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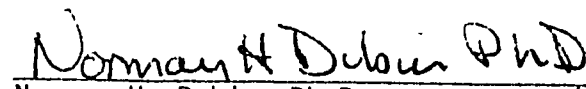
SIMPLIFIED TECHNIQUES OF FERTILITY MANAGEMENT  
NON-SURGICAL FALLOPIAN TUBE OCCLUSION STUDIES

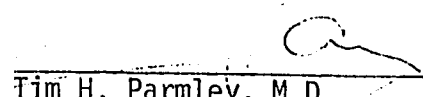
FINAL REPORT

October 1, 1982 - November 27, 1983

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## APPENDICES

<u>Reprint #1.</u>	Pharmacokinetic Studies on Quinacrine Following Intrauterine Administration to Cynomolgus Monkeys
<u>Reprint #2.</u>	Effect of Intrauterine and Intravascular Quinacrine Administration on Histopathology, Blood Chemistry, and Hematology in Cynomolgus Monkeys
<u>Reprint #3.</u>	Pharmacology of Quinacrine Hydrochloride with Emphasis on its Use as a Tubal Occluding Agent
<u>Reprint #4.</u>	Teratologic and Mutagenic Studies with Intrauterine Quinacrine Hydrochloride
<u>Reprint #5.</u>	Histologic Changes Following Intrauterine Administration of Quinacrine Hydrochloride
<u>Reprint #6.</u>	Quinacrine Hydrochloride: Future Research
<u>Reprint #7.</u>	Hysterosalpingography in Cynomolgus Monkeys
<u>Manuscript.</u>	Comparison of the Operating Microscope and Loupe for Microsurgical Tubal Anastomosis: A Randomized Clinical Trial

## I. Summary

The following report includes the results of our work completed from October 1, 1982 to November 30, 1983. The details of our studies with tetracycline analogues as possible tubal occluding agents is described. The results of these studies are encouraging to the degree that we will attempt through the auspices of Family Health International (FHI) to procure an IND from the FDA to initiate Phase I studies on pre-hysterectomy cases to evaluate the efficacy and safety of Intrauterine instillation of tetracycline pellets in women. To this end we are in the process of developing with FHI, a proposal to be submitted to the FDA. The current version of this proposal is included in this report (section III). Also, we have submitted a Research Protocol Notification (RPN) to the Johns Hopkins Committee on Clinical Investigations which has been reviewed and approved by the committee. The RPN is also included in this report (section III). Reprints of seven papers pertaining to transcervical sterilization which have been published during this year are appended to this report. A manuscript entitled "Comparison of the Operating Microscope and Loupe for Microsurgical Tubal Anastomosis: A Randomized Clinical Trial" by Dr. John Rock et al. appears in the appendix also. This manuscript, which describes work supported by this AID contract is currently in press.

## II. Effect of Intrauterine Chemical Agents on Cynomolgus Monkeys

### A. Introduction

Techniques for nonsurgical female sterilization have potential advantages over current surgical techniques and these have been discussed previously (Shelton, J.D. and Speidel, J.J.: In Female Transcervical Sterilization, Chapter 1, Harper & Row, Phila., 1983). Quinacrine and methylcyanoacrylate are currently in use in various countries and are apparently well accepted by certain populations of women. We have previously studied the toxicological effects of quinacrine on monkeys. The results of these studies and other reports in the literature have raised some concern over potential hazards of this drug (see Appendix reprints #3 and 6) although there have been little if any severe side effects reported by those who are currently administering the drug in clinical settings. We have, however, embarked on a study screening other potential chemical agents which may be as efficacious in causing sterilization, yet have less risk or a greater safety margin (therapeutic index) than quinacrine.

It has been noted that tetracycline, like quinacrine, has been used to treat pleural and peritoneal effusions. Because of this sclerosing property of tetracycline we examined the potential of this drug as a tubal closure agent in several species. In a previous progress report (October 1, 1981-September 30, 1982) we described studies in which intrauterine tetracycline solutions were capable of causing uterine or tubal necrosis in rabbits and rats and monkeys. The damage we observed in the rat was similar to that seen following quinacrine treatment.

Furthermore, while quinacrine resulted in acute mortality of some of the rats treated with this drug, no mortality occurred following tetracycline treatment. Transcervical injections in monkeys resulted in tubal damage in some of the monkeys treated. We also reported that certain tetracycline analogues (Fig. 1) were also effective in causing uterine damage in the rat. Because of these studies we embarked on the following study, screening the effects of tetracycline and tetracycline analogues in monkeys.

It should be noted that in our previously reported quinacrine studies no histopathology of the tube which could be construed as tubal closure was observed. However, in some cases necrosis of epithelial tissue and underlying stroma in the cornual region of the endometrium was noted. This is consistent with the type of damage which precedes the development of fibrous connections which lead to tubal closure, but it does not necessarily mean that closure will occur. It is possible that anatomical differences between monkeys and women prevent tubal closure from occurring in monkeys. It should also be noted that in women, quinacrine treatment in 3 consecutive cycles is needed to ensure a pregnancy rate of less than 5% (Zipper, J. et al.: In, Female Transcervical Sterilization, Chapter 9, Harper & Row, Phila., 1983).

In the following study, tetracycline induced lesions of the reproductive tract were compared to those induced by quinacrine in cynomolgus monkeys. While the study was primarily instituted to screen for efficacy of tetracycline and its analogues, blood levels of drugs, blood chemistry and hematology and liver and kidney pathology data were also collected as an index of toxicity.

Drug	Year introduced	Brand names*
Chlortetracycline	1948	<u>Auroclonin</u>
Oxytetracycline	1960	<u>Terramycin, Liguamycin,</u> <u>Aquachel</u>
▶ Tetracycline	1962	<u>Achroclonin, Panmycin, Steclin,</u> <u>Sumycin, Tetracyl, Robitet,</u> <u>Tetraz, Tetrachol</u>
▶ Demeclocycline (demethylchlortetracycline)	1969	<u>Declomycin</u>
Methacycline	1961	<u>Rondomycin</u>
▶ Doxycycline	1965	<u>Vibramycin, Doxycycline II,</u> <u>VibraVet†</u>
Minocycline	1972	<u>Minocin, Vectrin</u>

\* Brand names that are underlined are available for veterinary use. † VibraVet is a veterinary product, but it is not approved for use in the United States.

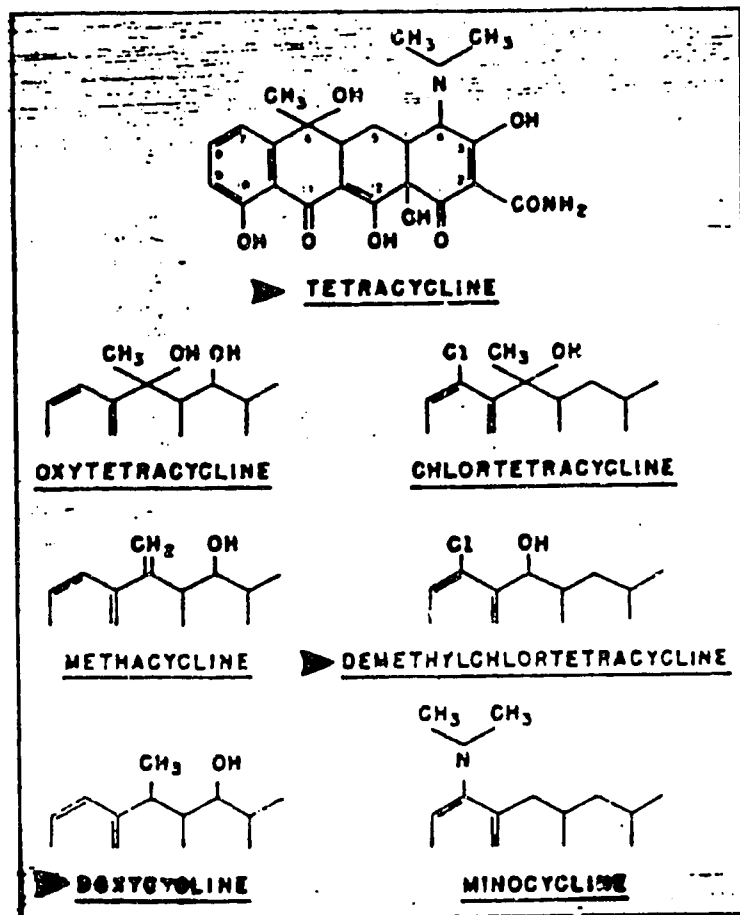


Figure 1. Structure and Brand Names of Tetracycline Analogues. Those used in the current study are marked with an arrowhead (from Aronson, A.L., JAMA 176:1061, 1980).

## B. Study Protocol

Female cynomolgus monkeys (Primate Imports) were housed separately and were observed daily for vaginal bleeding. When bleeding was observed, the monkey became a candidate for drug insertion within 14 days of bleeding. The monkeys received either intrauterine solutions or pellets of tetracycline hydrochloride (Sigma lot # 81F-0307) or doxycycline hydrochloride (Sigma lot # 90F-00251) or pellets of demeclocycline hydrochloride (Sigma lot # 99C-0077), or quinacrine hydrochloride. Pelletization of the antibiotics was performed by the Department of Pharmacology, University of Maryland, Baltimore, Md. Quinacrine pellets were obtained from Family Health International, Research Triangle Park, NC. The quinacrine pellet dose was 30 mg and is comparable to about 2 X the dose administered to women on a body weight basis and is also equivalent to the dose of quinacrine solution administered to monkeys in our previous study (see Appendix reprints # 1 and 2). The dose of tetracycline or analogues was 100 mg based upon the preliminary studies with tetracycline solution. This dose was used for both solution and pellets. All doses are expressed as free bases. Solutions were injected in a 1 ml volume. At time of drug application, the monkey was brought to the operating room under Ketaset anesthesia and was then prepared for abdominal surgery by anesthetization with halothane. Before surgery, blood was taken from the femoral vein for chemistry and hematology determinations and also drug concentration. For the pellet treated monkeys, a mid-abdominal incision was made through the skin and body wall. The uterus was exposed, a mid-ventral incision was made in the uterus and a pellet of either quinacrine, tetracycline, doxycycline, or demeclocycline, was inserted into the lumen of the uterus as close to the fundal region as possible. The



Incisions were then closed in the uterus, body wall, and skin. For monkeys receiving solutions of drugs, laparotomies were also performed and the drug was injected directly into the uterine lumen with a needle and syringe. The reason for this was to try to document spillage through the tube at the time of injection. However, in no case could we be sure that the drug even penetrated into the tube. Three monkeys receiving transcervical injections of tetracycline solution were also included in the data analysis.

At 4 and 24 hours an additional blood sample was obtained for drug analysis. Seven to 9 days after drug administration, depending upon scheduling of the operating room, the monkey was again anesthetized with halothane and a hysterectomy was performed. Uterine, cervical, tubal, and ovarian tissue were removed and fixed in Hartman's solution and later histopathology on these tissues was obtained. Blood was again obtained for blood chemistry, hematology, and drug determination. Liver and kidney biopsies were also obtained. Each of the above groups consisted of 4 monkeys.

Three control monkeys were also studied. Two of these received saline injection directly into the uterus under laparotomy. One was sham operated; the uterus was opened but nothing inserted and sutured as a control for the monkeys receiving pellets. These animals had blood drawn following the usual schedule and were operated upon one week later comparable to the treated monkeys.

### C. Assay Methods

Blood chemistry determinations were performed by Vet Path laboratories, Teterboro, NJ. The analyses included: serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase,

gamma-glutamyl transpeptidase, alkaline phosphatase, lactic dehydrogenase, cholesterol, triglyceride, total protein, albumin, globulin, blood urea nitrogen, creatinine, direct and total bilirubin, uric acid, glucose, iron, magnesium, sodium, potassium, calcium, phosphorus, and chloride.

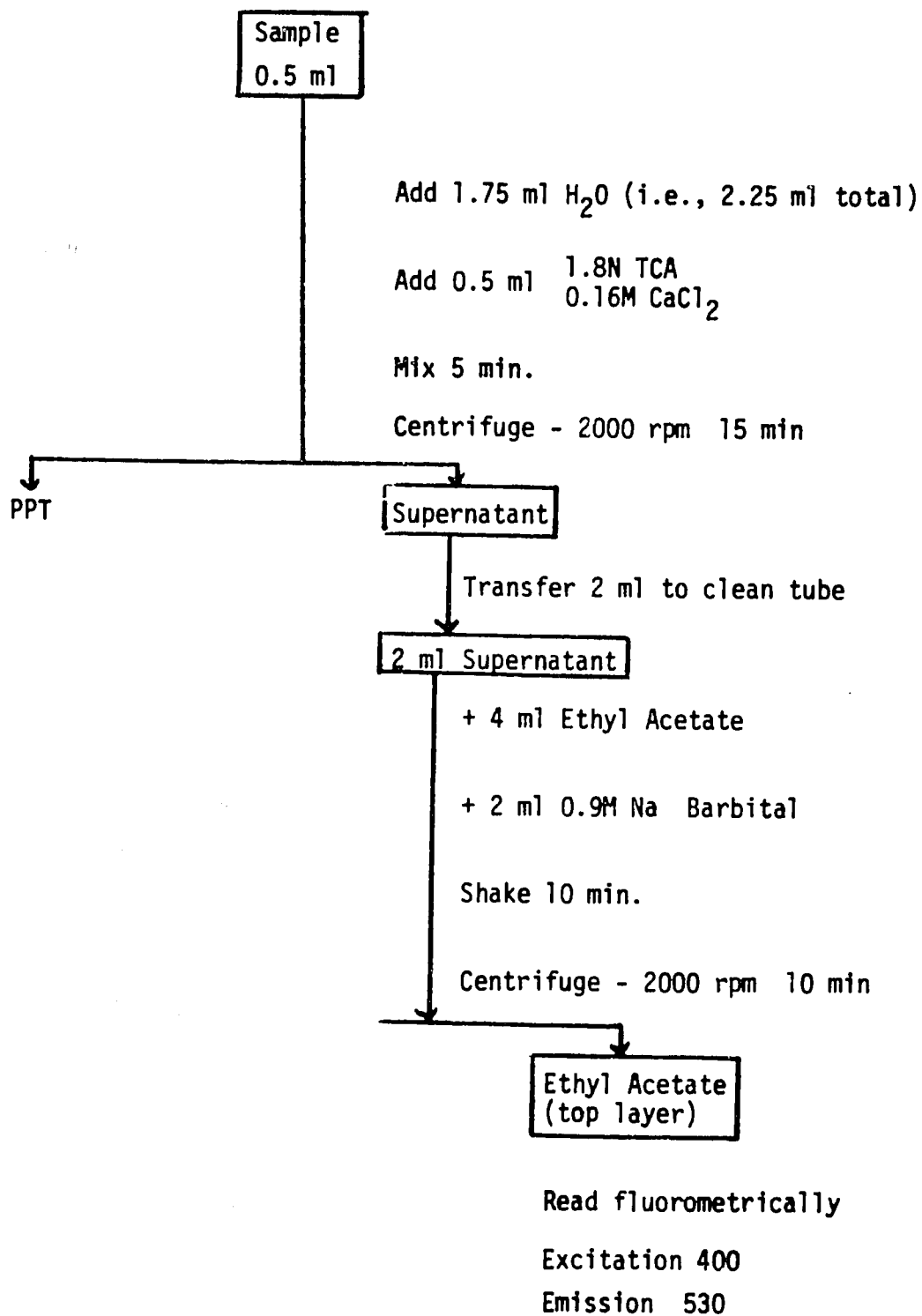
Hematology was performed by the Division of Comparative Medicine, The Johns Hopkins University School of Medicine. These analyses included: white blood cell count, neutrophils, lymphocytes, red blood cell count, hematocrit, and hemoglobin.

Plasma quinacrine was determined by a fluorescence procedure based upon Brodie and Udenfriend (J. Biol. Chem. 151:299, 1943) and described and validated by our laboratory as reported in a previously published paper (Dubin et al., Fertil. Steril. 38:735, 1982, included in the appendix, reprint #1).

Tetracycline was measured by a fluorescent procedure based upon that described by Kohn (Analytical Chemistry 33:862, 1961). A flow chart is presented in Fig. 2. Distilled water (1.75 ml) was added to 0.5 ml of serum in a conical centrifuge tube. To this was added 0.5 ml of a solution of 1.8N Trichloroacetic acid and 0.16N  $\text{CaCl}_2$ . The tube was stoppered and shaken mechanically at a medium speed for 5 minutes after which the solution was centrifuged for 15 min. at 2000 rpm. The supernatant (2 ml) was transferred to a clean tube and 4 ml ethyl acetate and 2 ml sodium barbital were then added. This tube was stoppered and shaken for 10 min. and centrifuged at 4°C at 2000 rpm. The ethyl acetate layer was then read on an Aminco Bowman Spectrophotometer with an excitation wavelength of 400 nm and an emission wavelength of 530 nm. The relative fluorescence was compared

# SERUM TETRACYCLINE

## Flow Sheet



against a standard curve prepared from tetracycline hydrochloride ranging in mass from 0.1 to 10 µg and run through the assay procedure. The standard curve represented as a log-log plot is depicted in Figure 3.

In preliminary studies, standard tetracycline was subjected to the extraction procedure and an excitation and emission spectra were obtained and found to be identical to that reported in the literature. Three µg of tetracycline HCl was added directly to control serum and subjected to the extraction procedure and the emission and excitation spectra were identical to that of the standard. Furthermore, a serum sample obtained from monkeys treated with tetracycline pellets at 4 hours and 1 day after treatment and which had been frozen for 5 months were also subjected to the procedure. The spectra derived from these samples exposed to the above procedure was also identical to the standard tetracycline spectra (Fig. 4).

Demeclocycline and doxycycline were measured by the same procedures using the appropriate compound in the standard curve (Fig. 3). Recovery for each antibiotic from serum based upon spiked samples were as follows: tetracycline, 100%; doxycycline, 55%; demeclocycline, 82%. Final results for doxycycline and demeclocycline were corrected for recovery.

#### D. Study Results

Comparing pathology between monkeys receiving pellet treatment, we found superficial necrosis in the uterus (Table 1) in quinacrine (4/4), tetracycline (2/4), doxycycline (3/4) and demeclocycline (1/4) treated monkeys. Some tubal damage was seen in the intramural section of one quinacrine-treated monkey (Fig. 5B) and one

