

HUMAN FERTILITY CONTROL BY TRANSVAGINAL APPLICATION OF QUINACRINE ON THE FALLOPIAN TUBE*

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The development of a technic for non-surgical sterilization having acceptable clinical effectiveness and a minimum of undesirable side effects is one of the principal objectives of physicians who are concerned about the demographic problems of the world. As noted by Corfman,¹ who recently reviewed the relevant historic aspects, transvaginal sterilization technics have been studied for more than 1 century. Froriep² in 1849 applied silver nitrate to the cornual areas of the tubal ostia. Kocks⁴ in 1878 attempted to effect tubal occlusion by transuterine electrocoagulation of the same areas. Nonspecific caustic agents such as phenol and solutions of iodine have been instilled into the endometrial cavity in attempts to produce tubal obstruction.^{5,8}

In two recent communications^{9,10} we described our experiences in humans using an intrauterine instillation of a 2% solution of formaldehyde in ethanol. Our preliminary observations with this nonspecific type of caustic material suggest that they should be dissolved in ethanol before their instillation. Their combination with ethanol enhances, and possibly hastens, the coagulation of the endometrial proteins, thus reducing to a minimum the quantity of caustic material which may escape through the fallopian tube into the peritoneal cavity. Retention of the caustic material within the tubal lumina is thereby extended over a longer period of time, permitting

progressive diffusion into the tubal epithelium and into the subepithelial tissues. In our previous studies we found that at least six successive monthly instillations with the formaldehyde-ethanol solution were necessary in order to effect tubal occlusion.

According to the Life Table method of analysis, we calculated that the cumulative rate of pregnancy/100 women after 30 months followup in women who had been shown to have tubal occlusion was 8.7. During the period of instillations it was 8.4. There was a dropout rate of 26.0 and a total continuation rate of 45.0¹⁰ (see Table 1). It is logical to assume that these same rates might prevail for other nonspecific caustic agents used in a similar manner.

The most important side effects observed in patients receiving instillations of this type of caustic material were hypomenorrhea and amenorrhea. This indicates that the functional capacity of the endometrium is either partially or completely suppressed as a result of the instillations. Our observations also clearly indicated that this "local" functional effect was only temporary, and was invariably followed by complete restoration to normalcy. As seen in Fig. 1 the histology of the deep layers of the endometrium in these patients was normal and in subsequent biopsies showed no consistent changes.⁹ On the other hand, histologic studies of the fallopian tubes showed rather marked fibrosis of the intramural zone (see Fig. 2).

These preliminary studies prompted us

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TABLE 1. *Cumulative Rates of Nonclosures and Closures per 100 Women**

	Rate after					
	3 mos.	6 mos.	9 mos.	12 mos.	24 mos.	30 mos.
Events (non-closures)						
Pregnancies						
After tubal obstruction	0.0	3.1	4.1	6.4	8.7	8.7
During treatment	1.0	4.1	8.4	8.4	8.4	8.4
Spontaneous recovery of tubal patency	0.0	0.0	1.0	1.0	8.9	8.9
Bleeding	0.0	0.0	1.0	1.0	1.0	1.0
Oligo and/or hypermenorrhea	1.0	3.4	5.6	7.8	10.2	10.2
Amenorrhea	0.0	1.4	3.5	6.8	10.5	10.5
P.I.D.	0.0	0.0	0.0	1.1	2.2	2.2
Medical relevant	1.0	1.0	1.0	1.0	1.0	1.0
Other medical	0.0	0.0	0.0	1.1	3.7	3.7
Personal relevant	2.0	2.0	2.0	2.0	2.0	2.0
Dropout	10.3	20.7	24.9	26.0	26.0	26.0
Method failure	0.0	0.0	2.1	3.2	3.2	3.2
Relevant events (closures)						
Total pregnancies	1.0	7.2	12.5	14.8	17.1	17.1
Spontaneous recovery of tubal patency	0.0	0.0	0.0	0.0	5.3	5.3
Bleeding	0.0	0.0	0.0	0.0	0.0	0.0
Oligo and/or hypermenorrhea	0.0	0.0	0.0	0.0	0.0	0.0
Amenorrhea	0.0	0.0	0.0	0.0	0.0	0.6
P.I.D.	0.0	0.0	0.0	0.0	0.0	0.0
Medical relevant	1.0	1.0	1.0	1.0	1.0	1.0
Personal relevant	2.0	2.0	2.0	2.0	2.0	2.0
Dropout	10.3	20.7	24.9	26.0	26.0	26.6
Method failure	0.0	0.0	2.1	3.2	3.2	3.2
Total closures	14.3	30.9	42.5	47.0	54.6	54.6
Continuation	85.5	68.8	57.2	52.7	45.0	45.6
Women-months of observation	265.5	483.5	660.0	805.5	1275.0	1336.5

