6%. Coefficient of variation

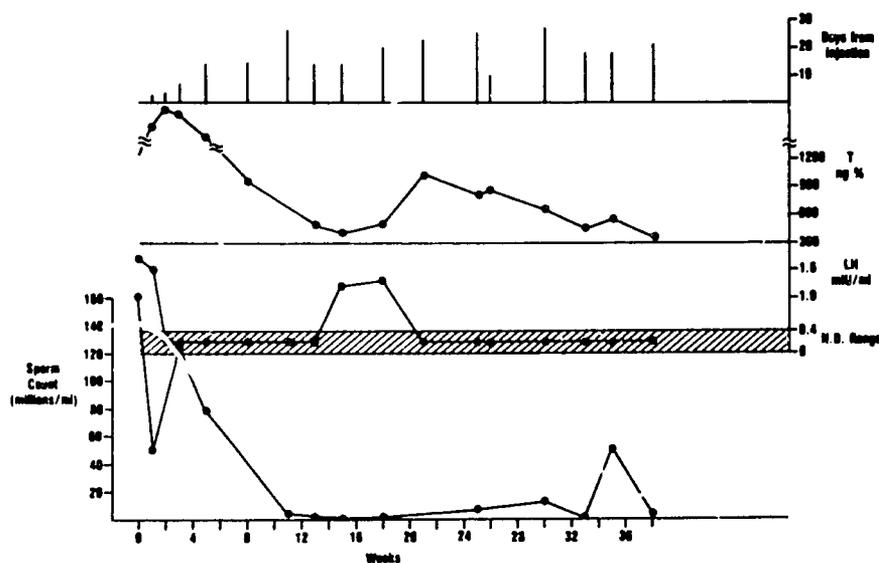


Figure 5. Relationship between sperm counts, LH and testosterone levels, and time intervals between TE injections, in a subject who showed a breakthrough of sperm production during the maintenance phase.

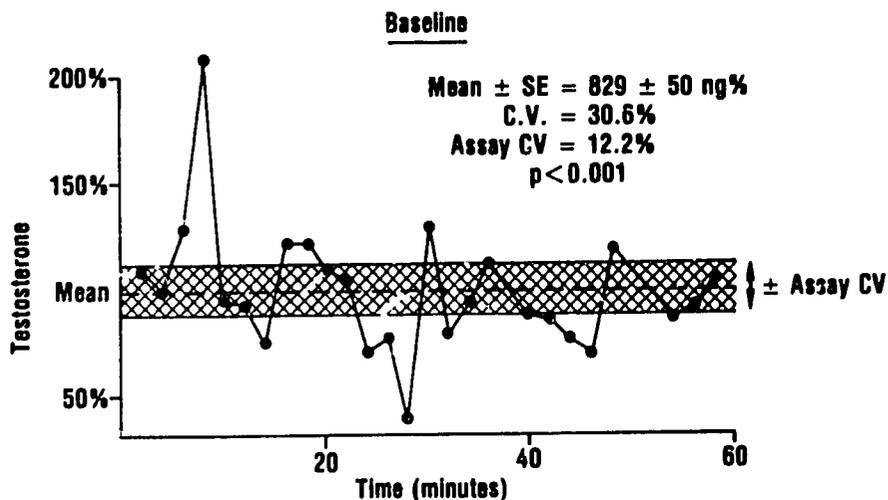


Figure 6. Plasma testosterone levels measured every 2 minutes for 1 hour in a normal adult male. The shaded area represents the confidence limits of the assay.

of the testosterone assay was 12.2%. The variations in plasma levels were significantly greater than would be expected from that of the assay variation ($P < 0.001$).

Three days after the injection the mean plasma level was 1680 ± 66 ng%, with a coefficient of variation of 21.2% (Figure 7). The coefficient of variation was smaller than during the baseline study, but was still significantly different from the variation of the assay ($P < 0.025$).

By the 7th day, the mean testosterone level had declined (1009 ± 40 ng%), and the coefficient of variation (21.2%) was still significantly different from the error of the assay ($P < 0.025$) (Figure 8).

By the 10th day, the mean level had dropped to 663 ± 18 ng% and the coefficient of variation was 14.2%, which was not different from the assay variation (Figure 9). Ten days after the injection, the oscillations were abolished and the plasma levels had dropped from a peak of 1680 ng% 3 days after the injection, to 663 ng%. These measurements, when compared with plasma measurements in the castrated individual, strongly suggest that most of the testosterone measured at this time interval was of exogenous rather than of endogenous origin (Figure 3).

Statistical analysis of the variances at various time intervals shows that there was a significant difference between the baseline and the 3rd and 7th day as well as between the 10th and the 3rd and the 7th days of studies (Table 1). This further demonstrates the progressive flattening of the oscillatory pattern prior to its disappearance by the 10th day. More sophisticated kinetic studies are in order, to establish with greater precision the relationship between the negative feedback mechanism, the plasma testosterone levels, and the pituitary LH. It appears that in a normal adult human male, a single injection of TE produces a suppression of circulating LH levels, followed by a marked decrease in intratesticular testosterone production. This decrease is most

pronounced between the 8th and 10th days after the injection.

The suppression of both LH and endogenous testosterone production is associated with a flattening of the oscillatory pattern of plasma testosterone levels. At the time when the oscillatory pattern is abolished, the testosterone level is close to the individual's pretreatment level and is definitely within normal adult male range for integrated plasma testosterone levels. It is of particular interest that the physiologic and kinetic changes reach their maximum between the 8th and 10th days after the injection. This time interval is similar to the injection interval required for maintenance of azoospermia during chronic treatment with TE.

Other TE Studies

Several groups of investigators have studied TE as a potential contraceptive agent for the human male. Mauss and co-workers administered 250 mg of TE weekly to 7 men for a period of 21 weeks (a total of 22 injections, or 5500 mg TE) (7, 8). Consistent azoospermia did not occur in any of the subjects. In 7 individuals, the sperm counts were less than 5 million/ml and in 4 of the 7 they were below 1 million/ml. During the hormone administration, serum testosterone levels almost doubled, and FSH and LH levels were suppressed. These changes were reversed when the injections were discontinued. During the recovery period, sperm counts gradually returned to pretreatment levels.

Paulsen and co-workers treated a group of 42 men with TE, 200 mg/week, for 6 months (17). Sperm counts below 5 million/ml were found in 39 men, and 20 of these subjects developed azoospermia. It is unclear whether each subject received weekly injections for the entire 6 months, or only until azoospermia appeared. If one assumes that subjects were treated for the entire time period, then each man received a total of 5200 mg of TE. This report was a preliminary summary of data

3 Days after 200 mg Testosterone Enanthate

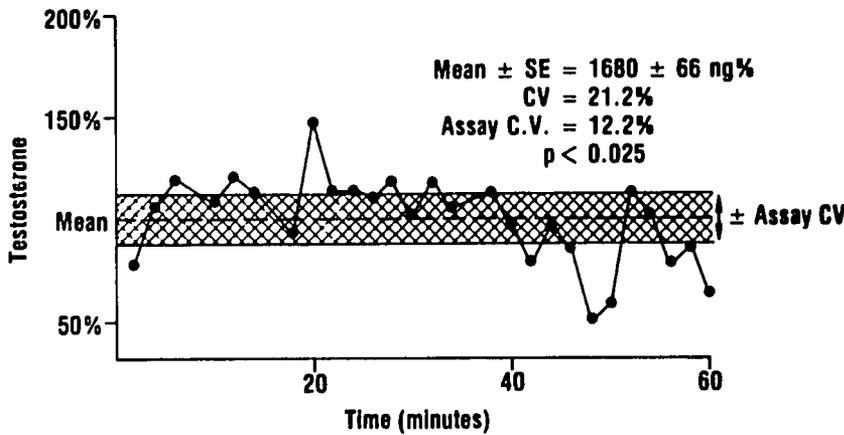


Figure 7. Plasma testosterone levels measured every 2 minutes for 1 hour in a normal subject, 3 days after a single injection of 200 mg TE. The shaded area represents the confidence limits of the assay.

and did not provide complete information on blood hormone levels. Serum testosterone levels appeared to be above control levels in samples obtained 2 days after testosterone administration, and near the baseline 10 days after injection. Sperm production returned after discontinuation of therapy.

Swerdloff and co-workers administered 200 mg of TE weekly for 16 weeks to 17 subjects (31). A total of 3300 mg of TE was administered to each subject. Azoospermia was induced in 10 men. In one man, sperm counts dropped below 0.3 million/ml; in another, sperm counts dropped to between 0.3 and 5 million/ml. In 5 subjects, the sperm counts were not suppressed below 5 million/ml, but an additional 1- to 4-week therapy regimen resulted in suppression below 5 million/ml in 4 of these 5 men. The mean serum testosterone was elevated 64% above the control value, while FSH levels were totally suppressed.

Cunningham and co-workers treated 17 men with 200 mg of TE weekly for 12 weeks (3). Data on 16 subjects

revealed that in 9 men, the sperm counts were below 1 million/ml, in 3 men, sperm counts were above 10 million/ml, and in the other 4 men, sperm counts were between 1 and 10 million/ml. No subject achieved azoospermia. The mean serum testosterone levels were about 50% above control values, while serum FSH and LH were suppressed.

The major difference between the studies reported from our laboratories and those reported by other investigators is that we utilized an induction phase schedule, followed by a maintenance schedule of injections every 10 days, resulting in azoospermia or severe oligospermia (less than 300,000/ml) in all subjects. LH was suppressed to undetectable levels.

While it is difficult to make a comparison between the study from our laboratories, described here, and studies reported by other investigators, one can contrast the total dose of testosterone administered with the degree of suppression of sperm production. The total doses of TE were calculated from the dose schedules reported in the

	Baseline (CV* = 30.6%)	3 days (CV = 21.2%)	7 days (CV = 21.2%)	10 days (CV = 14.2%)
p < 0.001	p < 0.025	p < 0.025	p > 0.1	Assay (CV = 12.2%)
	p < 0.05	p < 0.05	p < 0.001	Baseline (CV = 30.6%)
		p > 0.1	p < 0.025	3 days (CV = 21.2%)
			p > 0.025	7 days (CV = 21.2%)

* CV = Coefficient of variation.

Table 1. Statistical analysis of the variances of plasma testosterone levels at several time intervals after a single injection of 200 mg TE.

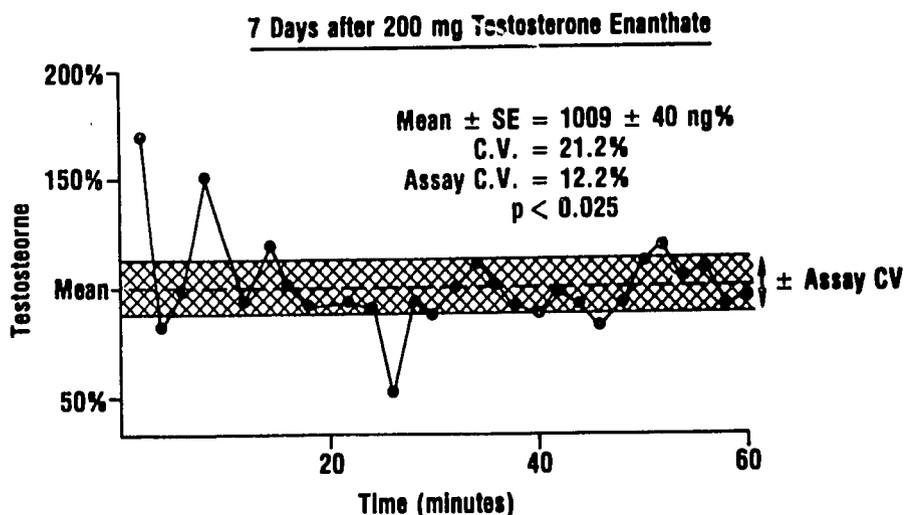


Figure 8. Plasma testosterone levels measured every 2 minutes for 1 hour in a normal subject, 7 days after a single injection of 200 mg TE. The shaded area represents the confidence limits of the assay.

various publications. Utilization of the schedule reported from our laboratory resulted in a more consistent suppression of sperm counts; in addition, the suppression was accomplished at a total dose of TE which was not higher than that used in other studies. Sustained suppression of serum LH occurred in our subjects, despite only brief elevation (2 weeks) of plasma testosterone levels. Since the only major difference in the dose schedules between our studies and those reported by others was the frequent injection schedule during the first 2 weeks of the study, it is possible that this difference was related to the differences in the degree of suppression of sperm production. Data from our laboratory, and data reported by other investigators, clearly demonstrate that administration of 200 mg of TE at time intervals greater than 10 days results in an escape of suppression of sperm production in a significant number of subjects.



During the past 3 decades, the quest for a systemic male contraceptive has resulted in the discovery of a number of organic compounds capable of arresting the spermatogenic process. However, most of these compounds were found to have either mutagenic or carcinogenic properties. At present, the only exceptions to this generalization are the reports from China concerning gossypol. While these reports appear promising, since apparently thousands of men have been treated and the success rate was reported to be better than 95%, with no attendant serious side effects, further studies of gossypol will be needed to confirm these findings. It should be noted, however, that too few systematic investigations of

10 Days after 200 mg Testosterone Enanthate

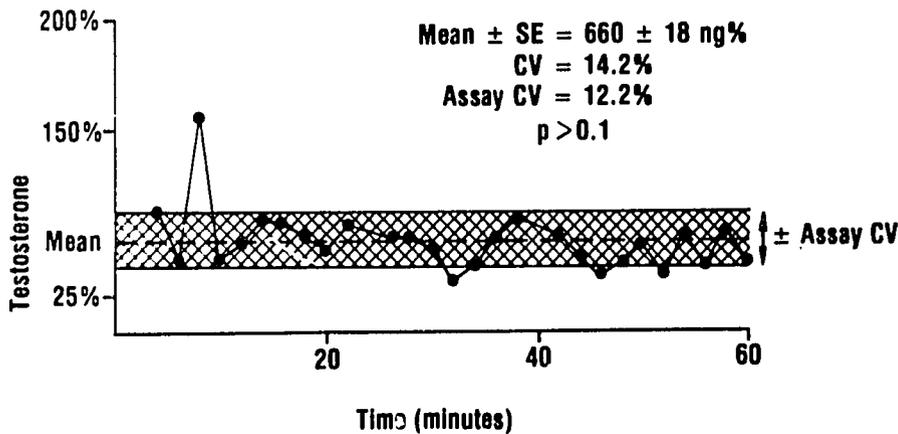


Figure 9. Plasma testosterone levels measured every 2 minutes for 1 hour in a normal subject, 10 days after a single injection of 200 mg TE. The shaded area represents the confidence limits of the assay.

analogues or derivatives of the various organic compounds shown to be effective antispermatogenic agents in lower species have been conducted to warrant the conclusion that none of these classes of organic compounds holds promise for the development of an effective and safe male contraceptive in the human.

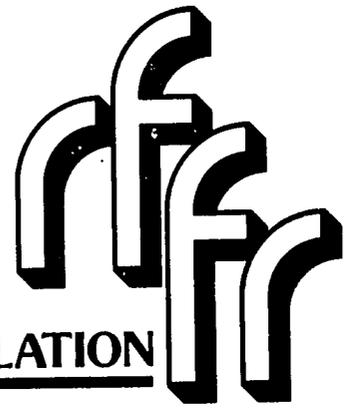
An approach similar to hormonal contraception in the female is available for the male. Effective antispermatogenic doses of testosterone preparations may not necessarily be associated with abnormally high circulating levels and undesirable side effects. Considerable evidence has been presented that administration of testosterone at a proper dose and at an appropriate time interval will induce azoospermia which can be maintained for substantial periods of time, and can be achieved with doses of testosterone which will not result in significant elevation of plasma testosterone levels.

Data have been published which demonstrate that the kinetics of negative feedback may allow the maintenance of LH suppression and spermatogenic arrest in the presence of steady plasma testosterone levels within normal range. The physiologic basis for this approach has been demonstrated. Pharmaceutical studies are now needed that consider the synthesis of an appropriate testosterone preparation which, when orally or parenterally administered, will maintain a uniform plasma testosterone level.

Other avenues, such as combined therapy with testosterone and progestin, also hold promise, once the appropriate progestin, or its analog, is developed. Such progestin must possess no side effects and, in combination with low-dose testosterone, should reduce production of spermatozoa to an essentially azoospermic level.

REFERENCES

1. Coulston F, Beyler AL, Drobeck HP: The biologic actions of a new series of bis(dichloroacetyl)diamines. *Toxicol Appl Pharmacol* 2:715, 1960.
2. Coulston F, Silvestrini B: Reversible inhibition of spermatogenesis in rats and monkeys with a new class of indazole-carboxylic acids. *Proc Simposio Internazionale la Ricerca Scientifica Nell' Industria Farmaceutica*. Roma, October, 1975, p 1045.
3. Cunningham GR, Silverman VE, Thornby J, Kohler PO: The potential for an androgen male contraceptive. *J Clin Endocrinol Metab* 49:520, 1979.
4. Heller CG, Moore DJ, Paulsen CA: Suppression of spermatogenesis and chronic toxicity in men by a new series of bis(dichloroacetyl)diamines. *Toxicol Appl Pharmacol* 3:1, 1961.
5. Landing BH, Goldin A, Noe HA: Testicular lesions in mice following parenteral administration of nitrogen mustard. *Cancer* 2:1075, 1949.
6. Leonard JM, Paulsen CA: Contraceptive development studies for males: Oral and parenteral steroid hormone administration. In Patanelli DJ (ed): *Hormonal Control of Male Fertility*. US Department of Health, Education, and Welfare, Bethesda, Maryland, p 223, DHEW Publ No (NIH) 78-1097, 1978.
7. Mauss J, Borsch G, Bormacher K, Richter E, Leyendecker G, Nocke W: Effect of long-term testosterone oenanthate administration on male reproductive function: clinical evaluation, serum FSH, LH, testosterone and seminal fluid analyses in normal men. *Acta Endocrinol* 78:373, 1975.
8. Mauss J, Borsch G, Richter E, Bormacher K: Investigation on the use of testosterone oenanthate as a male contraceptive agent. *Contraception* 10:281, 1974.
9. Moore CR, Price D: Gonad hormone functions and reciprocal influence between gonads and hypophysis with its bearing on problem of sex hormone antagonism. *Am J Anat* 50:13, 1932.
10. Morse HC, Heller CG: Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. 6th Annual Meeting of the Society for Study of Reproduction, Athens, Georgia, 1973, p 101.
11. National Coordinating Group on Male Antifertility Agents: Gossypol—A new antifertility agent for males. *Chinese Med J* 4:417, 1978.
12. Nelson WO, Bunge RG: Effect of therapeutic dosages of nitrofurantoin (Furadantin) upon spermatogenesis in man. *J Urol* 77:275, 1957.
13. Nelson WO, Steinberger E: The effect of furadroxyl upon the testis of the rat. *Anat Rec* 112:367, 1952.
14. Nelson WO, Steinberger E: Effects of nitrofurantoin compounds on the testis of the rat. *Fed Proc* 12:103, 1953.
15. Nelson WO, Steinberger E, Boccabella A: Recovery of spermatogenesis in rats treated with nitrofurans. *Anat Rec* 118:333, 1954.
16. Patanelli DJ, Nelson WL: A quantitative study of inhibition and recovery of spermatogenesis. *Rec Progr Horm Res* 20:491, 1964.
17. Paulsen CA, Leonard JM, Burgess EC, Ospina LF: Male contraceptive development: re-examination of testosterone enanthate as an effective single entity agent. In Patanelli DJ (ed): *Hormonal Control of Male Fertility*. US Department of Health, Education, and Welfare, Bethesda, Maryland, p 17, DHEW Publ No (NIH) 78-1097, 1978.
18. Prasad MRN, Rajalakshmi M, Gupta G, Dinaker N, Arora R, Karkun T: Epididymal environment and maturation of spermatozoa. In Mancini RE, Martini L (eds): *Male Fertility and Sterility*. Academic Press, London, 1974, p 459.
19. Prasad MRN, Rajalakshmi M, Gupta G, Karkun T: Control of epididymal function. *J Repro Fertl (Suppl)* 18:215, 1973.
20. Scheerer SB: The use of progestins and androgens as a male contraceptive. In Patanelli DJ (ed): *Hormonal Control of Male Fertility*. U.S. Department of Health, Education, and Welfare, Bethesda, Maryland, p 257, DHEW Publ No (NIH) 78-1097, 1978.
21. Skoglund RD, Paulsen CA: Danazol-testosterone combination; a potentially effective means for reversible male contraception. A preliminary report. *Contraception* 7:357, 1973.
22. Steinberger E: Recent advances in regulation of male fertility. Ninth Harold G. Mack Symposium, Detroit, October, 1973.
23. Steinberger E: Recent advances in regulation of male fertility. In Moghissi KS, Evans T (eds): *Regulation of Human Fertility*. Detroit, Wayne State University Press, 1976, p 274.
24. Steinberger E, Boccabella A, Nelson WO: Cytotoxic effects of 5-chlor-2-acetyl thiophen (Ba 11044) on the testis of the rat. *Anat Rec* 125:312, 1956.
25. Steinberger E, Smith KD: Effect of chronic administration of testosterone enanthate on sperm production and plasma testosterone, follicle-stimulating hormone, and luteinizing hormone levels: a preliminary evaluation of a possible male contraceptive. *Fertil Steril* 28:1320, 1977.
26. Steinberger E, Smith KD: Suppression and recovery of sperm production subsequent to administration of testosterone enanthate. Morphologic and hormonal evaluation. In Patanelli DJ (ed): *Hormonal Control of Male Fertility*. US Department of Health, Education, and Welfare, Bethesda, Maryland, p 195, DHEW Publ No (NIH) 78-1097, 1978.
27. Steinberger E, Smith KD: Testosterone enanthate. A possible reversible male contraceptive. *Contraception* 16:261, 1977.
28. Steinberger E, Smith KD, Rodriguez-Rigau LJ: Suppression and recovery of sperm production in men treated with testosterone enanthate for one year. A study of a possible reversible male contraceptive. *Int J Androl (Suppl)* 2:748, 1978.
29. Steinberger E, Steinberger A, Sanborn BM: Molecular mechanisms concerned with hormonal control of the seminiferous epithelium. In Fabbrini A, Steinberger E (eds): *Recent Progress in Andrology*. Proceedings of the Sero Symposium, Vol. 14. London, Academic Press, 1978, p 143.
30. Swerdloff RS, Campfield LA, Palacios A, McClure RD: Suppression of human spermatogenesis by depot androgen: potential for male contraception. *J Steroid Biochem* 11:663, 1979.
31. Swerdloff RS, Palacios A, McClure RD, Campfield LA, Brosman SA: Male contraception: clinical assessment of chronic administration of testosterone enanthate. *Int J Androl (Suppl)* 2:731, 1978.



RESEARCH FRONTIERS IN FERTILITY REGULATION

INHIBITION OF PROGESTATIONAL ACTIVITY FOR FERTILITY REGULATION

Robert T. Chatterton, Ph.D.
*Professor, Department of Obstetrics and Gynecology
Northwestern University Medical School
Chicago, Illinois*

This review will describe some of the areas of current research on postovulatory contragestational techniques, and will mention certain factors that must be considered in the evaluation of potential new contragestational agents. It is not an exhaustive review; rather, it focuses upon some of the more promising new antiproggestational agents.

This is becoming one of the most important areas of research in the field of fertility regulation. The development of a safe and effective early postconceptive method is highly desirable, and holds considerable promise for providing control of fertility with relatively few untoward side-effects. Such a method could be self-administered; it would not require physician intervention; it would be effective on a postcoital or hindsight basis; it would not require high levels of motivation; and supply and distribution problems would be minimal.

POINTS OF ATTACK

Pregnancy depends on the availability of progesterone to the uterus; withdrawal of progesterone results in breakdown of the secretory endometrium. After implantation, the decidua which develops from the endometrial stroma is shed, along with the embryo, in the absence of sufficient progesterone. Progesterone is also required to maintain quiescence of the myometrium; a deficiency results in an increase in the amplitude of spontaneous contractions and a greater sensitivity of the uterus to oxytocin and prostaglandins. Progesterone has also been shown to diminish immunological recognition of histocompatibility antigens; this action of progesterone may serve to prevent cell-mediated rejection of fetal tissues, which are derived in part from the paternal genotype.