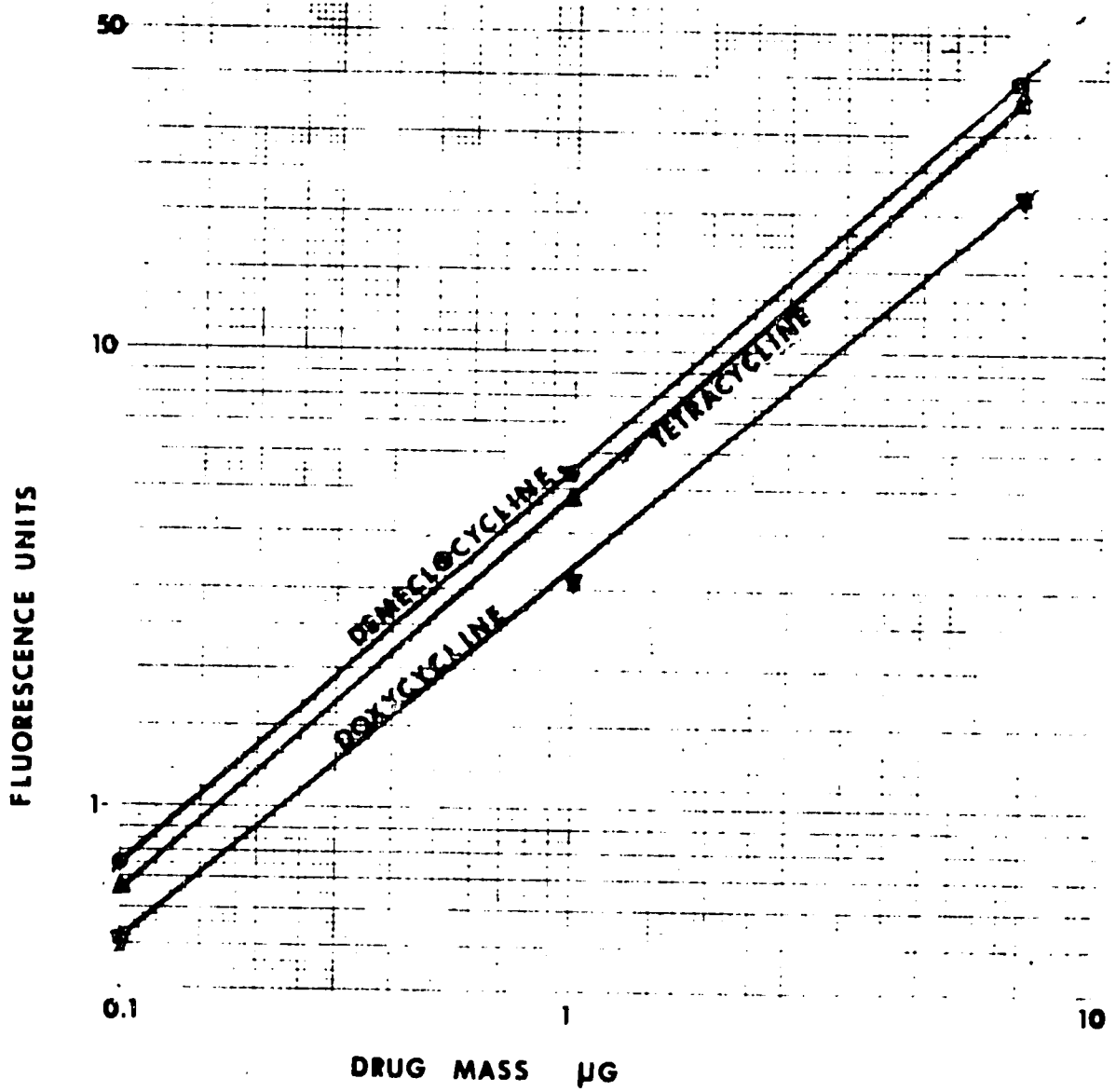


Figure 3. Standard Curves for Tetracycline, Demeclocycline and Doxycycline

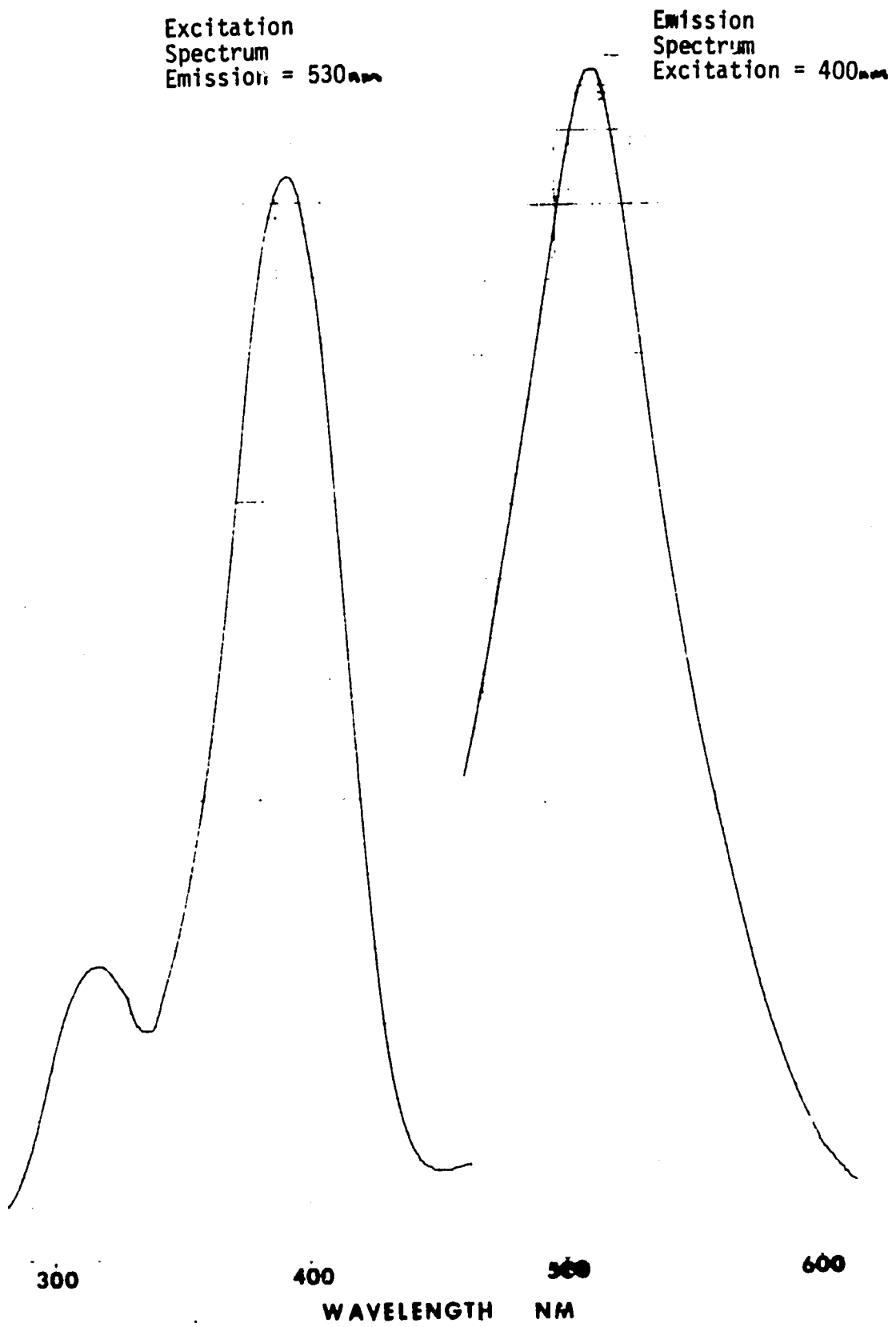
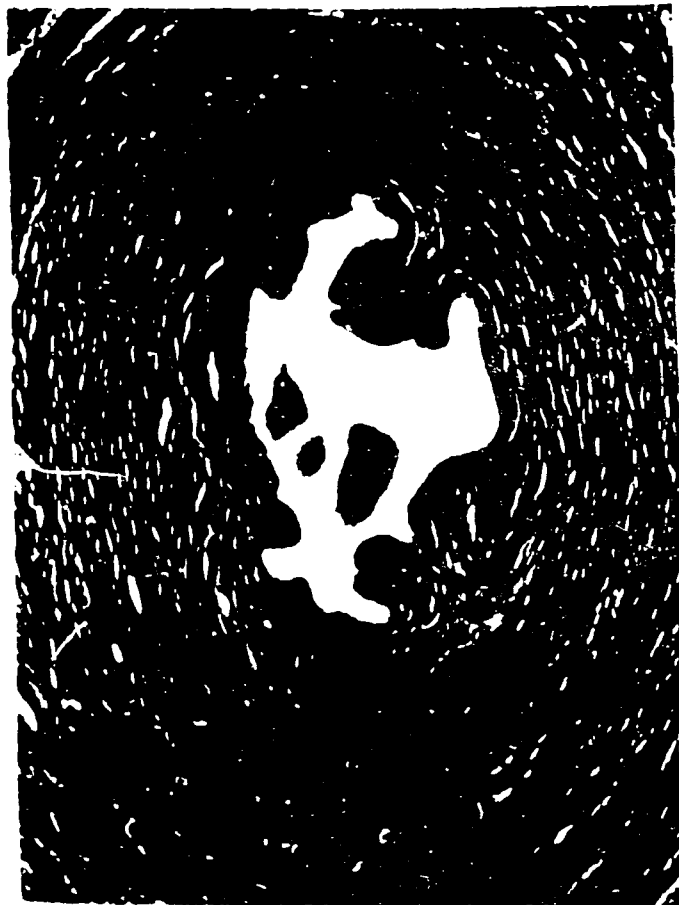


Figure 4. Excitation and Emission Spectra for Standard Tetracycline

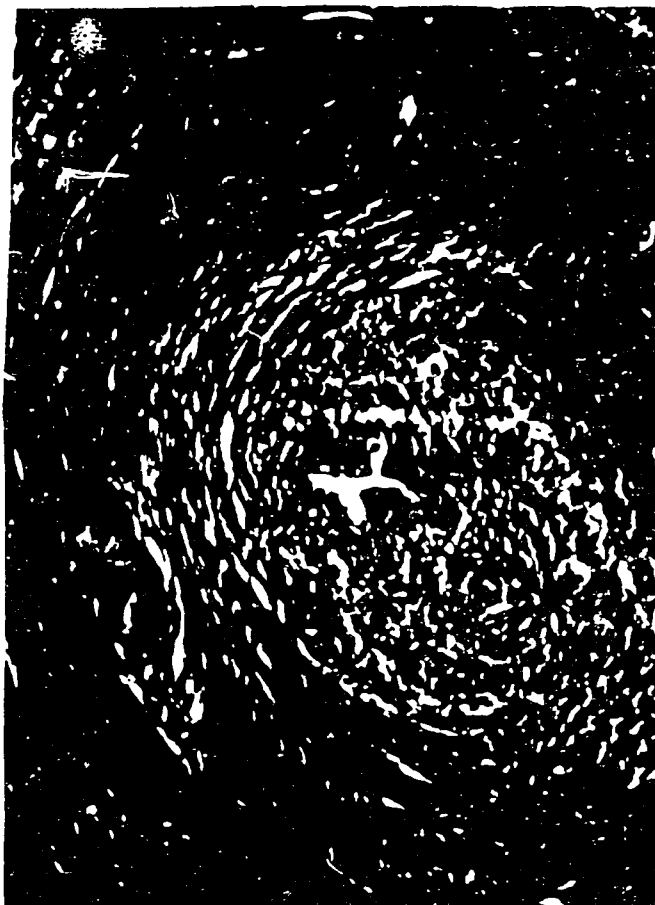
Table 1. Histopathology of Reproductive Organs after Intrauterine Pellet Administration

<u>Quinacrine</u>	<u>Tube</u>		<u>Uterus</u>	<u>Cervix</u>	<u>Ovaries</u>
	<u>Left</u>	<u>Right</u>			
W-72	damaged epithelium	old damage regenerating	endometrial necrosis	WNL	WNL
W-54	WNL	WNL	superficial damage	WNL	WNL
W-74	WNL	WNL	widespread superficial necrosis	widespread superficial necrosis	WNL
W-75	WNL	WNL	endometrial necrosis	WNL	WNL
<u>Tetracycline</u>					
W-76	WNL	WNL	marked superficial autolysis	focal superficial autolysis	WNL
U-44	WNL	WNL	WNL	WNL	WNL
W-68	WNL	WNL	superficial necrosis & repair	WNL	WNL
U-46	WNL	WNL	WNL	WNL	WNL
<u>Declomycin</u>					
W-73	WNL	WNL	WNL	WNL	WNL
U-47	WNL	WNL	WNL	WNL	WNL
U-52	WNL	WNL	superficial endometrial damage	WNL	WNL
U-37	WNL	WNL	WNL	WNL	WNL
<u>Doxycycline</u>					
U-43	WNL	WNL	inflammation & repair focally at surface	marked superficial autolysis	WNL
S-49	WNL	WNL	WNL	focal superficial necrosis & repair	WNL
W-69	WNL	WNL	endometrial necrosis	superficial & deep necrosis	WNL
R-18	superficial damage but a lumen persists	damage but a lumen persists	endometrial necrosis	superficial necrosis	WNL



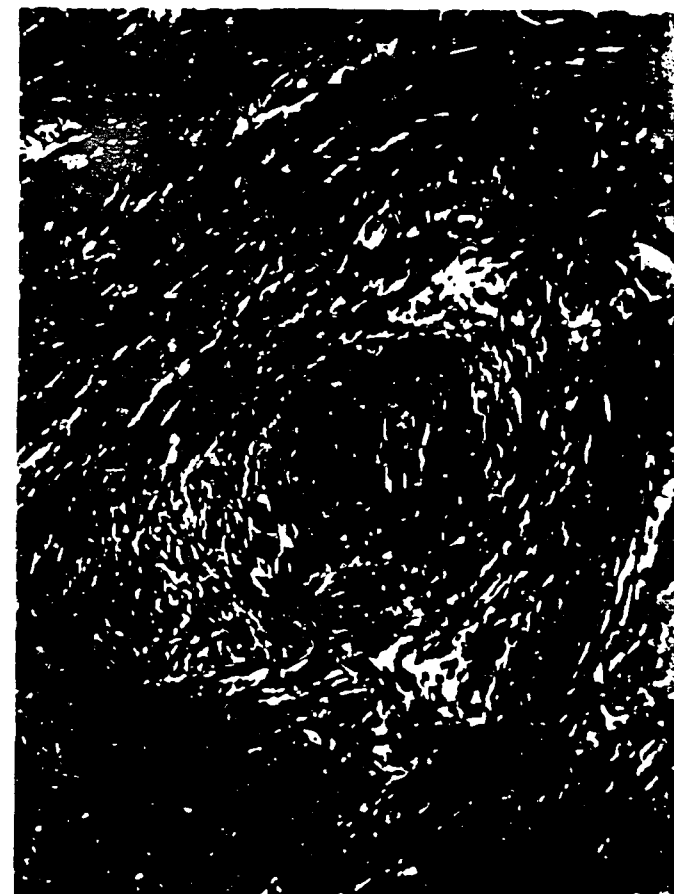
CON

A. Section through intramural portion of a normal tube



Q

B. Quinacrine induced necrosis of intramural section of tube (W-72)



DOXY

C. Doxycycline induced necrosis of intramural section of tube (R-18)

Figure 5

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doxycycline-treated monkey (Fig. 5C). Cervical damage was observed in monkeys treated with quinacrine (1/4), tetracycline (1/4) and doxycycline (4/4). No ovarian pathology was observed in any of the monkeys so treated.

In monkeys receiving tetracycline solution tubal damage was observed in 2/4 monkeys, but not in monkeys receiving doxycycline solution (Table 2). Endometrial damage was seen in 2/4 monkeys receiving tetracycline solution and 3/4 monkeys receiving doxycycline solution. No tissue damage was seen in the reproductive tract of any of the control monkeys. Table 3 summarizes this data.

A number of non-specific lesions were noted in a number of the liver and kidney biopsies, including the controls. A variety of the changes were consistent with changes associated with tetracycline drugs including fat deposition and chronic interstitial nephritis. However, the nephritis was thought to be of longer duration than the time lapse from treatment to biopsy time. Although fatty infiltration has been associated with tetracycline toxicity, it may also be due to numerous non-specific causes. In any case the changes observed were mild. The absence of blood chemistry changes associated with liver or kidney damage reinforce our opinion that these changes are non-specific and minor.

Few differences were observed comparing blood chemistries from samples taken before and after treatment with pellets (see Table 4). With demeclocycline, cholesterol was significantly ( $P < 0.05$ ) as was total protein concentration ( $P < 0.01$ ). Hematology studies showed that hemoglobin was reduced by all drugs, but only significantly ( $P < 0.05$ ) with quinacrine, doxycycline, and demeclocyclin (Table 5).

Table 2. Histopathology of Reproductive Organs after Intrauterine Solution Administration and Controls

Tetracycline Solution	Tute		Uterus	Cervix	Ovaries
	Left	Right			
U-45	WNL	Scar tissue lumen open	superficial inflammation	WNL	WNL
S-57	Scar lumen open	WNL	endometrial necrosis	WNL	WNL
U-35	WNL	WNL	WNL	not available	not available
R-12	WNL	WNL	WNL	WNL	WNL
<hr/>					
Doxycycline Solution					
S-52	WNL	WNL	endometrial necrosis	superficial autolysis of lower uterine segment	WNL
U-42	WNL	WNL	WNL	WNL	WNL
W-71	WNL	WNL	endometrial necrosis	WNL	WNL
W-52	WNL*	WNL*	endometrial necrosis	WNL	WNL
<hr/>					
Control					
S-51	WNL	WNL	WNL	WNL	WNL
W-57	WNL	WNL	WNL	WNL	WNL
W-70	WNL	WNL	WNL	WNL	WNL

\*cornual damage but does not reach tube

Table 3

EFFECT OF INTRAUTERINE DRUGS ON  
REPRODUCTIVE TISSUE IN CYNOMOLGUS  
MONKEYS 7 DAYS FOLLOWING ADMINISTRATION

Drug	Tube	Uterus	Cervix
Quinacrine (36 mg)	1/4* (25%)	4/4 (100%)	1/4 (25%)
Tetracycline (100 mg)**	2/8 (25%)	4/8 (50%)	1/7 (14%)
Doxycycline (100 mg)**	1/8 (13%)	6/8 (75%)	5/8 (63%)
Demeclocycline (100 mg)	0/4 (0%)	1/4 (25%)	0/4 (0%)
Control	0/3 (0%)	0/3 (0%)	0/3 (0%)

\* proportion of monkeys demonstrated necrosis in this area

\*\* solution and pellet data combined

**Table 4. Effect of Intrauterine Pellets on Blood Chemistry**

	<u>Quinacrine</u>		<u>Tetracycline</u>		<u>Doxycycline</u>		<u>Declomycin</u>	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
Albumin/Globulin Ratio	1.07±0.12	0.97±0.07	1.14±0.13	1.05±0.12	1.02±0.05	1.00±0.05	0.99±0.06	0.95±0.07
Albumin Micro gm/dl	3.88±0.09	3.65±0.05	3.88±0.18	3.70±0.18	3.60±0.16	3.48±0.17	3.83±0.13	3.50±0.12
Alk. Phos. Micro i.u./l	30.80±112.2	249.8±66.3	197.5±18.25	226.0±15.61	181.3±17.77	212.5±23.28	180.5±10.38	206.0±25.24
Bilirubin Tot Micro mg/dl	0.15±0.05	0.15±0.03	0.20±0.06	0.23±0.13	0.15±0.05	0.15±0.03	0.25±0.07	0.15±0.03
BUN micro mg/dl	17.0±2.12	18.0±1.58	16.5±1.76	17.8±1.80	19.8±1.49	18.6±0.48	17.5±0.29	16.5±0.65
BUN/Creatinine Ratio	19.86±2.05	21.84±1.09	24.75±5.73	51.65±26.3	27.89±3.56	25.58±3.58	19.07±2.02	58.41±40.54
Calcium Micro mg/dl	9.65±0.23	9.43±0.13	9.58±0.22	9.38±0.23	9.80±0.19	9.88±0.36	9.68±0.24	9.25±0.16
Chloride Micro mmol/l	109.0±1.08	109.5±0.87	110.3±1.44	109.8±0.68	111.3±1.44	113.8±1.03	109.0±0.91	111.0±1.35
Cholesterol Micro mg/dl	105.5±16.09	113.3±17.89	125.0±17.23	132.0±18.65	132.8±10.52	130.0±11.53	137.7±2.43	125.3±3.61*
GGTP	49.5±4.57	48.5±16.24	47.3±4.98	39.3±6.33	55.0±15.04	39.0±15.01	57.67±10.48	34.3±5.04
Creatinine Micro mg/dl	0.85±0.03	0.83±0.06	0.73±0.09	0.63±0.19	0.75±0.12	0.78±0.10	0.95±0.10	0.70±0.20
Globulin mg/dl	73.75±8.53	59.25±8.04	67.25±3.45	64.5±2.33	58.3±6.34	61.5±5.95	58.5±9.04	61.75±9.66
LDH Micro i.u./l	393.5±61.83	586.0±233.9	398.0±57.87	360.3±29.54	485.5±103.3	463.3±94.96	354.5±60.74	339.3±39.07
Phosphate Micro mg/dl	4.88±0.87	4.88±0.30	4.23±0.62	4.30±0.27	4.35±0.48	3.40±0.16	4.90±0.70	4.08±0.36
Potassium Micro mmol/l	3.83±0.22	3.93±0.41	3.98±0.19	3.80±0.20	3.85±0.37	3.80±0.44	4.0±0.27	3.78±0.10

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Table 4 (continued)

	Quinacrine		Tetracycline		Doxycycline		Declomycin	
	Before	After	Before	After	Before	After	Before	After
SGOT, i.u./l	38.25±16.03	44.75±23.09	26.5±4.5	21.0±1.47	29.0±9.44	31.8±3.90	24.3±1.49	24.5±3.23
SGPT, i.u./l	36.25±14.28	55.0±26.77	37.3±9.59	47.25±6.33	51.5±19.0	53.5±15.95	74.5±35.53	45.5±6.44
Sodium Micro mmol/l	147.3±1.75	145.5±2.53	148.0±2.48	147.5±0.29	146.3±0.95	149.8±1.97	146.0±1.78	147.3±2.32
Total Protein Micro gm/dl	7.65±0.42	7.48±0.33	7.35±0.16	7.35±0.19	7.15±0.27	6.96±0.31	7.70±0.1	7.22±0.05
Triglycerides Micro mg/dl	33.5±5.25	36.75±11.90	27.75±10.26	57.0±10.48	48.5±8.95	41.5±8.15	40.5±3.40	38.5±2.25
Uric Acid Micro mg/dl	0.40±0.09	0.23±0.05	0.33±0.05	0.3±0.0	0.23±0.48	0.30±0.07	0.38±0.05	0.25±0.07
Bilirubin Dir Micro mg/dl	0.03±0.03	0.03±0.03	0.03±0.03	0.03±0.03	0.08±0.03	0.03±0.03	0.0	0.0

On this and following tables: \* = P < 0.05

\*\* = P < 0.01

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Table 5. Effect of Intrauterine Pellets on Blood Hematology

	<u>Quinacrine</u>		<u>Tetracycline</u>		<u>Doxycycline</u>		<u>Declomycin</u>	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
WBC / mm <sup>3</sup>	5828±1495	6422±723	6633±1428	7494±1838	6860±951	7909±2087	7715±1270	8455±1342
Total Neut %	54.0±6.82	54.75±5.55	48.0±4.60	50.25±8.07	38.5±5.90	43.5±7.24	35.25±7.59	41.0±4.81
Lympho %	32.25±6.37	35.25±6.24	38.75±4.66	41.75±8.44	54.75±8.04	49.50±7.24	50.75±6.52	50.5±6.2
Mono %	2.25±0.95	4.0±1.29	5.0±2.0	3.5±2.0	2.5±1.19	3.75±0.25	3.75±0.95	2.5±1.19
Eosine %	10.75±5.21	5.75±3.45	8.25±4.27	5.67±4.18	4.25±1.70	3.25±1.11	10.25±4.80	8.0±4.04
RBC X 1000	5223±290	4478±99	4498±233	4313±345	5178±452	4778±414	5625±409	4463±369
HCT	34.88±11.78	32.38±0.63	31.63±1.60	29.13±0.97	36.88±1.98	32.13±1.26	37.25±1.97	31.75±1.18
Hemoglobin gm %	10.93±0.28	9.6±0.32 <sup>*</sup>	10.08±0.51	8.7±0.40	11.65±0.49	9.45±0.34 <sup>*</sup>	11.6±0.69	9.33±0.44 <sup>*</sup>
Total Protein gm %	7.75±0.27	7.55±0.19	7.43±0.29	7.45±0.10	7.48±0.11	7.2±0.14	7.8±0.31	7.55±0.10
MCV μ <sup>3</sup>	66.35±0.91	72.40±2.06 <sup>*</sup>	70.76±4.37	68.72±5.64	71.87±2.74	68.20±4.12	66.56±2.41	72.01±3.69
MCH uug	21.02±0.61	21.46±0.74	22.57±1.56	20.59±1.84	22.81±1.10	20.04±1.02	20.57±0.83	21.1±0.96
MCHC %	31.44±0.80	29.63±0.44	31.87±0.49	29.33±0.91 <sup>*</sup>	31.65±0.37	29.46±0.76 <sup>*</sup>	31.10±0.23	27.36±2.33

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Calcium was significantly ( $P < 0.05$ ) lower after treatment with tetracycline solution from  $9.83 \pm 0.22$  to  $8.73 \pm 0.30$  mg/dl (Table 6). Phosphate was significantly lower ( $P < 0.01$ ) following treatment with doxycycline solution from  $5.43 \pm 0.35$  to  $3.5 \pm 0.18$  mg/dl. The hematocrit and hemoglobin concentration were decreased ( $P < 0.05$ ) in monkeys receiving tetracycline solution (Table 7). Similar decreases were seen in the control monkeys and probably this change is related to the blood sampling rather than any drug treatment.

One monkey (W-71) needs particular attention. No remarkable changes occurred during the week following administration of doxycycline solution and at the time of hysterectomy. Seven days after treatment, the monkey appeared healthy. Blood levels of doxycycline were  $0.68 \mu\text{g/ml}$  at 4 hours and  $0.42 \mu\text{g/ml}$  at one day post administration which was somewhat less than the average concentration for the group as a whole. Levels were near baseline at 1 week. Blood chemistries did not reveal any remarkable changes except a slight rise in LDH from  $250 \text{ iu/l}$  to  $565 \text{ iu/l}$ . No increase was seen in SGOT, SGPT, or  $\gamma$ -GTP, nor were there any other changes to indicate liver or kidney damage at that time. Biopsy results on day 7 revealed mild fatty infiltration of the liver. No lesions were visible in the kidney although only cortex was on the slide. During the hysterectomy procedure there was considerable blood loss and the difficulties following the operation were initially thought to be the result of the operation. The animal stopped eating 3 days after the operation and became dehydrated. It was given subcutaneous fluids on days 4 and 5 after the operation. The animal appeared to improve but then was found dead in the cage 8 days after the operation.