* Two per cent solution of formaldehyde in ethanol was used.

to search for nontoxic water-soluble substances which would have rather selective specific effects upon the epithelium and intramural portion of the fallopian tubes and which would be relatively innocuous if they came in contact with the peritoneum by escape from the tubes. From the morphologic standpoint the cornual portion of the tubal lumen is most vulnerable to occlusion because it is a very narrow canal and is surrounded by dense and highly tonic uterine muscles resembling a sphincter. For these reasons it is logical to assume that a water-soluble chemical which has antimetabolic or cytotoxic action might possess the quality of altering the function of the fallopian tube if in-

stilled through the endometrial cavity into the canal of the tube. Occlusion of more distal portions of the tube is less likely because the lumen becomes larger as it approaches the fimbriated end and offers less resistance and reduced time of contact with the instilled solution before its escape into the peritoneal cavity. We believe that the occlusive action of the cytotoxic agents will increase with increased time of contact with the epithelium and stroma of the tube. The endometrium is considerably more resistant to the cytotoxic drugs, presumably because of its relative thickness and because of periodic shedding and renewal. The resistance of the endometrium to locally applied cyto-

toxic agents is advantageous because cytotoxic agents which would produce changes in bleeding patterns are seldom produced as a result of the instillations.

In our search for potentially effective tubal occlusion, the rat served as our experimental model.^{11,12} One of the most interesting observations in our studies with the cytotoxic agents in the rat was that their influence upon fertility was not necessarily related to demonstrable histologic changes. It was possible to reduce fertility with one agent for considerable periods of time in the absence of histologic evidence of abnormality, whereas with another drug massive uterine obstruction occurred and this presumably was the cause of the reduced fertility.¹¹

Quinacrine, a derivative of acridine, has been studied intensively by us¹² and has been shown to produce an intense morphologic change in the endometrium of the rat when instilled into the uterine cavity. The uterine cavity becomes obstructed as a result of hyperplasia of the endometrium and subepithelial stroma, and there is a subsequent prolonged reduction in fertility. It seems likely that the obstructive reaction plays an important role in reducing the fertility. The extent and duration of the obstruction increase with the quantity of quinacrine which is instilled into the uterine cavity. It appears that with large



FIG. 1. Section of human uterus (hysterectomy specimen) 48 hr. after instillation with a 2% solution of formaldehyde in ethanol.

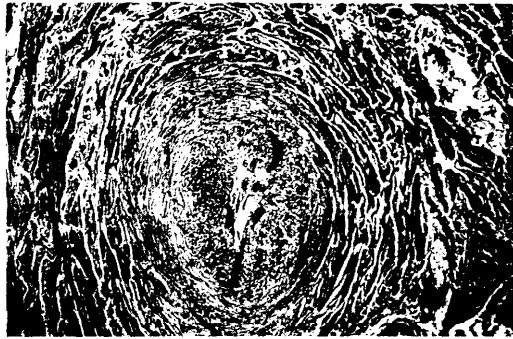


FIG. 2. Histologic aspect of the lesion induced by a 2% solution of formaldehyde in ethanol in the human fallopian tube. X 100.

doses the obstruction does not spontaneously reverse itself. We have shown that if estradiol and/or progesterone are given systemically at the time when the quinacrine is instilled, the obstructive action of the quinacrine is prevented. Also, the systemic administration of either estradiol or progesterone will reverse the uterine obstruction produced by a previous instillation of quinacrine, thus restoring patency of the corresponding uterine horn.