Availability of progesterone may be interfered with at sev-

eral levels (*Figure 1*). Pregnancy may be terminated at an early stage by substances that inhibit progesterone biosynthesis; increase the clearance of progesterone from the blood; compete with progesterone for receptors in the uterus; or indirectly oppose the action of progesterone on the myometrium.

Some advances have been made in recent years in all of these areas, however evaluation of new methods of progesterone inhibition is not a simple process. Many aspects of the action of new substances must be considered, in order to select one that has a low failure rate, while at the same time producing minimal residual and side effects. In addition to its contragestational activity, each compound must be tested for its uterotrophic, androgenic, and glucocorticoid activities, and for antagonism of the biologic effects of estradiol, testosterone, and progesterone. Since many compounds that inhibit the effects of progesterone have opposing activities of their own, it is important that these compounds be tested for possible defeminizing properties and for some of the deleterious side-effects of estrogens, such as the tendency to increase blood pressure and clotting.

During the first trimester of pregnancy, when the contribution of the ovary is essential, and while the ovary depends on gonadotropins for progesterone secretion, disruption of pregnancy may be achieved in several ways:

- 1) Binding of gonadotropins to their receptors may be interfered with by compounds that compete for binding or cause a loss of available receptors (down-regulation).
- 2) The biosynthesis of progesterone in the corpus luteum may be interfered with by inhibitors of steroidogenesis or of conversion of pregnenolone to progesterone.

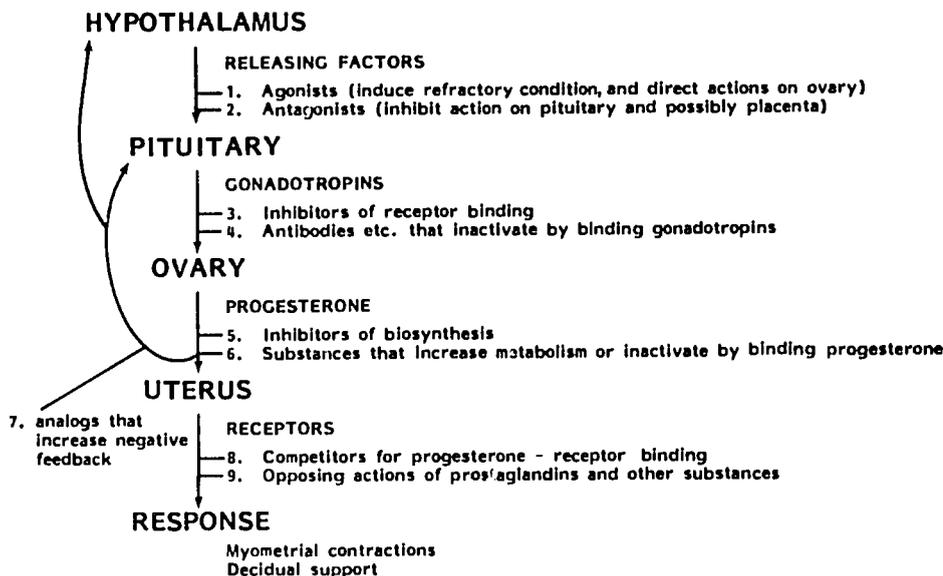


Figure 1. Points of interference with progesterone actions.

- 3) Progesterone may be removed from the circulation before it reaches its target organs, by increasing its metabolism or clearance from the body.
- 4) Progesterone may be interfered with at the cellular level by inhibitors that compete for receptor binding, and in this way progesterone is unable to exert its effects on its target cells.
- 5) Progesterone may be interfered with by substances or compounds that exert opposing actions. Typically, the substance would be an estrogen or prostaglandin, but compounds with fewer side-effects and greater specificity are being sought.

FACTORS IN TESTING CONTRAGESTATIONAL AGENTS

The following section will touch upon some of the problems associated with carrying out the evaluation and testing of new contragestational agents, providing an overview of the procedures and citing selected examples. A more detailed consideration of individual compounds will be presented in a later section.

Assessment of biological activities

With respect to the utility of a given animal species to serve as a model for studying the effects of candidate substances in the human, no single species is adequate to demonstrate effectively the quantitative relationships between dose and response, or even the relative potencies of the substances for the several biological effects being examined. Small laboratory animals probably should be used only to gain information about which organs are affected by a substance, and eventually about the molecular biology of the response. Laboratory animals can be used to provide information about the specificity and the mechanism of action of a compound. For example, is the compound a

competitive inhibitor of progesterone in the uterus or an inhibitor of steroid biosynthesis?

The irrelevance of extrapolating quantitative drug responses among species is obvious when one considers the extreme sensitivity of the rat to estrogens, an effect that has been related to the levels of circulating estradiol. At the preovulatory period, estradiol levels in the rat are only one-tenth those of women. The hamster is probably a better animal model for studying estrogen-related effects.

Predicting responses to pharmacologically modified steroids may be particularly difficult because, in addition to prolonging the half-life of the compound, structural modifications may result in altered binding to receptor molecules. There is evidence that the synthetic steroids produce different receptor complexes (1) and exert effects that may be intermediate between, or different from, those of any of the natural steroids. The way that an animal of any given species responds to a modified compound may differ significantly from the responses of other species; for example, the response to medroxyprogesterone acetate in rabbits differs from that of guinea pigs (24).

Dose requirements and mode of action

After the biological characteristics of the contragestational steroid are established, the minimal effective dose, or the dose to which 50% of the test animals respond (ED_{50}) should be established, along with the dose that is lethal to 50% of the animals (LD_{50}). Further elucidation of the time of pregnancy (rat) during which the substance is most effective provides information about whether the substance acts to interfere with the reproductive process before implantation, during implantation, while the pituitary is still required for gestation, or later.

If concomitant administration of progesterone, in doses that sustain pregnancy in the ovariectomized animal, is

capable of overcoming the contragestational activity, the substance may be interfering with the biosynthesis of progesterone. This interference may occur either by blocking the action of luteotropic hormones, or by inhibiting an enzyme in the biosynthesis of progesterone. Assays of serum progesterone will assist in the interpretation of the effects. LH assays will not be of value in pregnancy. Prolactin assays may be useful if the extreme diurnal variation is taken into account (59), but the applicability of this information to the human is questionable. In primates, a useful procedure for evaluation of luteolytic factors is determination of the ability of the substance to shorten the luteal phase of the menstrual cycle with and without administration of human chorionic gonadotropin (hCG). Not all compounds that interfere with corpus luteum function appear to be able to overcome the sustaining effect of hCG, as exemplified by studies of oxymetholone (32) and LH-RH (11).

If the substance being tested acts during implantation and thereafter, and is not associated with a decrease in serum progesterone, or if the action of the substance is not reversed by doses of progesterone that normally maintain pregnancy, a competitive inhibitor may be involved that prevents the progesterone from exerting its effects on maintenance of the decidua or on myometrial quiescence. However, the substance could also act as an estrogen to promote excitability of the myometrium, or it could stimulate synthesis of prostaglandins (or inhibit their catabolism), which would stimulate uterine contractions.

The rat is not the best model for such studies, because it does not respond to progesterone withdrawal or to prostaglandins with expulsion of the conceptus, as is the response in the human. In the rat, fetuses are resorbed, a process that takes about 4 days. Fetal death in the rat after progesterone withdrawal or prostaglandin administration in fact may be the result of uterine contractions, but the fetuses are retained.

The effects of progesterone withdrawal and prostaglandin administration can be most readily monitored in the rabbit or guinea pig, respectively, as these species have sufficiently pliable cervixes to allow expulsion of the fetuses during pregnancy. The guinea pig may be particularly useful for studying direct actions of prostaglandins on the uterus, since there is only a short span, from days 12 to 28 of the 60-day gestation period, during which the influence of the ovary is required to maintain pregnancy. An abortifacient action that is independent of the ovary can be readily studied either during the first 12 days or after midgestation. Conversely, the guinea pig is not a good model for studying actions of substances that decrease progesterone secretion, because in this species, suppression of uterine contractility is largely reliant upon relaxin and does not depend upon the influence of progesterone.

By contrast, in the rabbit, rat, mouse, and hamster,

maintenance of pregnancy depends upon ovarian progesterone until very late in gestation. None of these species may be considered good models for studies of prostaglandins as contragestational substances, however, because their ovaries are much more sensitive than are human ovaries to the luteolytic activity of prostaglandins. Inferences about contragestational substances, relative to the phase of human gestation in which pregnancy is dependent upon the ovary as a source of progesterone, can probably be made only from studies using other primates, particularly if the substance is known to have a direct action on the ovary.

Duration of effect

The next consideration in the testing of a contragestational substance is the duration of the effect. The plasma half-life ($T_{1/2}$) can be calculated in several animal species using the radioactively labeled compound. Obviously, a compound with a very short half-life will not be effective unless it is administered frequently. The half-life of a steroid may be improved in some cases by adding an ethinyl group, for example. Lan and Katzenellenbogen (38) have shown that, by the addition of an ethinyl group, estriol is made as active as estradiol-17 β in the assay in which uterine dry weight is the end-point. In evaluating the half-life of a compound that causes a decrease in serum progesterone, assessing the concentration of unbound (free) progesterone may be important. It is particularly important to measure the free steroid, when the possibility exists that the treatment may alter the serum concentration of corticosteroid-binding globulin (CBG), a property common to compounds possessing estrogenic activity. In the case of agents that act by causing a decrease in serum progesterone, it is apparent from several studies that for effectiveness, the duration of depletion of the progesterone is just as important as the degree of depletion.

The active form of the substance being tested is often not the form that is administered. Esters and even ethers are frequently cleaved *in vivo*, to give the form of the compound that is capable of binding to a receptor protein in a target cell. For example, the methoxy group of mestranol must be cleaved to a hydroxyl to produce ethinyl estradiol before it will become capable of exerting its effect. In studying properties of the contragestational agent, knowing the form of the compound that is active at the cellular level may be important; implants of the compound may be completely inactive if placed in a target tissue that does not have the enzymes necessary for conversion of the compound to its active form.

Route of administration

The active form and the half-life of the compound are considerations in establishing the optimal route of administration and the most appropriate vehicle in which to give the compound. The oral route may be most desirable to

the patient, but if the compound is metabolized to inactive products by the flora of the intestine, or is poorly absorbed or rapidly metabolized by the liver, using this route may not be feasible.

Drug tolerance and side-effects

Development of resistance to the medication: is also obviously an important deterrent to continued use, especially with substances that must be administered frequently. Polypeptides, especially those with molecular weights greater than 10,000, can be immunogenic. The developing antibody titer against the substance will not only inactivate it, but the recipient could experience an anaphylactic reaction to the material.

The body may develop other kinds of compensatory reactions which may decrease the effectiveness of the administered compounds. An increase in the patient's tolerance to some drugs may involve the development of mixed function oxidases in the liver that inactivate the drug as part of the general detoxification process.

Side-effects of the contragestational drugs are most frequently related to vascular, gastrointestinal, and psychogenic actions. Certainly, organ weights and careful autopsies should be obtained in conjunction with toxicity studies.

For consideration of specific agents, contragestational substances have been divided into two groups: 1) those substances that act in some way to decrease the concentration of the progesterone that reaches the uterus; and 2) those substances that either directly antagonize the progesterone at the receptor site, or indirectly oppose the action of progesterone.

SUBSTANCES THAT INHIBIT PROGESTERONE SECRETION OR PROMOTE ITS CLEARANCE

Failure to maintain progesterone in the blood at levels required for maintenance of early pregnancy may be caused by a decrease in progesterone secretion by the ovary, or by an increased rate of metabolism and excretion of circulating progesterone. When the mechanism of action of a drug is well understood, there is no ambiguity about the cause of progesterone withdrawal, as detected by a decrease in the serum concentration of the hormone. However, even when a compound is known to decrease the rate of secretion of progesterone, the process by which it does this may not be known.

Prostaglandins

Although there is considerable evidence that prostaglandins (PG) produced by the uterus or administered to sheep or rats are luteolytic (46), that is, they bring about regression of the corpus luteum, there is little evidence that they

have a similar effect in the human, at least not with doses of prostaglandins that can be tolerated. In some studies, transient decreases in progesterone have been observed, when $\text{PGF}_{2\alpha}$ was given during the luteal phase of the menstrual cycle, but the extension of the menstrual cycle caused by injection of hCG was not abolished by $\text{PGF}_{2\alpha}$ administration (2). Serum 17-hydroxyprogesterone, which is indicative of ovarian secretion, decreased before serum progesterone levels declined, when women were given $\text{PGF}_{2\alpha}$ in early pregnancy. Nevertheless, some derivatives of the prostaglandins may have selective effect on the ovary. McCracken, Einer-Jensen, and Fried concluded that some 13-dehydro analogs of $\text{PGF}_{2\alpha}$ have very weak smooth muscle-stimulating activity, but are potent luteolytic agents in both the sheep and monkey (47). If these compounds can be shown to prevent progesterone secretion by the human ovary in the presence of hCG, they may be effective in menstrual induction, without causing the gastrointestinal side effects typical of the primary natural prostaglandins. However, altering the dosage and route of administration may also reduce the vomiting and diarrhea that are common side-effects of some of the analogs that apparently act on the myometrium.

The 15-methyl $\text{PGF}_{2\alpha}$ methyl ester is effective in 68% to 97% of cases, with an "acceptable" degree of side-effects, when given intravaginally or by intrauterine instillation (10), but gastrointestinal effects are reduced to a greater extent by 16,16-dimethyl PGE_2 , and even more by 16,16-dimethyl-*trans*- Δ^2 - PGE_1 methyl ester and 16-phenoxy- ω -tetranor- PGE_2 methylsulphonylamide, without a loss of effectiveness (10).

The primary, if not exclusive, action of the prostaglandins that have been tested clinically appears to be the effect of the prostaglandins on myometrial contraction, since doses that fail to affect ovarian function result in abortion. Continued tonic contraction of the myometrium may result in ischemia, which deprives the conceptus of essential nutrients and oxygen. In this respect, the prostaglandins used as contragestational agents should be classified as indirect antagonists of progesterone. Nevertheless, it is possible that local concentrations in the ovary are elevated to luteolytic levels. Prostaglandin levels may be increased locally in the ovary by analogs that have a particular affinity for the ovary, by substances that stimulate ovarian PG production, or by substances, such as the triazole compounds that are considered later, that inhibit PG catabolism.

LRF agonists

Interestingly, luteinizing hormone releasing hormone (LRF), and particularly its more potent agonists (11), as well as antibodies to LRF (36), are both capable of interfering with normal ovarian function in somewhat similar ways. The LRF "super" agonists can inhibit steroid produc-

tion by the ovary of hypophysectomized rats (45) and by luteal cells *in vitro* (16); however, apparently by interfering with binding of LH to its receptor, the agonists also induce a refractory state in which the pituitary *in situ* fails to produce LH after an initial response (18). Casper and associates studied the contragestational properties of the LRF agonist [D-Trp⁶,Pro¹⁰NEt]-LRF (11). This peptide, (LRF-Ag), is 144 times more potent in stimulating LH and FSH release than is natural LRF, because of its resistance to enzymatic degradation, and its increased uptake and retention by pituitary tissue. It was tested by the subcutaneous administration of 50 μ g of LRF on 2 successive days during the luteal phase of the menstrual cycle in 5 normal women. Luteolysis, judged by serum progesterone and onset of menses, was hastened in 17 of 28 cycles. The LRF agonist was effective when given 6 or more days from the LH surge, in agreement with other studies (12, 41). Unfortunately, the luteolytic effect was completely overcome in the presence of hCG at levels of 85 mIU/ml or less. When tested in women who were less than 8 weeks pregnant, LRF was also not effective (11). It would seem, at least with the agonists that have been tested so far, that LRF analogs do not offer a means of inducing menstruation in the presence of hCG. Other applications for LRF agonists, such as inhibition of ovulation by administration as a nasal spray, have shown promise, however (5).

Immunization against hCG

The immunological approach has been used to prevent rescue of the corpus luteum after conception. Immunization of the female against hCG would appear to be the most successful of the immunological methods. It has the advantage over methods that utilize antigens associated with ova (58) or sperm (29) in that humeral antibodies are available to combine with an antigen that passes through the blood. To combine with the fertilized ovum or sperm, the antibodies must be secreted into the lumen of the reproductive tract. Antibodies directed against antigens characteristic of the ovum may interfere with the development or even the survival of primary oocytes in the ovary, and thus bring about permanent sterilization. Another risk is that the induction of antibodies to antigens from ova or other specific cell types may result in development of an autoimmune disease that is not restricted to the particular cell or tissue that was the origin of the antigen. A third danger is that immune complexes deposited in the kidney can cause kidney failure. In the case of hCG, this problem is minimal, although it is still a concern, since many normal cells apparently produce very small amounts of this glycoprotein (15). Only the syncytiotrophoblast produces amounts having biological significance, however.

Active immunization with hCG has been studied extensively by Talwar and associates in New Delhi (63), and by Stevens in Columbus, Ohio (62). In a study of baboons actively immunized with partially purified baboon

chorionic gonadotropin (bCG), Stevens found that pregnancies were not sustained. Evidently, the bCG reaching the ovary was insufficient to promote the necessary steroid production. Curiously, when a highly purified bCG was used as the antigen, no effect on fertility was observed. One explanation is that the large amount of bCG secreted by the trophoblast in early pregnancy was more than sufficient to neutralize the circulating antibody. High titers of antibody are obviously required.

Specificity of the antigen is important to eliminate cross-reaction with LH. If the method is to be practicable, pituitary LH should not be neutralized. This is important from two points of view. First, LH is a "self" antigen produced constantly, albeit at different rates, and antigen-antibody complexes may become deposited in the kidney and vasculature if antibody titers remain elevated. Second, normal menstrual cycles probably would not continue, and reversal of the sterilizing effect may be impossible. Ideally, the active immunization procedure would be reversible, if progesterone were provided to maintain pregnancy once conception had occurred until the placenta had begun to produce sufficient progesterone. To allow for normal menstrual cycles and potentially, conception, baboons (61) and women (64) have been immunized with the β -subunit of hCG. In some cases, animals have been immunized with only the C-terminal 45 amino acid residues of the β -subunit, in order to produce antisera that are specific for the part of hCG that is most distinct from hLH (23). Methods using the hCG fragment conjugated to tetanus toxoid, or methods using Freund's adjuvant, have produced highly specific antisera. So far, successful contragestational immunizations have not been achieved in the few women who have been treated, but the β -hCG immunization has been successful in baboons (61).

Lithospermic acid

Other methods for interfering with gonadotropin support of the ovary have some potential as well, although none has been studied sufficiently to characterize its effectiveness, particular benefits, or risks. One such substance is extracted from *Lithospermum ruderale*, and has been used as a "tea" by Indians of the Shoshone tribe to reduce fertility (26). In rats and chicks, cold water extracts, especially those allowed to oxidize in the crude extract, have caused a reduction in gonadotropic effects. When studying the dose response of extracts in the chick, Breneman and Zeller found that with increasing doses, the pituitary content of gonadotropin increased, but at the highest dose of the extract, the pituitary content was reduced to levels below those seen in control animals (8). This was interpreted as inhibition of gonadotropin release with suppression of gonadotropin synthesis at the highest doses. Cold water extracts of *L. ruderale* also impaired development of gonads and accessory sex organs of the immature rat (26).

Such extracts can also inhibit the ovarian and uterine

weight increases caused by pregnant mare serum gonadotropin. The extract may act by inhibiting gonadotropin binding or by direct effects on the ovary. Either the active principle has effects on both the pituitary and the ovary, or it acts differently in the rat and the chick. It does not, however, compete with steroids for their receptors (25). Further study is required to distinguish between the alternatives. A polyphenol that has been named lithospermic acid has been isolated and chemically described (35). An oxidation product of lithospermic acid apparently is the active substance in plant extracts, but more work must be done on the structure of the biologically active form before a chemically pure substance will be available for pharmacologic studies.

Pineal peptides

Another substance that acts to suppress pituitary gonadotropin secretion was purified as a low molecular weight polypeptide from the pineal gland (51). At least two different peptides have been separated from melatonin and are capable of blocking the compensatory ovarian hypertrophy after hemiovariectomy. The preovulatory LH surge and ovulation can also be suppressed by these substances (52), and there is some evidence for postovulatory suppression of ovarian function as well. Perhaps a peptide substance that directly inhibits pituitary gonadotropin secretion rather than acting indirectly, as LRF does, could be identified with a minimum expenditure of time.

Inhibitors of ovarian steroid synthesis

Aminoglutethimide. The other types of compounds that act by interfering with progesterone secretion are those that inhibit steroidogenesis in the ovary and placenta. Aminoglutethimide is a compound that blocks the conversion of cholesterol to pregnenolone. Glasser and co-workers found that aminoglutethimide phosphate, when given to rats as a single intraperitoneal injection (150 mg/kg) after implantation, caused abortion in only 25% of the animals, although plasma progesterone was depressed by 80% within 30 minutes (28). Recovery of normal

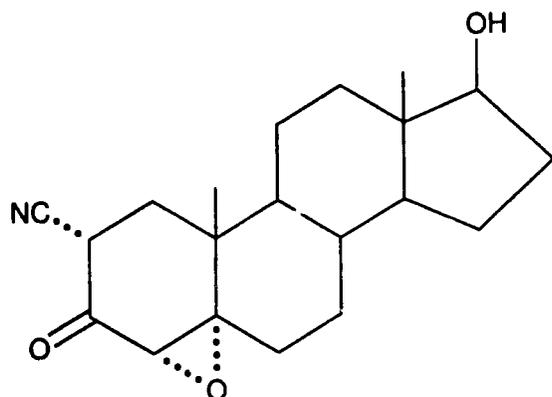


Figure 2. Trilostane

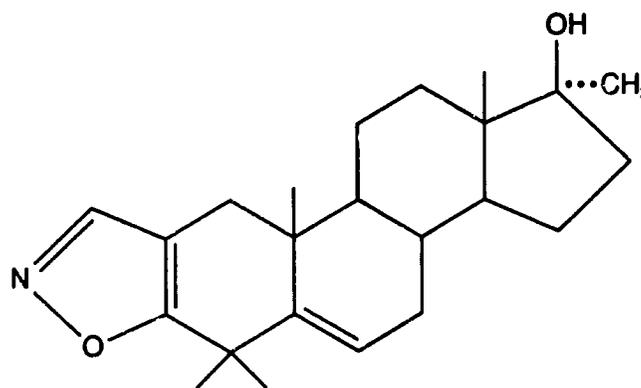


Figure 3. Azastene

plasma progesterone occurred within 48 hours. However, when three injections of the same dose were given, abortion occurred in all rats within 72 hours. This effect could be blocked by the administration of 5 mg of progesterone/rat/day, but LH, hCG, prolactin, and 20 α -dihydroprogesterone did not block the effect. The importance of both the degree and duration of progesterone depletion for interruption of pregnancy is well illustrated in this study.

In another study of aminoglutethimide in baboons, a similar effect was noted. The drug was given for 1 to 3 days to 6 baboons that were pregnant for 31 to 99 days. Serum progesterone was reduced to as little as 3.2% of the initial concentration in one animal, and to less than 20% of initial concentration in 4 of 5 of the remaining baboons, but pregnancy continued for at least 3 weeks. The fact that peripheral blood levels may not reflect the concentration at the uteroplacental junction was emphasized, particularly if the inhibitor is more effective in inhibiting ovarian than placental progesterone biosynthesis. Here, also, the duration of depletion must be considered as well.