Table 6. Effect of Intrauterine Solution on Blood Chemistry

	<u>Tetracycline</u>		<u>Doxycycline</u>		<u>Control</u>	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
Albumin Micro gm/dl	4.10 $\pm$ 0.15	3.77 $\pm$ 0.37	3.50 $\pm$ 0.17	3.68 $\pm$ 0.14	3.67 $\pm$ 0.09	3.67 $\pm$ 0.12
Globulin gm/dl	4.53 $\pm$ 0.99	2.77 $\pm$ 0.26	3.50 $\pm$ 0.26	3.35 $\pm$ 0.12	3.83 $\pm$ 0.35	3.43 $\pm$ 0.09
Albumin/Globulin Ratio	0.99 $\pm$ 0.21	1.38 $\pm$ 0.15	1.01 $\pm$ 0.09	1.10 $\pm$ 0.09	0.97 $\pm$ 0.10	1.07 $\pm$ 0.05
Alk Phos Micro i.u./l	157 $\pm$ 73.66	91.3 $\pm$ 23.5	194 $\pm$ 29.67	200 $\pm$ 34.36	199 $\pm$ 23.03	186 $\pm$ 19.20
Bilirubin Tot Micro mg/dl	0.24 $\pm$ 0.04	0.17 $\pm$ 0.04	0.30 $\pm$ 0.07	0.20 $\pm$ 0.04	0.27 $\pm$ 0.08	0.27 $\pm$ 0.07
Bilirubin Dir Micro mg/dl	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.03	0.05 $\pm$ 0.03	0.03 $\pm$ 0.03	0.03 $\pm$ 0.03
BUN Micro mg/dl	26.3 $\pm$ 50.4	20.7 $\pm$ 4.33	16.5 $\pm$ 2.18	15.5 $\pm$ 1.94	19.0 $\pm$ 1.00	17.0 $\pm$ 1.53
BUN/Creatinine Ratio	36.3 $\pm$ 3.67	25.2 $\pm$ 2.59	15.5 $\pm$ 0.80	15.0 $\pm$ 1.57	17.4 $\pm$ 1.23	16.4 $\pm$ 1.23
Creatinine Micro mg/dl	1.00 $\pm$ 0.12	0.80 $\pm$ 0.10	1.08 $\pm$ 0.16	1.05 $\pm$ 0.12	1.10 $\pm$ 0.06	1.03 $\pm$ 0.03
Calcium Micro mg/dl	9.83 $\pm$ 0.22	8.73 $\pm$ 0.30*	9.13 $\pm$ 0.16	9.68 $\pm$ 0.36	9.13 $\pm$ 0.35	9.33 $\pm$ 0.22
Cholesterol Micro mg/dl	159 $\pm$ 7.42	129 $\pm$ 16.6	1230 $\pm$ 15.8	136 $\pm$ 3.64	121 $\pm$ 14.6	119 $\pm$ 23.2
Chloride Micro mmol/l	108 $\pm$ 2.31	109 $\pm$ 1.72	112 $\pm$ 1.30	111 $\pm$ 0.91	110 $\pm$ 0.88	110 $\pm$ 1.53
GGPT	60.6 $\pm$ 33.7	24.3 $\pm$ 0.67	44.3 $\pm$ 7.06	52.3 $\pm$ 11.3	43.0 $\pm$ 8.08	46.6 $\pm$ 11.1
Glucose Micro mg/dl	69.0 $\pm$ 17.6	70.7 $\pm$ 4.41	60.5 $\pm$ 2.90	61.5 $\pm$ 2.87	73.0 $\pm$ 4.93	67.7 $\pm$ 2.91
LDH Micro i.u./l	396 $\pm$ 60.5	345 $\pm$ 92.9	379 $\pm$ 56.1	331 $\pm$ 105.4	404 $\pm$ 58.9	614 $\pm$ 187.5
Phosphate Micro mg/dl	5.73 $\pm$ 0.07	4.73 $\pm$ 1.31	5.43 $\pm$ 0.35	3.50 $\pm$ 0.18**	5.10 $\pm$ 0.96	3.63 $\pm$ 0.76
Potassium Micro mmol/l	3.80 $\pm$ 0.15	3.77 $\pm$ 0.17	3.48 $\pm$ 0.25	3.68 $\pm$ 0.15	3.77 $\pm$ 0.03	4.60 $\pm$ 0.56
Total Protein Micro gm/dl	8.63 $\pm$ 0.84	6.53 $\pm$ 0.50	7.00 $\pm$ 0.30	7.03 $\pm$ 0.21	7.50 $\pm$ 0.35	7.10 $\pm$ 0.20
SGOT i.u./l	50.0 $\pm$ 4.04	24.7 $\pm$ 12.1	28.3 $\pm$ 7.61	34.8 $\pm$ 6.41	28.3 $\pm$ 9.53	31.0 $\pm$ 11.6
SGPT i.u./l	62.0 $\pm$ 7.00	39.3 $\pm$ 19.4	32.0 $\pm$ 11.6	27.5 $\pm$ 3.59	64.3 $\pm$ 49.6	31.3 $\pm$ 13.3
Sodium Micro mmol/l	143 $\pm$ 2.00	140 $\pm$ 3.85	146 $\pm$ 0.96	147 $\pm$ 0.75	145 $\pm$ 1.45	145 $\pm$ 2.19
Triglycerides Micro mg/dl	82.3 $\pm$ 47.5	41.3 $\pm$ 13.5	33.5 $\pm$ 8.99	28.5 $\pm$ 3.12	62.3 $\pm$ 7.27	39.0 $\pm$ 16.9
Uric Acid Micro mg/dl	0.30 $\pm$ 0	0.23 $\pm$ 0.07	0.33 $\pm$ 0.09	0.18 $\pm$ 0.09	0.43 $\pm$ 0.15	0.50 $\pm$ 0.40

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Table 7. Effect of Intrauterine Solution on Hematology

	<u>Tetracycline</u>		<u>Doxycycline</u>		<u>Control</u>	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
WBC / mm <sup>3</sup>	5459 <sub>±</sub> 2201	6405 <sub>±</sub> 1543	7243 <sub>±</sub> 1252	6306 <sub>±</sub> 646	7800 <sub>±</sub> 970	8224 <sub>±</sub> 1463
Total Neut %	34.3 <sub>±</sub> 3.84	45.5 <sub>±</sub> 2.96	45.3 <sub>±</sub> 8.16	39.8 <sub>±</sub> 7.66	50.7 <sub>±</sub> 4.91	52.7 <sub>±</sub> 8.45
Lympho %	54.0 <sub>±</sub> 0.0	48.3 <sub>±</sub> 4.36	48.0 <sub>±</sub> 9.27	52.0 <sub>±</sub> 5.96	43.0 <sub>±</sub> 3.61	39.0 <sub>±</sub> 6.09
Mono %	6.67 <sub>±</sub> 2.03	3.50 <sub>±</sub> 0.50	3.50 <sub>±</sub> 0.29	2.75 <sub>±</sub> 0.63	3.00 <sub>±</sub> 0.58	4.67 <sub>±</sub> 0.68
Eosino %	5.00 <sub>±</sub> 2.0	2.75 <sub>±</sub> 1.80	3.50 <sub>±</sub> 0.96	5.50 <sub>±</sub> 2.53	3.33 <sub>±</sub> 1.33	3.68 <sub>±</sub> 2.19
RBC x 1000	5260 <sub>±</sub> 319	4168 <sub>±</sub> 314	5288 <sub>±</sub> 356	4913 <sub>±</sub> 195	5473 <sub>±</sub> 122	4590 <sub>±</sub> 344
HCT	33.8 <sub>±</sub> 0.17	28.0 <sub>±</sub> 1.47	34.1 <sub>±</sub> 1.83	31.5 <sub>±</sub> 1.76	37.7 <sub>±</sub> 1.45	29.3 <sub>±</sub> 2.33
Hemoglobin gm %	9.80 <sub>±</sub> 0.21	8.13 <sub>±</sub> 0.45	11.1 <sub>±</sub> 0.59	10.1 <sub>±</sub> 0.68	11.7 <sub>±</sub> 0.23	9.40 <sub>±</sub> 0.55
Total Protein gm %	7.60 <sub>±</sub> 0.91	7.15 <sub>±</sub> 0.92	6.95 <sub>±</sub> 0.19	7.28 <sub>±</sub> 0.28	7.43 <sub>±</sub> 0.07	6.87 <sub>±</sub> 0.46
MCV U <sup>3</sup>	64.8 <sub>±</sub> 3.72	67.6 <sub>±</sub> 2.70	64.9 <sub>±</sub> 3.25	64.3 <sub>±</sub> 3.33	68.9 <sub>±</sub> 3.27	64.0 <sub>±</sub> 2.45
MCH μg	1.34 <sub>±</sub> 0.77	19.6 <sub>±</sub> 0.41	21.2 <sub>±</sub> 1.22	20.6 <sub>±</sub> 0.94	21.4 <sub>±</sub> 0.65	20.6 <sub>±</sub> 0.88
MCHC %	29.0 <sub>±</sub> 0.66	29.0 <sub>±</sub> 0.98	32.6 <sub>±</sub> 0.30	32.2 <sub>±</sub> 1.42	31.1 <sub>±</sub> 0.61	32.1 <sub>±</sub> 0.64

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The autopsy report (Fig. 6) revealed kidney and liver pathology, primarily fatty infiltration, compatible with doxycycline toxicity. It seems unlikely that the changes seen from hysterectomy to death were due to acute doxycycline exposure 7 days prior to hysterectomy, but the possibility cannot be excluded and is hereby reported. It should be noted that fatty infiltration often occurs in response to fasting.

Intrauterine instillation of 100 mg tetracycline to monkeys results in an increase in serum tetracycline levels observed at 4 hours after administration (Fig. 7). Levels were higher following administration of solution than pellets. With tetracycline solution, serum concentration decreases to about 10% of the 4-hour level, one day after treatment. In pellet treated monkeys, the concentration at one day was about 40% that of the 4-hour level suggesting that there is a slower release rate with pellets than with solutions, as might be expected.

Serum levels at 4 and 24 hours of doxycycline were equivalent regardless of the mode of delivery (Fig. 8). Serum levels of demeclocycline following pellet administration were much lower than those observed for tetracycline or doxycycline at comparable times. Plasma quinacrine levels at 4 and 24 hours were also equivalent regardless of the mode of delivery (Fig. 7). The solution data for this comparison is taken from previously published studies done by our laboratories (Appendix reprint #1). For all drugs, serum or plasma levels were near to or below the limit of detection for the respective assays by one week after administration.

#### E. Conclusions

This study indicates that tetracycline and doxycycline are capable of inducing lesions in the reproductive tract 7 days following



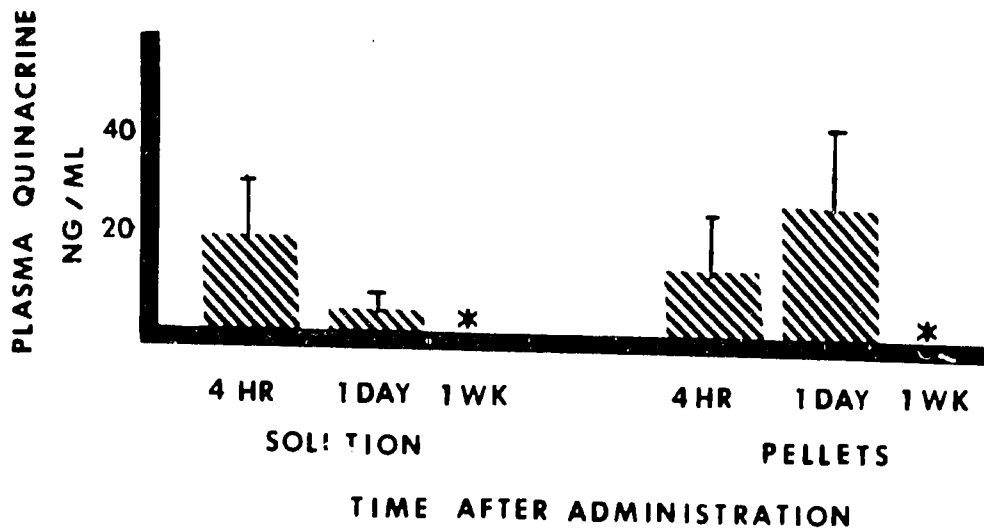
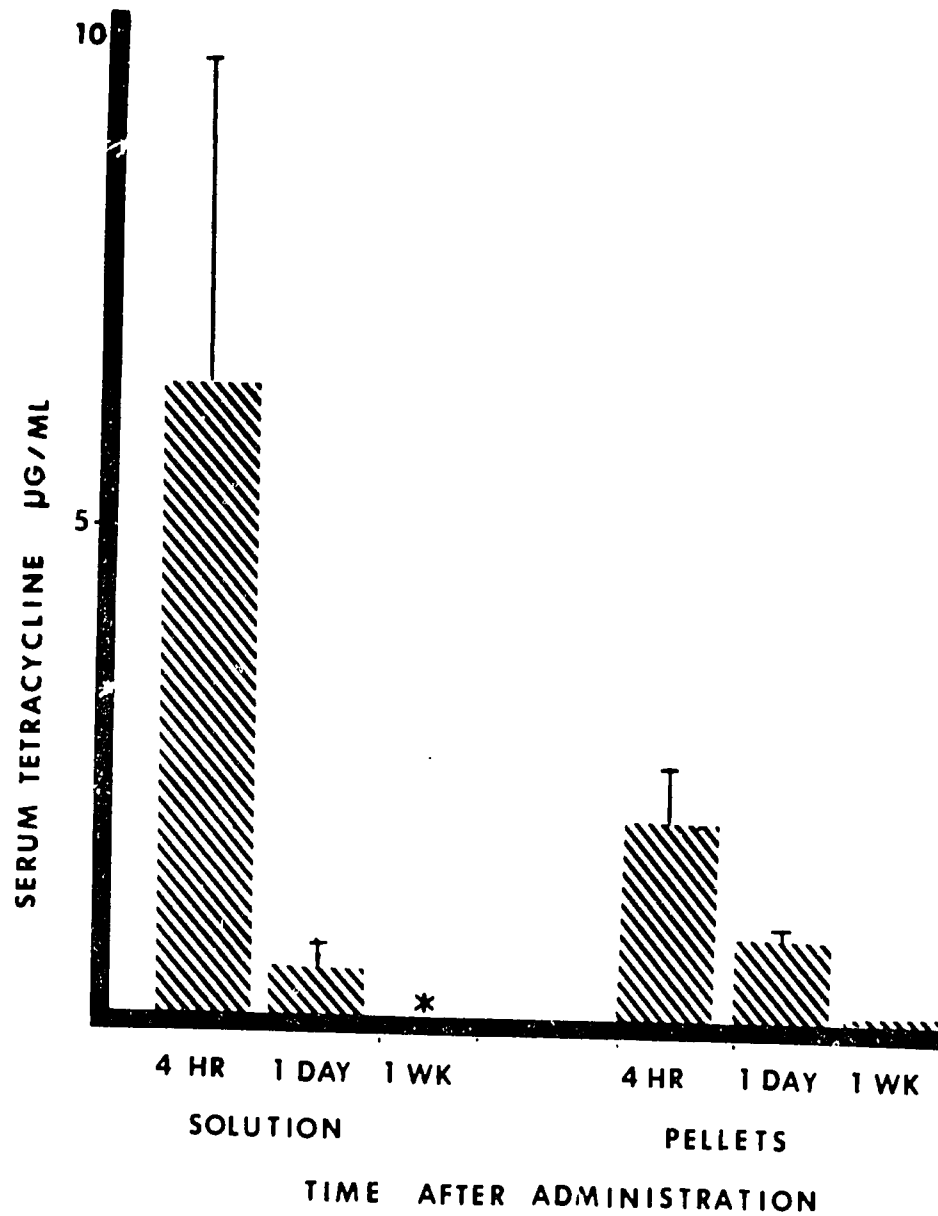


Figure 7. Drug levels after Intrauterine Administration: Tetracycline and Quinacrine.

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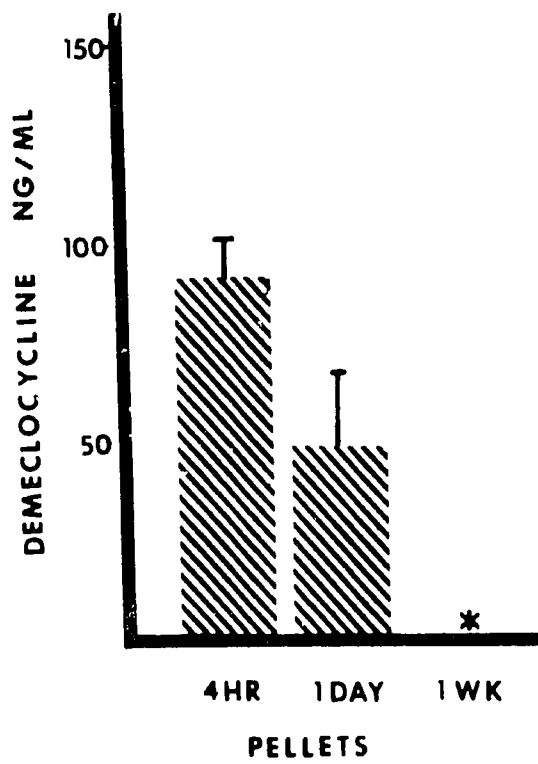
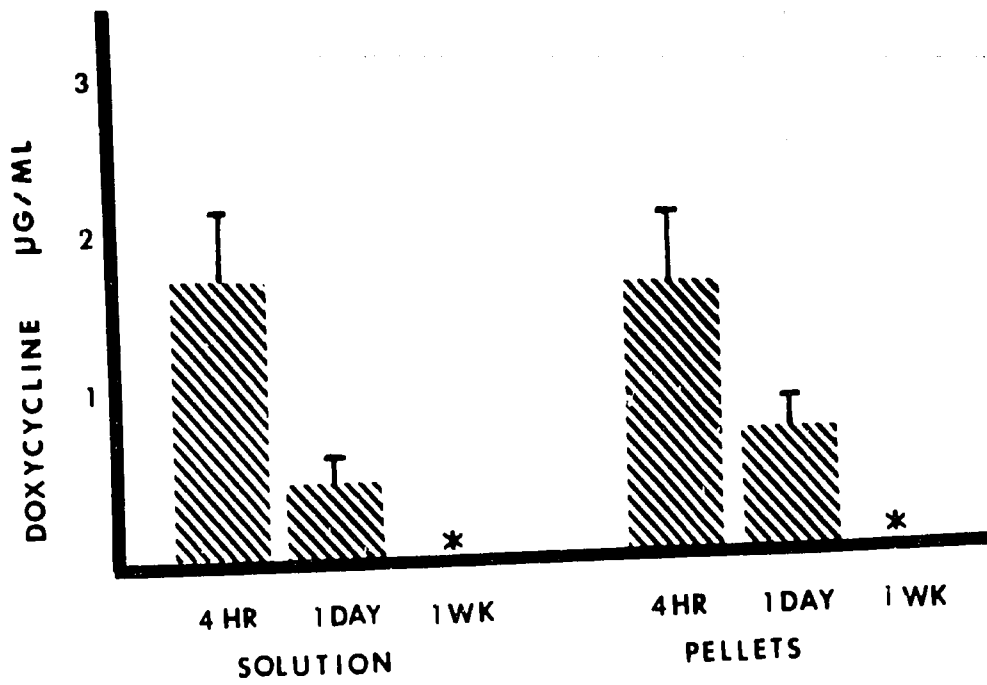


Figure 8. Drug levels after Intrauterine Administration: Doxycycline and Demeclocycline.

Intrauterine administration of the drug in cynomolgus monkeys. These lesions are comparable to those caused by administering intrauterine quinacrine pellets in the same species. Blood chemistry, hematology tests, and liver and kidney biopsies indicate that this dosage is non-toxic. Blood levels achieved in monkeys following intrauterine pellets or solutions of 100 mg tetracycline are comparable to those levels achieved when 2 gm of tetracycline are administered via the intraperitoneal route in women. Thus it is expected that intrauterine instillation of tetracycline instillation of 1 gm in humans will result in blood levels well within a safe range, even in the event of intraperitoneal spillage. Because of these results and the extensive literature on tetracycline toxicity, an IND is being prepared for Phase I testing of intrauterine tetracycline in women who are about to undergo hysterectomy (see next section).

Phase I Clinical Pharmacology Protocol  
Tetracycline Hydrochloride

Investigators: Tim Parmley, MD  
Norman Dubin, PhD

Investigational site: Johns Hopkins University  
School of Medicine

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## I. Introduction

The demand for female sterilization far exceeds the ability of most countries to provide services. The development of a rapid, effective, safe method that can be performed without surgery remains a high priority. Considerable research has focused on tubal occlusion techniques involving the injection of pharmacologic agents and adhesive materials into the oviduct, either through a hysteroscope or through a blind transcervical delivery system. In this study tetracycline hydrochloride will be investigated as a nonsurgical method of occluding the Fallopian tubes.

Chemistry. Tetracycline hydrochloride, a derivative of naphthacene carboxamide, is an antimicrobial agent which has also been proved effective as a sclerosing agent.

Animal Toxicology. In acute and chronic studies, tetracycline hydrochloride had no apparent carcinogenic or mutagenic activity. Teratological effects are related to the process of cartilage formation and calcification of bone if given during the second trimester of pregnancy; it appears to be relatively safe in the first trimester. Oral doses of tetracycline can result in esophageal, gastric and intestinal irritations leading to nausea, vomiting and gastric burning.

The Johns Hopkins University has recently completed a toxicologic and pharmacokinetic study of intrauterine tetracycline HCl in monkeys. The results showed that tetracycline-induced damage to the reproductive tract is limited to the endometrium with minimal effect on the myometrium. The blood levels were in the range of two ~~ug~~  $\mu\text{g}$ /ml at four hours after 100 mg of



tetracycline pellets were administered intrauterinely. Women in this study will receive one gm tetracycline which, on a body weight basis, is about half the dose given to monkeys and is within the range recommended for oral dosing in severe infections in humans.

Pharmacology. Tetracycline hydrochloride inhibits protein synthesis. Tetracycline concentrates in the liver and is excreted in urine and feces. It binds plasma proteins. Plasma concentrations are affected by a number of compounds, especially milk products and gels containing aluminum hydroxide, sodium bicarbonate and calcium and magnesium salts.

#### Summary of Clinical Experience.

##### 1. Antimicrobial Studies

Tetracycline hydrochloride has been approved by the FDA for use as an antibiotic.

##### 2. Pleural Cavity Studies

Tetracycline hydrochloride (HCl) has been used successfully as a sclerosing agent, primarily in the pleural cavity for the control of pneumothorax (see attachment 1).

#### II. Objective of study

This is a single site study. This protocol is designed to evaluate the effect and safety of the transcervical insertion of 1000 mg tetracycline hydrochloride pellets on the human uterus. The study population will be hospitalized female patients awaiting hysterectomy. The systemic absorption of the drug and changes in blood chemistry and

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hematology over the 24-hour interval between pellet insertion and hysterectomy will be determined.

### III. Length of study

Each patient will be under intensive study for the 24 hour interval between pellet insertion and hysterectomy.

### IV. Number of patients to be studied

A total of 10 women will participate in the study.

### V. Informed consent

The risks and benefits of participating in the study will be explained to each potential subject prior to entering into the study. Written informed consent is also required prior to study entry (Appendix A). The signing of informed consent will be recorded in each subject's case record. The signed consent form will be retained by the investigator. The physician who advises the patient to have a hysterectomy for treatment of her condition will not be the same physician who recruits her for this study.

### VI. Institutional review

Prior to the shipment of study medication and initiation of the study, the investigator will provide FHI with written documentation that the study protocol, case record forms (if required) and informed consent forms have

been approved by the appropriate Institutional Review Board. The names and occupations of the IRB members or the General Assurance Number (DHHS) will be provided.

#### VII. Criteria for subject selection

Source. Subjects will be women scheduled for a medically indicated hysterectomy.

Complete medical and gynecologic history, and complete medical and gynecologic exam, including bimanual pelvic exam, will be performed and recorded before entry into the study. If a Pap smear has not been performed within six months prior to entry, or if the record of it is not available, a Pap smear will be performed and evaluated prior to entry into the study. To be included in this study, the results of the Pap smear should not indicate a cytology reading greater than mild to moderate (Class II) dysplasia.

#### Inclusion Criteria

1. from 21-50 years of age
2. 6-12 days post onset of menses
3. normal pelvic exam (except cervical atypia and/or dysplasia)
4. negative pregnancy test (RIA for beta subunit of HCG)

#### Exclusion Criteria

1. D&C within past six months
2. use of oral contraceptives in past 30 days
3. use of IUD in past 30 days

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4. invasive cancer
5. previous surgical sterilization
6. history or evidence of pelvic inflammatory disease
7. history of significant disease of the cardiovascular, renal, hepatic or central nervous system
8. breastfeeding
9. hypersensitivity to tetracycline

#### VIII. Experimental protocol

Study design. This is a single site study of tetracycline hydrochloride. Women will receive 1000 mg of the study drug in pellets which will be administered transcervically via two sequential sterile pellet insertions (500 mg per insertion 10 minutes apart). Hysterectomies will be performed 24 hours after the insertion of tetracycline. Samples of saliva and venous blood (antecubital stick) will be obtained at nine points over a 48-hour interval ranging from 24 hours before to 24 hours after hysterectomy using a heparin lock (immediately before pellet insertion and 1/2, 1, 2, 3, 4, 10, 24 and 48 hours after pellet insertion). The total blood volume will not exceed 150 ml. An additional sample will be obtained just before hospital discharge. Total urine will be collected and tampons will be inserted in the vagina in order to recover any unabsorbed drug during the 24-hour period between tetracycline insertion and hysterectomy. At the time of hysterectomy, the peritoneal cavity will be irrigated with 10 ml saline and the fluid will be collected and assayed for tetracycline. Appendix B shows the schedule of tests and procedures in detail.