Quinacrine was selected by us for study in humans because it has been injected with safety and without secondary effects into the peritoneal and pleural cavities of humans for the control of effusions resulting from metastatic lesions.^{3,7} Our preliminary observations with its use as a means of producing tubal occlusion in humans were described in 1969.¹⁰ It is our purpose in this paper to present the results of additional and more detailed clinical studies of the intrauterine instillations of solutions of quinacrine to control fertility.

MATERIALS AND METHODS

Quinacrine hydrochloride (Winthrop Laboratories, New York, N. Y.) was prepared in two different concentrations for use in two separate groups of patients. For each group a saturated solution of quinacrine hydrochloride was prepared in distilled water and additional crystalline quinacrine was added to the solution. This

mixture of a saturated solution and a suspension was freshly prepared each time immediately before use. The two mixtures were different only in the quantity of the crystalline suspension and the total volume administered per patient. The patients in Group A were given a regimen consisting of a 2-ml. suspension containing 250 mg. of quinacrine directly into the endometrial cavity. A maximum of three such instillations was given. Each instillation was administered within 3 or 4 days after the termination of the menstrual flow. The regimen for the patients in Group B was a 4-ml. suspension containing 1 gm. of quinacrine as an intrauterine instillation. A maximum of two such instillations was given per patient.

Technic of Intrauterine Instillation.

Only gynecologically normal women were included in the program. The anterior lip of the cervix was grasped with a tenaculum and the direction and length of the uterine cavity were found. A biopsy cannula was then introduced to the top of the endometrial cavity and the suspension of quinacrine was slowly injected through the cannula from an attached 10-ml. syringe.

The injection was completed in approximately 1 min. The cannula was withdrawn from the uterus 1-2 min. after completion of the injection to minimize reflux of the suspension into the vagina.

Group A. Eighty-five patients were admitted to this series. An attempt was made to insufflate the tubes or to obtain a hysterosalpingogram after each instillation. When the tubes were found to be occluded, no additional instillations were done. In those instances when tubal patency persisted after the third instillation, the patient was tabulated as a method failure. If a patient missed two or more clinic visits after her last instillation, she was designated in the tabulations as a dropout.

Four patients in this series who had tubal occlusion requested surgical sterili-

zation. Salpingectomies were done on these four at various intervals up to 1 year after the last uterine instillation and the tubes were studied histologically. In addition, endometrial biopsies were obtained from approximately 20 of the patients within this series.

Group B. Thirty-seven patients were admitted to this series. Tubal insufflation with CO₂ was attempted in each patient after the first uterine instillation. If the tubes were occluded, no additional instillations were done and the patient was examined monthly for tubal patency and for pregnancy. When tubal patency was found after the first instillation, a second was performed. When the second instillation did not occlude the tubes, the patient was tabulated as a method failure. Any patient who failed to return within 2 months after the first instillation and was subsequently found to have patent tubes or to be pregnant was tabulated as a dropout.

Analysis of Data. The Life Table technique was applied to the rather complex events and closures which occurred in Group A. Because the pattern of treatment for the patients in Group B was much less complicated, the results are expressed in percentage of tubal occlusion subsequent to one and two instillations, and to contraceptive use-effectiveness in relation to time. The Life Table method was not used for Group B.