Csapo and Erdos showed that after administration of anti-progesterone antiserum (APA) to rats, the effect could still be blocked if progesterone was given 3 hours later, but that the progesterone depletion induced irreversible changes in most animals by 6 hours, with the result that progesterone administration could not rescue the pregnancy (22). Unfortunately, the dose of aminoglutethimide could not be increased because of the CNS-depressing activity of the drug. Attempts to modify the structure to obtain greater specificity for inhibition of steroidogenesis have not been successful, but the congeners that have been tested by Glasser and colleagues are only those with modifications of the piperidine moiety (27). Aminoglutethimide at relatively low doses greatly enhanced the abortifacient activity of PGF_{2 α} in the rat, however (27), a finding that may be worthy of further investigation.

Oxymetholone. Oxymetholone was one of several compounds originally tested as substances that might increase progesterone clearance by increasing its metabolism (7).

The drug successfully shortened the luteal phase of the menstrual cycle, but this shortening apparently was achieved by an inhibition of progesterone biosynthesis. Oxymetholone has also been used as an anabolic steroid, and treatment of women after ovulation suppressed serum progesterone by 50% to 80%, and shortened the cycle by 6 to 8 days (19). The drug did not interfere with luteal function in women who also received hCG (4), nor did it terminate pregnancy when given for 7 to 10 days in cumulative doses of 250 to 3000 mg (9). This experience emphasizes the importance of testing contragestational drugs initially in the presence of hCG, if their mechanism is luteolysis, before testing their abortifacient activity in pregnant women.

Trilostane and azastene. Trilostane and azastene (Figures 2 and 3) are competitive inhibitors of 3β -hydroxysteroid dehydrogenase (HSD) (55). Inhibition of this enzyme presumably will block conversion of dehydroepiandrosterone to androstenedione as well as of pregnenolone to progesterone. Since conversion of pregnenolone to progesterone occurs in the adrenal as well as in the ovary and placenta, production of steroids, including cortisol and aldosterone, that succeed progesterone in the metabolic pathways of these organs, will be decreased. Some organ-specificity of these inhibitors, however, has been observed. Trilostane appears to be primarily a suppressant of adrenal steroidogenesis. Given orally to rats, it inhibits corticosterone and aldosterone production, and elevates circulating levels of pregnenolone at doses that are lower than those that produce adrenal hypertrophy or that inhibit gonadal steroidogenesis (57). In the monkey, also, serum cortisol is decreased at doses that are less than those necessary to terminate pregnancy.

Azastene appears to be equipotent in inhibiting the adrenal and ovary of the rat. However, Azastene produced a luteolytic effect and terminated pregnancy in the monkey at 500 to 1000 mg/day for 5 days, even though no effect on corticoid production was detectable (56).

Concurrent progesterone administration prevents the abortifacient action as expected. Schane and Creange

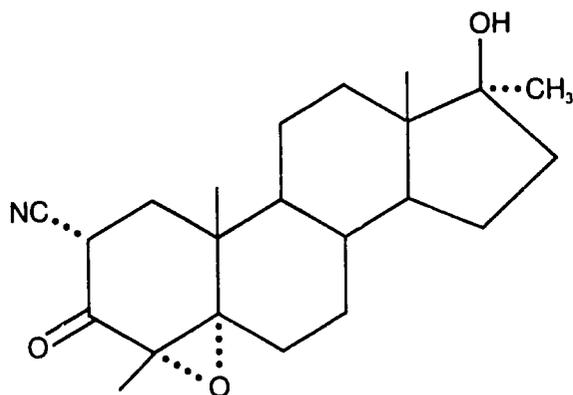


Figure 4. Win 32,729

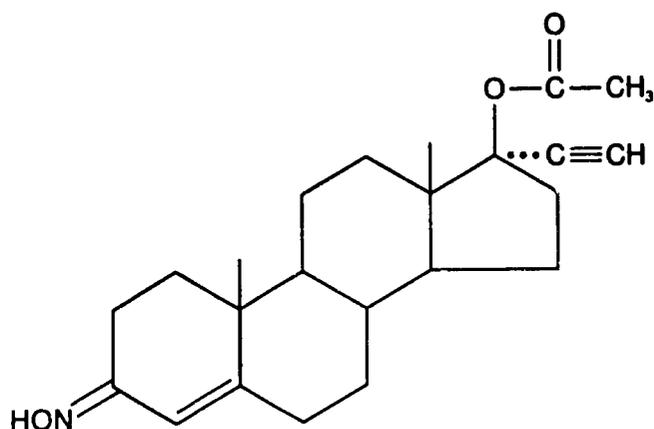


Figure 5. 17β-acetoxy-17α-ethinyl-4-androstene-3-one-(3-oxime) (ORF 9371)

used an interesting technique to distinguish between primary effects of this and other HSD inhibitors on progesterone secretion from secondary effects that decrease progesterone only as a consequence of disrupted placental circulation (55). They have administered a synthetic progestogen such as Provera (which can be distinguished from progesterone in the radioimmunoassay) along with an HSD inhibitor, to maintain pregnancy. Decreases in serum progesterone, other than those that can be attributed to Provera alone, must be due to the effect of the inhibitor on steroidogenesis. By this criterion, the HSD inhibitors are directly inhibitory to progesterone biosynthesis.

The undoing of Azastene, the recently most promising HSD inhibitor, is the by-now-familiar inability of the drug, in the presence of hCG, to induce luteolysis in women. The discrepancy between the effectiveness of Azastene in the pregnant monkey and in the human is puzzling. Perhaps the drug is metabolized differently. It may be inactivated more rapidly in the human, or an active metabolite may be produced more efficiently in the monkey.

Currently, investigators at Sterling-Winthrop have focused their attention on Win 32,729 (Figure 4), a related steroidal compound that is more potent than Azastene in the monkey by a factor of 10. Its interceptive activity is also prevented by progesterone, and cortisol is suppressed in the monkey when only 5 times the effective abortifacient dose is used.

Danazol. A compound that has found use in endometriosis, because of its activity in suppressing steroid-mediated actions on the uterus, is Danazol. This compound is related to ethinyl testosterone, by the addition of an isoxazole ring. It not only causes suppression of FSH and LH secretion, but it apparently also decreases progesterone secretion by a direct effect on the ovary. In addition, it binds to progesterone and androgen receptors, antagonizing the actions of these hormones. Postovulatory treatment causes a se-

vere depression in serum progesterone levels and shortens the luteal phase to 10 days; hCG treatment does not bring about recovery of luteal function (3). Thus, Danazol has real potential for occasional use, but the daily dose required for contraceptive protection is minimally 200 mg (17), a dose of steroid that would certainly burden liver detoxification ability.

Passive immunization against progesterone

The last of the progesterone-suppressing methods is one that we have been investigating at Northwestern University. This is still in the developmental stage, but in principle what we are attempting to do is to prepare a specific, high-affinity absorbent that will remove a sufficient amount of progesterone from the body to cause involution of the endometrium and promote contractility of the myometrium. To this end, we have produced a large volume of antiprogesterone antiserum (APA) that is capable of inducing abortion in rats. This approach was reported by Csapo and associates (21) and Raziano, Ferin, and Vande Wiele (54) previously.

Although the concentrations of free progesterone measured in serum of these rats by the equilibrium dialysis method are not detectably different, progesterone is decreased in uterine tissue within 36 hours. In the interim, the APA injection causes a surge of LH and FSH release and a transient increase in concentrations of progesterone in the ovary, uterus, and other tissues (14).

Whether this burst of gonadotropin activity, presumably induced by an initial depletion of progesterone in the pituitary, is an obligatory component of the subsequent contragestational decline in progesterone is not known. In any event, the dose of APA which was sufficient to bind 6 μg of progesterone was sufficient to bring about an eventual depletion of progesterone, and abortion.

The next step toward development of a practical application of this absorbent is to encapsulate APA in a manner that prevents its absorption into blood, but permits entry of progesterone into the microcapsules to bind to the entrapped APA. Ideally, such microcapsules would be effective

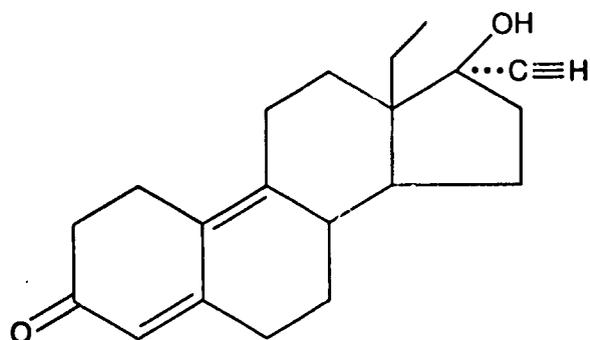


Figure 6. Norgestrienone (R2323)

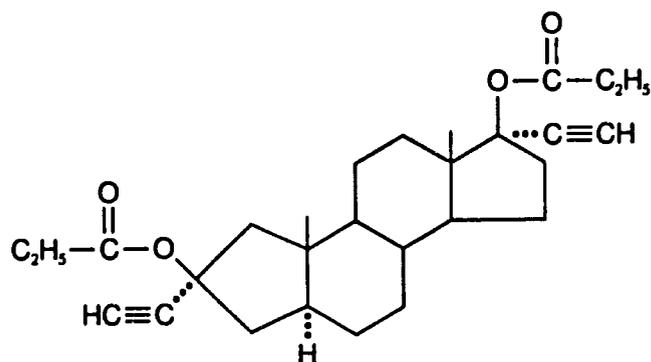


Figure 7. Anordrin

orally, absorbing progesterone that passes through the enterohepatic circulation or that diffuses into the gut.

Such an encapsulated preparation has been injected intraperitoneally, to compare the effect with that of the soluble antiserum injected into the same site. No detectable leakage of APA from the microcapsules was noted, as determined by the absence of antibody in blood. In contrast to a greater than 3-fold increase in serum progesterone in rats injected with soluble APA, a decline in serum progesterone occurred within 48 hours, falling to less than 4 ng/ml in rats receiving the encapsulated APA. Interestingly, a transient decrease in serum progesterone occurred in rats receiving the encapsulated polymer without APA, reflecting the relatively weak but abundant potential binding of progesterone to this material. This transient decrease was sufficient to reduce the number of viable fetuses by 50%. In this initial test, the polymer with APA was lethal to all rats, whereas all rats given the polymer alone survived the experiment. Further work is underway to study dose responses, to evaluate more hydrophilic polymers, and to investigate the oral route of administration.

PROGESTERONE ANTAGONISTS

Compounds included in this category are those that interact with progesterone competitively or noncompetitively for receptor binding, or that oppose an action of progesterone on the uterus. Only a few compounds have been found that act by competition for the progesterone receptor.

ORF 9371

One such compound is a steroidal antiprogesterin from Ortho Pharmaceutical Co., ORF 9371, which has the structure 17 β -acetoxy-17 α -ethinyl-4-androstene-3-one-(3-oxime) (Figure 5). This compound is neither estrogenic nor antiestrogenic, and it has no inhibitory effect on gonadotropin secretion (31). It does inhibit uterine proliferation induced by progesterone in rabbits, and it prevents decidual development and implantation in rats. It does not bind to the estrogen receptor, although the

analog without the acetate group binds weakly, and it may be a metabolite *in vivo*.

R2323

A compound with similar properties is norgestrienone (R2323) (Figure 6). However, Mora and associates found no abortifacient activity of this compound in doses of 100 mg to 400 mg, when it was administered to 57 women shortly after missed menstrual periods (48). It does suppress serum progesterone in nonpregnant women, but hCG reverses this effect, which suggests that R2323 acts to suppress gonadotropin secretion.

Anordrin

Another compound that may act by antagonizing progesterone is Anordrin (Figure 7). This compound was considered an antiprogesterin by Pincus and Banik as a result of their search for orally active contraceptive agents in the early 1960s (53). Recently, a report from China has disclosed extensive clinical investigations of Anordrin as a postcoital contraceptive; as the author noted, "The rate of protection against [conception] reached more than 99% ..." (37). Clinical trials showed that women who received Anordrin did not have the excessive proliferation of the endometrium that is seen with postcoital estrogens. In most cases, suppression of the endometrial proliferation was seen, and some women had atrophic changes of the endometrium (37). Animal studies of the drug suggest that it has both luteolytic and estrogenic activity (20, 30), yet the great species differences and non-parallel dose-response curves require additional studies to adequately define the mode of action of Anordrin.

ORF 3858 and other estrogenic compounds

Other compounds, such as 2-methyl-3-ethyl-4-phenyl- Δ^4 -cyclohexene carboxylic acid (ORF 3858) (Figure 8), that have abortifacient activity, and are structurally related to diethylstilbestrol (DES) (Figure 9), have been found to be converted *in vivo* to products that have estrogenic properties. A deliberate effort has been made to prepare derivatives of DES and hexestrol that structurally resemble estriol, that is, that have hydroxyl groups on two adjacent carbon atoms in one ring, but not in both rings

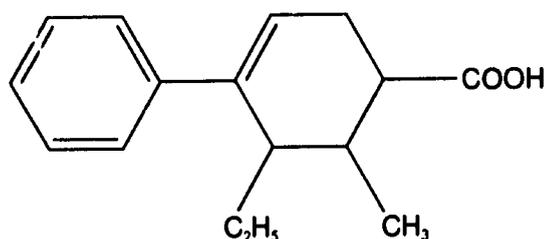


Figure 8. 2-methyl-3-ethyl-4-phenyl- Δ^4 -cyclohexene carboxylic acid (ORF 3858)

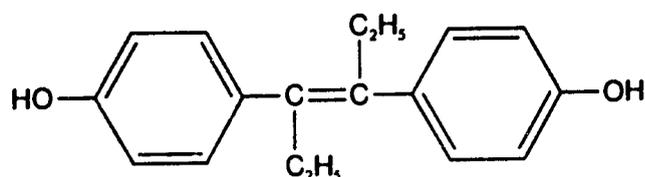


Figure 9. Diethylstilbestrol (DES)

(65). These compounds have reduced estrogenic activity in relation to their interceptive activity, but since they retain significant estrogenic activity, they have not been tested clinically.

Estrogens themselves, however, have been used as post-ovulatory interceptives, on the assumption that the infrequent use after unprotected coitus will not present the health hazard associated with long-term exposure to estrogens. In one study, pregnancy could be adequately prevented by giving women 50 mg of DES/day, 30 mg/day of conjugated equine estrogens, or 5 mg of ethinyl estradiol (EE)/day, for 5 consecutive days, if treatment was begun within 72 hours after unprotected coitus at midcycle (6). Nausea was experienced by about half of the patients, but without serious side-effects. In macaques given marginally abortifacient doses of estrogen, no fetal abnormalities were observed (49). Some improvement in this procedure is believed to accrue with the combined EE/ Δ^4 -norgestrel treatment (60, 66). In the latter study, in which two tablets were taken immediately after unprotected intercourse, followed by two tablets within 12 hours (0.05 mg EE and 0.5 mg Δ^4 -norgestrel per tablet) no failures were observed among the two-thirds of patients who returned for follow-up. Still, about 12% experienced severe nausea and 8% reported vomiting. The mechanism for the estrogen effect presumably is in counteracting the progesterone action on the myometrium as well as the decidua; some lowering of plasma progesterone has been observed with DES, but only when treatment was begun on the day of the LH peak (40). The length of the luteal phase was unaltered, however, and an effect on secretion of progesterone by the intact corpus luteum seems unlikely. Progestins have also been administered without the estrogenic component for interceptive purposes. Norethindrone in particular has been well studied in this manner, but has proven ineffective (50).

Some evidence has been obtained for prostaglandin biosynthesis, as a mechanism by which the estrogens act to terminate pregnancy. Auletta and co-workers have shown that at least the luteolytic effect of estrogens in the rhesus monkey can be prevented by simultaneous administration of indomethacin (4). Administration of prostaglandins themselves in different forms and by different routes has proven to be an effective method of terminating early pregnancy, as discussed above and reviewed extensively elsewhere (33, 34). Most forms of prostaglandins act to

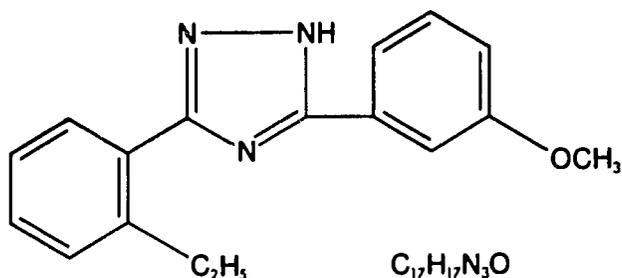


Figure 10. 3-(2-ethylphenyl)-5-(3-methoxyphenyl)-1H-1,2,4-triazole (dl-111-IT)

intercept pregnancy in the human at the uterine level, although some of the newer forms, particularly the 13-dehydro derivatives, may also have a luteolytic action that will contribute to their effectiveness in terminating pregnancy, as discussed above (47).

The triazole compounds

The difficult problem in applying prostaglandins to fertility regulation has been to limit the effects as much as possible to the reproductive organs.

One method of doing this may be to selectively promote synthesis or prevent metabolism of prostaglandins in organs of the reproductive tract. The triazole compounds being studied by Lerner and co-workers may have this potential, since they act to prevent metabolism of prostaglandins and thereby increase their availability to the organs in which they are produced, although presently available compounds do not have this degree of specificity (63).

Lerner has selected one triazole compound produced by Lepetit of Milan for further study. The compound is 3-(2-ethylphenyl)-5-(3-methoxyphenyl)-1H-1,2,4-triazole, and it has been assigned the symbol dl-111-IT (Figure 10). This compound is most effective in the monkey when injected intramuscularly between days 34 and 38 of gestation, at a dose of 10-25 mg/kg body weight. With a treatment on 5 successive days, 2 to 5 mg/kg/day was effective. In hamsters and rats the abortifacient dose remained effective in all animals when 4 mg/day of progesterone was given concomitantly. Thus, abortion does not depend on the luteolytic effect of the drug.

In this respect, and with regard to the side effects, dl-111-IT appears to act as a prostaglandin; metabolism of prostaglandins is inhibited in the reproductive tract, in the lung, and probably in other organs as well (42). The compound has no estrogenic, androgenic, or progestogenic activity, nor does it inhibit these activities of other compounds. It is relatively nontoxic, the LD_{50} being 300 times the ED_{50} in the hamster, but its greatest shortcoming, aside from its lack of specificity for the reproductive tract, may be its lack of activity by the oral route.

ORF 5513

Some other nonsteroidal compounds that have contragestational activity have been investigated. One relatively new compound is the salt of 3,5-bis-(dimethylamino)-1,2,4-dithiazolium chloride (ORF 5513) (31) (Figure 11). It is an unusual compound, in that it acts at several levels to interrupt the reproductive process; it inhibits ovulation, it inhibits implantation, and it is abortifacient. The compound lacks hormonal activity and has no apparent luteolytic effect. It is effective in preventing ovulation at doses of from 0.01 to 0.1 mg/kg body weight/day, but only if it is started 3 days or more before ovulation. Effective abortifacient doses in the rat were lowest between days 10 and 13 of pregnancy, when 5 mg/kg was effective in producing cellular changes in the chorionic villi and chorionic-fetal vessels.

Trichosanthin

Other interesting compounds under investigation are natural products that have been used for many centuries as aqueous extracts or "teas" to induce menstruation. Trichosanthin was isolated by Chinese scientists from the root of *Trichosanthis kirilowii*. It is a basic protein with a molecular weight of 18,000; it is said to induce abortion and to have an ameliorating effect on choriocarcinoma (13). Interestingly, it does not induce abortion in the two most commonly used laboratory species, the rat and the hamster, or in other species in early pregnancy. Hahn and associates have studied Trichosanthin in the guinea pig and the mouse (31). It has no effect on contractions of the nonpregnant guinea pig uterus, but it induces contractions in the pregnant animal when given intraperitoneally as a single injection of 200 μ g at the end of the first trimester (16-22 days). It has been postulated that Trichosanthin induces prostaglandin action within the uterus, and preliminary data show that indomethacin inhibits its effect. If this is an organ-specific induction of prostaglandin biosynthesis it would, of course, have substantial advantages over administration of prostaglandins for elective abortions.

Zoapatanol

Zoapatanol is another natural product that has been investigated recently by the group at Ortho. Teas made from the

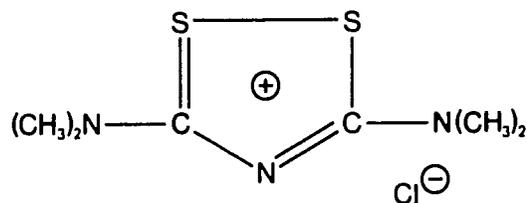


Figure 11. 3,5-bis-(dimethylamino)-1,2,4-dithiazolium chloride (ORF 5513)

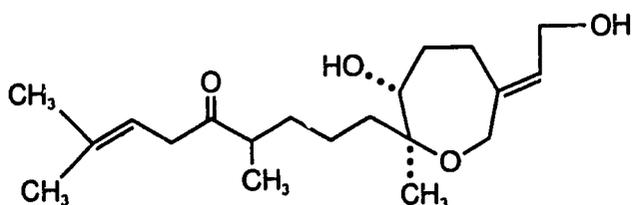


Figure 12. Zoapatanol

Montanoa tomentosa (zoapatle) plant have been used in Mexico for facilitation of childbirth, stimulation of menses, and termination of early pregnancy (39, 44). The diterpene structure containing an oxepane ring has been reported recently (Figure 12). In rats and hamsters, this compound inhibits implantation when given on days 1 through 6 of pregnancy. It also has an effect after implantation in the guinea pig; when given at the end of the first trimester, intrauterine death occurred. Landgren administered zoapatle orally to 6 women in early pregnancy (39). Administration resulted in menstrual-like cramps and a significant dilatation of the cervix in all subjects, but the zoapatle did not cause luteolysis, based on assays of plasma progesterone. No changes were detected in the cardiovascular system or in blood lipids, proteins, or electrolytes, and hematologic, liver, kidney, and thyroid function tests were all normal. Since definite uterotonic contractions are produced, this compound shows promise as a compound that may produce selective stimulation of prostaglandin secretion. Zoapatanol differs from Trichosanthin in that it is not a protein, and it acts earlier in pregnancy than does the Trichosanthin.

SUMMARY

Some new methods have been developed that act at each of the areas in which progesterone biosynthesis or action can be inhibited. Some of the new "super" agonists of LRF that may be absorbed intranasally interfere with gonadotropin-mediated ovarian secretions. If sufficient down-regulation of ovarian gonadotropin receptors can be achieved to prevent rescue of the corpus luteum by hCG, LRF agonists could prove to be an excellent once-a-month medication or menstrual inducer. The agonists are highly specific and free from unpleasant side-effects. Gonadotropins may be suppressed by pineal peptides as well, but a postovulatory application seems unlikely; perhaps these compounds, as well as LRF antagonists, could be used during the follicular phase of the cycle to suppress gonadotropins to the extent that only follicular luteinization occurs, without ovulation.

At the level of the ovary, hope has not completely faded for a luteolytic prostaglandin derivative, such as one of the 13-dehydro prostaglandins, that has only minor actions on smooth muscle. On the other hand, some possibility seems to exist of relatively specific actions on uterine musculature by the 16,16-dimethyl prostaglandins and other C-16 derivatives; these prostaglandins also have substantially reduced gastrointestinal effects. There is now evidence that the combining of an inhibitor of ovarian steroidogenesis with a prostaglandin may produce a synergistic interceptive action. Some inhibitors of steroidogenesis, such as Azastene, display a high degree of specificity for the ovary, and have minimal effects on the adrenal gland. Azastene itself has not proven effective in terminating pregnancy, but other similar compounds, which have a longer biological half-life and potentially greater activity in inhibiting placental steroidogenesis, are being developed. Other compounds, primarily natural products that have been used as "teas" for fertility control in different cultures around the world, also are being purified, synthesized, and investigated.

The target organs and sites of action of these compounds have not been well-characterized to date, but some, such as lithospermic acid, appear to suppress ovarian secretions, while others, such as zoapatanol, may stimulate endogenous production of prostaglandins. Endogenous prostaglandins may also be the active agent in the triazole compounds that were developed from psychoactive drugs; in this case, the drug causes a build-up of prostaglandins by decreasing their metabolism. The triazole compounds are active in primates, but they suffer somewhat from lack of specificity, causing gastrointestinal and respiratory side-effects.