Laboratory Evaluation. Tetracycline will be measured spectrophotofluorimetrically in saliva, blood, urine, and peritoneal wash and also in a number of uterine segments. Vaginally inserted tampons will also be assayed for the drug. SMA-12s will be performed on blood samples at 0, 24 and 48 hours postinsertion of tetracycline pellets and an SMA-6 on all ~~other~~ blood samples.

All original laboratory report slips must be available for evaluation by FHI on request.

Adverse experiences. Patients will be observed for abdominal pain, tenderness and fever over the 24-hour postinsertion interval. No other alterations will be made in the usual pre and postoperative management of these patients. All adverse experiences that occur during the study must be recorded in detail. Necessary and sufficient history, physical exam and laboratory evaluations must be performed to document the nature and extent of the adverse experience and its relationship to the study drug.

#### IX. Medication

Study medication. Each tetracycline hydrochloride pellet is cylindrical and has a diameter of less than 4mm. The pellets are compacted to contain 10 mg tetracycline per millimeter of length.

Insertion is accomplished with a preloaded sterile plastic insertion device, 500 mg being delivered at each of 2 insertions separated by 10 minutes. The insertion procedure is essentially the same as that for inserting an IUD.

Concurrent medication. Other medications may be taken, provided that the drugs and dosages are recorded.

Storage and accounting of study medication. The investigator will store all study medications in a secure area. Study medications will be dispensed under the direct supervision of the investigating physician or study nurse. The amount of drug and dose dispensed will be recorded on the study medication inventory form. All unused study medications will be returned to FHI.

#### X. Criteria for efficacy evaluation

Efficacy evaluation will include a histological analysis of the effect of the study drug on the tissues of the uterus and Fallopian tubes.

#### XI. Safety

Adverse Experiences. Each patient will be carefully monitored for adverse experiences by the medical personnel. All volunteered, recorded or observed adverse experiences will be recorded by type, severity, time of onset in relationship to drug administration and the investigator's judgment as to whether or not related to the study drug or to another cause.

All serious or unanticipated adverse reactions are to be reported immediately to Family Health International.

Any patient discontinued from the study must have a detailed record of the circumstances.

All patients withdrawn from the study because of serious, limiting adverse experiences will be followed until the adverse experience resolves and a return to normal or to the patient's baseline condition has occurred.

All patients may be discontinued from the study at any time if, in the judgment of the investigator, continuation in the study would be detrimental to the physical or mental health of the patient.

Laboratory parameters. Safety will be monitored via the laboratory tests previously described. The systemic absorption of the drug and changes in blood chemistry and hematology over the 24-hour interval between pellet insertion and hysterectomy will be determined.

#### XII. Materials supplied to investigator

25 prepackaged sterile inserters, each containing 500 mg tetracycline hydrochloride pellets.

#### XIII. Other

1. All data will be recorded on the attached forms (Appendix <sup>c</sup> ~~1~~) or the usual patient records of the hospital where the study will be conducted and will be reported to FHI for each patient.
2. All unused study medication is to be returned to FHI.

3. Source documents specifically related to the clinical study, such as originals of laboratory report slips, must be available for inspection by FHI on request.
4. The investigator will be responsible for the study analysis.
5. The investigator will submit a report of the completed study to FHI.
6. A copy of the state license for the clinical laboratory will be supplied to FHI. Normal limits for laboratory tests for this laboratory will be provided.
7. A copy of the state license for the physician-investigator and all study nurses will be supplied to FHI.
8. The investigator and associate investigators will submit an F.D.A. Form 1573 with curriculum vitae to FHI.



XIV. Investigator consent

I agree to the above protocol as written.

_____	_____
Investigator	Date

_____	_____
Associate Investigator	Date

_____	_____
Associate Investigator	Date

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*Appendix*  
Attachment A

SUBJECT CONSENT FOR PARTICIPATION IN AN INVESTIGATION  
OF A BIOLOGICAL EVALUATION OF  
INTRAUTERINE ADMINISTRATION OF TETRACYCLINE

You are being asked to participate in a research study. The study is being done to find out what effect the antibiotic tetracycline has on the uterus. Preliminary studies indicate that tetracycline may close off the tube which carries the egg to the womb, and therefore, might serve as a sterilizing agent.

If you agree to participate, the following will happen: Approximately 24 hours before you are scheduled to have your uterus removed, several pellets of tetracycline will be inserted into your uterus by passing a small tube through your cervix. This may cause some cramps. The tube will be removed leaving the pellets behind and a tampon will be placed in your vagina to trap any of the drug which might leak out. We will obtain blood nine times over the next two days through the same IV so that you are stuck only once. The amount of blood taken each time will only be about a tablespoonful. While this small amount of blood will not be any problem for you, the needle sticks cause some minor pain and might cause some minor bruising. We will ask for one more blood sample just before you leave the hospital. We will also ask you to spit into a tube at about the same time the blood sample is being obtained. We will ask you to save all of your urine in a bottle which will be provided. The blood, saliva, and urine will be tested to see how much of the drug is in them.

After your uterus has been removed and given the routine examination, it will be further evaluated for the effect of tetracycline.

We don't expect that you will notice any of the effects of this drug, nor do we expect that it will cause you any harmful effect. However, no drug is entirely safe and some side effects of tetracycline are damage to the liver or kidneys or changes in your normal bacteria. A slight risk of uterine perforation at the time of insertion of the tetracycline exists but this would not be expected to have any harmful effects as you are scheduled to have a hysterectomy. There will be no change in the way that your surgery is performed and, therefore, participation in this study should not affect the risk of having your uterus removed.

There is no benefit to you if you participate.

If you sign this form, you are willing to join the research project described on the other side of this page. Your doctors did explain the other kinds of treatment that are available to you and to others. You should ask any questions you have about this research study. You may ask questions in the future if you do not understand something that is being done.

The records from this research study will not be given to anyone who is not helping on this study unless you agree to have the records given out. The study uses a drug that is under the jurisdiction of the Food and Drug Administration (FDA) and, therefore, the FDA government officials may look at the relevant part of your medical records as part of their job to review new drug studies.

If you want to talk to anyone about this research study because you think you have not been treated fairly or you think you have been hurt by joining the study, you should call \_\_\_\_\_ (Principal Investigator) at \_\_\_\_\_ (phone), or call the Office of the Joint Committee on Clinical Investigation at \_\_\_\_\_. Either ~~he~~ investigator or the people in the Committee office will help to find medical care for the injury you feel you have suffered. You should understand that The Johns Hopkins University, The Johns Hopkins Hospital, and the Federal Government do not have any program to provide compensation for you if you experience injury or other bad effects which are not the fault of the investigators.

You may withdraw from the research study at any time. Even if you do not want to join the study, or if you withdraw from it, you will still have the same quality of medical care available to you at Johns Hopkins.

If you agree to join this study, please sign your name below.

\_\_\_\_\_  
Subject's signature

\_\_\_\_\_  
Signature of parent of guardian  
(when applicable)

\_\_\_\_\_  
Witness to above signatures

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Date

NOTE: Signed copies of this consent form must be: a) retained on file by the Principal Investigator; b) deposited in the patient's medical record; and c) given to the patient.

Appendix B

Calendar for Various Tests and Procedures

	Preinsertion	Insertion	<i>Hours after insertion</i>							Hospital Discharge	
			1/2	1	2	3	4	10	24		48
Saliva	x		x	x	x	x	x	x	x	x	x
SMA-6	x		x	x	x	x	x	x	x	x	x
SMA-12	x									x	x
Tetracycline assay	x		x	x	x	x	x	x	x	x	x
Urine collection										x	x
Insert tampon		x									x
Observation sheet				x				x	x	x	
Remove tampon										x	
Hysterectomy										x	
Peritoneal fluid sample										x	
Uterine lumen wash										x	
Peritoneal wash										x	

11

**Appendix C**

**Supplementary Data Collection Forms**

Family Health International  
Phase I Clinical Pharmacology Study  
Tetracycline Hydrochloride

Patient Identification \_\_\_\_\_

Date: \_\_\_\_\_  
          day      month      year

Admission Criteria

Yes    No

from 21 to 50 years of age  
6-12 days post onset of menses  
normal pelvic exam (except cervical atypia and/or  
dysplasia)  
negative pregnancy test (investigators will use any of  
the sensitive tube tests capable of detecting levels of  
HCG of less than 0.4 I.U. HCG/ml. urine)

If no, patient cannot enter study

Exclusion Criteria

Yes    No

D&C within past six months  
use of oral contraceptives in past 30 days  
use of IUD in past 30 days  
invasive cancer  
previous surgical sterilization  
history or evidence of pelvic inflammatory disease  
history of significant disease of the cardiovascular,  
renal, hepatic or central nervous system  
breastfeeding  
hypersensitivity to tetracycline

If yes, patient cannot enter study

Investigator's signature \_\_\_\_\_ Date \_\_\_\_\_

4/5

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time 0 - Before inserting tetracycline:

- \_\_\_ Saliva 1-3 ml
- \_\_\_ Urine - empty bladder (urine cup)
- \_\_\_ Establish heparin lock in antecubital vein stick
- \_\_\_ Draw blood for SMA-12
- \_\_\_ Draw blood for SMA-6
- \_\_\_ Draw blood for tetracycline - 3 ml
- \_\_\_ Temperature \_\_\_\_\_
- \_\_\_ Pulse \_\_\_\_\_
- \_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_ Respiration \_\_\_\_\_

- \_\_\_ Insert tetracycline pellets
- \_\_\_ Insert tampon
- \_\_\_ Instruct patient to collect all urine in container provided

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

47

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 1/2 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline - 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

48



Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 1 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline - 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 2 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline - 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 3 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline - 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 4 hr

- \_\_\_ Saliva 1-3 ml
- \_\_\_ Blood for SMA-6
- \_\_\_ Blood for tetracycline - 3 ml
- \_\_\_ Temperature \_\_\_\_\_
- \_\_\_ Pulse \_\_\_\_\_
- \_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_  
Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 10 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline - 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 24 hr (or time of hysterectomy)

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-12
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline \_ 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = 48 hr

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-12
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline \_ 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

DATA COLLECTION CHECK SHEET

Time = Hospital Discharge

- \_\_\_\_\_ Saliva 1-3 ml
- \_\_\_\_\_ Blood for SMA-6
- \_\_\_\_\_ Blood for tetracycline \_ 3 ml
- \_\_\_\_\_ Temperature \_\_\_\_\_
- \_\_\_\_\_ Pulse \_\_\_\_\_
- \_\_\_\_\_ Blood pressure \_\_\_\_\_
- \_\_\_\_\_ Respiration \_\_\_\_\_

Additional comments: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

Signature \_\_\_\_\_

56



Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

TETRACYCLINE STUDY

Mode of Hysterectomy (Abdominal/Vaginal)

Pathologic Examination

Gross

A. external evaluation of the excised uterus

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

B. naked eye examination of the uterus opened to visualize the endometrial cavity

\_\_\_\_\_

Histology

A. four quadrant sections of the cervix

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_
4. \_\_\_\_\_

B. three transfundal sections which include anterior and posterior wall endometrium

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_

C. three sections of the intramural fallopian tube taken from both right and left sides

- |                  |                 |
|------------------|-----------------|
| 1. (right) _____ | 1. (left) _____ |
| 2. (right) _____ | 2. (left) _____ |
| 3. (right) _____ | 3. (left) _____ |

D. three sections from each available adnexa\* including ovary and fallopian tube

1. (right) \_\_\_\_\_ 1. (left) \_\_\_\_\_

2. (right) \_\_\_\_\_ 2. (left) \_\_\_\_\_

3. (right) \_\_\_\_\_ 3. (left) \_\_\_\_\_

\* In some cases it will not be clinically appropriate to remove the adnexae.

\_\_\_\_ Attach copy of patient history form

WHEN FORM IS COMPLETE, DELIVER TO SUE SCHEPER (X 5764, Park Bldg., Rm: B202)

Signature \_\_\_\_\_

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Patient Name \_\_\_\_\_ Hospital History # \_\_\_\_\_

Date \_\_\_\_\_

TETRACYCLINE STUDY

Tissue Measurement Data Sheet (Dr. Dubin's Laboratory)

cervix surface \_\_\_\_\_

deep \_\_\_\_\_

endometrium \_\_\_\_\_

myometrium \_\_\_\_\_

tube\* isthmus \_\_\_\_\_

ampulla \_\_\_\_\_

fimbria \_\_\_\_\_

ovary\* \_\_\_\_\_

\*in some cases it will be clinically appropriate to remove the adnexae.

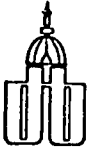
peritoneal fluid \_\_\_\_\_

lumen wash \_\_\_\_\_

Signature \_\_\_\_\_



THE JOHNS HOPKINS UNIVERSITY  
SCHOOL OF MEDICINE  
and  
THE JOHNS HOPKINS HOSPITAL  
BALTIMORE, MARYLAND 21205



Joint Committee on Clinical Investigation

Please address reply to:  
Administration 127  
School of Medicine  
720 Rutland Avenue

TEL. (301) 955-3008

December 13, 1983

Timothy H. Parmley, M.D.  
Associate Professor of OB/GYN  
Nelson 2-104  
The Johns Hopkins Hospital

Dear Dr. Parmley:

I am pleased to inform you that the Research Project Notification (RPN) for your protocol entitled:

"Phase I Clinical Trial of Tetracycline Hydrochloride as an Intrauterine Sterilizing Agent" (with Drs. Dubin, Rosepshein, King and Blake)- NEW

was reviewed and approved on December 13, 1983.

If unanticipated adverse effects are encountered in the course of the study, or if new information becomes available which could change the perception of a favorable risk:benefit ratio, the investigator is responsible for informing the Committee of this situation promptly so that the JCCI may review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Enclosed is the approved consent form to be used in the study. (from 12/13/83 to 12/13/84). All previous versions of the consent form should be destroyed.

The approval is valid for one year. Renewal should be requested at least one month before this approval expires. The reference number in the upper right corner has been assigned to your study for the convenience of the Committee, and we request that you cite this number in future correspondence.

Sincerely yours,

Thomas R. Hendrix, M.D.  
Chairman - J.C.C.I.  
c/o 127 S/M Administration Bldg.

APPROVAL OF THE  
PHARMACY & THERAPEUTICS COMM  
HAS BEEN GRANTED

TRH:hm  
Enclosure  
cc: P&T Committee

CLINICAL INVESTIGATION CONSENT FORM  
The Johns Hopkins Medical Institutions

Title of Project: PHASE I CLINICAL TRIAL OF  
TETRACYCLINE HYDROCHLORIDE AS AN INTRAUTERINE  
STERILIZING AGENT

Patient I.D. Plate

Explanation of Project to Subject:

You are being asked to participate in a research study because you are scheduled to have a hysterectomy in the next few days. The study is being done to find out what effect the antibiotic tetracycline has on the uterus. Preliminary studies indicate that tetracycline may close off the tube which carries the egg to the womb, and therefore might serve as a sterilizing agent.

If you agree to participate, the following will happen: Approximately 24 hours before you are scheduled to have your uterus removed, several pills of tetracycline will be inserted into your uterus by passing a small tube through your cervix. This may cause some cramps. The tube will be removed leaving the pellets behind and a tampon will be placed in your vagina to trap any of the drug which might leak out. We will obtain blood ten times over the next two days through the same needle so that you are stuck only once. The total amount of blood taken will be about a cup. While this small loss of blood will not be any problem for you, the needle sticks cause some minor pain and might cause some minor bruising. We will ask for one more blood sample, a tablespoonful, just before you leave the hospital. We will also ask you to spit into a tube at about the same time that the blood sample is being obtained. We will ask you to save all of your urine in a bottle which will be provided. The blood, saliva, and urine will be tested to see how much of the drug is in them.

After your uterus has been removed and given the routine examination, it will be further evaluated for the effect of tetracycline. At your routine follow-up visit, 4-6 weeks after the operation, we will ask you for an additional blood sample for repeat laboratory tests.

We don't expect that you will notice any of the effects of this drug, nor do we expect that it will cause you any harmful effect. However, no drug is entirely safe, and some side effects of tetracycline are allergic reactions or changes in your normal bacteria. A slight risk of uterine perforation at the time of insertion of the tetracycline exists but this would not be expected to have any harmful effects as you are scheduled to have a hysterectomy. There will be no change in the way that your surgery is performed and therefore participation in this study should not affect the risk of having your uterus removed.

There is no definite benefit for you if you participate.

6/1

If you sign this form, you are willing to join the research project described on the other side of this page. Your doctors did explain the other kinds of treatment that are available to you and to others. You should ask any questions you have about this research study. You may ask questions in the future if you do not understand something that is being done.

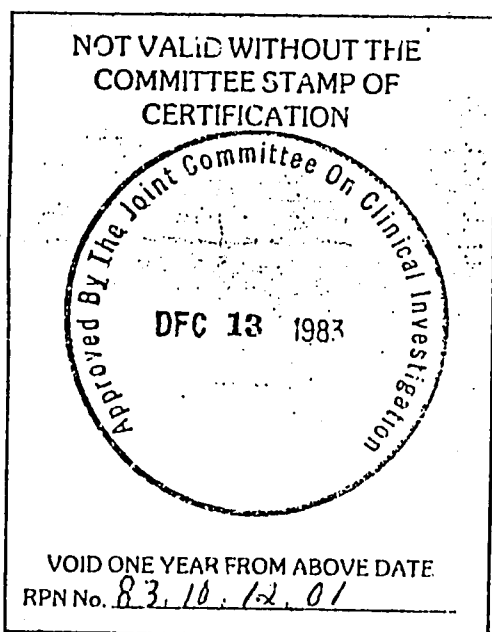
The records from this research study will not be given to anyone who is not helping on this study unless you agree to have the records given out. If the study uses a drug that is under the jurisdiction of the Food and Drug Administration (FDA), the FDA government officials may look at the relevant part of your medical records as part of their job to review new drug studies.

If you want to talk to anyone about this research study because you think you have not been treated fairly or you think you have been hurt by joining the study, you should call \_\_\_\_\_

Dr. Timothy Farnley (Principal Investigator) at 955-6710 (phone), or call the Office of the Joint Committee on Clinical Investigation at 955-3008. Either the investigator or the people in the Committee office will help to find medical care for the injury you feel you have suffered. You should understand that The Johns Hopkins University, The Johns Hopkins Hospital, \_\_\_\_\_ and the Federal Government do not have any program to provide compensation for you if you experience injury or other bad effects which are not the fault of the investigators.

You may withdraw from the research study at any time. Even if you do not want to join the study, or if you withdraw from it, you will still have the same quality of medical care available to you at Johns Hopkins.

If you agree to join this study, please sign your name below.



\_\_\_\_\_  
Subject's signature  
(Including children, when applicable)

\_\_\_\_\_  
Signature of Parent or Guardian (when applicable)

\_\_\_\_\_  
Witness to above signatures

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Date

NOTE: Signed copies of this consent form must be: a) retained on file by the Principal Investigator;  
b) deposited in the patient's medical record; and c) given to the patient

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## **Pharmacokinetic Studies on Quinacrine Following Intrauterine Administration to Cynomolgus Monkeys**

NORMAN H. DUBIN, PH.D., DAVID A. BLAKE, PH.D., MARIA C. DIBLASI, B.A., TIM H. PARMLEY, M.D.,  
AND THEODORE M. KING, M.D., PH.D.



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## Pharmacokinetic studies on quinacrine following intrauterine administration to cynomolgus monkeys\*

Norman H. Dubin, Ph.D.†  
David A. Blake, Ph.D.  
Maria C. DiBlasi, B.A.  
Tim H. Parmley, M.D.  
Theodore M. King, M.D., Ph.D.

*Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland*

*Recent efforts have been made to develop a chemical oviductal occluding agent. Intrauterine quinacrine has been used in certain areas of the world with moderate success in effected tubal closure. This report presents the pharmacokinetics of a quinacrine solution (30 mg) as administered to cynomolgus monkeys via the intrauterine route, compared with intravascular injection. The data show rapid transfer of the drug from the uterine to the vascular compartment and uptake by almost all tissues examined. Although plasma concentrations disappear within 24 hours, levels can be detected in most tissues for at least 1 week following intrauterine injection. After 28 days, however, tissue levels of the drug are absent or near the limit of detection. Fertil Steril 38:735, 1982*

In recent years there have been efforts toward development of chemical methods of tubal occlusion.<sup>1</sup> One such method, that of intrauterine quinacrine administration, has been pioneered by Zipper et al.,<sup>2</sup> and, depending upon the precise mode of administration, has had an acceptable degree of efficacy.<sup>3</sup> While the procedure is used widely in certain parts of the world today (e.g., Chile), the lack of basic pharmacologic and toxicologic studies prevents use of the drug in the United States. This report presents the pharmacokinetics of the drug as administered by the intrauterine route, compared with intravascular administration, as performed in cynomolgus monkeys.

Received May 10, 1982; revised and accepted July 27, 1982.

\*Supported by the International Fertility Research Program Subcontracts 601 and 603.

†Reprint requests: N. H. Dubin, Ph.D., Park Building, Room B202, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

### MATERIALS AND METHODS

#### EXPERIMENTAL APPROACH

Adult female cynomolgus monkeys were purchased from Primate Imports, Port Washington, NY. They were housed in individual cages and fed Charles River Primate Formula (Agway Company, Waverly, NY) supplemented with fresh fruit. Water was available ad libitum. The monkeys were followed daily for signs of vaginal bleeding by the use of cotton swabs.