RESULTS

Group A. As seen in Table 2 tubal obstruction was produced in 60 of the 85 patients (70.5%). In 68 patients tubal insufflation was performed after each instillation in consecutive cycles in order to determine tubal patency. Tubal obstruction was found after the first cycle following the initial instillation in 24 of the 68 studied patients (35.2%). It can be seen that the cumulative tubal occlusion rate was 88.2% after three consecutive instillations.

The rate of pregnancies/100 women-years in relation to the number of instillations which produced tubal obstruction is shown in Table 3. The pregnancy rate dropped from 4.9 after one instillation to 1.2 after three instillations.

A summary of the relevant data on events and closures over a 2-year period is shown in Tables 4 and 5. The rate of pregnancy, after tubal obstruction had been diagnosed, was 8.6. This rate includes those patients having one, two, and three instillations. During the treatment period the rate of pregnancies in women who had patent tubes was 5.8. There was a dropout rate of 10.6. At the end of 31 months there was a 62.8 rate of continuation.

Menstrual patterns were recorded on all of the patients and were found to continue in a normal manner. Although some minor variations occurred they were not considered to be significant.

The fallopian tubes from the 4 patients who had salpingectomies were sectioned serially and studied microscopically. The

TABLE 2. *Effect of Number of Instillations on Tubal Patency; Partial and Cumulative Rates (Quinacrine Regimen A) **

No. of instillations	No. of patients	Nonpatent patients		Patent patients	Cumulative rates of obstruction
		No.	Percentage		
1	55	24	43.6	31	35.2
2	41	24	58.5	17	70.5
3	20	12	60.0	8	88.2

* Cumulative rates calculated from 68 studied patients (60 nonpatent and 8 patent).

TABLE 3. *Cumulative Rates of Pregnancies After 31 Months in Relation to the Number of Instillations Which Produce Tubal Obstruction (Quinacrine Regimen A)*

No. of instillations	No. of patients	Pregnancies	Rate/100 women
1	24	4	4.9
2	24	2	2.5
3	12	1	1.2

TABLE 4. *Cumulative Rates of Nonclosures per 100 Women (Quinacrine Regimen A)*

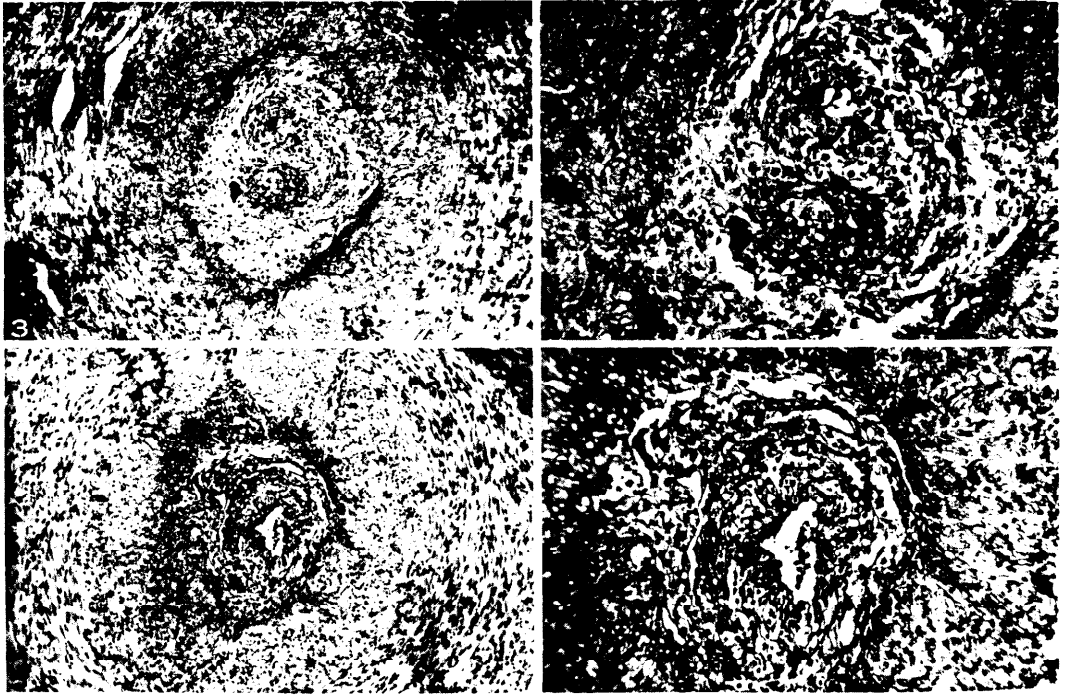
Events	Rate after			
	3 mos.	9 mos.	12 mos.	31 mos.
Bleeding	1.1	3.6	3.0	3.6
Oligo and/or hypomenorrhea	0.0	1.2	2.6	2.6
Amenorrhea	6.0	0.0	0.0	0.0
P.I.D.	1.1	2.4	2.4	2.4
Medical relevant	0.0	0.0	0.0	0.0
Other medical	1.1	1.1	1.1	1.1
Personal relevant	0.0	0.0	0.0	0.0