Secreted progesterone may be inhibited in its actions by

increasing its clearance, by interfering with its binding to receptors, or by modifying the end-organs in such a way that the progesterone cannot exert its effects. Passive immunization to progesterone, while a useful tool for investigators, is limited because of immunological and other problems associated with repetitive injections of proteins.

Attempts are now being made to encapsulate antiprogestosterone antibodies in a form that will allow the steroid to be bound, without allowing release of antibodies into the circulation. Steroid hormone analogs, such as norgestrienone, that bind to progesterone receptors, are capable of preventing the biologically active hormone from exerting its side-effects, but so far, the only drug that has been effective in the presence of hCG is Danazol; the main shortcoming of Danazol in this respect is the very large dose required and the attendant and potential additional

side-effects.

Other compounds that are competitive inhibitors may be nearing the time when clinical testing can be done. Substances interfering indirectly with the physiological function of progesterone, such as the estrogens, certainly also have a place in the treatment of unprotected midcycle coitus.

As many new compounds are discovered or rediscovered, and are investigated with the objective of promoting their optimal contraceptational effect with a minimum of undesirable side-effects, more methods for regulation of fertility will become available. Methods more appropriate to the circumstances of gestational age and health needs of women of different cultures and convictions will increase the acceptability and use of contraception among couples interested in regulating their own fertility.

REFERENCES

- Agarwal MK: Evidence that natural vs synthetic steroid hormones bind to physicochemically distinct cellular receptors. *Biochem Biophys Res Commun* 73:767-772, 1976.
- Arrata WSM, Chatterton RT: Effect of prostaglandin $F_{2\alpha}$ on the luteal phase of the cycle in nonpregnant women. *Am J Obstet Gynecol* 120:954-959, 1974.
- Asch RH, Fernandez EO, Silerkhodr TM, Bartke A, Pauerstein CJ: Mechanism of induction of luteal phase defects by Danazol. *Am J Obstet Gynecol* 136:932-940, 1980.
- Auletta FJ, Caldwell BV, Speroff L: Estrogen-induced luteolysis in the rhesus monkey, reversal with indomethacin. *Prostaglandins* 11:745, 1976.
- Bergquist C, Nilius SJ, Wise L: Intranasal gonadotropin-releasing hormone agonist as a contraceptive agent. *Lancet* 2:215-217, 1979.
- Blye RP: The use of estrogens as post-coital contraceptive agents. *Am J Obstet Gynecol* 116:1044, 1973.
- Bolch OH, Warren JC: Induction of premature menstruation with catatoxic steroids. *Am J Obstet Gynecol* 111:1107, 1971.
- Breneman WR, Zeller RJ: *Lithospermum* inhibition of anterior pituitary hormones. *Biochem Biophys Res Commun* 65:1047-1053, 1975.
- Brenner PF, Mishell D: A study of the abortifacient effect of oxymetholone in early gestation. *Contraception* 11:669, 1975.
- Bygdeman M: Menstrual regulation with prostaglandins. In Karim SMM (ed): *Practical Applications of Prostaglandins and their Synthesis Inhibitors*. Baltimore, University Park Press, 1979, pp 267-282.
- Casper RF, Sheehan K, Erickson G, Yen SSC: Neuropeptides and fertility control in the female. In Zatuchni GI, Lobbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.
- Casper RF, Yen SSC: Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. *Science* 205:408-410, 1979.
- Chang MC, Saksens SK, Lau IF: Induction of mid-term abortion by trichosanthen in laboratory animals. *Contraception* 19(2):175, 1980.
- Chatterton RT, Cheesman KL, Mehta RR, Venton DL: Post-ovulatory interception. In Zatuchni GI, Lobbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.
- Chen HC, Hodgen GD, Matsuura S, Lin LJ, Gross E, Reichert LE, Birken S, Canfield RE, Ross GF: Evidence for a gonadotropin from nonpregnant subjects that has physical, immunological and biological similarities to human chorionic gonadotropin. *Proc N Acad Sci* 73:2885-2889, 1976.
- Clayton RN, Harwood JP, Catt KJ: Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. *Nature* 242:90-92, 1979.
- Colle ML, Greenblatt RB: Contraceptive properties of Danazol. *J Reprod Med* 17:98, 1976.
- Corbin A, Beattie CW, Tracy J, Jones R, Roell TJ, Yardley J, Rees RWA: The anti-reproductive pharmacology of LH-RH and agonistic analogues. *Int J Fertil* 23:81, 1978.
- Cox SW, Heinrichs WL, Paulsen CA, Conrad SH, Shiller HS, Henzl MR, Hermann WL: Perturbations of the human menstrual cycle by oxymetholone. *Am J Obstet Gynecol* 121:121, 1975.
- Crabbe P, Fillion H, Letourneaux Y, Diczfalusy E, Aedo A-R, Goldzieher JW, Shaikh AA, Castracane VD: Chemical synthesis and bioassay of anordrin and dinordrin I and II. *Steroids* 33:85-96, 1979.
- Csapo AI, Dray F, Erdos T: The biological effects of injected antibodies to estradiol-17 β and to progesterone in pregnant rats. *Endocrinology* 97:603, 1975.
- Csapo AI, Erdos T: The critical control of progesterone levels and pregnancy by antiprogesterone. *Am J Obstet Gynecol* 126:598-601, 1976.
- Das C, Talwar GP, Ramakrishnan S, Salahuddin M, Kumar S, Hinorani V, Coutinho E, Croxatto H, Hemmingson E, Johansson E, Luukkainen T, Shahani S, Sundaram K, Nash H, Segal SJ: Discriminatory effect of anti-Pr- β -hCG-TT antibodies on the neutralization of the biological activity of placental and pituitary gonadotropins. *Contraception* 18:35-50, 1978.
- Feil PD, Bardin CW: The use of medroxyprogesterone acetate to study progestin receptors in immature, pregnant, and adult rabbit uterus. *Adv Exp Med Biol* 117:241-254, 1979.
- Findley WE, Jacobs BR: The antigonadotropic activity of *Lithospermum ruderale*. I. The lack of steroid-like activity at the receptor level. *Contraception* 21:199, 1980.
- Gassner FX, Hopwood ML, Jochle W, Johnson G, Sunderly SG: Antifertility activity of an oxidized polyphenolic acid from *Lithospermum ruderale*. *Proc Soc Exp Biol Med* 114:20-25, 1963.
- Glasser SR: Personal communication, 1980.
- Glasser SR, Northcutt RC, Chytil F, Strott CA: The influence of an antisteroidogenic drug (aminoglutethimide phosphate) on pregnancy maintenance. *Endocrinology* 90:1363, 1972.
- Goldberg E: Sperm specific antigens and immunological approaches for control of fertility. In Talwar P (ed): *Recent Advances in Reproduction and Regulation of Fertility*. Amsterdam, Elsevier/North Holland Biomedical Press, 1979, p 281.
- Gu Z, (Ku C-P), Chang MC: A-nor steroids as post-coital contraceptives in the hamster with special reference to the transport and degeneration of eggs. *Contraception* 20:549-557, 1979.
- Hahn DW, McGuire JL, Chang MC: Contraceptives. In Zatuchni GI, Lobbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.
- Henzl MR, Segre EJ, Nakamura RM: The influence of oxymetholone on the hCG stimulated corpus luteum. *Contraception* 8:515, 1973.
- Karim SMM (ed): *Obstetric and Gynaecological Uses of Prostaglandins*. Baltimore, University Park Press, 1976.
- Karim SMM (ed): *Practical Applications of Prostaglandins and their Synthesis Inhibitors*. Baltimore, University Park Press, 1979.
- Kelley CJ, Mahajan JR, Brooks LC, Neubert LA, Breneman WR, Carmack M: Polyphenolic acids of *Lithospermum ruderale*—Douglass ex Lehmann (Bonginaceae). I. Isolation and structure determination of lithospermic acid. *J Org Chem* 40:1804-1815, 1975.
- Koch Y: Effects of antibodies against luteinizing hormone-releasing hormone on reproduction. In James VHT (ed): *Endocrinology*, Vol I. Amsterdam, Excerpta Medica, 1977, p 34.
- Ku C-P, Chu M-K, Chiang H-C, Chao S-H, Pany T-W, Tsou K: Pharmacological studies of a contraceptive drug-anordrin. *Chinese Med J* 2:177-184, 1976.
- Lan NC, Katzenellenbogen BS: Temporal relationships between hormone receptor binding and biological responses in the uterus: Studies with short and long-acting derivatives of estrin. *Endocrinology* 98:220-227, 1976.
- Landgren BM, Aedo AR, Hagenfeldt K, Diczfalusy E: Clinical effects of orally administered extracts of *Montanoa tomentosa* in early human pregnancy. *Am J Obstet Gynecol* 135:480, 1979.
- Lehmann F, Just-Nastanski I, Behrendt B, Czygan P-J, Bettendorf G: Effect of post-ovulatory administered oestrogens on corpus luteum function. *Acta Endocrinol* 79:329, 1975.
- Lemay A, Labrie F, Ferland L, Raynaud JP: Possible luteolytic effects of luteinizing-hormone releasing hormone in normal women. *Fertil Steril* 21:29-34, 1979.
- Lerner LJ, Carminati P: Effect of day of pregnancy and pregnancy terminating agents on prostaglandin synthesis and metabolism and histone metabolism in the rat utero-placental fetal complex and lung. *J Steroid Biochem* 8:395, 1977.
- Lerner LJ, Galliani G, Omodei-Sale A, Asandri A, Luzzani F, Gallico L, Grant AM: A new agent for the pharmacological control of pregnancy. In Zatuchni GI, Lobbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.
- Levine SD, Adams RE, Chen R, et al: Zoapatanol and montanol, novel oxepane diterpenoids, from the Mexican plant zoapatle (*Montanoa tomentosa*). *J Am Chem Soc*

101:3404, 1979.

45. MacDonald GJ, Beattie CW: Pregnancy failure in hypophysectomized rats following LHRH administration. *Life Sci* 24:1103-1110, 1979.

46. McCracken JA, Barcikowski B, Carlson JC, Green K, Samuelsson B: The physiological role of prostaglandin $F_{2\alpha}$ in corpus luteum regression. *Adv Biosci* 9:599-624, 1973.

47. McCracken JA, Einer-Jensen N, Fried J: Prostaglandin $F_{2\alpha}$ and its 13-dehydro analogs: comparative luteolytic effects *in vivo*. In Channing CP, Marsh JM, Sadler WA (eds): *Ovarian Follicular and Corpus Luteum Function*. New York, Plenum Publishing Company, 1979, pp 577-601.

48. Mora G, Faundes A, Johansson EDB: Lack of clinical contraceptive efficacy of large doses of R2323 given before implantation or after a missed period. *Contraception* 12:211-220, 1975.

49. Morris JM, Van Wagenen G: Interception: the use of post-ovulatory estrogens to prevent implantation. *Am J Obstet Gynecol* 115:101, 1973.

50. Nygren K-G, Johansson EDB, Wide L: Post-ovulatory contraception in women with large doses of norethindrone. *Contraception* 5:445-456, 1972.

51. Orts RJ, Bensen G: Inhibitory effects on

serum and pituitary LH by a melatonin-free extract of bovine pineal glands. *Life Sci* 12:513-519, 1973.

52. Orts RJ, Kocan KM, Johnson RP: Antifertility properties of bovine pineal extracts: reduction of ovulation and preovulatory luteinizing hormone in the rat. *Acta Endocrinol* 85:225-234, 1977.

53. Pincus B, Banik UK: Anti-progestins and implantation. *Excerpta Medica Int Congr Series* 72:558-562, 1963.

54. Raziano J, Ferin M, Vande Wiele RL: Effects of antibodies to estradiol- 17β and to progesterone on nidation and pregnancy in rats. *Endocrinology* 90:1133-1138, 1972.

55. Schane HP, Creange JE: An overview of luteal phase contraception. In Zatuchni GI, Labbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.

56. Schane HP, Creange JE, Anzalone AJ, Potts GO: Interceptive activity of Azastene in rhesus monkeys. *Fertil Steril* 30:343, 1978.

57. Schane HP, Potts GO, Creange JE: Inhibition of ovarian, placental and adrenal steroidogenesis in the rhesus monkey by trilostane. *Fertil Steril* 32:464, 1979.

58. Shrivvers CA: Antigens of the ovum as a potential basis for the development of contraceptive vaccine. In World Health Organiza-

tion Symposium on Development of Vaccines for Fertility Regulation. Copenhagen, Scriptor, 1976, pp 81-91.

59. Smith MS, Neill JD: Termination at mid-pregnancy of the two daily surges of plasma prolactin initiated by mating in the rat. *Endocrinology* 98:696, 1976.

60. Smith RP, Ross A: Post-coital contraception using dl-norgestrel ethinyl estradiol combination. *Contraception* 17:247-252, 1978.

61. Stevens VC: Antifertility effects from immunization with intact, subunits, and fragments of hCG. In Edwards RG, Johnson MH (eds): *Physiological Effects of Immunity Against Reproductive Hormones*. Cambridge, Cambridge University Press, 1975, p 249.

62. Stevens VC: Anti-pregnancy immunization. In Zatuchni GI, Labbok Mfi, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper and Row, 1980.

63. Talwar GP: Anti-hCG immunization. Introduction. *Contraception* 18:19, 1978.

64. Talwar GP: Immunology in reproduction. *J Reprod Med* 22:61, 1979.

65. Wotiz H: Personal communication, 1980.

66. Yuzpe AA, Lance WJ: Ethinyl estradiol and dl-norgestrel as a post-coital contraceptive. *Fertil Steril* 29:932, 1977.

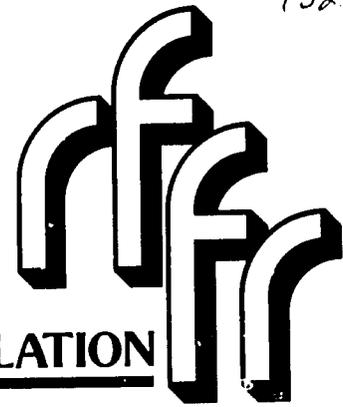
9320346/23

May 1981, Volume 1, Number 4



Northwestern University
1040 Passavant Pavilion
303 East Superior Street
Chicago, Illinois 60611

Editor: Gerald I. Zatuchni, M.D., M.Sc.
Managing Editor: Kelley Osborn
This publication is supported by AID/DSPE-C-0035



RESEARCH FRONTIERS IN FERTILITY REGULATION

GOSSYPOL: A POSSIBLE MALE ANTIFERTILITY AGENT REPORT OF A WORKSHOP

Gerald I. Zatuchni, M.D., M.Sc.
*Professor of Clinical Obstetrics and Gynecology
Northwestern University Medical School
Director, Program for Applied Research on Fertility Regulation
Chicago, Illinois*

Carolyn K. Osborn
*Editor
Department of Obstetrics and Gynecology
Northwestern University Medical School
Chicago, Illinois*

In the early 1970s, reports began to appear in Chinese medical journals that gossypol, a yellowish substance occurring in certain species of cotton plant, was being investigated as a possible male antifertility agent. Much of the information about gossypol research was anecdotal in nature, however, and the published reports lacked complete information on controls, preliminary physical and biochemical studies of subjects, and follow-up evaluations.

Interest in the antifertility capability of gossypol spread westward, and numerous animal experiments were organized to confirm or refute the Chinese experience. These initial experiments demonstrated considerable variability in toxicity and utility of gossypol as a male contraceptive agent. Accordingly, the Program for Applied Research on Fertility Regulation (PARFR), as an organization providing scientific and technical assistance for research in the development of new or improved means of fertility control, served as the sponsor of a workshop bringing together a small group of scientific experts who have done various types of research with gossypol.

The major objective of the workshop, which was held in Chicago, Illinois in March, 1980, was to provide a free exchange of ideas concerning early and ongoing research on the use of gossypol, and to evaluate the potential of the compound as a method of fertility control in the male. The meeting was structured to review the initial interest in gossypol relative to animal and human nutrition, and to discuss recent and on-going studies on the toxicity and efficacy of gossypol as a male contraceptive.

The reports presented at the workshop, and some subsequent developments in the evaluation of gossypol, are summarized here.

HISTORY OF GOSSYPOL AS AN ANTIFERTILITY AGENT

The antifertility effects of gossypol were first suggested by the findings of epidemiologic studies done in the People's Republic of China which showed that in certain areas, there were more than the expected number of cases of infertility. Environmental factors were eventually discounted, and food poisoning was suspected; after some investigation, cottonseed oil was implicated as a possible source of the problem. A contributing factor may have been that some farmers had changed the oil-processing technology. For years, Chinese farmers had processed cottonseed oil for use in food preparation by first heating the cottonseed and then pressing the oil out. Then, during the 1950s, a new method of preparing the oil was adopted, and farmers took their cottonseed to a central location where it was pressed without heating (36). After exposure to this crude cottonseed oil for periods of a year or more, a number of people developed a condition characterized by fever and dyspepsia; women developed amenorrhea and men became infertile. When use of the oil was discontinued, the amenorrheic women resumed normal menstrual cycles; many of the exposed men did not immediately regain fertility, however. Gossypol was identified as an active ingredient in the cottonseed oil and was be-

1

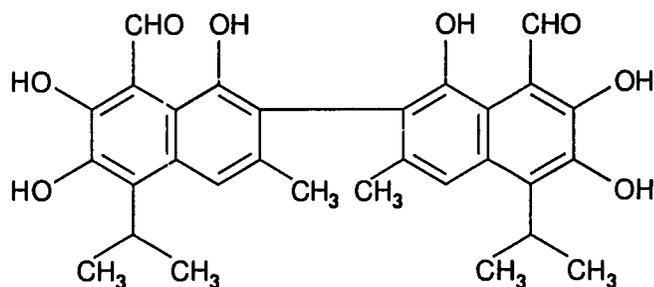
lieved to be responsible for both the toxic effects and the infertility. Demographic investigations during the 1960s provided further evidence to implicate gossypol as the significant factor.

In 1971, Chinese investigators initiated experiments using purified gossypol in several species of animals, and in the following year, clinical studies of gossypol as a male contraceptive agent were begun. In 1972, a number of pharmaceutical chemists, pharmacologists, and physicians organized the National Coordinating Group on the Male Antifertility Agent, Gossypol, and clinical studies on the antifertility effect, the site of action, pharmacokinetics, and toxicity of gossypol were carried out. The findings were summarized in an article appearing in the Chinese Medical Journal in 1978, "A New Male Contraceptive Drug — Cotton Phenol (Gossypol)" (43).

According to this report, some 4,000 Chinese men had used a gossypol contraceptive pill for at least six months, and some for more than four years. The efficacy was said to be 99.89%. The men usually recovered fertility by three months after discontinuation of treatment, and several births of apparently healthy babies were observed among the wives of men who stopped using gossypol (41, 43).

This article and other reports from China caused considerable excitement among investigators in the field of population and family planning, for if the data and conclusions were correct — namely, that gossypol constituted a new non-steroidal method of fertility control for the male that could be highly effective and reversible, fully safe, and inexpensive — then possibly a simple answer to the world's population problem had been discovered. An additional attraction was that with an estimated world production of more than 40 million tons of cottonseed per year, well over 100,000 tons of gossypol would thereby be available, or at least 25 kg for every male in the world.

Another part of the cotton plant, the cotton root bark (*gossypium* species), has had a long history in various parts of the world as an abortifacient and menses inducer, and contains a high concentration of gossypol. With the growing interest in the male antifertility potential of gossypol, some scientists are taking a closer look at cotton root bark as well, for information about its safety, efficacy, and general utility. Slocumb and co-workers have been investigating its extensive folkloric use as a supposed abortifacient among women in the Southwest United States (52). The bark is stripped and boiled for two hours and the resulting supernatant is used as an active elixir; the elixir contains high concentrations of gossypol. It is sold at herbalist stores for about \$3 to \$4 for a 4-oz. fluid extract. Preliminary survey data suggest that cotton root bark ingestion is associated with the onset of menstrual-like cramps and bleeding in a high percentage of women within four to 72 hours. About three-fourths of the women report moderate gastrointestinal symptoms and headaches. Surveillance



as a conjugate, or expired after decarboxylation. Although the peak concentration of gossypol in the testes was lower than that detected in other organs, at low doses the testicular tissue was highly sensitive to gossypol.

Recent investigations by Lee and Malling at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, suggest that gossypol works by inhibiting an enzyme that has a crucial role in the metabolism of sperm and sperm-generating cells (41). The investigators have provided some information as to the possible mechanism of action of gossypol, showing that its target enzyme is lactate dehydrogenase X. This finding indicates that gossypol does not affect either sex hormone levels or libido. It appears to inhibit to some extent each of several lactate dehydrogenases occurring throughout the body. Its greatest inhibitive effect, however, is on lactate dehydrogenase X, which is found only in sperm and testis cells. Gossypol appears to be a competitive inhibitor of a cofactor necessary for enzyme activity, thereby inhibiting sperm production. The agent also affects other enzymes. For example, in rodents, it can cause irreversible inactivation of malate dehydrogenase, but this effect has not been observed in human tissues. In rodents and humans, gossypol also inhibits glutathione S-transferase, an enzyme that participates in the detoxification of certain organic compounds, including potential carcinogens (41).

Gossypol in animal and human food

Although cottonseed is a by-product of cotton fiber production, processing the seeds is a major industry in the

cotton-producing areas of the world; oil obtained from cottonseed is useful in food preparation and cooking as salad oils, shortening, and margarine; cottonseed meal is used for animal feed and fertilizer; and high-protein cottonseed flour (e.g., Incaparina) is used to supplement protein-deficient diets in developing countries.

Gossypol is poisonous to nonruminant animals, including humans. This has inhibited the economic utilization of cottonseed products for nutrition. In 1915, Withers and Carruth established that gossypol was the factor responsible for the toxicity of cottonseed meal (61, 62). In order that the cottonseed oil and meal could be safely consumed by humans and domestic animals, extensive research was undertaken, focusing primarily on the removal or detoxification of gossypol. Two excellent recent reviews, one by Abou-Donia (1) and the other by Berardi and Goldblatt (5), document the laboratory animal and human research on gossypol that has been carried out toward this end.

Incaparina studies

In 1956, a research group at the Institute of Nutrition of Central America and Panama (INCAP) developed Incaparina, a high nutritive food intended for malnourished children, pregnant and lactating women, and adults suffering from protein/calorie malnutrition. Incaparina became commercially available in 1959 (6). Based on cottonseed protein, Incaparina contains cottonseed flour, corn flour, lysine, yeast, calcium carbonate, and vitamin and mineral supplements. In developing the flour, and determining its safety as food, the investigators studied the metabolic

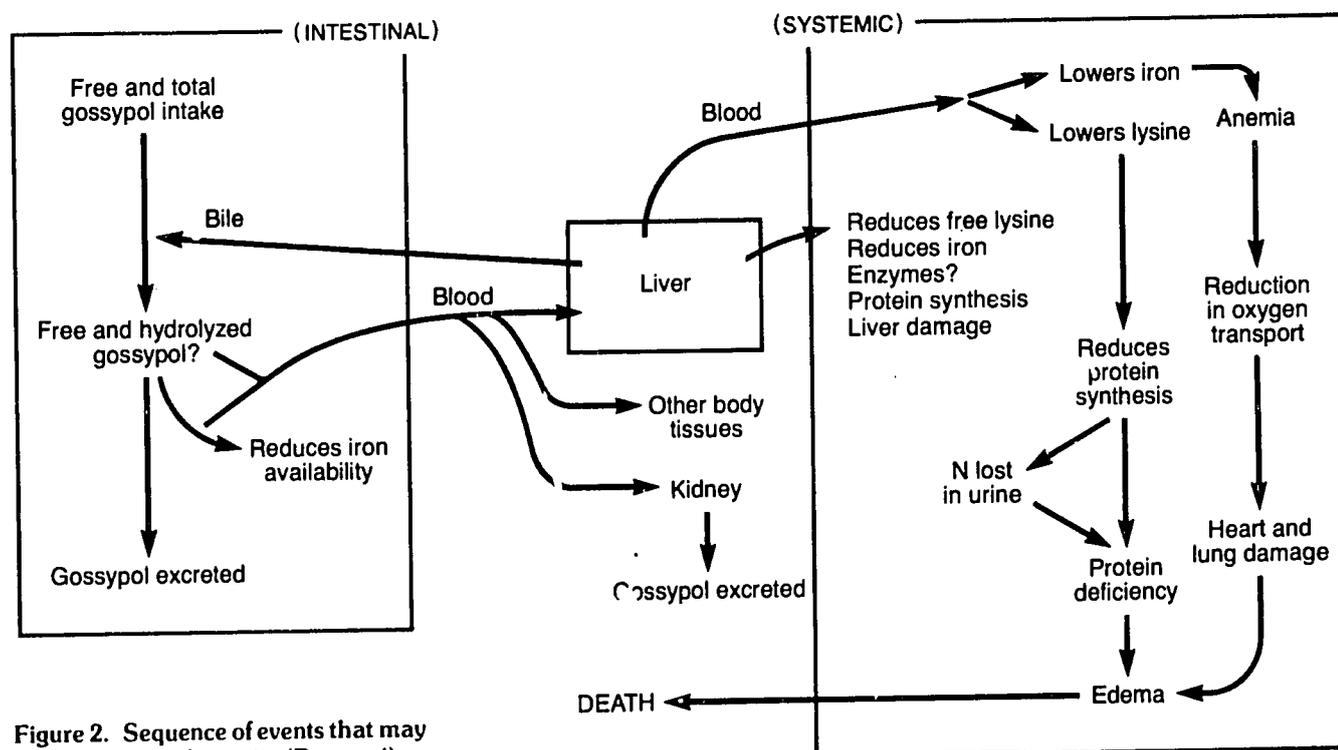


Figure 2. Sequence of events that may explain gossypol toxicity (Bressani).

activity of gossypol in experimental animals (swine, poultry, rats, and dogs) and in humans; the effects of cooking on the gossypol; and the long-range toxicity of gossypol.