Fifteen monkeys were studied. Three received intravascular (saphenous vein) injection of 30 mg quinacrine in a 1-ml volume of sterile water. The injection solution was freshly prepared on the day of injection. Nine monkeys received intrauterine injection of a solution of 30 mg quinacrine in 1 ml sterile water. The injection procedure has been previously described in detail.<sup>4</sup> The dose was determined by the solubility of quinacrine and the volume capacity of the uterus. Three monkeys received intrauterine injection in 1 ml normal

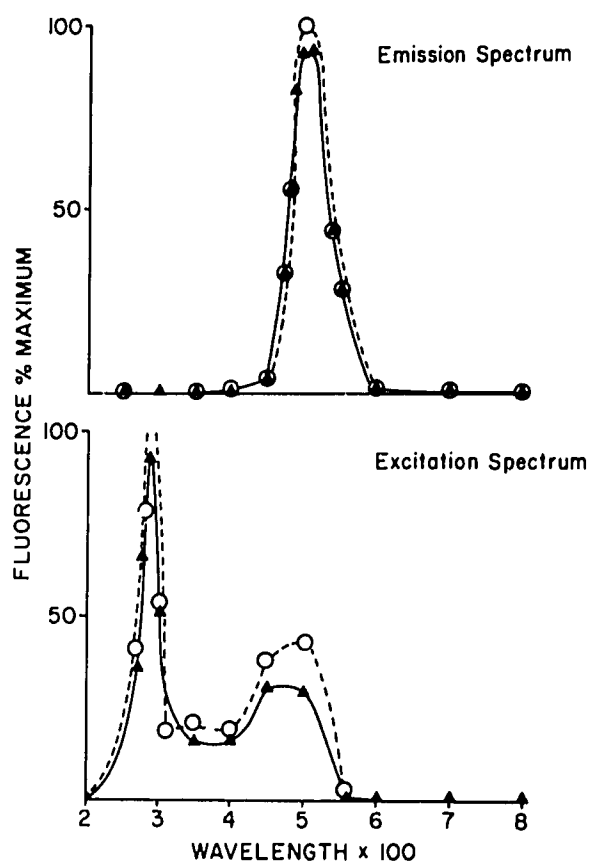


saline. We chose saline, rather than water, to eliminate the possible effects of a hypotonic solution. Before intrauterine injection was made, daily vaginal swabs were made of each monkey to determine cyclic bleeding, and injections were made within 14 days of the first day of bleeding (proliferative stage of cycle). Within 24 hours prior to treatment, blood samples were obtained from the femoral vein for baseline quinacrine levels. Blood was also taken for chemical and hematologic studies (results of which are described in an accompanying paper<sup>5</sup>). Food was withdrawn 24 hours prior to treatment, but water was still available. The animals were anesthetized with ketamine hydrochloride, and an indwelling catheter (Intracath, Deseret Company, Sandy, UT) was inserted into the saphenous vein. In those that received quinacrine intravenously, it was inserted in the vein contralateral to the injection. Lactated Ringer's solution (Cutter Injection USP, Berkeley, CA) was infused at approximately 0.25 ml/min with a Holter model 911 peristaltic pump (Extracorporeal Medical Specialties, Inc., King of Prussia, PA). Blood samples were obtained periodically with the use of a three-way stopcock. The bladder was also catheterized for continuous urine collection. The animal was restrained in a specially-designed chair for a 4-hour period so that blood and urine samples could be obtained. Following this procedure, the animal was returned to its cage and observed daily. Blood samples were obtained on days 1, 2, 3, 7, and 28 after treatment or until autopsy.

The three saline-treated monkeys and three of the monkeys receiving intravascular or intrauterine quinacrine were autopsied 24 hours after injection. Three other monkeys receiving intrauterine quinacrine were autopsied at 7 days and three others at 28 days after treatment.

#### QUINACRINE TEST MATERIAL

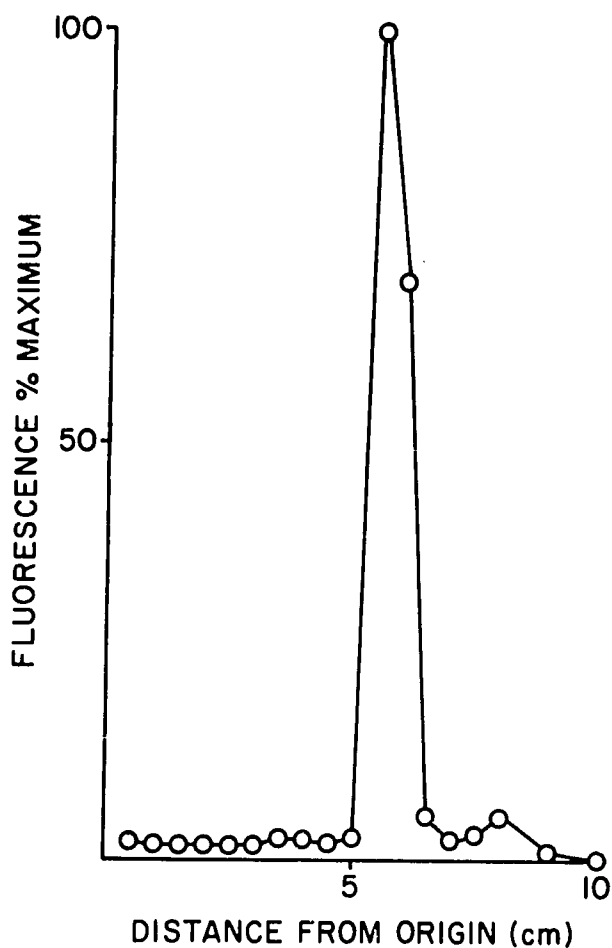
Quinacrine hydrochloride was obtained from Sigma Chemical Company, St. Louis, MO, and the lot number of the material used was 7-3-2. We also obtained a standard preparation from the United States Pharmacopeia. Two separate stock solutions were prepared for each standard (USP and Sigma), and a standard solution of various dilutions was prepared from each stock solution. The excitation and emission spectra for both USP and Sigma standards (1000 ng) are shown in Figure 1. The two curves show maximum excitation and emission wavelengths equivalent to each



**Figure 1**  
Emission and excitation fluorescence spectra of Sigma (O—O) and USP (▲—▲) standards. Emission spectra were scanned with an excitation wavelength of 285 nm. The excitation spectra were scanned with an emission wavelength of 500 nm.

other and in agreement with reported values. Mass spectra for both standards were found to be identical by the Mass Spectrometry Center, The Johns Hopkins University.

Thin-layer chromatography of these standards was performed on silica gel plates. Both Sigma and USP standards were spotted (approximately 1 µg) and chromatographed in 30% NH<sub>4</sub>OH: MeOH (1.5:100).<sup>6</sup> Both quinacrine samples ran with the same R<sub>f</sub> value and could be visualized as a yellow spot. Under an ultraviolet lamp, two other prominent spots could be visualized for both preparations. In one experiment, 5 µg of Sigma quinacrine was chromatographed, and 0.5-cm sections from the origin to the front were scraped into test tubes and eluted with solvent. The tubes were centrifuged, and supernatant fractions were dried under nitrogen. Lactic acid solution was added, and each tube was assayed for fluorescence at an excitation wavelength of 285 nm and an



**Figure 2**  
Relative fluorescence of the quinacrine standard as detected in eluted samples of 0.5-cm sections scraped from a chromatographic plate following development in  $\text{NH}_4\text{OH}:\text{MeOH}$  (1.5:100).

emission wavelength of 500 nm. In the peak corresponding to the quinacrine standard (Fig. 2), 98.5% of the total fluorescence in the chromatogram was found.

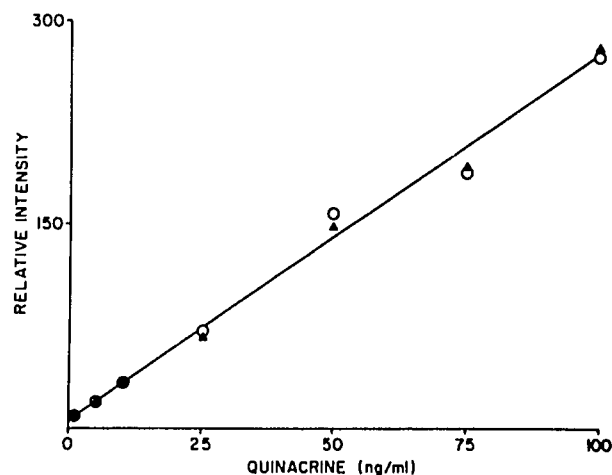
#### QUINACRINE ASSAY

Quinacrine was quantified by a fluorometric method based upon the procedure by Brodie and Udenfriend.<sup>7</sup> Blood was collected in 5-ml tubes containing 10 mg potassium oxalate and centrifuged at 2000 RPM for 10 minutes at 4° C, and plasma was separated. We added 0.3 ml 0.2 M  $\text{Na}_2\text{HPO}_4$  to 1 ml plasma and 3 ml ethylene dichloride. The solvent extract was then shaken with 0.1 ml distilled water and 0.9 ml 85% lactic acid for 15 minutes and centrifuged. The aqueous layer was transferred to a quartz cuvette and read fluorometrically in an Aminco-Bowman spectro-

photofluorometer (American Instrument Company, Silver Spring, MD) at an excitation wavelength of 285 nm and an emission wavelength of 500 nm. We then interpolated the fluorometric readings from a quinacrine standard curve to obtain quinacrine concentrations. Weighed tissues were prepared by homogenization with a Polytron PT 10-35 (Brinkmann, Westbury, NY) equipped with a PT 105T probe at 4° C in a minimum amount of distilled water. The concentration of homogenized tissue was diluted with distilled water to bring the final concentration to 100 mg tissue/ml. For urine and tissues, the ethylene dichloride layer was subjected to an additional wash with 2.5 N NaOH prior to addition of lactic acid. Background fluorescence was detected in body fluid and tissues of control monkeys, and these levels were used as the appropriate blank, which was subtracted from those values obtained from treated animals.

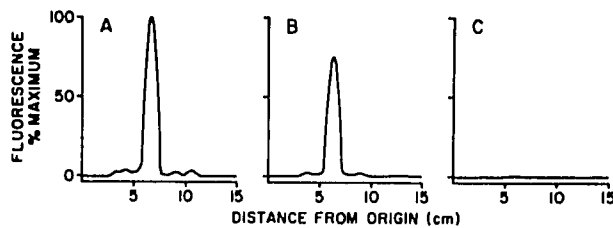
Standard solutions of both Sigma and USP quinacrine were prepared in 85% lactic acid solution. Figure 3 shows the relative fluorescence intensity of various concentrations of quinacrine. There was a linear relationship between quinacrine concentration and relative fluorescence intensity over a long range of concentrations. The linearity of the curve extends to at least 1000  $\mu\text{g}/\text{ml}$ . The higher concentrations are not shown in the figure. The sensitivity of the assay was 2.5 ng. Known concentrations of quinacrine added to plasma, which were then assayed, yielded a recovery of > 85%.

A chromatographic analysis of plasma from a quinacrine-treated monkey was also made (Fig.



**Figure 3**  
Standard curve of both Sigma (○) and USP (▲) quinacrine samples.

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**Figure 4**  
Chromatography of fluorescent material from (A) quinacrine standard; (B) extracted plasma of quinacrine-treated monkey plasma; (C) extracted plasma from a nontreated monkey.

4). Following extraction of plasma, thin-layer chromatography was performed as described. The developed chromatogram was scanned for fluorescence with the use of an Aminco-Bowman Thin-Film scanner (American Instrument Company, J4-8247) and recorded on a Harvard apparatus 10-speed chart mover. One major peak corresponding to that of authentic quinacrine was seen. Thus, it appears that the substance measured by the fluorescence assay is indeed quinacrine and not a metabolic product.

## RESULTS

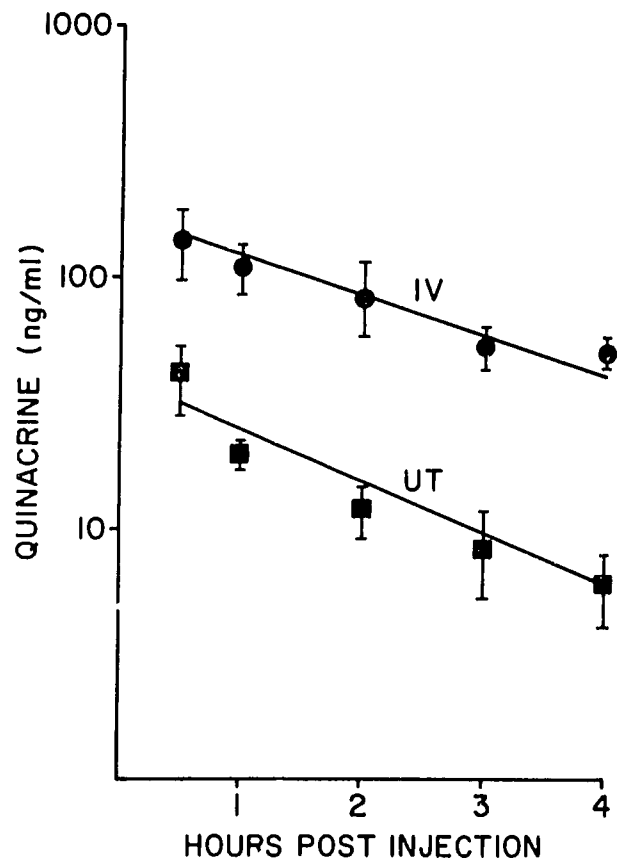
Following intravascular injection of 30 mg quinacrine, two of three monkeys vomited or retched within 3 minutes of the injection. This did not occur in monkeys receiving intrauterine injections of quinacrine or saline.

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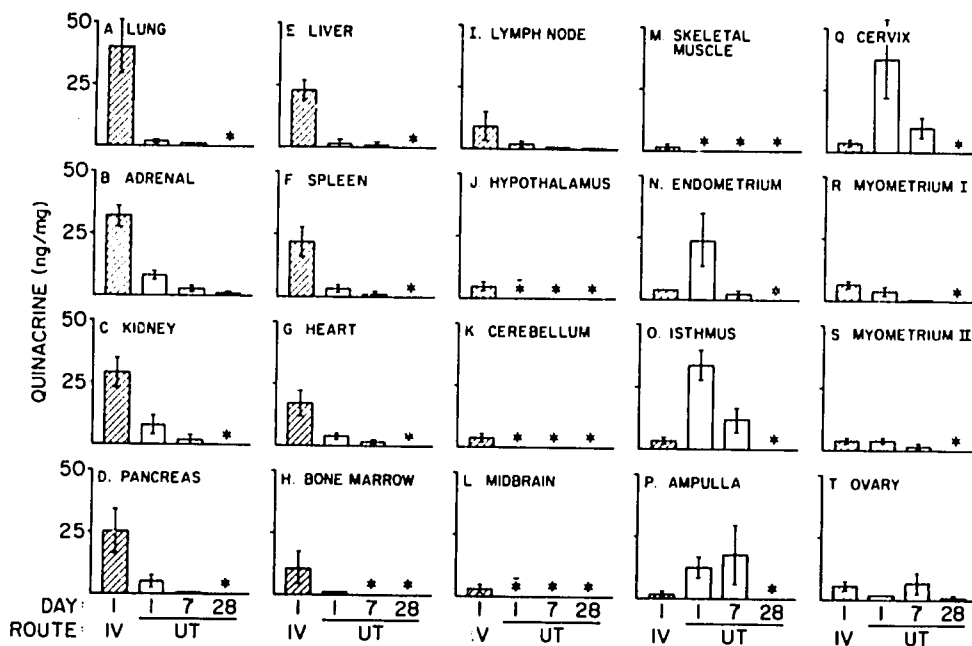
A total of  $248 \pm 32 \mu\text{g}$  quinacrine was found in a 4-hour posttreatment urine collection in those animals receiving intravascular injection, accounting for 0.8% of the original injection. In

those receiving an intrauterine injection,  $128 \pm 34 \mu\text{g}$  was found. The plasma concentration of quinacrine was  $23.5 \pm 8.5 \text{ ng/ml}$  24 hours after intravascular administration of the drug. In monkeys receiving intrauterine injections, plasma quinacrine was not detected 24 hours after administration.

Figure 6 represents the tissue levels of quinacrine in various treatment groups. One day after intravascular injection of 30 mg quinacrine, the highest levels were observed in lung ( $40.2 \pm 11.45 \text{ ng/mg}$ , mean  $\pm$  standard error of the mean [SEM]). All other organs studied had concentrations of quinacrine, the least being  $1.6 \pm 0.8 \text{ ng/mg}$  in skeletal muscle. Even this tissue had a tissue:plasma ratio of 68:1 1 day after injection. Twenty-four hours following intrauterine injection of 30 mg quinacrine, the highest concentrations were found in the isthmus ( $33.9 \pm 6.0 \text{ ng/mg}$ ) and the endometrium ( $23.5 \pm 11.5 \text{ ng/mg}$ ). High levels ( $> 10 \text{ ng/mg}$ ) were found also in the cervix and ampulla. Of the nonreproductive organs, the adrenal had the highest concentration



**Figure 5**  
Quinacrine concentration in the plasma of monkeys at various times after injection of 30 mg of quinacrine by an intravascular (IV,  $n = 3$ ) route or an intrauterine (UT,  $n = 8$ ) route.



**Figure 6**  
Tissue concentrations of quinacrine after either IV administration (hatched bars) or UT administration (solid bars) of 30 mg quinacrine. Vertical lines indicate  $\pm$  SEM ( $n = 3$ ). For UT injection concentrations are shown at 1, 7, and 28 days after injection. The asterisk indicates that levels were not significantly different from the background fluorescence of the control animals given injections of saline. The asterisk with the bar above it indicates that levels were less than 0.5 ng/mg but were significantly higher ( $P < 0.01$ ) than the corresponding control levels.

of quinacrine ( $8.4 \pm 2.9$  ng/mg) 1 day after intrauterine injection of the drug. Quinacrine was not detectable, or near the limit of detection, in all tissues at 28 days following intrauterine injection.

## DISCUSSION

Pharmacokinetic information is available for quinacrine following administration via the oral route because of its widespread use as an antimalarial agent during World War II. However, no data are available for its absorption and distribution following intrauterine administration. The cynomolgus monkey was chosen for this particular study because its reproductive anatomy is similar to that of man, and its cervical canal, although not straight like that of a woman, can be penetrated by a blunt-end needle<sup>1</sup> so that quinacrine can be directly deposited into the uterine cavity. A solution of quinacrine was used, since this was part of a broader toxicology study<sup>5</sup> and the "worst case" of introduction of quinacrine into the circulatory system was desired. The dose given (30 mg/ml) is approximately 2 times the 250-mg dose (on a per body weight basis) that would be given to women via the uterus at any one time of administration.

Quinacrine entered the blood very rapidly following intrauterine injection. The highest levels were observed at 30 minutes, when the first sample was taken. In humans, quinacrine is reported

to reach its peak from the gastrointestinal tract in 2 hours<sup>8</sup>

It is estimated that the blood volume is 80 ml/kg body weight. The average weight of the three monkeys receiving intravascular injection was approximately 3 kg. Thus, the estimated blood volume is 240 ml. If 30 mg quinacrine were distributed in this volume, the expected concentration at time zero would be 125  $\mu$ g/ml. The earliest plasma sample obtained 30 minutes after intravascular injection averaged  $149 \pm 42$  ng/ml, thus indicating that quinacrine was removed extremely rapidly from the blood, probably being concentrated in tissue. The tissue level data suggest that this was indeed the case. This situation, that of enormous apparent volume of distribution and rapid binding to tissue, has been reported in humans following intravascular or enteral absorption.<sup>8</sup>

Following intravascular administration, quinacrine could be detected in all organs examined 24 hours later. Following intrauterine injection, levels at 24 hours were highest, as expected, in the endometrium, cervix, and portions of the tube. However, quinacrine could also be detected at this time in all tissues except cerebellum and skeletal muscles. The highest concentration of quinacrine in a nonreproductive organ was the adrenal, which was not unexpected because it has a large blood supply on a tissue mass basis.

Many tissues from animals which were injected with intrauterine quinacrine and autopsied 1

week later also contained significant amounts of quinacrine, although generally the levels were less than the 24-hour levels. By 28 days, all tissue levels of quinacrine were undetectable or near the limit of detection.

Urinary excretion was very low, as illustrated by the fact that less than 1% of the injected dose was eliminated in the urine after 4 hours. Similarly, low excretion rates have been reported for humans. Significant amounts of quinacrine have been detected in the urine up to 2 months after dosing.<sup>9</sup> Based upon the rate of achievement of a steady-state plasma level with constant oral dosing, the elimination half-life has been estimated to be approximately 7 days.<sup>4</sup>

In summary, the study described illustrates that intrauterine injection of quinacrine results in a rapid entry into the circulatory system, followed by rapid and tenacious uptake by body tissues. Quinacrine can be detected in the reproductive tissues and some of the nonreproductive tissues 7 days after intrauterine injection. However, by 1 month all tissues have undetectable levels of quinacrine or levels near the limit of detection. In our accompanying report,<sup>5</sup> we demonstrate the lack of pathologic changes in any of the nonreproductive organs examined at either 1, 7, or 28 days, despite the uptake and retention of quinacrine.

*Acknowledgments.* The authors gratefully acknowledge Ralph Cox for his technical assistance, Doris Cummings for help in validation of the quinacrine assay, and Sue Scheper for preparation of the manuscript.

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## **Pharmacokinetic Studies on Quinacrine Following Intrauterine Administration to Cynomolgus Monkeys**

NORMAN H. DUBIN, PH.D., DAVID A. BLAKE, PH.D., MARIA C. DIBLASI, B.A., TIM H. PARMLEY, M.D.,  
AND THEODORE M. KING, M.D., PH.D.



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## Pharmacokinetic studies on quinacrine following intrauterine administration to cynomolgus monkeys\*

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*Recent efforts have been made to develop a chemical oviductal occluding agent. Intrauterine quinacrine has been used in certain areas of the world with moderate success in effected tubal closure. This report presents the pharmacokinetics of a quinacrine solution (30 mg) as administered to cynomolgus monkeys via the intrauterine route, compared with intravascular injection. The data show rapid transfer of the drug from the uterine to the vascular compartment and uptake by almost all tissues examined. Although plasma concentrations disappear within 24 hours, levels can be detected in most tissues for at least 1 week following intrauterine injection. After 28 days, however, tissue levels of the drug are absent or near the limit of detection. Fertil Steril 38:735, 1982*

In recent years there have been efforts toward development of chemical methods of tubal occlusion.<sup>1</sup> One such method, that of intrauterine quinacrine administration, has been pioneered by Zipper et al.,<sup>2</sup> and, depending upon the precise mode of administration, has had an acceptable degree of efficacy.<sup>3</sup> While the procedure is used widely in certain parts of the world today (e.g., Chile), the lack of basic pharmacologic and toxicologic studies prevents use of the drug in the United States. This report presents the pharmacokinetics of the drug as administered by the intrauterine route, compared with intravascular administration, as performed in cynomolgus monkeys.

### MATERIALS AND METHODS

#### EXPERIMENTAL APPROACH

Adult female cynomolgus monkeys were purchased from Primate Imports, Port Washington, NY. They were housed in individual cages and fed Charles River Primate Formula (Agway Company, Waverly, NY) supplemented with fresh fruit. Water was available ad libitum. The monkeys were followed daily for signs of vaginal bleeding by the use of cotton swabs.