TABLE 5. *Cumulative Rates of Closures per 100 Women (Quinacrine Regimen A)*

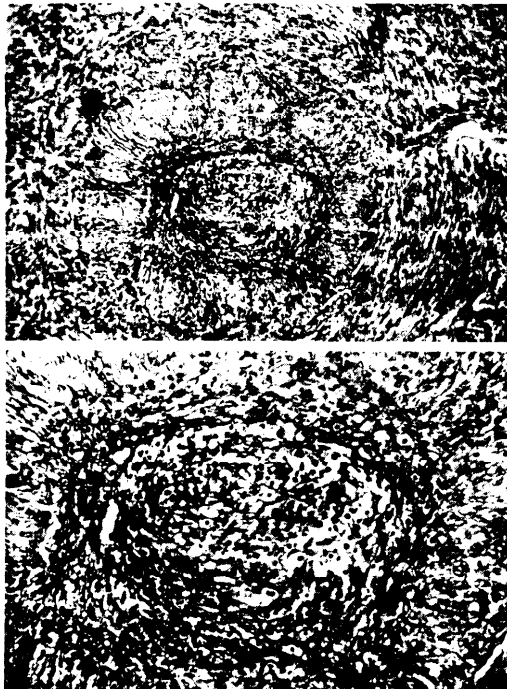
Relevant events	Rate after			
	3 mos.	9 mos.	12 mos.	31 mos.
Pregnancies				
After diagnosed tubal obstruction	0.0	6.1	8.6	8.6
During treatment	5.8	5.8	5.8	5.8
Spontaneous recovery of tubal patency	0.0	0.0	2.6	2.6
Dropout	9.4	10.6	10.6	10.6
Method failure	9.4	9.4	9.4	9.4
Continuation	75.4	68.0	62.8	62.8
Women-months of observation	220.5	565.5	712.5	1068.6

histologic changes were most marked in the cornual portion of the tubes and diminished rapidly and progressively for a distance of 2-3 mm. In most instances the remaining portions of the tubes were found to be normal. Within the affected area the lumen was obstructed by granular tissue consisting of lightly stained cells having regular normal nuclei. No significant abnormalities were detected in the tubal musculature. This histologic picture was similar in all 4 patients and remained unchanged after 1 year (see Figs. 3-8).

Group B. Tubal obstruction was found after the first cycle following the initial instillation in 24 of the 37 patients (64.8%). There were 9 patients who had a second instillation and 5 of these were shown to be obstructed during the succeeding cycle (55%). The cumulative rate of obstruction for the entire group was 84.3 (see Table 6). The 29 patients who had demonstrable



FIGS. 3-6. Serial section of human fallopian tube 60 days after a single instillation of quinacrine regimen A. X 100.



FIGS. 7 and 8. Serial section of human fallopian tube 1 year after a single instillation of quinacrine regimen A. X 100.