In their early Incaparina investigations, Bressani and associates proposed that gossypol toxicity, or its antiphysiologic effects, could be explained on the basis of its activity at the metabolic level. They developed a working scheme of gossypol toxicity action, as shown in *Figure 2* (6).

In 1959, the investigators ran long-term feeding tests to detect any possible harmful physiologic effects of gossypol, especially with regard to lactation and reproduction. The levels of gossypol used in these studies were very small, about 3.5 mg/rat/day. No negative effects on reproduction were seen; cottonseed flour-fed rats had relatively high fertility, possibly owing to the high level of protein fed, which may have introduced some protection against the toxic effects of gossypol; litters were of normal birth weight. At the end of the study, organ weights were normal, and no pathologic effects were detected. The investigators concluded that the flour was safe for human consumption.

With the growing interest in the antifertility effects of gossypol, some investigators are considering extension of the Incaparina research to include follow-up evaluations of the 3- to 6-year old children studied by Bressani's group in the

1950s to look at their present fertility levels, and epidemiologic-fecundity studies of the populations now depending mainly upon Incaparina for their nourishment.

TOXICITY

Extensive research has been performed to determine the toxic effects of gossypol in different species. Gossypol is often toxic to dogs, cats, swine, chickens, rats, mice, rabbits, guinea pigs, and other nonruminant animals (18, 26). The toxicity is greatly increased when gossypol is administered intravenously or intraperitoneally. In ruminant animals, oral gossypol is relatively non-toxic, probably because of bacterial metabolism in the rumen which results in the binding of gossypol to protein (1).

The mechanisms by which gossypol causes tissue damage are as yet poorly understood. Toxicity may be due to the action of gossypol on specific enzymes, or interference with amino acid, protein, or iron metabolism (5).

The pathologic symptoms of gossypol toxicity are many and varied, depending upon animal species. The common manifestations in a variety of laboratory and farm animals are depressed appetite, loss of body weight, and inefficient protein utilization (5). Cardiac irregularity is the most common acute toxic effect. In subacute reac-

SPECIES (REFERENCE)	SYMPTOMS	POSTMORTEM FINDINGS
RATS (12, 24, 28)	appetite loss; growth rate depression; diarrhea; anorexia; hair loss; anemia	intestinal dilation, impaction; hemorrhagic congestion of stomach, intestines; congestion in lungs, kidneys
CATS (49)	spastic paralysis, usually of hind legs; rapid pulse; dyspnea; cardiac irregularity	edema of lungs, heart; heart enlargement; degeneration of sciatic nerve
DOGS (22, 23, 59)	posterior incoordination; stupor; lethargy; diarrhea; anorexia; weight loss; vomiting	lung edema; hypertrophy and edema of heart; congestion, hemorrhages of liver, small intestine, stomach; fibrosis of spleen, gall bladder; congestion of splanchnic organs
RABBITS (34, 49)	stupor; lethargy; loss of appetite; diarrhea; spastic paralysis; decrease in litter weights	hemorrhages in small intestine, lungs, brain, leg bones; enlarged gallbladder; edema, impaction of large intestine
SWINE (14, 15, 30, 55)	"thumps" or labored breathing; dyspnea; weakness; emaciation	widespread congestion, edema of many organs; fluid in body cavities; edematous bladder, thyroid gland; flabby, dilated heart with microscopic lesions; renal lipidosis; atrophied spleens, myocardial injury

(Modified from Berardi LC, Goldblatt LA: Gossypol. In Liener IE [ed]: Toxic Constituents of Plant Foodstuffs. New York, Academic Press, 1969, p 251)

Table 1. Symptoms and postmortem findings attributed to chronic toxicity of gossypol in cottonseed meals, selected nonruminants.

		REVERTANTS PER PLATE				
		TA 98	TA 100	TA 1535	TA 1537	TA 1538
GOSSYPOL (1.8-125 μ g per plate)	-S-9	16-29	78-114	7-10	3-6	22-23
	+S-9	26-45	101-117	8-12	6-9	29-30
NEGATIVE CONTROL (solvent only)	-S-9	27	100	12	6	22
	+S-9	28	106	13	7	26

Positive controls used: 2-nitrofluorene — strains TA 98, TA 1538
2-acetylaminofluorene, methyl methanesulfonate — TA 100, TA 1535
9-aminoacridine — TA 1537

Doses of gossypol >125 μ g/plate were toxic to these strains

Table 2. Gossypol acetic acid (Sigma Chemical Co., St. Louis, Mo) and gossypol formate (Shanghai Institute of Materia Medica) tested in the Ames *Salmonella mutagen* bioassay.

tions, pulmonary edema occurs, and in chronic reactions, malnutrition and growth retardation are seen. Table 1 indicates the toxic effects of chronic gossypol administration in cottonseed meal to selected nonruminant animals.

A variety of studies suggest that tolerance levels for gossypol are definitely associated with the species and even the strain of animal, the mode of administration, the mineral and protein content of the diet, the cumulative dose of free gossypol, and the type of gossypol administered. It is not certain whether the toxic metabolic effects of gossypol are related to its infertility effects. Most gossypol studies in animals have been done with cottonseed meal, rather than with pure gossypol. An intriguing finding is that more rigorous purification of the gossypol

seems to lead to loss in activity, suggesting that the antifertility effect might be associated with a highly potent trace constituent of cottonseed rather than with gossypol itself (21).

In studies with laboratory and farm species, the major dietary factors employed to modify gossypol toxicity expression have been iron and protein quantity and/or quality. In 1913, Withers and Brewster observed that iron salts alleviated cottonseed toxicity (60). In 1928, Clark proposed that gossypol reacted with the free amino groups of protein to form an insoluble complex (11). After a survey of the literature, Harper and co-workers concluded that the physiologic effects of ingested gossypol may be reduced or eliminated, within limits, by increasing the dietary level or quality of pro-

	DOSE (μ g per plate)	REVERTANTS PER PLATE			
		G 428		TR 2705	
		-S-9	+S-9	-S-9	+S-9
GOSSYPOL ACETIC ACID (Sigma)	2	34	58	22	37
	20	43	48	24	37
	200	39	59	26	33
GOSSYPOL FORMATE (Shanghai)	2	36	68	21	30
	20	45	91	30	25
	200	29	44	22	39
GOSSYPOL ACETATE (Peking)	2	42	66	31	58
	20	37	48	24	60
	200	40	50	34	82
SOLVENT ONLY (DMSO)		42-48		27-49	
STREPTONIGRIN	10	918	959	1017	703

Table 3. Gossypol tested in Ames *Salmonella* strains positive for streptonigrin.

tein, and that a major effect of protein is reduced gossypol absorption (32).

Most investigative studies with minerals have involved iron salts. Clawson and co-workers clarified the role of iron in preventing gossypol toxicity, and provided support for Clark's hypothesis that iron forms an insoluble complex with gossypol in the pig gut, since liver iron was inversely related to dietary gossypol, and dietary iron did not prevent injected gossypol toxicity (14, 15, 54). When high levels of iron (in 2:1 and 3:1 ratios of iron to gossypol) were added to the diet, pigs were able to survive, ingesting potentially lethal levels of gossypol, and gossypol accumulation in the tissues could be greatly reduced by dietary management (7).

Soluble iron salts added to a gossypol-containing diet fed to rats greatly improved survival rate and body weight gain, and the free and bound gossypol concentrations in the liver were reduced in direct relation to the amount of iron supplied. An inverse relationship was noted between the level of dietary protein and concentration of free and bound gossypol in the tissues of pigs, suggesting that gossypol apparently became bound to protein while in the digestive tract, and the bound form was not absorbed (50).

If the human reaction to gossypol is comparable to the reaction in test animals, a diet lacking adequate levels of protein, iron, or certain minerals could affect either the efficacy or the safety of its administration as an antifertility drug, and malnutrition could severely affect gossypol toxicity and its antifertility factors (32).

GENETIC TOXICITY OF GOSSYPOL

Using short-term mutagenicity screen tests, de Peyster has investigated the potential of gossypol to interfere with normal genetic replication processes. Results from these tests are frequently used to assist in predicting the potential teratogenic and carcinogenic hazard of a chemical. Gossypol acetic acid (Sigma Chemical Co.), gossypol formate (Shanghai Institute of Materia Medica), and gossypol acetate (Peking, Dr. Liu Kuo-chen), were evaluated in a small battery of preliminary *in vitro* and *in vivo* tests, selected for their ability to detect different types of damage to cellular DNA. The three *in vitro* tests were the Ames *Salmonella*/mammalian-microsome test (3), the *B. subtilis* multi-gene sporulation test (39), and the *S. cerevisiae* D3 mitotic recombination test (51). The *in vivo* mouse sperm head abnormality test was also performed (63), using intraperitoneal doses of 2, 4, 8, and 16 mg/kg/day for five consecutive days. This test was of particular interest because of the Chinese reports of "malformed spermatozoa" in the male volunteers (43).

Gossypol did not appear to be mutagenic in the *in vitro* tests (19, 20). The most extensive testing was done in

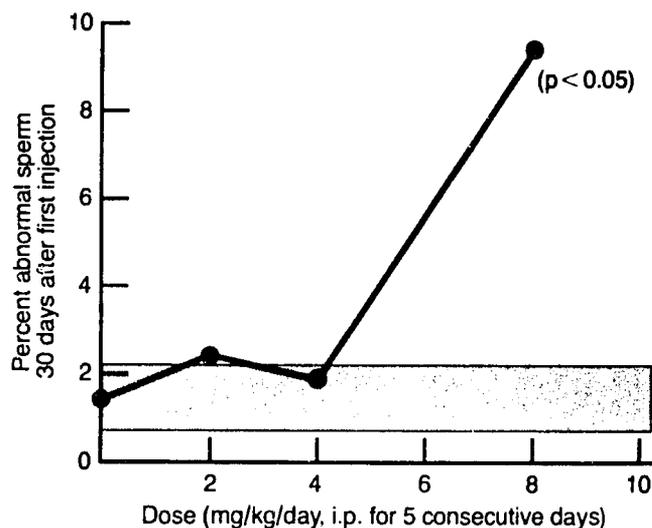


Figure 3. Induction of abnormal sperm head shape in B6C3 mice by gossypol acetic acid.

the standard test strains of *Salmonella* and in some newly developed *Salmonella* tester strains (Tables 2, 3). However, 8 mg/kg/day of gossypol acetic acid given to Charles River B6C3 mice did produce an increase in percentage of epididymal sperm with morphologically abnormal heads. At autopsy, an average of 9% abnormal shapes was seen in the caudae of treated mice, compared with 1.4% in corn oil-treated controls (Figure 3). Gossypol in doses of 16 mg/kg/day for five consecutive days was lethal to all mice in the group ($n = 5$). Using the same criteria for counting morphologically abnormal sperm, de Peyster found that methyl methanesulfonate, a potent mutagenic alkylating agent, given in daily doses of 80 mg/kg, produced an average of 20% abnormal sperm.

A wide variety of other chemicals with known mutagenic, carcinogenic, and teratogenic potential induce increased numbers of abnormal sperm (63), although the mechanisms by which abnormal shapes arise are not fully understood. Whether the effect of gossypol was due to direct action on DNA contained in the sperm head or to indirect biochemical or other interactions cannot be determined by this test alone. Regardless of the mechanism involved, de Peyster observes that the possible implications of increased numbers of abnormal sperm should be considered. An elevated proportion of abnormal sperm in the semen of fathers of spontaneous abortuses has been reported (27). Similarly, some investigators believe that structurally abnormal sperm are generally not viable and are associated with infertility, although this is not known for certain, and there is some experimental evidence to the contrary (53). Alternatively, abnormal sperm could also theoretically contain non-lethal mutations which end up in the gene pool of future live offspring. Observance of a high frequency of abnormal sperm heads in which damage may have

	DOSE	TIME PERIOD	EFFECT
MALE RATS	5 mg/kg/day	8 weeks	number of implantation sites in mated females not significantly decreased
	5-10 mg/kg/day	14 weeks	no toxicity; did not disturb sex drive or mating ability
	10 mg/kg/day	8 weeks	3 of 9 females mated to treated males not pregnant
	10 mg/kg/day	12 weeks	2 of 6 females mated to treated males not pregnant
	15 mg/kg/day	4 weeks	4 of 6 rats died
MALE HAMSTERS	5-15 mg/kg/day	6-14 weeks	no toxicity
	10 mg/kg/day	10 weeks	4 of 6 females mated to treated males not pregnant; in 2 pregnant hamsters, significantly reduced number of implantation sites compared to controls
MALE RABBITS	1.25-10 mg/kg/day	5-15 weeks	5 rabbits in good health, unchanged body weights; 1 rabbit died; effect on sperm production minimal or non-existent

Table 4. Results of oral administration of gossypol acetic acid in male rats, hamsters and rabbits; males were periodically mated with proestrous females that were subsequently killed and number of implantation sites recorded (10).

occurred through chemical interaction with DNA could also reflect concurrent DNA interaction in somatic cells, eventually leading to tumors or cell death.

Although these preliminary results and those of other investigators (16) would seem to indicate that gossypol does not have obvious mutagenic potential, de Peyster concludes that more extensive testing is warranted if safety with regard to genetic effects is to be assured.

ANTIFERTILITY STUDIES

Much remains to be learned about gossypol toxicity and efficacy, the reversibility of gossypol-induced infertility, and the mechanism of action of gossypol on spermatogenesis. To this end, a number of studies have been initiated using small animals and sub-human primates. Some of these studies are described below.

Animal studies

M. C. Chang, Gu, and Saksena studied the antifertility effects of orally administered gossypol acetic acid in male rats, hamsters, and rabbits (10). The summary of these studies is shown in *Table 4*. The male hamsters appeared to be more sensitive to the antifertility effects of gossypol and less sensitive to the toxicity effects than were male rats. In male rabbits, although sperm motility was disturbed in some cases, the number and fertilizing capacity of sperm

were not adversely disturbed. In some gossypol-treated rats, the numbers of sperm in the vas deferens or epididymis were decreased, but this was not true in the male hamsters. Also, the motility of sperm in the hamster appeared to be more affected by gossypol than was the case in the rat (10).

Waller and Zaneveld found that rabbits were very susceptible to toxic effects of gossypol acetate, and rats were resistant to its antifertility effects (57) (*Table 5*). They noted, however, that if the rats had been dosed for several additional weeks, an antifertility effect may have been achieved. In other rat studies, Bardin, Sundaram, and C. C. Chang, using gossypol acetic acid, found that the majority of treated animals became infertile; sperm concentration was significantly reduced, and increased numbers of nonmotile and abnormal sperm were seen in the cauda epididymidis (4) (*Table 5*). The mating performance of the rats was normal, and histologic examination of the organs of gossypol-treated animals showed no deviation from normal.

Hoffer and associates compared the effects of two different samples of gossypol (gossypol monoacetate from China* and a pure form of gossypol†), since at least some of the

* Provided by Dr. Sheldon Segal at the Rockefeller Foundation (Peking Batch #1).

† Obtained through Dr. Guy Jividen, with the United States cotton industry.

toxicity of gossypol may reside in contaminating compounds (33). The investigators looked for differences in purity, toxicity, or effect on the morphology of reproductive organs between these two compounds, when given to male rats at the lowest dose of gossypol (7.5 mg/kg) reported by the Chinese to be effective in producing infertility. No differences were seen in the morphologic effects of gossypol or gossypol monoacetate on the testis or epididymal sperm, at the doses and time intervals studied.

In another study, Hoffer studied the ultrastructural effects of higher doses of gossypol (20 and 30 mg/kg/day), fed by gavage to rats for seven weeks (Table 5). The animals were

sacrificed weekly and the testes and epididymides excised. Damage was seen in the seminiferous epithelium in the form of intercellular vacuoles, Sertoli cell vacuolization, and atrophic seminiferous tubules, but only in relatively few seminiferous tubules (Figure 4). By contrast, there was widespread damage to epididymal sperm, easily detectable by electron microscopy at week five (Figure 5). No morphologically demonstrable effects on the epididymal or vasal epithelium were observed. The discrepancy between the widespread damage to epididymal sperm and the more limited extent of testicular damage was intriguing to the investigators. Although some defective spermatids could be detected in the testis with the electron micro-

SPECIES (REFERENCE)	AMOUNT OF GOSSYPOL & DURATION OF TREATMENT	EFFECTS & MORPHOLOGICAL FINDINGS	CONCLUSIONS
RABBIT (57)	40 mg/kg/day 33 days	weight loss, death; epididymal sperm unchanged; severe liver and lung congestion, edema, intraperitoneal fluid	no antifertility effects, probably because toxic effects so severe
RAT (57)	40 mg/kg/day 28 days	normal health throughout; epididymal sperm appeared normal	no antifertility effects, but if study continued longer, antifertility effects might occur
RAT (4)	7.5 mg/kg/day 12 weeks	sperm concentration much reduced; nonmotile, abnormal sperm in cauda epididymides; sperm broken, sperm heads separated from tails, tails sharply bent; mating normal; serum LH, FSH, T normal	majority of rats infertile; return to fertility slow (12 wks post-treatment)
RAT (33)	20 and 30 mg/kg/day 7 weeks	damage to seminiferous epithelium; widespread damage to epididymal sperm by wk 5; no morphologically demonstrable effects on epididymal or vasal epithelium; no statistically significant changes in serum FSH, LH, T, or rates of fatty acid and cholesterol synthesis	total inhibition of sperm motility by 7 wks of treatment
ADULT RHESUS MONKEY (4)	0, 5, 20 mg/animal/day 3 months	no changes in blood chemistry values, CBC, or androgen, gonadotropin levels; serum potassium levels normal; no change in sperm count, although decapitated sperm frequent	animals resistant to antifertility effects; 20 mg/animal dose, 5× antifertility dose in humans, compared on body weight basis
	80 mg/animal/day 7 weeks	no effect on testes function; plasma LH and T normal; sperm count, motility, morphology normal	
STUMPTAIL MACAQUES (56)	10, 25, 50, 100 mg/ml gossypol/PVP co-precipitate	motility of sperm in vaginal fluid of mated females decreased with increasing concentrations of gossypol	80% + spermatazoa immotile at doses of 50 mg/ml co-precipitate

Table 5. Findings of selected gossypol studies in small animals and subhuman primates.

scope, deleterious changes were much more apparent in sperm which had already passed into the epididymis. The investigators theorized that the flagellar components in the testis might be destabilized by gossypol treatment, and that this instability manifests itself during epididymal transit (33).

In biochemical studies, Hoffer found no statistically significant changes either in serum FSH, LH, or testosterone in gossypol-treated rats, or in rates of testicular fatty acid and cholesterol synthesis. Sperm motility was examined at seven weeks, and total inhibition of sperm motility was noted. Bardin, Sundaram, and Chang (4) also examined effects of gossypol on serum levels of LH, FSH, and testosterone in rats and found that they were normal.

Hoffer is also looking at the antifertility properties and morphological effects of a number of analogs and deriva-

tives of gossypol including apogossypol hexacetate, which is reported to be less toxic than gossypol, with the idea that the antifertility action of gossypol could be separated from its toxicity by modifying its structure.

Sundaram and associates found the male rhesus monkey resistant to the toxicity and antifertility effects of gossypol (4) (Table 5). After administration of gossypol acetic acid to the monkeys for three months, no changes were seen in blood chemistry values, complete blood count, androgen or gonadotropin levels, or serum potassium levels. Sperm counts were normal, although decapitated sperm were frequent.

Hahn found that the hamster was much more sensitive to the antifertility effects of gossypol than rats or mice (29). High levels of gossypol administered to male mice caused toxic effects before any antifertility effect was observed



Figure 4. In a small proportion of seminiferous tubules of rats treated with gossypol, deleterious effects of gossypol treatment including the occurrence of atrophic seminiferous tubules and the presence of large intra- and intercellular vacuoles in the seminiferous epithelium can be observed. This is a light micrograph of an entirely atrophic seminiferous tubule from a rat treated with 7.5 mg/kg/day of gossypol for 4 weeks. Most of the cells seen here are Sertoli cells, and Sertoli cell nuclei can be recognized by their prominent nucleoli. (With permission of Anita P. Hoffer)



Figure 5. Electron micrograph of sperm in the lumen of the vas deferens of a gossypol-treated rat, showing sections through the sperm tail. This is from the vasa lumen after 4 weeks of 7.5 mg/kg/day of gossypol. In general, five different types of defect can be identified: Supernumerary or displaced outer dense fibers (ODFs), missing ODFs and/or doublets in principal piece, early signs of mitochondrial degeneration in midpiece, profiles consisting only of ODFs but devoid of mitochondria or fibrous sheath, and double tails. In the micrograph shown here, missing or displaced ODFs, double tails, vacuolated mitochondria and cytoplasmic droplets containing large numbers of ODFs can be observed. (With permission of Anita P. Hoffer)

SPECIES	AMOUNT OF GOSSYPOL & DURATION	EFFECTS
MALE MOUSE	40 mg/kg/day 5 wks	toxicity before antifertility effect achieved
	20 mg/kg/day 5 wks	no antifertility effect, little toxicity
	10 mg/kg/day 5 wks	no antifertility effect
MALE HAMSTER	10 mg/kg/day 5-6 wks	partial anti-fertility effect
	20 mg/kg/day 3 wks	total antifertility effect
FEMALE RAT	80 mg/kg/ on each of 3 days prior to expected ovulation	no inhibition of ovulation
FEMALE MOUSE	40 mg/kg on days 1-13 of pregnancy	26% of litter nonviable 4% of control nonviable
	80 mg/kg on days 1-13 of pregnancy	100% of litter nonviable

Table 6. Summary of findings by Hahn et al. with gossypol acetate in rats, mice, and hamsters (29).

(Table 6). At lower dose levels, there was less toxicity but no antifertility effect. In male hamsters, however, a partial antifertility effect was noted at five to six weeks, while at a higher dose level, a total antifertility effect was noted at three weeks.

In fertility studies of the female rat, a dose of gossypol on each of the three days prior to expected ovulation failed to inhibit ovulation (29). In the female mouse, administration of 40 to 80 mg/kg gossypol during days one to 13 of pregnancy resulted in nonviable offspring (26%-100% of the litters).