Fifteen monkeys were studied. Three received intravascular (saphenous vein) injection of 30 mg quinacrine in a 1-ml volume of sterile water. The injection solution was freshly prepared on the day of injection. Nine monkeys received intrauterine injection of a solution of 30 mg quinacrine in 1 ml sterile water. The injection procedure has been previously described in detail.<sup>4</sup> The dose was determined by the solubility of quinacrine and the volume capacity of the uterus. Three monkeys received intrauterine injection in 1 ml normal

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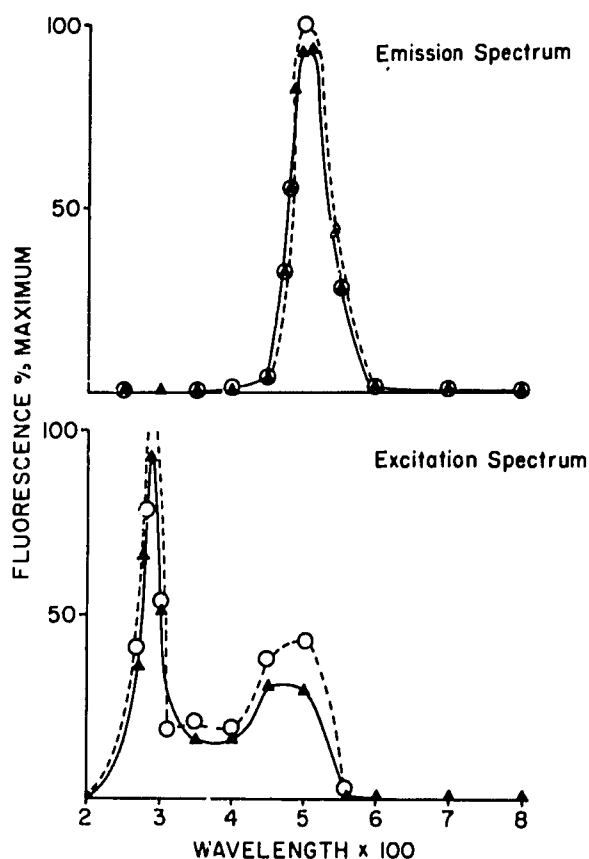
†Reprint requests: N. H. Dubin, Ph.D., Park Building, Room B202, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

saline. We chose saline, rather than water, to eliminate the possible effects of a hypotonic solution. Before intrauterine injection was made, daily vaginal swabs were made of each monkey to determine cyclic bleeding, and injections were made within 14 days of the first day of bleeding (proliferative stage of cycle). Within 24 hours prior to treatment, blood samples were obtained from the femoral vein for baseline quinacrine levels. Blood was also taken for chemical and hematologic studies (results of which are described in an accompanying paper<sup>5</sup>). Food was withdrawn 24 hours prior to treatment, but water was still available. The animals were anesthetized with ketamine hydrochloride, and an indwelling catheter (Intracath, Deseret Company, Sandy, UT) was inserted into the saphenous vein. In those that received quinacrine intravenously, it was inserted in the vein contralateral to the injection. Lactated Ringer's solution (Cutter Injection USP, Berkeley, CA) was infused at approximately 0.25 ml/min with a Holter model 911 peristaltic pump (Extracorporeal Medical Specialties, Inc., King of Prussia, PA). Blood samples were obtained periodically with the use of a three-way stopcock. The bladder was also catheterized for continuous urine collection. The animal was restrained in a specially-designed chair for a 4-hour period so that blood and urine samples could be obtained. Following this procedure, the animal was returned to its cage and observed daily. Blood samples were obtained on days 1, 2, 3, 7, and 28 after treatment or until autopsy.

The three saline-treated monkeys and three of the monkeys receiving intravascular or intrauterine quinacrine were autopsied 24 hours after injection. Three other monkeys receiving intrauterine quinacrine were autopsied at 7 days and three others at 28 days after treatment.

#### QUINACRINE TEST MATERIAL.

Quinacrine hydrochloride was obtained from Sigma Chemical Company, St. Louis, MO, and the lot number of the material used was 7-3-2. We also obtained a standard preparation from the United States Pharmacopeia. Two separate stock solutions were prepared for each standard (USP and Sigma), and a standard solution of various dilutions was prepared from each stock solution. The excitation and emission spectra for both USP and Sigma standards (1000 ng) are shown in Figure 1. The two curves show maximum excitation and emission wavelengths equivalent to each



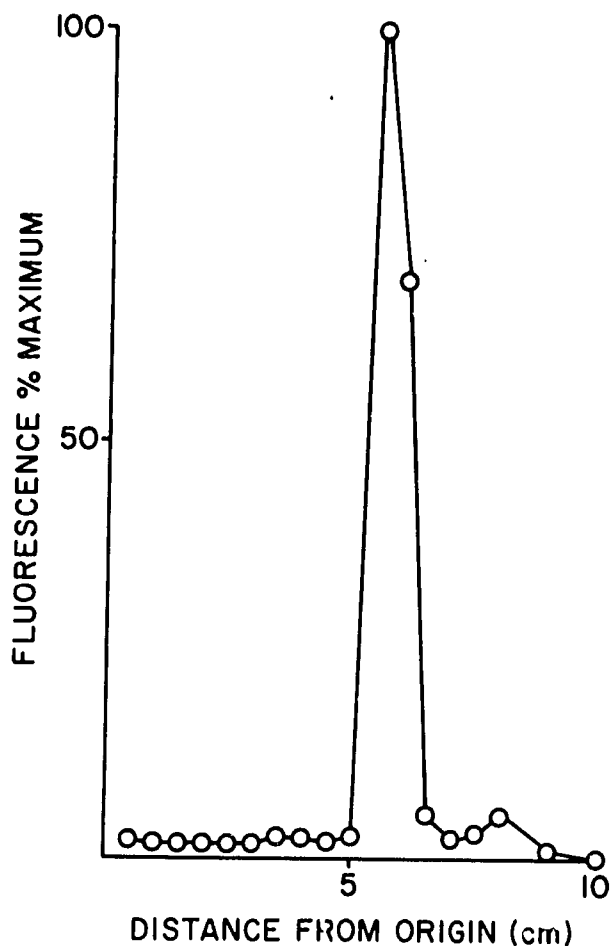
**Figure 1**  
Emission and excitation fluorescence spectra of Sigma (○—○) and USP (▲—▲) standards. Emission spectra were scanned with an excitation wavelength of 285 nm. The excitation spectra were scanned with an emission wavelength of 500 nm.

other and in agreement with reported values. Mass spectra for both standards were found to be identical by the Mass Spectrometry Center, The Johns Hopkins University.

Thin-layer chromatography of these standards was performed on silica gel plates. Both Sigma and USP standards were spotted (approximately 1  $\mu$ g) and chromatographed in 30%  $\text{NH}_4\text{OH}$ : MeOH (1.5:100).<sup>6</sup> Both quinacrine samples ran with the same  $R_f$  value and could be visualized as a yellow spot. Under an ultraviolet lamp, two other prominent spots could be visualized for both preparations. In one experiment, 5  $\mu$ g of Sigma quinacrine was chromatographed, and 0.5-cm sections from the origin to the front were scraped into test tubes and eluted with solvent. The tubes were centrifuged, and supernatant fractions were dried under nitrogen. Lactic acid solution was added, and each tube was assayed for fluorescence at an excitation wavelength of 285 nm and an

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**Figure 2**  
Relative fluorescence of the quinacrine standard as detected in eluted samples of 0.5-cm sections scraped from a chromatographic plate following development in  $\text{NH}_4\text{OH}:\text{MeOH}$  (1.5:100).

emission wavelength of 500 nm. In the peak corresponding to the quinacrine standard (Fig. 2), 98.5% of the total fluorescence in the chromatogram was found.

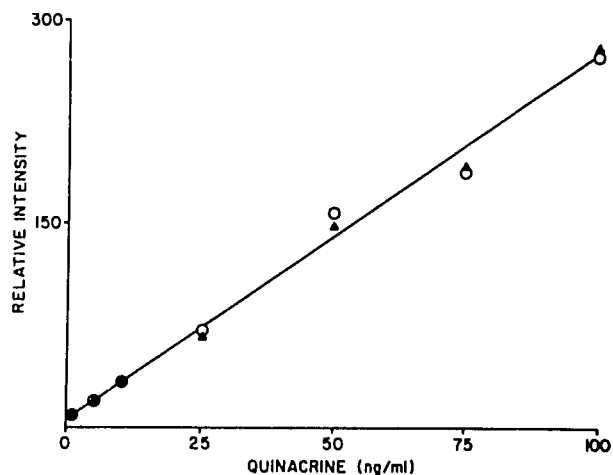
#### QUINACRINE ASSAY

Quinacrine was quantified by a fluorometric method based upon the procedure by Brodie and Udenfriend.<sup>7</sup> Blood was collected in 5-ml tubes containing 10 mg potassium oxalate and centrifuged at 2000 RPM for 10 minutes at 4° C, and plasma was separated. We added 0.3 ml 0.2 M  $\text{Na}_2\text{HPC}_4$  to 1 ml plasma and 3 ml ethylene dichloride. The solvent extract was then shaken with 0.1 ml distilled water and 0.9 ml 85% lactic acid for 15 minutes and centrifuged. The aqueous layer was transferred to a quartz cuvette and read fluorometrically in an Aminco-Bowman spectro-

photofluorometer (American Instrument Company, Silver Spring, MD) at an excitation wavelength of 285 nm and an emission wavelength of 500 nm. We then interpolated the fluorometric readings from a quinacrine standard curve to obtain quinacrine concentrations. Weighed tissues were prepared by homogenization with a Polytron PT 10-35 (Brinkmann, Westbury, NY) equipped with a PT 105T probe at 4° C in a minimum amount of distilled water. The concentration of homogenized tissue was diluted with distilled water to bring the final concentration to 100 mg tissue/ml. For urine and tissues, the ethylene dichloride layer was subjected to an additional wash with 2.5 N NaOH prior to addition of lactic acid. Background fluorescence was detected in body fluid and tissues of control monkeys, and these levels were used as the appropriate blank, which was subtracted from those values obtained from treated animals.

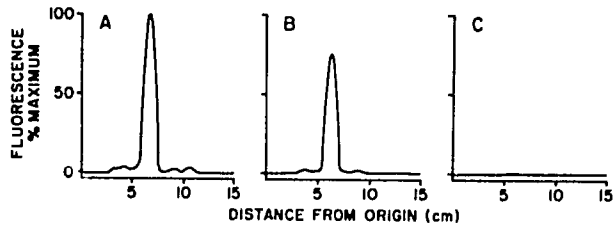
Standard solutions of both Sigma and USP quinacrine were prepared in 85% lactic acid solution. Figure 3 shows the relative fluorescence intensity of various concentrations of quinacrine. There was a linear relationship between quinacrine concentration and relative fluorescence intensity over a long range of concentrations. The linearity of the curve extends to at least 1000  $\mu\text{g}/\text{ml}$ . The higher concentrations are not shown in the figure. The sensitivity of the assay was 2.5 ng. Known concentrations of quinacrine added to plasma, which were then assayed, yielded a recovery of > 85%.

A chromatographic analysis of plasma from a quinacrine-treated monkey was also made (Fig.



**Figure 3**  
Standard curve of both Sigma (○) and USP (△) quinacrine samples.

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**Figure 4**  
Chromatography of fluorescent material from (A) quinacrine standard; (B) extracted plasma of quinacrine-treated monkey plasma; (C) extracted plasma from a nontreated monkey.

4). Following extraction of plasma, thin-layer chromatography was performed as described. The developed chromatogram was scanned for fluorescence with the use of an Aminco-Bowman Thin-Film scanner (American Instrument Company, J4-8247) and recorded on a Harvard apparatus 10-speed chart mover. One major peak corresponding to that of authentic quinacrine was seen. Thus, it appears that the substance measured by the fluorescence assay is indeed quinacrine and not a metabolic product.

## RESULTS

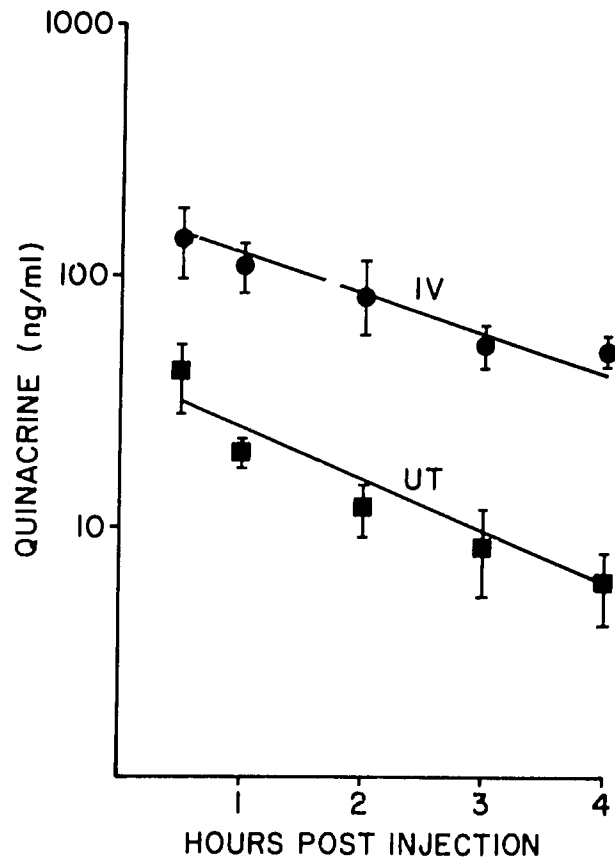
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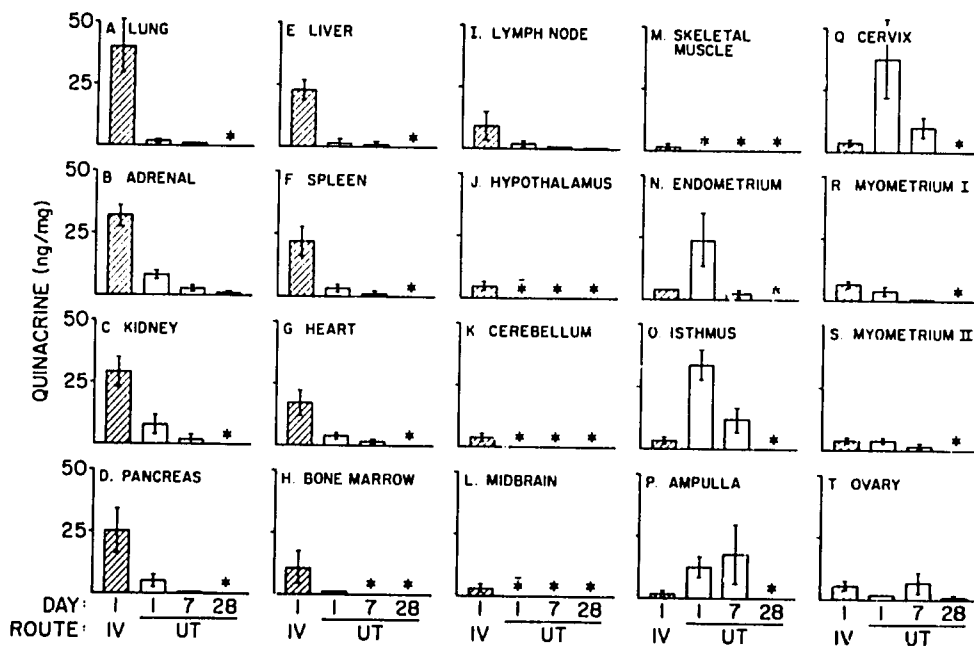
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**Figure 6**  
Tissue concentrations of quinacrine after either IV administration (hatched bars) or UT administration (solid bars) of 30 mg quinacrine. Vertical lines indicate  $\pm$  SEM ( $n = 3$ ). For UT injection concentrations are shown at 1, 7, and 28 days after injection. The asterisk indicates that levels were not significantly different from the background fluorescence of the control animals given injections of saline. The asterisk with the bar above it indicates that levels were less than 0.5 ng/mg but were significantly higher ( $P < 0.01$ ) than the corresponding control levels.

of quinacrine ( $8.4 \pm 2.9$  ng/mg) 1 day after intrauterine injection of the drug. Quinacrine was not detectable, or near the limit of detection, in all tissues at 28 days following intrauterine injection.

## DISCUSSION

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In summary, the study described illustrates that intrauterine injection of quinacrine results in a rapid entry into the circulatory system, followed by rapid and tenacious uptake by body tissues. Quinacrine can be detected in the reproductive tissues and some of the nonreproductive tissues 7 days after intrauterine injection. However, by 1 month all tissues have undetectable levels of quinacrine or levels near the limit of detection. In our accompanying report,<sup>5</sup> we demonstrate the lack of pathologic changes in any of the nonreproductive organs examined at either 1, 7, or 28 days, despite the uptake and retention of quinacrine.

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# 6

## Pharmacology of Quinacrine Hydrochloride With Emphasis on its Use as a Tubal Occluding Agent

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An intensified program for identification of synthetic antimalarial drugs was conducted in Germany during the 1920s. Quinacrine was one of 12,000 synthetic chemicals found to be effective in treating malaria and was widely used prior to World War II. Quinine remained the chief antimalarial drug, however, until the Japanese invasion of the South Pacific cut supplies and expanded US production of quinacrine became imperative, increasing from a prewar level of 1200 lb/year to almost one ton/day.<sup>9</sup> Much of the pharmacologic knowledge about quinacrine was acquired from observations and studies made during that time.<sup>16</sup>

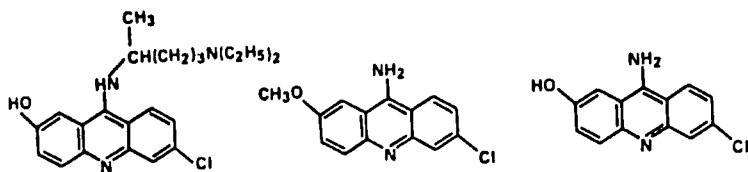
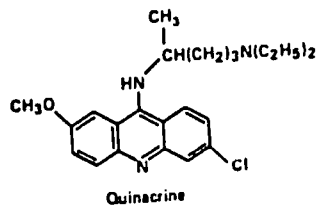
Quinacrine is a bright yellow crystalline powder. The structure of quinacrine hydrochloride is shown in Figure 6-1. The metabolic fate of quinacrine is incompletely understood. In humans, urinary products include the unmetabolized form as well as the O-demethylated derivative, the side-chain-cleaved amine derivative, and an O-demethylated side-chain-cleaved amine.<sup>16</sup> The pharmacokinetics of quinacrine has been studied primarily in regard to ingestion of the drug.<sup>15</sup> Because the mode of administration may alter the absorption of a drug and its distribution, we investigated the pharmacokinetic properties of quinacrine following intrauterine injection. The data are presented later in this chapter.

Besides its use in the management of malaria, quinacrine has been used to

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These studies were supported by contracts from the International Fertility Research Program (IFRP) #601 and #603 and AID DSPE/C 0054.

*Pharmacology of Quinacrine Hydrochloride as a Tubal Occluding Agent / 61*



**FIG. 6-1.** Structure of quinacrine and urinary metabolites. Metabolites (left to right) are O-demethylated, side-chain cleaved, and O-demethylated side-chain cleaved derivative (based on Steck EA: Chemotherapy of Protozoan Disease, vol III, pp 23-172. Walter Reed Army Institute of Research, US Government Printing Office, 1982)

treat tapeworm infection, cutaneous leishmaniasis, and lupus erythematosus and to control neoplastic effusions in the pleural and peritoneal cavities.

Numerous toxic effects of quinacrine have been reported. some of which are listed below:

**Skin:**

- Lemon-yellow pigmentation
- Dermatitis
- Atrophy of sebaceous and sweat glands

**Gastrointestinal tract:**

- Nausea
- Vomiting
- Abdominal cramps
- Diarrhea

**Cardiovascular system:**

- Lowering of blood pressure
- Decreased cardiac output
- Bradycardia

**Central nervous system:**

- Motor acceleration
- Epileptiform convulsions
- Delusions
- Hallucinations

**Other:**

- Fever
- Headache

The occurrence of these symptoms depends on dose, length of treatment, mode of delivery, and patient susceptibility. For a more detailed discussion

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of side-effects. the reader is referred to Goodman and Gilman<sup>9</sup> and an extensive review by Findlay.<sup>7</sup> The toxic effects of quinacrine as a tubal occluding agent are discussed later in this chapter.

## QUINACRINE ADMINISTRATION TO MONKEYS

### INTRAVASCULAR VERSUS INTRAUTERINE INJECTIONS

Quinacrine was administered to cynomolgus monkeys by means of intravascular or intrauterine injections.<sup>4,5</sup> The cynomolgus monkey was chosen for these studies because its reproductive anatomy is similar to that of the human, and its cervical canal, although more convoluted than that of a woman, can be penetrated by a blunt-end needle.<sup>13</sup> Thus, quinacrine can be deposited directly into the uterine cavity. A solution of quinacrine was used, so that "worst case" exposure to quinacrine could be evaluated. The dose given (30 mg in 1 ml) is approximately two times the dose, on a body weight basis, that would currently be placed in a woman's uterus at any one time (250 mg is the current human dose).

Following intravascular injection of a 1-ml solution of 30 mg quinacrine, 149 ± 42 ng/ml (n = 3) was observed in the plasma at 0.5 hours (Fig. 6-2). The level declined during the next 3½ hours, with an estimated half-life of 2 hours. Less than 1% of the administered drug was found in the urine over

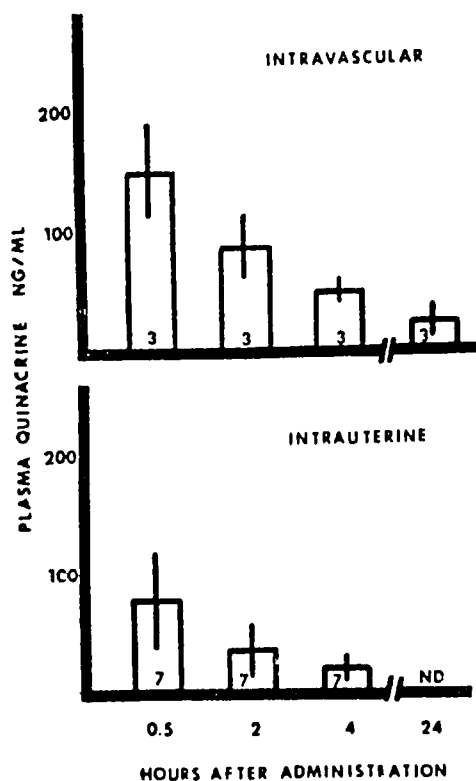


FIG. 6-2. Plasma quinacrine concentrations at various times after intravascular or intrauterine administration of 30 mg quinacrine hydrochloride solution to nonpregnant monkeys. (ND, not detectable; number in bars represents number of observations; Vertical line is ± SEM)

the first 4-hour interval. At 24 hours, the plasma level was  $23.5 \pm 8.5$  ng/ml. Tissues obtained following necropsy at this time were found to have high concentrations of quinacrine. The tissue-plasma ratios are indicated in Table 6-1.

Following intrauterine injections of the 30 mg quinacrine solution, plasma quinacrine levels were  $77.1 \pm 37.0$  ng/ml ( $n = 7$ ) and declined at a rate similar to that in the group receiving intravascular injections. Thus, quinacrine rapidly enters the circulation when deposited into the uterine cavity. Twenty-four hours after administration, quinacrine was found not only in the uterus and other reproductive organs but also in all organs listed in Table 6-1, except cerebellum and skeletal muscle. It is significant that high concentrations of quinacrine could be detected in the isthmus and ampulla of the oviduct, indicating the possibility of spill into the peritoneal cavity. Many of the tissues from animals that received intrauterine quinacrine and were necropsied 1 week later also contained significant amounts of quinacrine; however, these concentrations were, in general, significantly less than those concentrations found at 24 hours. By 28 days, all tissues had undetectable levels of quinacrine or levels near the limit of detection.

Following both intravascular and intrauterine injection of quinacrine, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and lactic dehydrogenase showed significant elevations compared with these measurements in control monkeys receiving 1 ml physiologic saline by way of the intrauterine route. The elevations were moderate and transient. re-

TABLE 6-1. Tissue-Plasma Ratio of Quinacrine 24 Hours After Intravascular Administration of 30 mg Quinacrine to Cynomolgus Monkeys

TISSUE	RATIO
Lung	1676:1
Adrenal	1345:1
Kidney	1211:1
Pancreas	1041:1
Liver	994:1
Spleen	904:1
Heart	723:1
Bone marrow	445:1
Lymph node	378:1
Myometrium	256:1
Ovary	219:1
Hypothalamus	200:1
Endometrium	184:1
Cerebellum	171:1
Uterine cervix	158:1
Midbrain	138:1
Isthmus (oviduct)	131:1
Ampulla (oviduct)	94:1
Skeletal muscle	68:1

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turning to preinjection levels within 48 hours. Histologic examination of tissues listed in Table 6-1 revealed no pathology, except for the reproductive tissue in those animals receiving intrauterine injections of quinacrine.<sup>7</sup>

Following intravascular injection of 30 mg quinacrine, two of three monkeys vomited or retched within 3 minutes of the injection. While nausea has been reported following oral administration of quinacrine in humans, this has implied a direct gastrointestinal effect. Since in these monkeys, the symptoms were seen following intravascular administration, this effect appears to be indirect, possibly acting through the central nervous system. No other toxic effects were observed in these animals that could be attributed to quinacrine administration.