TABLE 6. *Effect of Number of Instillations on Tubal Patency; Partial and Cumulative Rates (Quinacrine Regimen B)*

No. of instillations	No. of patients	Nonpatent patients		Patent patients	Cumulative rates of obstruction
		No.	Percentage		
1	37	24	64.8	13	64.8
2	9	5	55.0	4	84.3

tubal obstruction were exposed to pregnancy for a total of 214 women-months. Over the period of observation there were no pregnancies in this latter group. There were 4 patients who received only one instillation and whose tubes remained patent. These patients became pregnant during the period of observation.

Complications. There was one complication attributed to the method in a total of more than 250 instillations of suspensions of quinacrine. In this instance, immediately after the instillation the patient

became hyperirritable in a manner suggestive of the central nervous system excitation which occasionally results from Novocain (Winthrop Laboratories) which has been accidentally administered intravenously. The signs and symptoms of this patient were readily reversed by the intravenous administration of a rapid acting barbiturate and some chlorpromazine. The entire episode lasted, approximately 4 hr. and there were no demonstrable sequelae.

There was no evidence of peritoneal irritation or peritonitis in any patient and no patient complained of abdominal or pelvic pain during or after the instillations.

There were no ectopic pregnancies among the pregnancies which resulted from method failure.

DISCUSSION

Our preliminary studies of antimetabolic agents in the rat¹² suggested that quinacrine, a member of the acridine class, might be suitable to produce tubal obstruction in the human when instilled into the uterine cavity. In the rat this material produces uterine obstruction by causing a granulomatous proliferation of the endometrium. The clinical studies reported here indicate that a similar reaction occurs in humans. It is interesting that the most pronounced effect is upon the cornual portion of the tubal epithelium. The quinacrine suspension has relatively little effect upon the more distal portions of the tubes or upon the endometrial epithelium. The systemic effects of these intrauterine instillations are minimal. It appears that the antifertility effect of these instillations can be attributable to the mechanical obstruction of the cornual portions of the fallopian tubes.

In Group A the results indicated that the pregnancy rate is directly related to the number of instillations which are required to produce tubal obstruction. This observation suggests that there is an ac-

cumulative effect from the drug. In women whose tubes have become obstructed by one instillation of a 2-ml. suspension of 250 mg. of quinacrine (Group A), pregnancy rate of 4.9/100 women-years is attained at the end of the study. When tubal obstruction is produced by three instillations, a pregnancy rate of 1.2/100 women-years is attained (Table 3). In this group the principal reasons for closure are (1) pregnancy during the course of treatments (5.8%), and (2) patient dropout (10.6%). With the second dosage (Group B) we obtained 65% tubal obstruction after the first instillation. With the dosage used in Group A only 24 of the 68 studied patients were obstructed (35.2%) after the first instillation. In Group B there have been no pregnancies as of this time (March 1970). The length of observation to date is an average of 7.3 months of exposure/woman.

Effectiveness. The efficiency of the instillation method was improved significantly by increasing the volume of the suspension which was used. The total effectiveness could probably be enhanced by protecting the patient from accidental pregnancy before the tubes had become occluded. It is our belief that the application of interim protection is essential in any sterilization technic which is not or may not be a one-time method.

Because of our success in the use of quinacrine as an agent for producing tubal obstruction via intrauterine instillation, we became interested in determining, if possible, whether this property was due to activity of the entire quinacrine molecule or perhaps could be attributed to a specific portion of the molecule. The incentive for extending our studies into the area of mechanism of action at the pharmacologic level was the possibility that more specific and efficient substances might be found. Our studies to date have included only three additional compounds-riboflavine, proflavine, and chloroquine.⁶ A comparison of the antimalarial and molecular