Waller, Cameron, and Zaneveld evaluated the vaginal spermicidal efficacy of a gossypol-polyvinylpyrrolidone (PVP) co-precipitate inserted into the vaginas of female *Macaca arctoides* (stumptail Macaques) (56). Immediately following insertion of the co-precipitate, the females were mated with males and a sample of vaginal fluid obtained. Spermatozoa were observed under a microscope for motility, and samples were tested with gossypol-PVP in concentrations of 10, 25, 50, and 100 mg/ml in a gelatin base. With increasing concentrations, a decrease in motility occurred. More than 80% of the spermatozoa were immotile at doses of 50 mg/ml or greater.

***In vitro* studies**

Waller, Zaneveld, and Fong studied the *in vitro* spermicidal activity (determined according to a modified Sander/Cramer method) of gossypol, gossypol acetic acid, and polyvinylpyrrolidone (PVP) (58). No decrease in sperm

motility occurred at concentrations as high as 150, 150, and 200 mg/ml, respectively. On the other hand, a gossypol-PVP co-precipitate caused complete immobilization of all spermatozoa at 5 mg/ml within three minutes, and total immobilization in 20 seconds at 40 mg/ml (Table 7).

An *in vitro* study by Pösö and co-workers in Helsinki, using freshly obtained human spermatozoa, also showed that small concentrations of gossypol (25 to 100 μ moles/liter) inhibited sperm motility and interfered with glucose and fructose utilization by the spermatozoa (44). A study of gossypol effects on sperm velocity and percentage of rapidly moving sperm in a semen sample indicated that gossypol in doses as little as 1 ng/ml produced a 90% reduction of sperm motility (47).

HUMAN STUDIES

Thus far, over 10,000 healthy men in China have taken either gossypol acetic acid or gossypol formic acid as contraceptive pills for more than six months, and more than half of them have been clinically observed for two years (41). Subjects were initially treated with 20 mg daily for about two months. After either reduction of sperm count to below 4 million/ml or production of necrospermia, the subjects were shifted to a maintenance dose of 75 to 100 mg twice a month.

Examination of semen showed nonmotile, malformed, and dead sperm, as previously observed in the rat

	CONCENTRATION* (mg/ml)	20 SECONDS	3 MINUTES
PVP	30	+	+
	70	+	+
	200	+	+
GOSSYPOL (FREE)	20	+	+
	40	+	+
	80	+	+
	100	+	+
	150	+	+
GOSSYPOL ACETIC ACID	20	+	+
	40	+	+
	80	+	+
	100	+	+
	150	+	+
GOSSYPOL- PVP	1	+	+
	5	+	-
	10	+	-
	20	+	-
	40	-	-

* amount titrated per milliliter of saline
+ motile spermatozoa present
- no motile spermatozoa found

(Modified from Waller DP, Zaneveld LJD, Fong HHS: *In vitro* spermicidal activity of gossypol. *Contraception* 22(2):185, 1980)

Table 7. Antimotility effect of PVP, gossypol, and gossypol derivatives.

studies. Exfoliated abnormal spermatids and spermatozoa were also present in the semen. Azoospermia was achieved as treatment continued.

Serum LH and testosterone levels remained within normal range. Some men developed elevated levels of pyruvate transaminase and mild changes in electrocardiographic patterns; others experienced lowered blood potassium levels, however these findings could not be linked conclusively to the use of gossypol.

Initially, some men experienced fatigue, increased or decreased appetite, gastrointestinal complaints, or decreased libido, but these symptoms gradually disappeared without discontinuation of pill use. The subjects usually recovered fertility after discontinuation of treatment for three months (41, 43).

Investigators at the Nanjing Institute of Materia Medica studied 148 men who had used gossypol as a contraceptive sometime between 1972 and 1977 (37, 45). They found that 4.7% suffered "apparent hypokalemic paralysis", a condition arising from a deficiency of potassium in the body. By comparison, only 0.1% of 8,482 married men of approximately the same ages and occupations, who had not used gossypol, experienced the deficiency disease during the same period. The incidence of the condition appeared to be regional, however; the greatest incidence was in Nanjing, while in many other districts, no cases were reported among gossypol users. A survey of the potassium content of princi-

pal foods in the Nanjing diet revealed an average potassium intake below the generally accepted nutritional requirement.

The investigators reported that when the diet of rats was modified to lower their potassium intake, gossypol appeared to affect their potassium metabolism (46). In human studies, the investigators compared gossypol-using patients with hypokalemia and normal controls who had never used gossypol, monitoring the amounts of dietary potassium that they excreted. They found that the total level of potassium in gossypol users' body tissues was lower than that of the controls, and that it remained lower even when the potassium in blood plasma and intercellular fluids returned to normal concentrations. Other experiments showed that gossypol was responsible for renal potassium loss, and that this mechanism was the cause of the onset of hypokalemic paralysis. When gossypol users in Nanjing who showed early warning symptoms of hypokalemia paralysis, such as fatigue and muscle weakness, took dietary supplements of potassium salt, they did not develop hypokalemia paralysis. The investigators concluded that increasing the level of potassium in users' diets would probably help to prevent gossypol-related hypokalemia (45).

Other than these Chinese studies, no human studies utilizing gossypol as a male contraceptive agent have been reported. Chinese investigators have started a new series

of human trials involving five clinical research centers. Approximately 1,000 men will have complete baseline studies performed, including blood counts, biochemistry, and semen analysis. Repeated studies will be done at regular intervals during gossypol initiation, and during the period of gossypol maintenance. Gossypol will be discontinued after varying months of use, following which semen analyses and other biologic parameters will be closely monitored to determine the time required for return to normal (reversibility).

CONCLUSION

There is no question that the oral administration of gossypol in appropriate doses causes severe oligospermia and azoospermia in certain susceptible species, including humans. Whether the original Chinese optimism regarding the potential of gossypol as a useful male contraceptive agent will prove correct will depend upon new research, in both animals and humans, that will address the significant issues of toxicity and reversibility. Laboratory investigations to determine the mechanism(s) of action of gossypol possibly could lead

to the development of synthetic compounds exhibiting the desired antifertility effect without the toxic effects so far noted in certain animals.

As it is obvious that no animal model can substitute effectively for the human, carefully designed clinical studies should be performed in settings that can provide additional information on dose-response, efficacy, side-effects, metabolic effects, and reversibility potential. Nutritional studies must be undertaken to determine the positive or negative effects on these parameters when gossypol is administered with supplements of iron, potassium, protein, or amino acids. The quality of diet is likely to be an important factor if gossypol is to be used as a male antifertility drug in developing countries.

Certainly, if developed into a practical contraceptive pill for men, gossypol will make a far-reaching contribution in fertility regulation, and it is likely to have an enormous impact on society and on the lives of individuals. The sources and supplies of gossypol from cotton and related plants are abundant. As a contraceptive drug, gossypol could be self-administered and convenient to use. If the safety of gossypol and the reversibility of its contraceptive action can be confirmed, the use of gossypol in fertility regulation will represent a great achievement in science, and will contribute to human welfare.

REFERENCES

1. Abou-Donia MB: Physiological effects and metabolism of gossypol. *Residue Reviews*. New York, Springer-Verlag, 1976, vol 61, pp 126-160.
2. Adams R, Geissman JA, Edwards JD: Gossypol, a pigment of cottonseed. *Chem Rev* 60:555-574, 1960.
3. Ames BN, McCann J, Yamasaki E: Methods for detection of carcinogens and mutagens with the *Salmonella/mammalian-microsome mutagenicity test* *Mutat Res* 31:347-364, 1975.
4. Bardin CW, Sundaram KS, Chang CC: Toxicology, endocrine and histopathologic studies in small animals and Rhesus monkeys administered gossypol. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
5. Berardi LC, Goldblatt LA: Gossypol. In Liener IE (ed): *Toxic Constituents of Plant Foodstuffs*. New York, Academic Press, 1969, pp 211-266.
6. Bressani R: Human nutrition and gossypol. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
7. Buitrago JA, Clawson AJ, Smith FH: Effect of dietary iron on gossypol accumulation in an elimination from porcine liver. *J Animal Sci* 31:554-558, 1970.
8. Campbell KN, Morris RC, Adams R: The structure of gossypol. I. *J Am Chem Soc* 59: 1723, 1937.
9. Carruth FE: Contribution to the chemistry of gossypol, the toxic principle of cottonseed. *J Am Chem Soc* 40:647, 1918.
10. Chang MC, Zhiping Gu (Chi-ping Ku), Saksena SK: Effects of gossypol on the fertility of male rats, hamsters and rabbits. *Contraception* 21(5): 461-469, 1980.
11. Clark EP: Studies on gossypol. I. The preparation, purification and some of the properties of gossypol, the toxic principle of cottonseed. *J Biol Chem* 75:725, 1927.
12. Clark EP: Studies on gossypol: a progress report. *Oil Fat Ind* 6:15-19, 1929.
13. Clawson AJ: Effects of gossypol on animal nutrition. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
14. Clawson AJ, Smith FH, Barrick ER: Accumulation of gossypol in the liver and factors influencing the toxicity of injected gossypol. *J Animal Sci* 21:911-915, 1962.
15. Clawson AJ, Smith FH, Osborne JC, Barrick ER: Effect of protein source, autotaxing and lysine supplementation on gossypol toxicity. *J Animal Sci* 20:547-552, 1961.
16. Colman N, Gardner A, Herbert V: Non-mutagenicity of gossypol in the *Salmonella/mammalian-microsome plate assay*. *Environ Mut* 1:315-320, 1979.
17. Dai R-X, Pang S-N, Lin X-K, Lui Z-L, Gong R-H: A study of antifertility effect of cottonseed. *Acta Biol Exp Sinica* 11:1-10, 1978.
18. Dai R-X, Pang S-N, Liu Z-L: Studies on the antifertility effect of gossypol. II. A morphological analysis of the antifertility effect of gossypol. *Acta Biol Exp Sinica* 11:27-30, 1978.
19. de Peyster A: Genetic toxicology and mutagenicity testing of gossypol. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
20. de Peyster A, Wang YY: Gossypol - proposed contraceptive for men passes the Ames test. *N Engl J Med* 301:275-276, 1979.
21. Djerassi C: The politics of contraception; the view from Beijing. *N Engl J Med* 303(6):334-336, 1980.
22. Eagle E: Chronic toxicity of gossypol. *Science* 109:361, 1949.
23. Eagle E: Effect of repeated doses of gossypol on the dog. *Arch Biochem Biophys* 26:68-71, 1950.
24. Eagle E, Bialek HF: Effect of single and repeated doses of gossypol on the rat. *Food Res* 15:232-236, 1950.
25. Edward JD Jr: Total synthesis of gossypol. *Am J Chem Soc* 80:3798-3799, 1958.
26. Fang VS: Personal communication, 1980.
27. Furujelm M, Jonson B, Lagergren C-G: The quality of human semen in spontaneous abortion. *Int J Fertil* 7(1):17-21, 1962.
28. Gallup WD: Further observations in eliminating the toxicity of cottonseed meal. *J Dairy Sci* 10:519-526, 1927.
29. Hahn DW, Rusticus C, Probst A, Homm R, Johnson N: Antifertility and endocrine activities of gossypol in rodents. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
30. Hale F, Lymann CM: Effect of protein level in the ration on gossypol tolerance in growing-fattening pigs. *J Animal Sci* 16:364-369, 1957.
31. Halverson JO, Smith FH: Estimation of gossypol in cottonseed meal — a modified method. *Ind Eng Chem* 5:29, 1933.
32. Harper GA, Phelps RA, Jones LA: Effects of diet and other factors upon the physiological effects of gossypol. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
33. Hoffer AP: Light and electron microscopic studies on the effects of gossypol in the male rat. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
34. Holley KT, Harms WS, Storherr RW, Gray SW: Cottonseed meal in swine and rabbit rations. *Georgia Agr Expt Sta Mimeo Ser 12[NS]*, 1955, pp 1-27.
35. Hsueh S-P, Tsong S-T, Su S-Y, Wu Y-W, Liu Y, Chou T-H, Ma H-H: Cytological, radioautographic and ultrastructural observations on the antispermatogenesis action of gossypol in the rat. *Sci Sinica* 9:915-923, 1979.
36. Lobl TJ, Bardin CW, Chang CC: Pharmacologic agents producing infertility by direct action on the male reproductive tract. In Zatzuchni GI, Labbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper & Row, 1980, pp 146-168.
37. Low potassium levels from use of gossypol linked to paralysis. *Fam Plann Perspectives* 13(1):44-45, 1981.
38. Lu G-S: Purification of gossypol and preparation of derivatives. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
39. MacGregor JT, Sachs LE: The sporulation system of *Bacillus subtilis* as the basis of a multi-gene mutagen screening test. *Mutat Res* 38: 271-286, 1976.
40. Marchlewski L: Gossypol, ein Bestandtheil der Baumwollsamensamen. *J Prakt Chem* 60:84, 1899.
41. Maugh TH II: Male "pill" blocks sperm enzyme. *Science* 212 (April 17): 314, 1981.
42. Merck Index, 8th Edition. Rahway, NJ, Merck & Co, 1968, p 505.
43. National Coordinating Group on Male Fertility: A new male contraceptive drug — cotton phenol (Gossypol). *Chinese Med J* 4:417-428, 1978.
44. Pösö H, Wichmann K, Jänne J, Luukkainen T: Gossypol, a powerful inhibitor of human spermatozoal metabolism. *Lancet* I (8173):885-886, 1980.
45. Qian S-Z, Jing G-W, Wu X-Y, Xu Y, Li Y-Q, Zhou Z-H: Gossypol related hypokalemia: Clinico-pharmacologic studies. *Chinese Med J* 93:477, 1980.
46. Qian S-Z, Xu Y, Chen C, Cao L-M, Sun S-G, Tang X-C, Wang Y-E, Shen L-Y, Zhu M-K: The influence of gossypol on the potassium metabolism of rats and the effect of some possible contributing factors (low-K and low-Mg intake). *Acta Pharm Sinica* 14:514, 1979.
47. Ridley A, Blasco L: Testosterone and gossypol effects on human sperm motility. *Abstract. Fertil Steril Abstr Suppl* 35(2):244, 1981.
48. Saksena SK, Salmosen R, Lau IF, Chang MC: Gossypol, a male antifertility agent: its toxicological and endocrinological effects in male rabbits. *Contraception (in press)*.
49. Schwartze EW, Alsberg CL: *Pharmacol-*

- ogy of gossypol. *J Agr Res* 28: 191-198, 1924.
50. Sharma MP, Smith FH, Clawson AJ: Effective levels of protein and gossypol and length of feeding period on the accumulation of gossypol in tissues of swine. *J Nutrition* 88:434-438, 1966.
51. Simmon VF: *In vitro* assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J Natl Cancer Inst* 62(4):901-909, 1979.
52. Slocumb J. Medical and sociological aspects of Gossypium use among women in Southwest United States. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
53. Smith DM, Oura C, Zamboni L: Fertilizing ability of structurally abnormal spermatozoa. *Nature* 227:79-80, 1970.
54. Smith FH, Clawson AJ: Effect of diet on accumulation of gossypol in the organs of swine. *J Nutrition* 87:317-321, 1965.
55. Smith HA: The pathology of gossypol poisoning. *Am J Pathol* 33:353-365, 1957.
56. Waller DP, Cameron SM, Zaneveld LJD: Spermicidal effect of gossypol in an *in vivo* model of vaginal contraceptives. *J Andrology* 2:32, 1981
57. Waller DP, Zaneveld LJD: *In vitro* and small animal studies on gossypol. Presented at PARFR Workshop on Gossypol. Program for Applied Research on Fertility Regulation, March 11, 1980, Chicago, Illinois.
58. Waller DP, Zaneveld LJD, Fong HHS: *In vitro* spermicidal activity of gossypol. *Contraception* 22(2):183-187, 1980.
59. West JL: Lesions of gossypol poisoning in the dog. *J Am Vet Med Assoc* 96:74-76, 1940.
60. Withers WA, Brewster FJ: Studies on cottonseed meal toxicity. II. Iron as an antidote. *J Biol Chem* 15:161-166, 1913.
61. Withers WA, Carruth FE: Gossypol — A toxic substance in cottonseed meal. A preliminary note. *Science* 41:324, 1915.
62. Withers WA, Carruth FE: Gossypol, the toxic substance in cottonseed meal. *J Agr Res* 7:261, 1915.
63. Wyrobek AJ, Bruce WR: The induction of sperm-shape abnormalities in mice and humans. In Hollaender A, de Serres FJ (eds): *Chemical Mutagens*, vol 5, New York, Plenum Publishing Corporation, 1978.

PARTICIPANTS: WORKSHOP ON GOSSYPOL

Chicago, Illinois, March 11, 1980

Mohamed B. Abou-Donia, M.D.
Department of Pharmacology
Duke University Medical Center
Durham, NC

Nancy J. Alexander, Ph.D.
Oregon Regional Primate Research Center
Beaverton, OR

C. Wayne Bardin, M.D.
Center for Biomedical Research
The Population Council
The Rockefeller University
New York, NY

Gabriel Bialy, Ph.D.
National Institutes of Health
Bethesda, MD

Ricardo Bressani, Ph.D.
Division of Agriculture Food Chemistry
INCAP
Guatemala City, Guatemala

Chin-Chuan Chang, Ph.D.
Center for Biomedical Research
The Population Council
The Rockefeller University
New York, NY

M. C. Chang, Ph.D., Sc.D.
Worcester Foundation for Experimental Biology
Shrewsbury, MA

A. J. Clawson, Ph.D.
Animal Science Department
North Carolina State University
Raleigh, NC

Elizabeth B. Connell, M.D.
Harrington Park, NJ

Joseph E. Davis, M.D.
New York, NY

Ann de Peyster, Ph.D.
Department of Biomedical and Environmental
Health Sciences
University of California
Berkeley, CA

Norman B. Farnsworth, Ph.D.
Department of Pharmacognosy and Pharmacology
University of Illinois at the Medical Center
Chicago, IL

Alfredo Goldsmith, M.D., M.P.H.
Department of Obstetrics and Gynecology
Northwestern University Medical School
Head, Research Project Development
Program for Applied Research on Fertility Regulation
Chicago, IL

Z. P. Gu, M.D.
Shanghai Institute of Materia Medica
Shanghai, China

John Gueriguian, M.D.
Food and Drug Administration
Bureau of Drugs
Rockville, MD

Do Won Hahn, Ph.D.
Reproductive Pharmacology
Ortho Pharmaceutical Corporation
Raritan, NJ

Garlon Harper
Director of Research and Education
National Cottonseed Products
Memphis, TN

Peter Heinstejn, Ph.D.
Department of Medicinal Chemistry
School of Pharmacy
Purdue University
West Lafayette, IN

Anita P. Hoffer, Ph.D.
Department of Anatomy
Harvard Medical School
Boston, MA

Lynn A. Jones, Ph.D.
National Cottonseed Products Association, Inc.
Memphis, TN

Miriam H. Labbok, M.D., M.P.H.
Office of Population
Development Support Bureau
Agency for International Development
USIDCA
Washington, DC

Thomas J. Lobl, Ph.D.
Fertility Research Division
Upjohn Pharmaceutical Corporation
Kalamazoo, MI

Gui-Shen Lu, Ph.D.
Chinese Academy of Medical Sciences
Beijing, China

Katherine Ch'iu Lyle, M.P.H.
The Rockefeller Foundation
New York, NY

Edward C. Mather, D.V.M., Ph.D.
Department of Large Animal Surgery and Medicine
College of Veterinary Medicine
Michigan State University
East Lansing, MI

Dolores J. Patanelli, Ph.D.
Contraceptive Development Branch
Center for Population Research and Human Development
Bethesda, MD

C. Alvin Paulsen, M.D.
University of Washington School of Medicine
Seattle, WA

Richard A. Phelps, Ph.D.
Anderson, Clayton and Company
Houston, TX

Dr. M. R. N. Prasad
Special Programme of Research in Human Reproduction
World Health Organization
Geneva, Switzerland

Carlos Schaffenburg, M.D.
Food and Drug Administration
Bureau of Drugs
Rockville, MD

John J. Sciarra, M.D., Ph.D.
Department of Obstetrics and Gynecology
Northwestern University Medical School
Program Director
Program for Applied Research on Fertility Regulation
Chicago, IL

John C. Slocumb, M.D.
Department of Obstetrics and Gynecology
University of New Mexico
Albuquerque, NM

Emil Steinberger, M.D.
Department of Reproductive Medicine and Biology
University of Texas Medical School
Houston, TX

Kalyan S. Sun Jaram, Ph.D.
Center for Biomedical Research
The Population Council
The Rockefeller University
New York, NY

S. D. Tsong, M.D.
Beijing Institute of Basic Medical Sciences
Beijing, China

Donald P. Waller, Ph.D.
Department of Pharmacognosy and Pharmacology
University of Illinois at the Medical Center
Chicago, IL

Lourens J. D. Zaneveld, D.V.M., Ph.D.
Department of Physiology and Biophysics
University of Illinois at the Medical Center
Chicago, IL

Gerald I. Zatuchni, M.D., M.Sc.
Department of Obstetrics and Gynecology
Northwestern University Medical School
Director of Technical Assistance
Program for Applied Research on Fertility Regulation
Chicago, IL

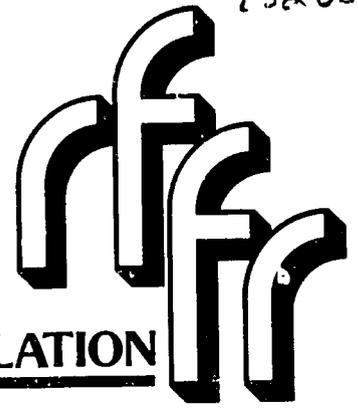
932 0546/23

December 1981, Volume 1, Number 5



Northwestern University
1040 Passavant Pavilion
303 East Superior Street
Chicago, Illinois 60611

Editor: Gerald I. Zatuchni, M.D., M.Sc.
Managing Editor: Kelley Osborn



RESEARCH FRONTIERS IN FERTILITY REGULATION

FEMALE STERILIZATION USING CHEMICAL AGENTS

Ralph M. Richart, M.D.

*Professor of Pathology
Columbia University College of Physicians and Surgeons
Director, Obstetrical and Gynecological Pathology and Cytology Laboratory
The Sloane Hospital for Women, New York*

Sterilization is a highly effective means of fertility regulation and ranks as the most popular method worldwide. Male sterilization using vasectomy can be accomplished with relative ease as a minor surgical procedure that does not invade the peritoneal cavity. Vasectomy can be performed on an outpatient basis by trained paramedical personnel and can be offered through large-scale programs. A man undergoing the procedure may experience local complications, such as incisional pain, hematoma, or wound infection, but because the peritoneal cavity is not entered, major complications seldom occur and deaths directly related to the procedure are rare. Infection with tetanus or anthrax, the principal cause of vasectomy-associated deaths, is most likely to occur in areas of the world where hygiene is poor.

Female sterilization using conventional techniques is always a surgical procedure requiring invasion of the peritoneal cavity. Whether ligation or electro-coagulation of the fallopian tubes is performed by means of a postpartum incision, a laparoscopic puncture, or a minilaparotomy speculum, the peritoneal cavity is exposed to potentially fatal invaders that inhabit the nonperitoneal world, and the surgical manipulation and treatment may cause major injuries to the abdominal viscera and blood vessels. Significant morbidity is difficult to avoid, especially in large series of patients, or in less sophisticated environments, and deaths, though rare, do occur.

Conventional female sterilization techniques cannot be offered on a broad scale, because most procedures require the considerable training usually possessed by a gynecologist or general surgeon; throughout the developing world, physicians, particularly those with specialty training, are concentrated in the cities, whereas 80% of

the population lives in rural areas. The few available physicians are unable to meet even the acute medical needs.

Many family planning specialists and policy makers believe that voluntary sterilization, because of its high effectiveness and acceptability, must become the mainstay of family planning programs. But, for the reasons mentioned, the conventional surgical approaches for female sterilization cannot be used in many areas of the world. Thus, a procedure is needed that can be performed without surgical entry of the woman's abdominal cavity, and is safe, effective, inexpensive, simple in design, and easy to learn.