#### INTRAUTERINE INJECTIONS DURING PREGNANCY

Intrauterine injections of quinacrine were also administered to pregnant monkeys between pregnancy days 34 and 46. Administration of 30 mg quinacrine resulted in plasma concentrations that were at least comparable to those in nonpregnant monkeys receiving intravascular administration of the same dose (Fig. 6-3). This may reflect increased vascularity of the uterus during pregnancy, and more rapid absorption of the drug. Following intrauterine administration of 3 mg of the drug, plasma concentrations were approximately 10-fold less than those in monkeys receiving the 30-mg dose.

One pregnant monkey was found dead in its cage 48 hours after intrauterine administration of 30 mg quinacrine. Autopsy did not reveal the cause of death. While there was no direct or statistical evidence that quinacrine was the cause of death, we were concerned, since we have never had a death in a pregnant monkey following intrauterine injections in our studies involving other drugs.<sup>2,3</sup> Also, the plasma quinacrine concentration at 30 minutes was 445 ng/ml, which was higher than any other 30-minute concentration, including concentrations following intravascular injections in nonpregnant monkeys. It is also significant that we were not able to obtain any urine from this animal, although its bladder was catheterized for the 4-hour postinjection collection period. Thus, impaired clearance of the drug may account in part for high plasma levels.

The effect of quinacrine administration on the fetuses of these pregnant monkeys is discussed in Chapter 7.

#### INTRAPERITONEAL ADMINISTRATION

A danger of intrauterine administration of drugs is either spill of the drug through the oviducts into the peritoneal cavity or perforation of the uterus with direct application of the drug into this cavity. To determine toxicity in case such an event occurs, the following study was performed.

Under ketamine anesthesia, a needle was inserted through the cervical canal and the uterus was punctured. A 1-ml solution containing 30 mg quinacrine was then applied directly into the peritoneal cavity. Three monkeys were subsequently observed for 3 days and showed no abnormal behavior. Laparotomy performed on day 3 revealed slight adhesion of one tube to the uterus in one monkey, but no other effects were observed.

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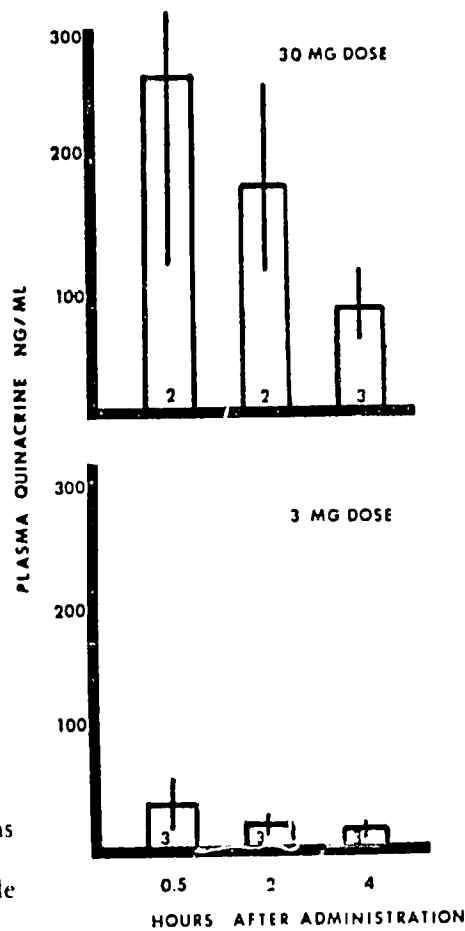


FIG. 6-3. Plasma quinacrine concentrations following intrauterine administration of either 30 or 3 mg quinacrine hydrochloride solution to pregnant monkeys.

A report by Chandra and Malaviya indicated that when high intraperitoneal doses of quinacrine suspension were administered to rhesus monkeys, death occurred within 10 minutes; lower doses caused hyperactivity. The same doses, placed in the uterus of those animals, had no effect.<sup>1</sup>

These studies prompted us to test higher doses of quinacrine in the peritoneal cavity of cynomolgus monkeys. Since pellets are the current form in which the drug is used in women, we elected to use pellets in this study. Three monkeys were studied at one time. Each was immobilized with ketamine and placed under halothane anesthesia. Each received a midabdominal incision and a pellet applicator (supplied by IFRP) was inserted through the incision and pellets were released into the peritoneal cavity. One applicator contained seven pellets (250 mg total dose), one contained 3½ pellets (125 mg), and one contained no pellets (sham control). The animals were then allowed to recover from anesthesia. Periodically, blood was taken from the femoral vein for quinacrine determination. This experimental procedure was repeated on different monkeys on two other occasions, so that each treatment group consisted of three animals.

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Of the three monkeys receiving 250 mg quinacrine, two died approximately 2 hours after administration. The third had a seizure, beginning at 2 hours after treatment, which lasted for approximately 1 hour. This animal recovered and appeared to be healthy thereafter. The three monkeys receiving 125 mg quinacrine all survived. One demonstrated slight tremors of the limbs while recovering from anesthesia. All animals receiving intraperitoneal quinacrine pellets, regardless of dose, demonstrated crouching behavior that may have been indicative of abdominal pain. No hyperactivity was observed. The sham monkeys showed more activity than did the treated animals during a 4-hour observation period following treatment.

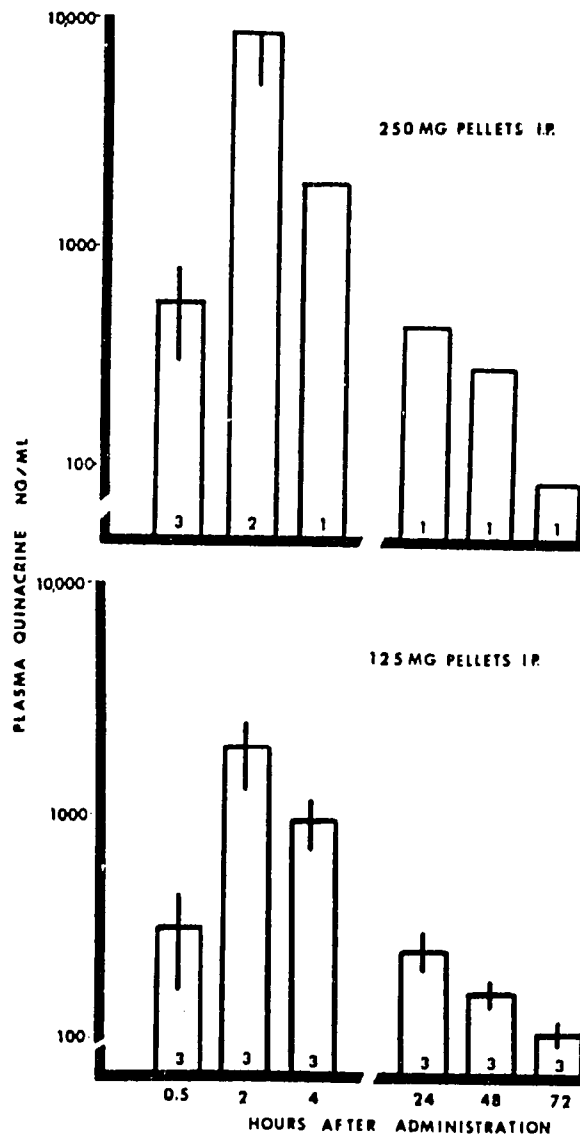


FIG. 6-4. Plasma quinacrine concentration following intraperitoneal administration of 250 or 125 mg quinacrine hydrochloride pellets to nonpregnant monkeys. Note that ordinate is logarithmic.

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**FIG. 6-5.** Peritoneal-inflammation and adhesions in monkey 3 days following intraperitoneal administration of 125 mg quinacrine pellets.

Plasma quinacrine levels were very high at 30 minutes after treatment (Fig. 6-4). These concentrations increased and we observed the highest levels at 2 hours, the time when the monkeys treated with the high dose died or had convulsions. A blood sample, obtained from one monkey at the time of its death, revealed a quinacrine level of 11,700 ng/ml. In the surviving monkeys treated with quinacrine, plasma concentrations of the drug remained elevated for 3 days after treatment, although they demonstrated a declining trend (see Fig. 6-4).

Surviving animals were autopsied 3 days after treatment. No pellet material could be identified in the peritoneal cavities. One monkey receiving 250 mg quinacrine and another receiving 125 mg quinacrine had an area of inflammation and adhesion directly below the area of incision, corresponding to the area of drug application (Fig. 6-5). Such a reaction could be a source of pain.

## COMMENTS

The studies with cynomolgus monkeys indicated three effects that cause concern about the use of quinacrine as a tubal occlusion agent: (1) abdominal pain, (2) central nervous system (CNS) stimulation, and (3) death following administration of the drug.

Some indication of the effect of applying quinacrine directly to the intraperitoneal cavity of humans can be acquired from studies in which quinacrine

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was used in the control of neoplastic effusions. In one study, 400-mg doses placed directly into the peritoneal cavity caused abdominal pain. The pain is thought to be due to an inflammatory response of the peritoneum to the drug.<sup>8</sup> In another study, instillation of less than 1200 mg resulted in minor toxic symptoms, such as pain or nausea.<sup>11</sup> As the dose exceeded 1200 mg, however, more severe toxic effects were reported, including transient CNS symptoms, such as hallucinatory episodes and "other cerebral difficulties."

The convulsions that occurred in one monkey in our study are of particular significance, because CNS effects were previously reported in people receiving oral quinacrine for treatment of malaria and in women receiving intrauterine quinacrine as a tubal occlusive agent.<sup>17</sup> CNS excitation following treatment of malaria with quinacrine had been noticed and characterized variously as psychic stimulation, motor acceleration, restlessness, insomnia, increased work capacity, and epileptiform convulsions.<sup>9</sup>

A "toxic psychosis" seems to be of two distinct types. In one, there is a sudden increase in motor activity, auditory and visual hallucinations, and delusions. The other type is characterized by gradual clouding of the sensorium, disorientation, amnesia for recent events, and confabulation.<sup>9</sup> That quinacrine, and not the disease, is the source of the symptoms has been demonstrated by the following studies using healthy subjects. In one study, 31 people who had never suffered from malaria were given increasing doses of quinacrine (from 100 mg to 1200 mg daily, orally).<sup>10</sup> Twenty-four reported CNS disturbances of varying degrees. Severe psychic disturbances occurred in 12 subjects, 3 of whom developed frank psychoses, with hallucinations and delusions. Control subjects receiving a placebo reported only mild symptoms, such as insomnia and tension.

In another study, five subjects were given oral doses of quinacrine, ranging from 200 to 1200 mg/day, until plasma levels of the drug exceeded 100 ng/ml.<sup>6</sup> Electroencephalograms were obtained from bipolar fronto-occipital tracings. Frequency of brain waves accelerated when the plasma level of drug exceeded 30 ng/ml. Psychological symptoms, from restlessness to acute panic reaction, occurred concurrent with acceleration in the electroencephalographic patterns.

Following instillation of quinacrine suspension into the uterus, "CNS excitation" was reported in 2% of the patients. However, the symptom has not been reported when pellets are administered.<sup>7</sup>

To date, more than 1000 women have received intrauterine administration of quinacrine, either in solutions or pellets, with no drug-related deaths reported. The studies in cynomolgus monkeys described above, and those in rhesus monkeys, emphasize that the pellet dose that caused death in the cynomolgus monkey was, on a body weight basis, 17 times the dose normally given to women, and the drug was placed directly into the peritoneal cavity.

A 250-mg dose of intraperitoneal quinacrine resulted in the deaths of two of the three monkeys. Assuming an approximate weight of 3 kg for a cynomolgus monkey, this would be about 80 mg/kg. In rats, the maximal tolerable dose is reported to be 250 mg/kg following intraperitoneal administration.<sup>12</sup> A 40-mg dose of quinacrine injected into the rat uterus resulted in a 32% death rate.<sup>11</sup> The mechanism by which death occurs in response to quinacrine is not known, but it appears to be related to the circulating levels of the drug.

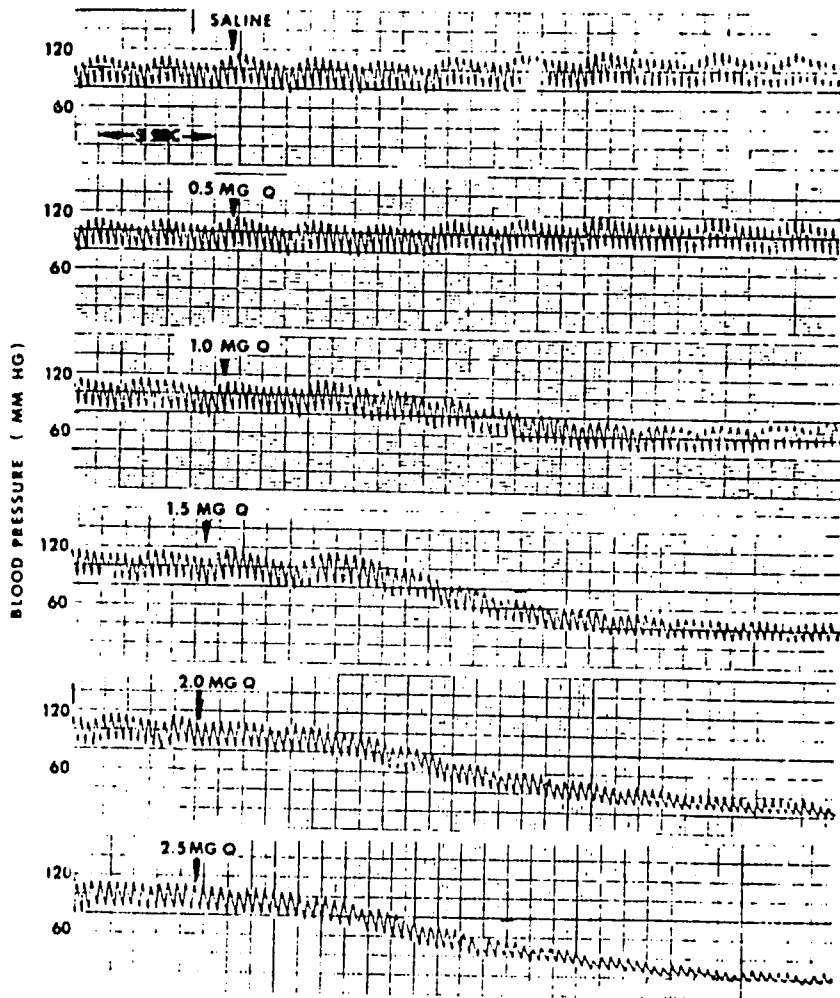


FIG. 6-6. Blood pressure in rat in response to increasing doses of intravascular quinacrine. Injection was made into jugular vein, and pressure recording was made from carotid artery by way of pressure transducer attached to polygraph. Each vertical division is 20 mm Hg. Recovery to preinjection pressure was allowed to occur before next dose was given. With 2.5 mg quinacrine, there was no recovery and animal died. (Q, quinacrine hydrochloride)

We have done preliminary studies in rats demonstrating that intravascular injections of quinacrine, beginning with 1 mg, will cause a decrease in blood pressure from 115/80 mm Hg (systolic/diastolic) to 70/45 mm Hg (Fig. 6-6). This occurs after a lag period of 6 or 7 seconds and is followed by complete recovery after 25 seconds. With increasing doses, there is a greater depression in blood pressure, with prolonged recovery time. Following a dose of 2.5 mg, however, blood pressure dropped to 20/15 mm. Hg, there was no recovery,

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and the animal died. This experiment was replicated in a second animal with the same outcome.

Clearly, there is some risk in the use of quinacrine as a tubal occlusion agent. The importance of these risks relative to the future use of this drug in fertility regulation will be discussed in Chapter 15.

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## 7

# Teratologic and Mutagenic Studies With Intrauterine Quinacrine Hydrochloride

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## TERATOLOGY

Since 1969, intrauterine quinacrine has been used clinically in various developing countries as a means of effecting tubal closure. The advantage of this procedure is that it is a nonsurgical method of accomplishing sterilization. Because of the possibility that some patients who receive quinacrine might be pregnant at the time of introduction of the drug into the uterus, it is necessary to consider the fetal outcome of such exposure. The following studies were directed toward determining the embryopathic effect and maternal toxicity of an intrauterine injection of quinacrine on pregnant monkeys and rats.

## MONKEYS

### Methods

Female cynomolgus monkeys were followed for cyclic bleeding and mated during a limited period (3 days) at Hazelton Texas Primate Center, Alice, Texas. If palpation on two occasions revealed that pregnancy had occurred, the monkeys were shipped to Baltimore at approximately 30 days of pregnancy and were placed in individual cages. Pregnancy was confirmed by our personnel by palpation, and treatment was instituted prior to 50 days of pregnancy. We gave a 1-ml intrauterine injection of either 0.9% saline, 3 mg

These studies were supported by contracts from the International Fertility Research Program #601 and #603.

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quinacrine HCl, or 30 mg quinacrine HCl (Quinacrine HCl; No. Q 0250FD, F&D lot No. 7-3-2, Sigma Chemical Co., St. Louis, Missouri). While the animal was under ketamine anesthesia, injections were made using a 12-cm 18-gauge blunt-end stainless steel needle inserted through the cervical canal.

Prior to treatment, blood was obtained for blood chemistry and hematology. Blood chemistry determinations included serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT),  $\gamma$ -glutamyl transpeptidase, alkaline phosphatase, lactic dehydrogenase (LDH), cholesterol, triglyceride, total protein, albumin, globulin, blood urea nitrogen, creatinine, direct and total bilirubin, uric acid, glucose, iron, magnesium, sodium, potassium, calcium, phosphorus, and chloride. Hematology analyses included leukocytes, neutrophils, lymphocytes, erythrocytes, hematocrit, and hemoglobin. Food was withdrawn 24 hours prior to treatment. Water was available at all times. Following the treatment, monkeys were returned to their cages and observed daily. Additional blood samples were obtained at 7 and 28 days after treatment for blood chemistry and hematology analyses. On day 28 after treatment, the monkey was repalpated to establish the progress of pregnancy. If pregnancy continued, at approximately 120 days' gestation, the fetus was removed surgically and examined.

### Results and Discussion

All monkeys received from the Texas Primate Center appeared in good health, although they appeared thin. Also, after arrival they did not eat for several days, possibly due to the new environment. Pregnancy in all animals was confirmed by palpation. Blood chemistry values prior to treatment were compared with those values obtained from nonpregnant cynomolgus monkeys (obtained from Primate Imports, Port Washington, NY). It was noticed that some values were significantly different. For example, the pregnant group of monkeys had higher SGOT ( $113.7 \pm 20.7$  vs.  $50.6 \pm 3.7$  IU/liter,  $p < 0.01$ ), LDH ( $857 \pm 136$  vs.  $404 \pm 34$  IU/liter,  $p < 0.001$ ), and  $\gamma$ -glutamyl transferase ( $42.8 \pm 4.7$  vs.  $26.1 \pm 2.0$  IU/liter,  $p < 0.001$ ), possibly resulting from the stress of the shipping procedure or from pregnancy.

Following injection of intrauterine physiologic saline, all three animals lost their pregnancies either by spontaneous abortion (*i.e.*, passed fetal tissue; No. 191N and No. 171N) or resorption of the fetus (No. 158N), as indicated by failure of the uterus to grow normally when palpated on day 28 after injection (Table 7-1). This was unexpected, because previous studies indicated that intrauterine physiologic saline injection resulted in a high percentage of normal pregnancies in cynomolgus monkeys (9/13, 70%).<sup>10,11</sup>

All monkeys receiving 3 mg quinacrine resorbed their pregnancies, as indicated by palpation 28 days after injection (see Table 7-1). Following an injection of 30 mg quinacrine one monkey (No. 161N) was found dead in the cage 2 days later. An autopsy disclosed focal peritoneal necrosis and peritonitis, which possibly could have been the result of quinacrine injection but were not believed to be extensive enough to cause death. The possibility that the uterus was perforated and injection made directly into the peritoneal cavity was considered. Because of this possibility, a separate study was instituted to examine the effect of intraperitoneal injection of quinacrine (see Chapter 6). The fetus and placenta showed signs of autolysis indicating that

TABLE 7-1. Summary of Treatment of Each Monkey and Pregnancy Outcome

ANIMAL NUMBER	INJECTION DOSE	PREGNANCY DAY OF INJECTION	OBSERVATION	FOLLOW-UP
			AT 28 DAYS POST INJECTION	
191N	Control	40	Uterus enlarged but hard	Passed tissue 30 days after injection
171N	Control	35	Aborted on day 27	High leukocyte count before and after abortion
158N	Control	45	Not pregnant	
170N	3 mg QHCl	39	Not pregnant	
200N	3 mg QHCl	36	Not pregnant	
180N	3 mg QHCl	46	Not pregnant	
176N	30 mg QHCl	42	Uterus growing but small	Deformed fetus at pregnancy day 115 obtained by hysterectomy
173N	30 mg QHCl	44	Not pregnant	
161N	30 mg QHCl	34	Day 2—mother dead	

QHCl, quinacrine hydrochloride

death of the fetus preceded that of the mother. This was most likely a result of the quinacrine injection.

When palpated on day 28 after injection, the fetus of the third monkey receiving 30 mg quinacrine (No. 176N) was "growing but smaller than expected" as described by the primate technician. The fetus was removed on pregnancy day 115 and examined. It was found to have multiple congenital deformities, including craniorhachischisis, scoliosis, epitheliogenesis imperfecta, atelectasis, ankylosis of the left tarsus, multiple skeletal abnormalities in the digits of paws and feet, and patent foramen ovale. It should be noted that the neural tube completes its closure by day 29 of development in monkeys and since the drug was administered on day 42, it is very unlikely that it could have been the causative factor. Defects such as ankylosis can occur at any stage of development; however, lower limb defects are often associated with neural tube defects in humans and thus there is a reasonable chance that the other defects observed were part of a spontaneous multiple congenital defect syndrome. The incidence of spontaneously occurring grossly observable abnormalities in newborn rhesus monkeys is approximately 1%.<sup>28</sup> Comparable data for cynomolgus monkeys are not available.

Some hematologic changes occurred following treatment. Since there were few animals in any one group, all pretreatment data were pooled, regardless of the treatment group to which the monkeys were assigned. This value was compared with all other data points by a t-test. The only differences that could be attributed to quinacrine were a decline in erythrocytes on day 7 for animals receiving 3 mg ( $4120 \pm 100 \times 10^3$  cells/cu mm) or 30 mg ( $4575 \pm 195 \times 10^3$  cells/cu mm) compared with the initial value ( $5688 \pm 337 \times 10^3$  cells/cu mm). Compared with pretreatment levels, there was a significantly lower hematocrit ( $22.8 \pm 1.2$  vs.  $36.8 \pm 1.8\%$ ,  $p < 0.001$ ) and hemoglobin concentration ( $6.4 \pm 0.7$  vs.  $10.2 \pm 0.5$  gm/dl,  $p < 0.01$ ) in monkeys 7 days

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after treatment with 3 mg quinacrine, but levels were normal 28 days after treatment. None of these effects was dose related.

Similar analyses were made with blood chemistries. Total protein was significantly less in monkeys treated with 3 mg quinacrine, compared with pre-treatment concentrations ( $6.4 \pm 0.2$  vs.  $7.3 \pm 0.1$  gm/dl,  $p < 0.01$ ). By day 28, this value had returned to normal.