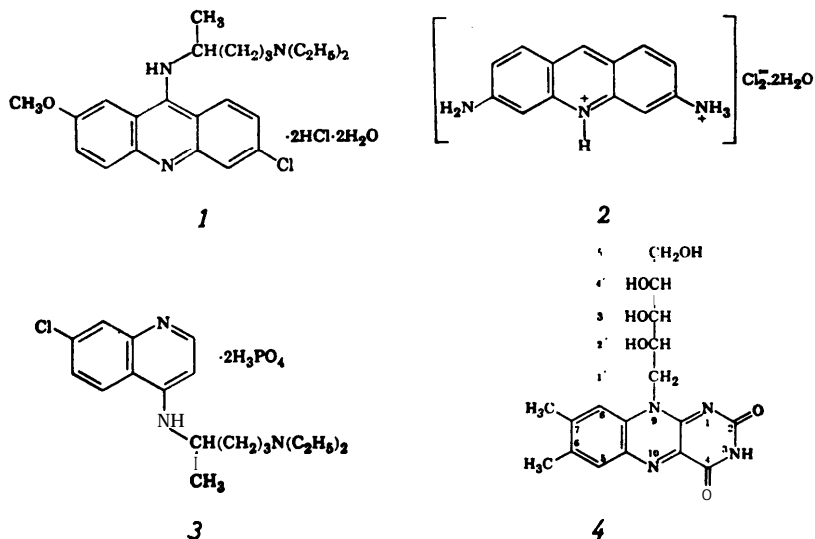


FIG. 9. Quinacrine, 1; Proflavine, 2; Chloroquine, 3; and Riboflavine, 4

structural relationships of these three substances with quinacrine is illustrated in Fig. 9. The pharmacologic effects of equal concentrations of these four substances upon the endometrial epithelium of the rat were studied.⁶ Of these only quinacrine and proflavine possessed endometriotropic properties. Chloroquine and riboflavin had no endometriotropic effects whatsoever in the concentrations used. Our tentative conclusion from this latter study is that the proliferative effects of quinacrine and proflavine are derived from the acridine nucleus. Much additional work will be necessary before more direct correlations can be drawn between chemical or molecular configuration and a specific effect upon the endometrial and tubal epithelium in both experimental animals and in humans. Studies along these lines have already begun.

Reversibility-Spontaneous and Induced.

During the first year after the demonstration of tubal obstruction our data indicated that there were a limited number of pregnancies and that tubal patency was occasionally restored. The incidence of accidental pregnancy did not increase during the second and third years and there

have been no new pregnancies registered since our last communication in October 1969.¹⁰ These data indicate that the rate of spontaneous reversal of the tubal obstruction and/or other antifertility factors is very low. With our present state of knowledge it is not possible to ascertain with any degree of confidence what errors have been made by us as to the diagnosis of tubal obstruction or to what extent, if any, hysterosalpingographies or tubal insufflations have contributed to the restoration of tubal patency. In any case, our clinical experience suggests strongly that spontaneous reversal of tubal obstruction occurs infrequently and only during the first year after treatment.

Experiments in rats¹² have shown that the hyperplastic reaction can be reversed by the administration of either an estrogen or a progestogen. Perhaps in the human the normal concentrations of estrogen or progestogen are insufficient to effect a reversal of the obstructive lesion. It seems possible that reversal could be produced in the human as in the rat by the systemic administration of either estrogens or progestogens, or perhaps by a combination of both. If tubal patency can be restored

clinically, then this procedure which was originally developed as a technic for non-surgical female sterilization could become a reversible method of contraception. Clinical studies to assess the reversibility of these antifertility effects by the administration of exogenous steroids have been planned.

Demographic Considerations. We consider it highly desirable to develop a nonsurgical technic for female as well as for male sterilization. There would be tremendous practical advantages of a technic such as described in this report because it can be readily performed by paramedical personnel with minimum danger to the patient. It would have even greater demographic implications if the method can be refined further such that a high degree of contraception could be consistently achieved by only one intrauterine instillation of the chemical agent responsible for the tubal obstruction and reduction in fertility.

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