The most promising nonsurgical female sterilization approach presently being evaluated is the transcervical introduction into the fallopian tubes of a pharmacologically active agent to produce tubal closure. Such a system has been sought for more than a century, and during the past two decades, many chemical agents have been tested, some in clinical trials.

A major problem with this approach is that most agents that are toxic to the tubal epithelium are also toxic to the peritoneum and the pelvic viscera. Finding a system that permits blind delivery of toxic agents to the fallopian tubes without allowing the agent to reach the peritoneal cavity has been difficult.

The major research efforts to develop a safe and effective nonsurgical means of blocking the fallopian tubes are described in the following sections, and clinical studies with several promising chemicals and delivery systems are detailed.

AGENTS FOR TUBAL CLOSURE

Chemical agents that have been tested for tubal blockade are numerous and fall into several major categories:

Caustic, sclerosing, granuloma-producing, and cytotoxic agents, acids and bases, tissue adhesives, and agents that are not pharmacologically active.

Strong caustic agents. In 1849, Froriep reported the use of a nitric acid-coated probe, passed transcervically on the tip of a cannula, to engage the tubal ostia and to cauterize the tubal lumen (20). Over the next hundred years, a number of caustic agents, such as silver nitrate, zinc chloride, copper sulfate, and formalin, used alone or in combination with ethanol, were tested for their ability to produce tubal closure in a number of animal species and in humans (Table 1).

AGENT	ANIMAL	REFERENCE(S)
SILVER NITRATE	Rabbit	(37, 48)
	Pig	(37)
	Monkey	(36)
	Human	(2, 45, 49)
ZINC CHLORIDE	Rabbit	(48)
	Rat	(58)
	Monkey	(36)
	Human	(2)
COPPER SULFATE	Rabbit	(51)
	Rat	(56)
FORMALIN (alone or with ETHANOL)	Rabbit	(51)
	Rat	(57)
	Human	(10, 57)

Table 1. Strong caustic agents used to block the fallopian tubes.

Silver nitrate, zinc chloride, and copper sulfate all produced similar reactions, regardless of the species tested: Immediate, extensive necrosis, inflammation, and edema. The extent of injury was dose-related and with higher doses of the agents, the entire wall of the fallopian tube became necrotic and the animals died, presumably of chemical peritonitis. Despite the massive injury, however, tubal closure was rarely achieved with these agents, and epithelial regeneration and re-establishment of the tubal lumen were common. The tubes were usually damaged severely, and complex intratubal adhesions simulating the follicular salpingitis found in humans were frequent following a healing phase.

Attempts to increase the closure rates using gelfoam, sponges, gauze strips, and surgical mesh repair materials were unsuccessful, as was the use of granuloma-producing chemicals and other agents to potentiate the action of these acute necrosis-producing agents (48).

Chronicity of exposure was important, and when silver nitrate was placed in a slow-release vehicle, it proved to be a technically satisfactory tube-closing agent. In human clinical trials, however, this combination proved unsatisfactory, because it was as caustic to the peritoneal

cavity as to the fallopian tube and produced a chemical peritonitis (45).

Formalin, alone or with ethanol, is a potent tube-closing agent and produces sclerosis of the endometrial cavity in small animals (51), but in clinical trials, the closure rate after multiple applications was insufficient to warrant continuing efforts (57).

Strong acids and bases. A variety of strong acids and bases have been used to treat the fallopian tubes of animals and humans. Sulfuric acid, salicylic acid, carbolic acid (phenol), and sodium hydroxide were all studied in the rabbit by Richart and co-workers (48). Phenol was used in combination with tincture of iodine in humans by Brazilian midwives (50), and in combination with a mucilago compound by investigators in the People's Republic of China (43).

These agents had an immediate effect on the epithelium of the fallopian tube and endometrial cavity, producing tissue destruction and both acute and chronic inflammation. As with the strong caustic agents, the effects were dose-related, and complete tubal necrosis could be produced with a sufficiently high concentration of these highly active chemicals. When delivered without a slow-release carrier, they were difficult to control and not infrequently spilled into the peritoneal cavity, where they produced acute chemical peritonitis and necrosis of pelvic structures. Phenol is the only agent in this group that has been used in humans, but it is effective only in a compound that limits its accessibility to the peritoneal cavity and apparently limits its release rate as well. When the agent is used alone and produces acute effects, tubal damage is the rule, but tubal closure is the exception.

Sclerosing agents. Sclerosing agents, commonly used by vascular surgeons for the obliteration of vessels, have also been applied to the endometrial cavity and fallopian tube in an effort to produce closure. Sodium morrhuate, sotradecol, and sodium lauryl sulfate have all been studied in animals (48), and sodium morrhuate has been used in humans as an adjunct to tubal ligation (15, 38). Sclerosing agents have not been useful in producing tubal blockade and appear not to be effective in producing epithelial necrosis or fibroblastic ingrowth.

Granuloma-producing agents. A large list of granuloma-producing agents, among them talc, asbestos, cellobiose, silica, diatomaceous earth, beryllium nitrate, quartz, and plant cells, have been studied in rabbits and monkeys (48, 51) and talc granules were used by Ringrose in an attempt to potentiate the action of silver nitrate in humans (49). These agents do produce granulomata in the wall of the uterus or fallopian tube, but the granulomata never reach sufficient size to produce tubal blockade, and the agents have no effect on the epithelial lining. If they reach the peritoneal cavity, they

also produce adhesions and granulomata on peritoneal surfaces and the pelvic viscera, a serious drawback to their use as potentiating agents in combination with more effective compounds.

Cytotoxic agents. ThioTEPA and quinacrine have been studied in animals (48, 55, 58, 60) and quinacrine has been studied extensively in humans (1, 3, 4, 9, 27, 30, 61). The mechanism of action of quinacrine in producing fallopian tube blockade is not known, but it appears to be species-specific and highly dose-related. Quinacrine binds to DNA, apparently intercalating between the coils of double-stranded nucleotides at the A-T base pairs. When the quinacrine is bound to DNA, the bound portions of the molecule are less likely to be replicated, and synthesis of DNA, RNA, and protein decreases. Histologically, in humans quinacrine appears to exert its effects only in the interstitial portions of the fallopian tube, where it produces subepithelial hyalinization and scarring, and destruction of the tubal lining epithelium. The effect appears to be highly localized and variable, and the precise mechanism is not known.

Cadmium, colchicine, and podophyllin have also been investigated but appear to be ineffective (58).

Tissue adhesives. Tissue adhesives from the cyanoacrylate series of compounds have been studied in a number of animals and in humans (6, 35, 36, 37, 48, 53) and the methyl derivative has been found to be effective in closing the fallopian tubes (6). Both ethyl and isobutyl cyanoacrylate cause less local toxicity than the methyl compound, and although both function as effective tissue adhesives, the failure to produce local necrosis makes them ineffective in closing the fallopian tubes (48). Gelatin resorcinol formalin (GRF) has been studied in experimental animals and, although it produced functional sterility in the rabbit, at histologic examination the majority of the tubes were patent, and GRF was not explored further (12, 13, 23).

Other agents. Although not pharmacologically active, silicone rubber and hot water have also been used to close the fallopian tubes, and both are potentially amenable to outpatient sterilization procedures (11, 14, 17, 24, 31, 32, 33, 39, 40, 41, 51) (Figures 1A and 1B). Silicone rubber was first studied by Hefnawi and co-workers in rabbits (24) and subsequently re-examined using different formulations by Erb and co-workers and Davis and co-workers in rabbits and monkeys (13, 17). The Erb formulation has also been applied in humans, using a special mixing device and a specially-designed hysteroscope for its administration (18, 40, 41). Rakshit reported human trials with blindly delivered silicone rubber techniques (39). Moulding and associates have reported the use of hot water to obliterate the tubal epithelium, but this approach has not been used clinically

(31, 32, 33). Droegemueller has described an apparatus for producing necrosis cryotherapeutically at the utero-tubal junction of the baboon; this apparatus has also been used in a limited clinical trial (16, 21) (Figure 2).

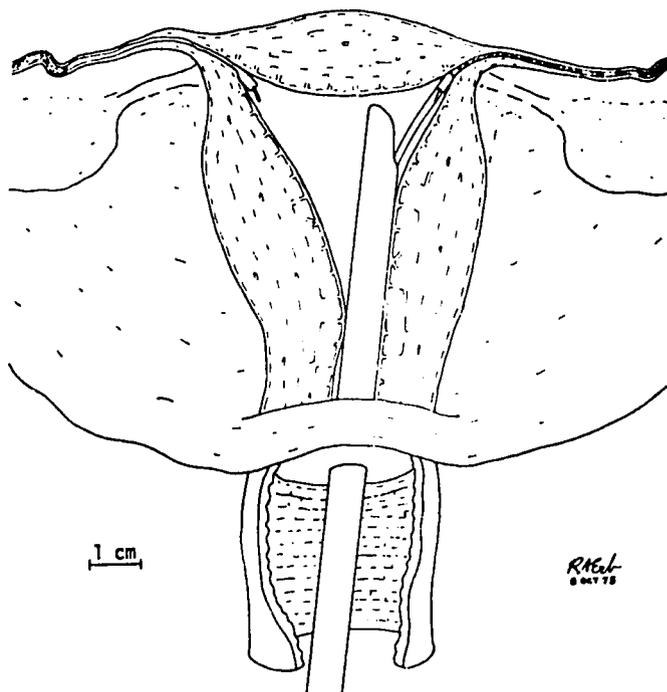


Figure 1A. Schematic drawing of the application of silicone rubber to the human fallopian tube using hysteroscope applicator system. (Illustration courtesy of Dr. T. P. Reed).

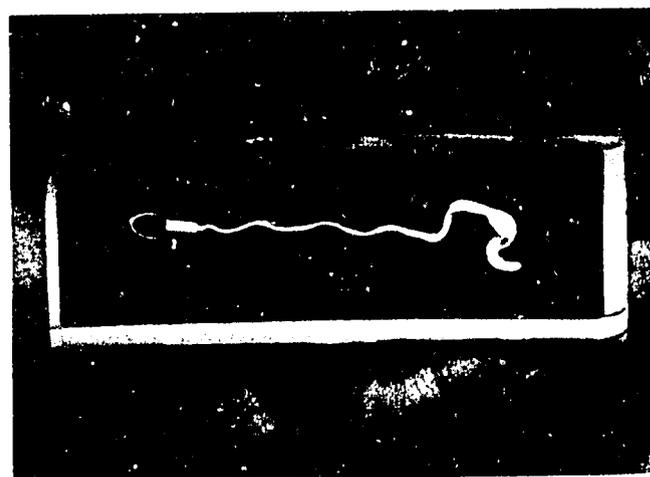


Figure 1B. Configuration of formed-in-place silicone rubber oviductal plug with retrieval loop on the left end. (Photograph courtesy of Dr. T. P. Reed).



Figure 2. Cryotherapy probe used to treat the cornual and lower interstitial segments of fallopian tube in humans. (Photograph courtesy of Dr. W. Droegemueller).

DELIVERY SYSTEMS

An important factor in developing a practical transcervical method of sterilization is to find a system whereby a toxic chemical can be delivered to the site where it is to produce the desired destruction without damaging surrounding tissues. Most of the agents mentioned above are toxic not only to the tubal epithelium, but to the peritoneum and pelvic viscera as well. Possible exceptions are quinacrine and methylcyanoacrylate (MCA), which seem not to be toxic in the peritoneal cavity unless introduced in very large amounts. Agents that produce immediate acute necrosis with wide-spread tissue damage and acute inflammation appear not to be satisfactory for tubal closure, since their flow cannot be controlled. They have consistently leaked from the fallopian tube into the peritoneal cavity, producing local or generalized peritonitis, depending on the volume reaching the peritoneal surfaces.

Attempts to control the flow of these cauterizing agents, by combining them with inert carriers or polymeric systems, have usually been unsuccessful, since the

agents are chemically so active that there are very few systems with which they can be utilized. No satisfactory slow-release system for delivering highly caustic agents has yet been devised that produces tubal closure without undesirable or dangerous side effects. Some of these agents would probably produce effective tubal closure, if it were possible to combine them in a slow-release delivery system, but efforts to develop such a system have not succeeded, and no new approaches are currently being studied. The problem is further compounded by the fact that most potentially useful carriers, such as thixotropic gels or carboxymethylcellulose, have a high viscosity that inhibits their easy flow through the interstitial portion of the tube.

Viscosity is an important variable in considering a method for delivering tube-closing agents, in the selection of the agents themselves, and in the development of active agents and carriers. The uterotubal junction is small and leads to a several-centimeter long, narrow, interstitial tubal segment which, in turn, is contiguous with the slightly wider isthmic tube and the increasingly wide ampulla and fimbrial sections. Since the tube is, for practical purposes, a fluid-filled potential space, and since the tubal orifice can be dilated to only 1 to 2 mm mechanically, considerable hydrostatic pressure is required to force a fluid into the tube for any significant distance.

Delivery is much easier with a substance of low viscosity, and becomes increasingly difficult as viscosity increases. Substances such as GRF (12) and the silver acetate/alginate mixture (22) are difficult to work with and to deliver using a catheter, a cannula, or other delivery technique. The hand-held cannula described by Corfman and Taylor (7), the balloon-tipped cannula systems described by Moulding and co-workers (34), and the tube-finding cannula developed by Battelle Laboratories, which has extendable arms designed to form a triangle (19), all were unable to pump viscous materials without being forced away from the cornua. The only systems that have delivered tube-closing agents successfully in a reproducible fashion have been those in which the tubal orifice is cannulated directly (1), or blindly (43), or those in which the uterus and fallopian tubes become a closed system and medication is forced into the tubes under pressure (47, 52).

Erb and his colleagues have used a specially-designed hysteroscope, shown in *Figure 1A*, to deliver silicone rubber to the fallopian tubes, but have reported that approximately 11% of patients cannot be treated with this system because the geometric configuration of the uterus precludes adequate tubal cannulation (42). If the catheter is not precisely placed in the uterotubal orifice, the high pressure required to drive the silicone rubber into the fallopian tube also causes leaking around the seal and inadequate application of the material.

Other agents, such as MCA and quinacrine, have also been delivered using hysteroscopy, and the investigators reporting on these attempts have noted similar difficulties in tubal cannulation (1, 29). Although the hysteroscope is useful in a variety of clinical applications, it is a difficult instrument to use, service, and maintain; considerable training and experience are required before an operator becomes skilled in its use, and a delivery system based on hysteroscopy will probably be too complicated for use either in the developing world or in most of the developed world.

Two systems for the application of MCA have been tested in clinical studies. Both produce a closed system by blocking off the uterine outflow tract with an inflatable balloon. With the blind delivery system described by Stevenson and Taylor, the MCA is applied under pressure, produced by injecting an excess of fluid into the uterine cavity through a cannula; the fluid then finds its way into the tubal orifice and the fallopian tubes (53). With this system, controlling the volume of fluid or delivering the chemicals with precision is difficult, but the system has been effective in clinical trials with MCA, whose polymerization time is only slightly inhibited, because the flow of the adhesive becomes self-limited as the viscosity rapidly increases and the monomer turns into a solid plastic.

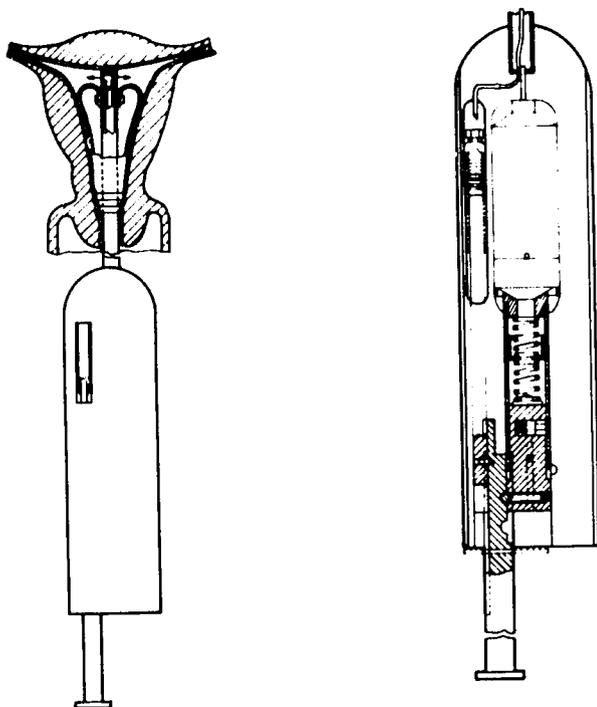


Figure 3. Schematic drawing of FEMCEPT device. A balloon-tipped cannula is passed transcervically to the uterine fundus, after which the plunger is pressed forward. The balloon partially expands, and MCA is expelled on the balloon's surface and is then forced into the tubes by the piston-like action of the advancing balloon.

The FEMCEPT device being studied by Richart and associates (Figure 3) uses a highly expandable balloon as a piston to drive the MCA into the fallopian tubes, and is capable of delivering a precisely measured volume of the material to the tubes without the danger of peritoneal spill or the need to leave large volumes of the chemical, which must eventually be expelled, behind in the uterus (47).

The delivery systems used for quinacrine initially comprised a blind system in which a catheter was passed transcervically to the fundus and the quinacrine solution was gently introduced using a lavage technique (61). Some investigators used a closed system with a cervical olive, attempting to determine whether failures occurred because the quinacrine did not reach the fallopian tubes, or because it was inactive as a tube-blocking agent (27). Subsequent studies indicated that multiple exposures to quinacrine were more efficacious than a single acute insult and that chronic exposure was the most effective (59).

On the basis of these findings, quinacrine pellets have been devised that can be placed in the uterus with an IUD inserter and left in place to diffuse into the fallopian tubes (25). Whether this approach will significantly increase the quinacrine closure rates is not yet known, but clinical investigation is underway.

In the most recently developed delivery system, the quinacrine is compressed onto the tips of a T-shaped or V-shaped IUD-like vector (28). The vector is inserted into the uterine cavity, and the tips, which lie in close proximity to the tubal ostia, theoretically enable the quinacrine to diffuse slowly into the tubes, thereby producing more complete closure.

Another method of delivery being considered is the incorporation of quinacrine into slow-release polymers, again, to extend the diffusion time and produce a higher rate of closure (28).

CLINICAL TRIALS

The only tube-closing agents that have been tested clinically in a significant number of patients are silver-based compounds, ethanol-formaldehyde, quinacrine, and methylcyanoacrylate. Some of the more interesting clinical trials are described in the following sections.

Silver-based compounds. Clinical trials, using silver nitrate compounded in a water-based cream, were undertaken in 14 patients by Richart and co-workers (45). In these patients, the fallopian tubes were brought into the vagina through a posterior colpotomy incision; a cannula was passed through the fimbriated end of the tube, and the silver nitrate cream was injected under direct vision. The tubes were then replaced in the abdomen and the vagina was closed.

The response of the patients was carefully studied. Although all the fallopian tubes in this series were closed, many of the patients experienced fever, leukocytosis, and significant pelvic pain ascribed to a chemical peritonitis. All these symptoms disappeared under treatment, and no long-term sequelae occurred.

Ringrose, in a separate study, used a blind cannula system to deliver various concentrations of a silver nitrate-based compound (10%, 15%, and 20%) to the fallopian tubes of 260 patients (49). The closure rates were approximately 50% with the 10% silver nitrate compound, and 70% with the 15% compound; the rate was not stated with the 20% compound. There was apparently a significant degree of intraperitoneal spill with all three concentrations, presumably due to the uncontrolled blind delivery system. The clinical sequelae increased with greater concentrations of silver nitrate: A number of patients suffered severe peritoneal signs, two had paralytic ileus, many required hospitalization, and some suffered additional complications not reported in the original publication (26).

In our recent attempts to incorporate the silver ion into a polymeric system that might be useful clinically, we found that even with a complicated alginate-based mixture, migration of the silver ion into the peritoneal cavity could not be prevented (22). In a recent series using baboons and *Cebus* monkeys, significant peritoneal damage and extensive necrosis occurred, and several animals died (44). It would appear that silver-based tube-closing compounds are potentially highly toxic, that further studies using these materials as a base should be undertaken with great care, and that thorough testing should be done before these compounds are used in clinical trials.

Ethanol-formaldehyde. In 1972, Zipper and co-workers, in a series of 93 women, used 2 ml of a 2% solution of formaldehyde in ethanol to lavage the uterus through a biopsy cannula (57). They determined tubal closure by insufflation or hysterosalpingography. Overall, non-patency was obtained in only 54 of the 97 women (58%), and the rate of closure increased steadily with serial injections. A number of women dropped out before completing the study, but those who had six instillations had a high rate of obstruction. The optimal number of treatments required to produce clinically satisfactory results was not shown conclusively with this study. At the two-year follow-up, 8.7% of the patients with supposed tubal closure had become pregnant (57).

Mucilago phenol. A group of investigators from the People's Republic of China have reported their findings with an extensive study involving 3,940 women whose fallopian tubes were treated with pharmacologically-active agents (43). They began their studies in 1970 with a phenol-based compound and modified the adminis-

tered agent through a series of trials extending through 1977. The rate of bilateral occlusion ranged from 77.6% in the initial series to 93.5% in the most recent series. The compound was administered using a hand-held plastic catheter inserted through a metal tube placed at the uterine tubal ostium. Normal saline was injected through the plastic catheter, and if there was no back-flow, 1 ml of air was injected, followed by 0.1 to 0.15 ml of mucilago phenol. Although the mucilago phenol was compounded differently in each of the separate trials, the details of the compounding are not given in the text, nor are the closure rates of the individual trials presented. The compound used most recently consisted of 35 ml liquid phenol, 5 g mucilago of tragacanth, 20 ml glycerin, and 100 ml or less of distilled water. Tubal patency was determined by hydrotubation.

The investigators also treated lactating women with the agent. The rate of successful location of both cornua was 92.8% in these women, as opposed to 82.5% in non-lactating women, but the rate of bilateral closure was 91% in 156 lactating women, as opposed to 94.9% in 275 non-lactating women.

A number of side effects occurred following the application of the phenol compound. Forty percent of the women had mild lower abdominal pain and back pain for 2 to 3 days and 1.2% were febrile. Thirty-five patients suffered acute pelvic inflammatory disease. The minor side effects disappeared spontaneously, the acute pelvic inflammatory disease was treated symptomatically, and all patients recovered without further problems. In three lactating women, the uterus was perforated. Ninety-six women underwent laparotomy after tubal occlusion for reasons not related to the sterilization procedure. Six patients developed adhesions between the tubes and the pelvic side wall or ovary, but five of these six patients had suffered acute inflammatory disease immediately after the operation.

The investigators followed 2,487 women for 2 to 7 years after bilateral tubal occlusion was achieved using the hydrotubation technique. Of these women, 64 became pregnant (a pregnancy rate of 2.6%). In 909 women, hydrotubation or hysterosalpingography was performed 2 to 7 years after a diagnosis of bilateral tubal obstruction. Of these patients, 15 were found to have one or both tubes patent (a patency incidence of 1.65%). Menstruation was unaffected.

The Chinese investigators also studied the relationship between the length of fallopian tube that was filled with the mucilago phenol compound and the rate of tubal closure, as determined by hysterosalpingogram (Figure 4). When more than 1 cm of tube was filled, 97% of the tubes were occluded. In contrast, if the agent was placed in the cornu alone, only 16% of the tubes were occluded.



Figure 4. Radio-opaque mucilago phenol in the fallopian tubes. When the entire length of fallopian tube is filled, bilateral closure rate is very high. (Photograph courtesy of Dr. Y. Wu).

At the time of the report, ten pregnancies had occurred, including one ectopic pregnancy, and two women were found to have patent fallopian tubes on hysterosalpingography. The total clinical failure rate was 0.7% after 7 years. An additional five procedures failed during years 7 to 9, making a total failure rate of 1%. There was a direct relationship between the length of fallopian tube filled and the pregnancy rate. If a 1 to 2 cm portion of tube was filled, the pregnancy rate was 7.1%. If 2 to 4 cm were filled, the rate decreased to 3.3%, and when the tube was filled to the ampulla, the rate decreased to 0.1%. There were no pregnancies if the entire fallopian tube was filled (Table 2).

Quinacrine. Quinacrine hydrochloride has been widely studied by a number of investigators (Table 3). In Zipper's initial study, the quinacrine was administered as a solution of 250 mg/2 ml, using a polyethylene catheter that was passed transcervically and placed at the fundus (61). In subsequent studies, the quantities of quinacrine were increased, and in some studies, attempts were

made to use a cervical olive to ensure that the quinacrine came in contact with the fallopian tubes. The closure rates using quinacrine lavage varied widely from study to study after a single application and, with the exception of Davidson's small series (9), seldom exceeded 70% unless multiple lavages were performed.

In the most recent studies, Zipper and his colleagues have compressed the quinacrine into pellets and introduced a number of them, totaling 250 mg, into the uterine fundus using an IUD inserter. In the initial studies, this process was repeated three times, and a bilateral closure rate in excess of 95% was achieved (25).

Laufe and associates, in addition to inserting compressed pellets into the uterus, have fashioned V-shaped and T-shaped IUD vectors that carry a compressed bolus of quinacrine in the tips of their arms (28). These arms are thought to deploy the quinacrine in the vicinity of the tubal ostia when the IUD is inserted into the uterus, ensuring that the quinacrine will be diffused in the region of the fallopian tube and produce the desired effect. Although only a few cases have been studied, the preliminary results are encouraging.

Quinacrine has been used widely to treat malaria and neoplastic effusions. Its mechanism of action in producing tubal closure is not known, but is thought to be related to its ability to intercalate with DNA, and to inhibit DNA, RNA, and protein synthesis. A puzzling aspect of the quinacrine studies has been the variability of the tubal lesions produced by the drug. Even in patients in whom the quinacrine was applied directly to the fallopian tubes, lesions might involve a wide area or be focal, and some tubes were undamaged. The drug is thought not to produce local complications in the peritoneal cavity, but has been associated with a variety of other side effects, the most serious of which is central nervous system excitation (Table 4). The fact that the few patients treated with quinacrine pellets have not experienced CNS excitation suggests that the pellets may be a more satisfactory mode of administration (25).

PORTION OF TUBE FILLED	NO. TUBES TREATED	NO. TUBES CLOSED	TUBES CLOSED (%)	PREGNANCY RATE (%)
CORNU ONLY	24	4	16	not given
INTERSTITIAL PORTION	27	23	85	not given
ISTHMIC PORTION (1-2 cm)	66	64	97	7.1
ISTHMIC PORTION (2-4 cm)	137	137	100	3.3
AMPULLA	222	222	100	0.1
FIMBRIA	34	34	100	0

Table 2. Length of tube filled by mucilago phenol, tubal occlusion (shown by HSG), and pregnancy rate (43).

TYPE OF STUDY/ REFERENCE	NO. PATIENTS (TOTAL 1,179)	QUINACRINE SUSPENSION DOSE	NO. INSTIL- LATIONS	BILATERAL OCCLUSION*	PER CENT
STERILIZATION Zipper, 1970 (61)	85	250 mg/2 ml	3	60	70.5
	37	1 g/4 ml	2	29	78.4
PREHYSTERECTOMY Davidson, 1973 (9)	10	1 g/6 ml	1	6	100.0
				4†	25.0
STERILIZATION Benoit, 1975 (3)	30	1 g/6 ml	3	25	83.3
STERILIZATION Zipper, 1975 (62)	638	130 @ 1.5 g	2	94	72.3
		508 @ 500 mg-3 g		343	68.4
STERILIZATION Israngkun, 1976 (27)	60	1 g/7 ml	1	23	44.2
STERILIZATION Mehtaji, 1977 (30)	98	1 g	3	70	71.4
PREHYSTERECTOMY Bhatt, 1977 (4)	12	1.5 g	1 or 2	5	41.6
STERILIZATION Zipper, 1979 (25)	139	250 mg‡	3	§	§

* Documented by HSG or insufflation. † Hysterectomy performed within 1 wk.
‡ Pellets employed; number per insertion not stated. § Status of tubes not evaluated.

(From King TM, Blake DA, Dubin NH: Internal document prepared for International Fertility Research Program [IFRP]; used with permission of authors and IFRP)

Table 3. Summary of clinical results in various quinacrine studies.

It has been reported that Xylocaine, epinephrine, cortisone, versinate, oxytocin, tetracycline, and copper potentiate the action of quinacrine (59). Sodium pentothal has been useful in solubilizing the quinacrine pellets, and in some patients in the pellet series, the quinacrine has been administered with pentothal as a carrier (25). Giving estrogen and progesterone following quinacrine therapy sometimes promotes recanalization, but it is not clear whether this effect has been important in human trials.

In most of the quinacrine studies, pregnancy has been used as an endpoint. To date, 69 pregnancies have been reported, all intrauterine (Table 5).

MCA. A number of investigators have studied the effects of MCA in the fallopian tubes (Table 6). MCA is an epitheliotoxic agent that releases acetocyanic acid and formaldehyde upon degradation, and produces necrosis of the tubal lining epithelium adjacent to the MCA. As the agent is gradually degraded over a period of 6 weeks,

DOSE/MODE OF ADMINISTRATION	COMPLICATION
SUSPENSIONS (1.0 - 3.0 g)	CNS excitation (3 cases) Vagal reaction (1 case) Abdominal/pelvic pain Abdominal distention Fever Chemical vaginitis Rash Death (2 reported in personal communications)
PELLETS (250 mg)	No CNS excitation (138 women, up to 3 instillations)

(From King TN, Blake DA, Dubin NH: Internal document prepared for IFRP; used with permission of authors and IFRP)

Table 4. Complications following transcervical quinacrine instillations.

STUDY/ REFERENCE	YEAR	PREGNANCIES (TOTAL 69)	COMMENTS
ZIPPER (61)	1970	7	8.6/100 woman years
DAVIDSON (9)	1973	0	Patients either had hysterectomy or used OCs
BENOIT (3)	1975	0	Many patients took OCs; 15 did not. No pregnancies reported, but duration of follow-up unclear
ZIPPER (62)	1975	9	In 2,294 woman months of follow-up
		41	In 12,383 woman months of follow-up
ISRANGKUN (27)	1976	6	Depo-provera contraception until tubal closure proven
MEHTAJI (30)	1977	2	In 680 woman months of follow-up
ZIPPER (25)	1979	4	In first year of follow-up of 53.9% of patients

(From King TM, Blake DA, Dubin NH: Internal document prepared for IFRP; used with permission of authors and IFRP)

Table 5. Pregnancies in patients with bilateral tubal occlusion following treatment with quinacrine.

an inflammatory infiltrate replaces the tubal epithelium and it, in turn, is replaced by young fibroblasts, which are gradually converted into dense scar tissue.

Clinical trials with MCA were initially reported by Stevenson and Taylor, who modified a Foley catheter for intrauterine instillation of the MCA (53). The size 12 catheter was passed through the cervix and a 5 mm balloon was inflated to block the uterine outflow tract. Following this, a 1- to 5- ml portion of MCA was injected through the narrow-bore polyethylene tube cemented into the catheter, and 15 seconds later, 2 ml of sterile

water were injected through the catheter to polymerize the remaining MCA in the uterine cavity. In the initial series, 12 patients were treated prior to hysterectomy, and the histological events taking place following MCA application were documented.

In a subsequent study, the technique was applied to 34 ambulatory women, 66% of whom had bilaterally closed fallopian tubes, as shown by hysterosalpingography 8 weeks following application (52). An additional 11 patients were treated a second time with MCA, and in 10 of these patients, both tubes were closed.

STUDY/ REFERENCE	YEAR	NO. PATIENTS	BILATERAL CLOSURE (%)	COMMENTS
STEVENSON, TAYLOR (53)	1972	12	—	Prehysterectomy application
STEVENSON (52)	1976	34	66	HSG at 8 weeks
		11	90	Reapplication of MCA
NEUWIRTH, ET AL (35)	1980	131	72	Closure rates of 54% to 78% in 3 series with various volumes of MCA (0.4-0.65)
		19	74	Reapplication of MCA
RICHART, NEUWIRTH (46)	1981	180	80	0.6 ml MCA; modified FEMCEPT device
		19	98	Reapplication of MCA following patency detected by HSG

Table 6. Results of clinical trials with MCA.

The largest series of MCA-treated patients studied to date has been reported by a group of collaborators using the FEMCEPT device (35, 47). These ongoing clinical studies are supported by the Program for Applied Research on Fertility Regulation (PARFR). The FEMCEPT device is an instrument that utilizes a balloon-tipped cannula to block the uterine outflow tract and to decrease uterine dead space. Following ejection of the MCA through the distal tip of the cannula, the balloon is further expanded and acts as a piston to force the MCA into the fallopian tubes.

In a series of 131 procedures (35), various volumes of MCA were applied to the uterus, and the bilateral closure rates, as determined by hysterosalpingography, ranged from 54% to 78%, with an overall closure rate of 72%. In 19 patients in whom one or both tubes were found to be patent and were subsequently retreated, the bilateral closure rate on retreatment was 74%.

In the latest series, using improved MCA and a slightly modified instrument, a bilateral closure rate of 80% was achieved after one application and 98% in those 19 patients who had a second application after tubal patency was detected by hysterosalpingography (46).

DISCUSSION

Certain principles have evolved from the studies of pharmacologically active agents applied to the fallopian tubes; these principles may be applicable to the design of future delivery techniques and tube-closing pharmacologically-active formulations. It is evident, because of the efficient regenerative capacity of the tubal lining epithelium, that acute injuries, even when they are massive, routinely fail to destroy all the epithelial cells, which may rapidly regenerate and reconstitute tubal patency during the period of healing and reconstruction. Those agents that are most effective in producing tubal closure are associated with a chronic long-term effect, because of their slow degradation rates (for example, MCA), or are released over a prolonged period of time (for example, mucilago phenol). It is also apparent that in the absence of chronicity, multiple applications are more effective than single applications and enhance the cumulative rate of tubal injury and closure (for example, quinacrine in solution or ethanol-formaldehyde). Most of the authors studying this problem at length have commented upon the necessity of meeting these requirements in order to produce a high rate of tubal closure.

It has been documented in the mucilago phenol studies from the People's Republic of China, and it was apparent in the studies of MCA done by our group on Rhesus monkeys, that another major determinant of tubal closure rates is the length of the fallopian tube exposed to the pharmacologically active agent. This is consistent with observational data from laparoscopic electrocoagulation studies, in which the early attempts at closure using

coagulation alone had a high failure rate, due to the small segment of tubal damage. As the length of injured segment was increased, tubal closure rates increased substantially, and it is generally thought that a 3 cm injury is optimal. It is apparent that the fallopian tube is more easily reached during the immediate postmenstrual and early proliferative phases of the cycle, when the endometrium has only begun its cyclic thickening, and it is probable that applications performed late in the cycle, or when the tubes are in spasm, will result in failure. It is also clear that there will be unavoidable failures in women with anomalous uteri or with intrauterine disease, such as uterine synechiae or submucous leiomyomata, which cannot readily be detected even by a carefully taken history and a pelvic examination.

The side effects of the agents that have been used to close the fallopian tubes relate principally to the ease with which their transit through the tube to the peritoneal cavity can be controlled, and to their local toxicity. It is difficult to control strong acids and bases or strong oxidizing and reducing agents, regardless of the manner in which formulation is attempted. Even when release rates are slow, the action of these generally highly corrosive ions is so intense that their spill into the peritoneal cavity produces serious sequelae. Even in the Chinese studies, in which attempts were made to limit the transit of the mucilago phenol, a significant proportion of the patients complained of pelvic pain, many had fever, and some suffered from acute pelvic inflammatory disease. This litany is similar to that reported by Ringrose, and seems to be characteristic of a syndrome of local pelvic peritonitis due to chemical spill. The series of patients treated with ethanol-formalin did not show serious side effects, but the action of the agent was so mild that five or six applications were required before significant tubal closure resulted.

CONCLUSION

The only two chemical agents for tubal closure that have been tested clinically and that appear to have minimal side effects are quinacrine and MCA. Both have been applied in a variety of settings, both produce roughly comparable closure rates, and both appear to be candidates, with modifications in the delivery system or the ability to monitor tubal penetration, for a clinically useful outpatient-based sterilization technique for women. The delivery systems appear to be relatively simple and easy to use. The efficacy must be increased in order to have the greatest utility without requiring multiple applications, and a substantial number of women must be followed for a significant period of time to determine that there are no long-term sequelae with these systems.

If these goals can be met, it is probable that tubal blockade can be accomplished using a safe, rapid, and relatively inexpensive outpatient procedure.

REFERENCES

1. Alvarado A, Quiñones R, Aznar R: Tubal instillation of quinacrine under hysteroscopic control. In Sciarra JJ, Butler JC Jr, Speidel JJ (eds): *Hysteroscopic Sterilization*. New York, Intercontinental Book Corporation, 1974, pp 85-94.
2. Babcock WW: Chemical hysterectomy. *Am J Obstet Gynecol* 7:693-696, 1924.
3. Benoit A, Melancon J, Gagnon M: Chemically-induced tubal occlusion in the human female using intrauterine instillation of quinacrine. *Contraception* 12:95, 1975.
4. Bhatt RV, Pathak ND, Chauhan LN, Shah DS, Bukapatamm VV: A study of transcervical instillation of quinacrine before hysterectomy to test tubal blockage. *Bombay, India Fertility Research Program*, September, 1977, p 171.
5. Chandra H, Nigam SC, Zutshi S, Malaviya B: Chemical occlusion of Rhesus monkey oviducts with chloroquine. *Contraception* 7:333, 1973.
6. Corfman PA, Richart RM, Taylor HC: Response of the rabbit oviduct to a tissue adhesive. *Science* 148:1348, 1965.
7. Corfman PA, Taylor HC: An instrument for transcervical treatment of the oviducts and uterine cornua. *Obstet Gynecol* 27:800, 1966.
8. Davidson OW: Quinacrine-induced tubal occlusion. In Sciarra JJ, Droegemueller W, Speidel JJ (eds): *Advances in Female Sterilization Techniques*. Hagerstown, Harper & Row Publishers, 1976, p 200.
9. Davidson OW, Wilkins C: Chemically-induced tubal occlusion in the human female following a single instillation of quinacrine. *Contraception* 7:333, 1973.
10. Davis JE: New methods of vas occlusion. In Zatzuchni GI, Labbok MH, Sciarra JJ (eds): *Research Frontiers in Fertility Regulation*. Hagerstown, Harper & Row Publishers, 1980, pp 252-261.
11. Davis RH, Erb R, Kyriazis GA, Balin H: Fallopian tube occlusion in rabbits with silicone rubber. *J Reprod Med* 14:56, 1975.
12. Davis RH, Fisher JS, McGowan L: Local antiphlogistic activity of l-phenylalanine and l-tryptophane. *J Endocrinol* 41:555, 1968.
13. Davis RH, McDonald J, Kyriazis G, Balin H: Effect on fertility of intrauterotubal injection of gelatin. *Obstet Gynecol* 42:446, 1973.
14. Davis RH, Moonka EK, Platt HA, Pitkow HS: Chronic occlusion of the rabbit fallopian tube with silicone rubber. *Obstet Gynecol* 6:142, 1978.
15. Dieckmann WJ, Harrod JP Jr: Tubal ligation (sterilization) by a modified Madlener method. *Am J Obstet Gynecol* 68:897-902, 1954.
16. Droegemueller W, Greer BE, Davis JR, Makowski EL, Chvapil M, Pollard A: Cryocoagulation of the endometrium at the uterine cornua. *Am J Obstet Gynecol* 131:1, 1978.
17. Erb RA, Davis RH, Kyriazis G, Balin H: Device and technique for blocking the Fallopian tubes. *Contemp OB/GYN*, February, 1974, p 92.
18. Erb RA, Reed TP: Hysteroscopic oviductal blocking with formed-in-place silicone rubber plugs. I. Method and apparatus. *J Reprod Med* 23:65-68, 1979.
19. Falb RD, Lower BR, Crowley JP, Powell TR: Transcervical fallopian tube blockage with gelatin-resorcinol-formaldehyde (GRF). In Sciarra JJ, Droegemueller W, Speidel JJ (eds): *Advances in Female Sterilization Techniques*. Hagerstown, Harper & Row Publishers, 1976, pp 208-215.
20. Froriep R: Zur vorbeugung der notwendigkeit des kaiserschnitts und der perforation. *Notizen Nat Neilkd* 211:9-10, 1849.
21. Greer BE, Droegemueller W, Bingham PE, Makowski EL: Uterine cryosurgery in baboons. In Sciarra JJ, Droegemueller W, Speidel JJ (eds): *Advances in Female Sterilization Techniques*. Hagerstown, Harper & Row Publishers, 1976, pp 231-238.
22. Gregor HP, Hsia HT, Palevsky S, Neuwirth RS, Richart RM: Fallopian tube cauterization by silver ion-polymer gels. In Paul DR, Harris FW (eds): *Controlled Release Polymeric Formulations*. New York, ACS Symposium Series #33, 1976.
23. Grode GA, Pavkov KL, Falb RD: Feasibility study on the use of a tissue adhesive for the nonsurgical blocking of fallopian tubes. *Fertil Steril* 22:552, 1971.
24. Hefnawi F, Fuchs A-R, Laurence KA: Control of fertility by temporary occlusion of the oviduct. *Am J Obstet Gynecol* 99:421, 1967.
25. IFRP Directions 1981-1982. Distributed by the international Fertility Research Program, Research Triangle Park, North Carolina.
26. Imlach A: Edmonton gynecologist at legal odds with provincial college. *Can Med Assoc J* 112:1345-1355, 1975.
27. Israngkun C, Phaovadi S, Neuwirth RS, Richart RM: Clinical evaluation of quinacrine hydrochloride for sterilization of the human female. *Contraception* 14:75-80, 1976.
28. Laufe L: Personal communication.
29. Lindemann H-J, Mohr J: Review of clinical experience with hysteroscopic sterilization. In Sciarra JJ, Droegemueller W, Speidel JJ (eds): *Advances in Female Sterilization Techniques*. Hagerstown, Harper & Row Publishers, 1976, pp 153-161.
30. Mehtaji S, Jadwani K, Goyal V: Chemical sterilization with quinacrine. *Fourth Transaction of Scientific Papers*. Bombay, India Fertility Research Program, September, 1977, p 167.
31. Moulding T, Norton W, Orr W: Preliminary studies for achieving transcervical oviduct occlusion by hot water or low pressure steam. *Adv Plann Parent* 12:79-85, 1977.
32. Moulding T, Sirota P: Hot water as a tubal occluding agent: Studies in detail. National Technical Information Service, PB293382.
33. Moulding TS, Sirota PS: Hot water as a tubal occluding agent. *Contraception* 19:433-442, 1979.
34. Moulding T, Straw W, Thompson H: The contrast pressure technique for female sterilization. *Contraception* 13:547-557, 1976.
35. Neuwirth RS, Richart RM, Stevenson TC, Bolduc LR, Zinser H, Baur H, Cohen J, Eldering G, Argueta-Rivas G, Nilsen PA: An outpatient approach to female sterilization with methylcyanoacrylate. *Am J Obstet Gynecol* 136:951-956, 1980.
36. Neuwirth RS, Richart RM, Taylor HC: Chemical induction of tubal blockade in the monkey. *Obstet Gynecol* 38:51-54, 1971.
37. Omran KF, Hulka JF: Tubal occlusion: A comparative study. *Int J Fertil* 15:226, 1970.
38. Pitkin RM: Sodium morrhuate for tubal sterilization. *Obstet Gynecol* 28:680, 1966.
39. Rakshit B: Attempts at chemical blocking of the Fallopian tube for female sterilisation. *J Obstet Gynecol India* 20:618, 1970.
40. Reed TP, Erb RA: Hysteroscopic oviductal blocking with formed-in-place silicone rubber plugs. II. Clinical studies. *J Reprod Med* 23:69-72, 1979.
41. Reed TP, Erb RA: Tubal occlusion with silicone rubber. *J Reprod Med* 25:25-28, 1980.
42. Reed TP, Erb RA, DeMaeyer J: Tubal occlusion with silicone rubber: An update. Presented at Lankeau Hospital, Hawaii, January, 1980.
43. Research Group of Tubal Occlusion by Drugs. Sterilization by occlusion of the Fallopian tubes with mucilago phenol: A seven years' clinical observation. *Chinese J Obstet Gynecol* 14:79, 1979.
44. Richart RM, Antonini-Filho R: Unpublished data.
45. Richart RM, Gutierrez-Najar AJ, Neuwirth RS: Transvaginal human sterilization: A preliminary report. *Am J Obstet Gynecol* 111:108-110, 1971.
46. Richart RM, Neuwirth RS: Unpublished data.
47. Richart RM, Neuwirth RS, Bolduc L: Single Application Fertility Regulating Device: Description of a new instrument. *Am J Obstet Gynecol* 127:86-90, 1977.
48. Richart RM, Taylor HC, Neuwirth RS: Experimental studies of fallopian tube occlusion. In Richart RM, Prager D (eds): *Human Sterilization*. Springfield, Charles C Thomas Publisher, 1972, pp 360-367.
49. Ringrose C: Office tubal sterilization. *Obstet Gynecol* 42:151, 1973.
50. Salgado C: Sterilisation induced by intrauterine caustic injections. *An Brasil Genet* 503, June, 1941.
51. Schenker JG, Polishuk JG: Regeneration of rabbit endometrium following intrauterine instillation of chemical agents. *Gynecol Invest* 4:1-13, 1973.
52. Stevenson TC: Methyl Cyanoacrylate (MCA) for tubal occlusion. In Sciarra JJ, Droegemueller W, Speidel JJ (eds): *Advances in Female Sterilization Techniques*. Hagers-

town, Harper & Row Publishers, 1976, pp 216-224.

53. Stevenson TC, Taylor DS: The effect of methylcyanoacrylate tissue adhesive on the human fallopian tube and endometrium. *J Obstet Gynecol* 79:1028, 1972.

54. Zipper J, Cole LP, Goldsmith A, Wheeler R, Rivera M: Quinacrine hydrochloride pellets: Preliminary data on a nonsurgical method of female sterilization. In preparation.

55. Zipper J, Insunza S: Pharmacological agents that potentiate or inhibit the occlusive action of quinacrine in the rabbit tube and rat uterus. In Duncan GW, Falb RD, Speidel JJ (eds): *Female Sterilization: Prognosis for Simplified Outpatient Procedures*. New York, Academic Press, 1972, pp 131-149.

56. Zipper J, et al: Human fertility control through the use of endouterine metal antagonisms of trace elements. *Control of Human Fertility*. Nobel Symposium 15, Stockholm, Almqvist & Wiksell, 1970.

57. Zipper J, Medel M, Pastene L, Rivera M: Intrauterine instillation of chemical cytotoxic agents for tubal sterilization and treatment of functional metrorrhagias. *Int J Fertil* 14:289, 1969.

58. Zipper J, Medel M, Prager R: Alterations in fertility induced by unilateral intrauterine instillation of cytotoxic compounds in rats. *Am J Obstet Gynecol* 101:971, 1968.

59. Zipper J, Medel M, Stachetti E, Pastene L, Rivera M, Prager R: Chemical agents for transvaginal sterilization. In Richart RM,

Prager DJ (eds): *Human Sterilization*. Springfield, Charles C Thomas Publisher, 1972, pp 339-352.

60. Zipper J, Prager R, Medel M: Biological changes induced by unilateral intrauterine instillations of quinacrine in the rat and their reversal by either estrogen or progesterone. *Fertil Steril* 24:48, 1973.

61. Zipper JA, Stachetti E, Medel M: Human fertility control by transvaginal application of quinacrine on the fallopian tube. *Fertil Steril* 21:581, 1970.

62. Zipper J, Stachetti E, Medel M: Transvaginal chemical sterilization: Clinical use of quinacrine plus potentiating adjuvants. *Contraception* 12:11, 1975.

*This publication was supported by the United States Agency for International Development (USAID).
The contents do not necessarily reflect USAID policy.*