There was a slight, but significant ( $p < 0.05$ ) elevation in SGPT at day 7 in monkeys receiving either dose level of quinacrine.

**RATS**

**Methods**

Virgin female Sprague-Dawley rats were bred by Charles River Breeding Labs, Wilmington, Massachusetts, or Harlan-Sprague Dawley of Indianapolis, Indiana. In each of the seven treatment groups, approximately 70% of the rats were obtained from Harlan-Sprague Dawley. The day on which a sperm-positive specimen was obtained was designated as day 0 of gestation. The animals were housed in a constant temperature and humidity room and were fed Charles River RMH-1000 formula. Water was provided *ad libitum* from an automatic system. Cages were lined with "Sani-Chips" bedding. The pregnant dams were weighed on arrival at our facility (day 6 of gestation), on the day of treatment, on gestation day 15, and on the day of necropsy (day 19).

Quinacrine hydrochloride (QHCl) was obtained from the same lot used in the study with monkeys. Quinacrine was dissolved in 0.2M sodium phosphate buffer, pH 7.4. Because of limited solubility, the highest dose given in the maximum injectable 0.1-ml volume was 4.0 mg. The low dose was 0.4 mg in a 0.1-ml volume.

Laparotomy was performed with the rat under ether anesthesia. The number of visible implantations in each horn was recorded. Intrauterine injections were made with a Hamilton syringe (0.1-ml capacity). In all treatment groups, one horn of each animal served as a control, receiving only the buffer vehicle. Those rats treated early in gestation (day 8) received a single 0.1-ml injection of quinacrine at the cervical end of the uterine horn. The contralateral horn received a single 0.1-ml injection of the buffer. Those rats treated later in gestation (day 12) received the drug in two 0.05-ml injections, one at the cervical end of the horn and the other at the ovarian end. The contralateral horn was treated similarly with buffer. The incision was sutured and the skin was closed with "autoclave." A buffer-sham treatment group had one horn treated with the buffer (0.1 ml) and the contralateral horn subjected to sham injections (insertion of the needle only).

To provide a positive control treatment group, pregnant rats were treated with 4-nitroquinoline-N-oxide (4NQNO; K&K Labs, Plainview, NY) at a dosage of 50 mg/kg by intrauterine injection on day 12 of gestation. The contralateral horn received 0.1 ml of the vehicle dimethyl sulfoxide (DMSO; Fisher Scientific Co, Silver Spring, MD).

All animals were killed by decapitation on day 19 of gestation. The fetuses were delivered by laparotomy/hysterotomy, measured (crown to rump), weighed, and examined for external anomalies. Approximately one of every three fetuses was fixed in Bouin's fluid (Allen's Modification B-15) for visceral

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examination by Wilson's technique. The remaining fetuses were cleared by glycerin-potassium hydroxide and stained with alizarin red S for skeletal study. Uteri were examined for resorption sites. Ovaries were examined for number of corpora lutea. Each uterine horn with attached ovary was fixed in 10% neutral buffered formalin.

The occurrence of resorptions, fetal death, and skeletal and visceral anomalies in specific treatment groups was examined by  $\chi^2$  analysis using the Yates correction.

**Results and Discussion**

**Resorption.** QHCl caused dose-related resorptions when given as a direct intrauterine injection on either day 8 or day 12 of gestation (Tables 7-2 and 7-3). Because there was an unequal number of implantations in the treated

TABLE 7-2. Teratogenicity Evaluation of Quinacrine Hydrochloride (QHCl) Following Intrauterine Injection on Day 8 in Rats

	TREATMENT*					
	BUFFER	SHAM	BUFFER	QHCl 0.4 MG	BUFFER	QHCl 4 MG
Number of uterine horns evaluated	20	20	20	20	20	20
Implantations						
Total number	126	106	108	114	103	137
Mean ( $\pm$ SE)/horn	6.3 $\pm$ 0.3	5.3 $\pm$ 0.4	5.4 $\pm$ 0.4	5.7 $\pm$ 0.4	5.2 $\pm$ 0.2	6.9 $\pm$ 0.5
	(p < 0.05)				(p < 0.01)	
Resorptions						
Total number	19	14	20	35	19	71
Mean ( $\pm$ SE)/horn	1.0 $\pm$ 0.2	0.7 $\pm$ 0.2	1.0 $\pm$ 1.9	1.8 $\pm$ 0.3	1.0 $\pm$ 0.2	3.55 $\pm$ 0.4
Percent/horn†	15.0 $\pm$ 2.4	12.6 $\pm$ 3.8	18.4 $\pm$ 3.3	31.2 $\pm$ 5.3	18.4 $\pm$ 3.9	51.8 $\pm$ 5.2
			(p < 0.05)		(p < 0.001)	
Viable fetuses						
Total number	107	92	87	78	84	65
Mean ( $\pm$ SE)/horn	5.4 $\pm$ 0.3	4.6 $\pm$ 0.4	4.4 $\pm$ 0.4	3.9 $\pm$ 0.4	4.2 $\pm$ 0.3	3.3 $\pm$ 0.4
Percent/horn‡	85.0 $\pm$ 2.4	87.4 $\pm$ 3.8	80.6 $\pm$ 5.2	68.0 $\pm$ 5.2	81.6 $\pm$ 3.9	47.6 $\pm$ 5.2
					(p < 0.001)	
Malformations						
Total number of malformed fetuses	6	5	8	6	1	2
Percent malformed/horn§	6.0 $\pm$ 3.3	6.3 $\pm$ 2.9	10.3 $\pm$ 4.5	5.9 $\pm$ 2.8	1.0 $\pm$ 1.0	6.0 $\pm$ 5.0

\*Injections made directly into uteri of anesthetized rats. For each rat, one uterine horn was treated with buffer and the other with buffer plus QHCl. Sham treatment involved insertion of the needle but no injection.

†Percent resorptions = number of resorptions/number of implantations  $\times$  100.

‡Percent viable = number of living/number of implantations  $\times$  100.

§Percent malformed = number of fetuses malformed/number viable  $\times$  100.

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TABLE 7-3. Teratogenicity Evaluation of Quinacrine Hydrochloride (QHCl) Following Intrauterine Injection on Day 12 in Rats

	TREATMENT*							
	BUFFER	SHAM	BUFFER	QHCl 0.4 MG	BUFFER	QHCl 4 MG	DMSO	4NQNO 1.25 MG
Number of uterine horns evaluated	19	19	18	18	19	19	19	19
Implantations								
Total number	127	97	87	120	93	125	97	114
Mean ( $\pm$ SE)/horn	6.7 $\pm$ 0.3	5.1 $\pm$ 0.4 ( $p < 0.01$ )	4.8 $\pm$ 0.4	6.7 $\pm$ 0.3 ( $p < 0.01$ )	4.9 $\pm$ 0.4	6.6 $\pm$ 0.4 ( $p < 0.01$ )	5.1 $\pm$ 0.5	6.0 $\pm$ 0.4
Resorptions								
Total number	18	22	18	34	28	86	48	92
Mean ( $\pm$ SE)/horn	1.0 $\pm$ 0.2	1.2 $\pm$ 0.3	1.0 $\pm$ 0.3	1.9 $\pm$ 0.4	1.5 $\pm$ 0.3	4.5 $\pm$ 0.7 ( $p < 0.001$ )	2.5 $\pm$ 0.5	4.8 $\pm$ 0.3 ( $p < 0.001$ )
Percent/horn†	14.9 $\pm$ 3.3	22.5 $\pm$ 6.0	23.3 $\pm$ 6.0	27.0 $\pm$ 0.5	27.6 $\pm$ 4.4	65.5 $\pm$ 8.3 ( $p < 0.001$ )	45.0 $\pm$ 7.9	85.0 $\pm$ 4.9 ( $p < 0.001$ )
Viable fetuses								
Total number	109	75	69	86	64	39	49	22
Mean ( $\pm$ SE)/horn	5.7 $\pm$ 0.4	3.9 $\pm$ 0.5 ( $p < 0.01$ )	3.8 $\pm$ 0.5	4.8 $\pm$ 0.3	3.4 $\pm$ 0.3	2.1 $\pm$ 0.5 ( $p < 0.05$ )	2.6 $\pm$ 0.4	1.2 $\pm$ 0.4 ( $p < 0.05$ )
Percent/horn‡	85.1 $\pm$ 3.3	77.5 $\pm$ 5.0	76.7 $\pm$ 6.0	73.0 $\pm$ 5.0	71.3 $\pm$ 4.4	34.4 $\pm$ 8.3 ( $p < 0.001$ )	55.0 $\pm$ 7.9	15.0 $\pm$ 4.9 ( $p < 0.001$ )
Malformations								
Total number of malformed fetuses	10	7	4	9	2	1	1	4
Percent malformed/horn§	9.9 $\pm$ 4.1	6.8 $\pm$ 3.3	5.5 $\pm$ 3.0	9.1 $\pm$ 3.3	2.6 $\pm$ 1.8	1.3 $\pm$ 1.3	5.3 $\pm$ 5.3	10.0 $\pm$ 5.8

\*Injections made directly into uteri of anesthetized rats. For each rat, one uterine horn was treated with buffer and the other with buffer plus QHCl or sham; except for 4NQNO, which was dissolved in DMSO.

†Percent resorptions = number of resorptions/number of implantations  $\times$  100.

‡Percent viable = number of living/number of implantations  $\times$  100.

§Percent malformed = number of fetuses malformed/number viable  $\times$  100.

DMSO, dimethyl sulfoxide; 4NQNO, 4-nitroquinoline-N-oxide.

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and control horns in most treatment groups, it was necessary to analyze these data as absolute values and also after normalizing the data by expressing as a percentage of implantations observed at the time of the treatment. A significant effect was observed in the high-dose groups when calculated either way. Susceptibility to QHCl-induced resorption appeared to be somewhat greater in early pregnancy since on day 8, the low dose (0.4 mg) caused a significant effect, whereas on day 12 this dose was ineffective. The magnitude of the resorption effect caused by the high dose of QHCl (4 mg) was similar on day 8 and day 12, taking into account the differences in control (buffer-induced) resorption rates at these gestational ages; the effect attributable to the drug was approximately 34% and 38% for day 8 and day 12 treatments, respectively. Injection of the buffer vehicle had no significant effect, since the resorption rate was not different between buffer and sham-treated (*i.e.*, insertion of needle without delivery of any liquid) groups at either stage of gestation.

Because 4NQNO is known to be a direct-acting mutagen (as is quinacrine), it was used as a positive control.<sup>19</sup> An intrauterine dose of 1.25 mg given on gestation day 12 had a marked resorption effect (see Table 7-3). DMSO was the vehicle used for 4NQNO, and it also appeared to have a significant resorption effect, although not enough to account for the entire effect observed with DMSO and 4NQNO.

**Malformations.** A low rate of malformation, less than 10% of viable fetuses, was observed in control (buffer, DMSO, sham) groups (Tables 7-2 and 7-3). There was no observed increase in this overall malformation rate in any of the groups treated with QHCl or 4NQNO.

In order to be certain that a teratogenic effect of a specific type had not been obscured by grouping all malformed fetuses, we also analyzed the results according to separate classification by type of malformation (skeletal, Table 7-4; visceral, Table 7-5). No evidence of treatment-related malformations emerged from this analysis. Some malformations are not listed in these tabulations because they were observed only in control groups, perhaps because of their greater number of fetuses.

The malformation data are presented in a manner appropriate for evaluating the possible occurrence of multiple malformation syndromes in treatment groups in Table 7-6. This analysis also failed to identify a teratogenic effect of either quinacrine or 4NQNO.

This study has demonstrated that intrauterine administration of solutions of QHCl to pregnant rats during the period of embryogenesis results in a marked and dose-related resorption effect. The potency (*i.e.*, dose required to produce a significant effect) of quinacrine for inducing resorptions appeared to be greater in early embryogenesis, whereas the efficacy of the drug (magnitude of effect at a maximal dose) was similar in early and late embryogenesis. There was no evidence of any malformations associated with quinacrine treatment. All viable fetuses were carefully examined for gross evidence of malformations. Skeletal and visceral examinations were performed on two thirds and one third, respectively. Therefore, the drug appears to have no teratogenic (*i.e.*, malformation-inducing) effect. Likewise, the positive control, 4NQNO, did not have a teratogenic effect, although like quinacrine, it did induce resorptions.

TABLE 7-4. Results of Skeletal Examinations: Specific Malformations

TREATMENT	NUMBER OF VIABLE FETUSES EXAMINED	NO STERNABRAE	INCOMPLETE SKULL BONES	METATARSALS NOT OSSIFIED	METACARPALS NOT OSSIFIED	14 PAIRS OF RIBS	ADACTYLE
Control							
Sham	112	7 (6.3%)*	1 (0.9%)				
Buffer	353	25 (7.1%)	4 (1.1%)			1 (0.9%)	
Total	465	32 (6.9%)	5 (1.1%)	2 (0.6%)	1 (0.3%)	1 (0.3%)	
				2 (0.4%)	1 (0.2%)	2 (0.4%)	
QHCl							
Day 8							
0.4 mg	50	5 (10.0%)	2 (4.0%)				
4.0 mg	44	1 (2.3%)		1 (2.0%)	1 (2.0%)	1 (2.0%)	
Total day 8	94	6 (6.4%)	2 (2.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	
Day 12							
0.4 mg	58	4 (6.9%)	1 (1.7%)	1 (1.7%)			
4.0 mg	26	1 (3.9%)					
Total day 12	84	5 (6.0%)	1 (1.2%)	1 (1.2%)		1 (1.7%)	
Total	178	11 (6.2%)	3 (1.7%)	2 (1.1%)	1 (0.6%)	1 (1.2%)	2 (1.1%)
DMSO control	32	1 (3.1%)	1 (3.1%)	1 (3.1%)			
4NQNO	16	2 (12.5%)	1 (6.3%)	1 (6.3%)	1 (6.3%)		1 (6.3%)

\*Numbers in parentheses are the percentage of number examined with abnormality.

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TABLE 7-5. Results of Visceral Examinations: Specific Malformations

TREATMENT	NUMBER OF VIABLE FETUSES EXAMINED	CLEFT PALATE	CAVITY IN ADRENAL	MISSING ONE KIDNEY, OVARY, AND UTERINE HORN	HYDRO-NEPHROSIS	ANENCEPHALY
Control						
Sham	55		1 (1.8%)		2 (3.6%)	
Buffer	167					
Total	222		1 (0.5%)		2 (0.9%)	
QHCl						
Day 8						
0.4 mg	28				1 (3.6%)	1 (3.6%)*
4.0 mg	21				2 (9.5%)	
Total day 8	49				3 (6.1%)	1 (2.0%)
Day 12						
0.4 mg	28			1 (3.6%)	2 (7.1%)	
4.0 mg	13					
Total day 12	41			1 (2.4%)	2 (4.9%)	
Total	90			1 (1.1%)	5 (5.6%)	1 (1.1%)
DMSO	17					
4NQNO	6	1 (16.7%)				

\*Only fetus from the pregnancy, no corresponding control horn.  
 (%), percent malformed/number examined.

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TABLE 7-6. Results of Skeletal Examinations: All Malformations

TREATMENT	NUMBER OF VIABLE FETUSES EXAMINED	NUMBER OF MALFORMATIONS				
		1 OR MORE	2 OR MORE	3 OR MORE	4 OR MORE	5 OR MORE
Control						
Sham	112	9 (8.0%)*	1 (0.9%)			
Buffer	353	29 (8.2%)	7 (2.0%)	2 (0.6%)	1 (0.3%)	
Total	465	38 (8.2%)	8 (1.7%)	2 (0.4%)	1 (0.2%)	
QHCl						
Day 8						
0.4 mg	50	6 (12.0%)	2 (4.0%)	2 (4.0%)	1 (2.0%)	
4.0 mg	44	1 (2.3%)				
Total day 8	94	7 (7.5%)	2 (2.1%)	2 (2.1%)	1 (1.1%)	
Day 12						
0.4 mg	58	6 (10.3%)	1 (1.7%)			
4.0 mg	26	1 (3.9%)				
Total day 12	84	7 (8.3%)	1 (1.2%)			
Total	178	14 (7.9%)	3 (1.7%)	2 (1.1%)	1 (0.6%)	
DMSO	32	1 (3.1%)	1 (3.1%)	1 (3.1%)	1 (3.1%)	1 (3.1%)
4NQNO	16	3 (18.8%)	1 (6.3%)	1 (6.3%)	1 (6.3%)	

\*Numbers in parentheses are the percentage of number examined with abnormality.

## MUTAGENICITY

### BACTERIA

#### Materials and Methods

The following compounds (obtained from Sigma Chemical Co., St. Louis, MO) were tested in the *Salmonella*/mammalian-microsome mutagenicity assay: acridine, chloroquine diphosphate, primaquine diphosphate, and quinacrine hydrochloride. Five dose levels (1,000 µg, 100µg, 10µg, 1µg, and 0.1µg/plate) were assayed with and without exogenous metabolic activation. Higher doses of chloroquine (10,000µg and 2,500µg/plate) were also tested to achieve the maximum noninhibitory dose. All test compounds except chloroquine diphosphate were dissolved in DMSO (Fisher Scientific, Fair Lawn, NJ). Chloroquine was dissolved in double distilled water and then filter-sterilized using a 0.22µm disposable unit (Millipore Corp, Bedford, MA). All dilutions of test drugs were prepared immediately prior to use. Purified sodium azide (NaN<sub>3</sub>) (Fisher Scientific, Fair Lawn, NJ) and 4NQNO (K&K Labs, Plainview, NY) were used as direct-acting mutagens. Benzo(a)pyrene (BP; Aldrich Chemical Co., Milwaukee, WI) and 2-anthramine (Sigma Chemical Co., St. Louis, MO) were used as positive controls requiring metabolic activation.

The *Salmonella typhimurium* strains used (TA 98, TA 100, TA 1535, TA 1537, and TA 1538) were provided by Dr. Bruce N. Ames, University of California, Berkeley. On receipt of the strains, the characteristic markers of each were checked as outlined by Ames and associates.<sup>4</sup> Overnight cultures

were prepared from frozen ( $-70^{\circ}\text{C}$ ) stocks by incubation with 2.5% Oxoid nutrient broth (KC Biologicals, Inc, Lenexa, Kansas) for 16 to 17 hours at  $37^{\circ}\text{C}$ .

Rat liver S9 was used as a source of exogenous metabolic activation. Livers were obtained aseptically from adult male Sprague-Dawley rats, 5 days after intraperitoneal treatment with Aroclor 1254 (Monsanto Chemical Co, St. Louis, MO) at a dosage of 250 mg/kg. The excised tissue was homogenized at  $4^{\circ}\text{C}$  with three volumes of 0.15M KCl using a Brinkmann Polytron. The homogenate was centrifuged at  $9000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . The supernatant fraction (S9) was stored in a  $-70^{\circ}\text{C}$  Revco freezer. Protein content was determined by a modified Lowry procedure.<sup>22</sup> The cofactor mixture contained per milliliter:  $\text{MgCl}_2$  (8  $\mu\text{mole}$ ), KCl (33  $\mu\text{mole}$ ), glucose-6-phosphate (5  $\mu\text{mole}$ ), NADP (4  $\mu\text{mole}$ ), and sodium phosphate, pH 7.4 (100  $\mu\text{mole}$ ). The S9 was diluted with 0.15M KCl so that when added to the cofactor, the final concentration of S9 protein was 1.0 mg per milliliter of S9-cofactor mix.

The assays were performed as suggested by Ames and associates using Vogel-Bonner Medium E-minimal glucose agar plates.<sup>1</sup> Briefly, 0.1 ml of the overnight broth culture, containing approximately  $10^7$  organisms, was added to 2 ml molten top agar supplemented with 0.5% NaCl, 15 $\mu\text{g}$  histidine, and 22 $\mu\text{g}$  biotin. The appropriate dose of test compound, solvent, or positive control in a 0.1 ml volume was then added. For metabolic activation assays, the molten agar received 0.5 ml of the rat liver S9-cofactor mix. This molten agar overlay was vortex-mixed after each addition and then poured over the base plate. Hardened plates were inverted and incubated at  $37^{\circ}\text{C}$  for 48 to 60 hours. Colonies were counted by direct inspection using a colony counter (Fisher Scientific, Fair Lawn, NJ). Results are expressed as the ratio of the number of revertant colonies in the presence of test compound to the average number of revertant colonies in the absence of test compound (spontaneous revertants).

## Results and Discussion

The results of *Salmonella* mutagenicity tests with acridine, chloroquine, primaquine, and quinacrine are shown in Table 7-7. Acridine, chloroquine, and primaquine had no detectable mutagenic activity with or without a rat liver metabolizing system. Quinacrine was clearly mutagenic to TA 1537, a frameshift test strain; the mutagenic activity appeared to be slightly enhanced by rat liver activation. Acridine, chloroquine, and primaquine are chemically related to quinacrine, and the latter two compounds are commonly used antimalarial agents. A review of published studies on the mutagenic activity of quinacrine is presented in Table 7-8. These results provide evidence of significant genetic toxicity of quinacrine, particularly in bacterial systems. Quinacrine is known to intercalate in deoxyribonucleic acid, and this property is utilized in many karyotyping laboratories for banding of chromosomes. Mammalian mutagenicity tests did not show a high level of activity, and conflicting results appear in the literature. Our unpublished study of karyotypes of lymphocytes from quinacrine-treated monkeys failed to demonstrate any chromosomal abnormalities (see below).

In summary, quinacrine is a direct-acting frameshift mutagen in bacterial systems. Its genotoxic potential in mammalian cells is equivocal. The lack of

TABLE 7-7. *Salmonella typhimurium* Mutagenicity Tests: Ratio of Revertants With Test Compound to Spontaneous Revertants

COMPOUND	μG/ PLATE	TA98		TA 100		TA 1535		TA 1537		TA 1538	
		NA*	S9†	NA	S9	NA	S9	NA	S9	NA	S9
Acridine	1,000	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	100	0.38	0.55	0.53	0.69	1.23	0.89	1.26	1.00	1.00	1.14
	10	0.57	0.83	0.92	0.79	1.23	0.89	0.85	1.00	0.71	1.00
	1	0.74	0.83	1.05	0.91	0.82	1.04	1.00	1.00	0.81	0.71
	0.1	0.74	0.62	1.05	0.82	0.86	1.14	0.74	1.00	0.62	1.07
Chloroquine	10,000	0.29	0.48	0.34	0.66	0.52	0.65	Toxic	1.18	Toxic	0.61
	2,500	0.82	0.66	0.96	0.96	1.16	0.59	0.64	1.12	0.78	1.04
	100	0.92	0.83	0.88	0.94	ND	ND	1.50	1.00	0.85	1.00
	10	1.04	0.93	0.85	0.94	ND	ND	0.50	0.67	1.08	1.13
	1	0.88	1.10	1.02	0.91	ND	ND	0.63	0.58	0.92	0.93
Primaquine	0.1	0.92	0.90	1.00	0.99	ND	ND	1.00	0.75	0.92	1.13
	1,000	Toxic	0.40	Toxic	0.41	Toxic	0.68	Toxic	1.18	Toxic	Toxic
	100	1.14	0.86	0.98	1.10	0.64	0.89	1.00	0.73	0.71	1.21
	10	1.14	0.74	0.76	0.88	0.82	0.71	0.67	0.91	1.00	0.86
	1	0.95	0.64	0.76	1.06	1.00	1.29	0.44	0.64	1.29	1.21
Quinacrine	0.1	0.91	0.74	0.66	1.03	1.50	0.71	0.56	0.55	0.64	1.07
	1,000	Toxic	0.86	Toxic	Toxic	Toxic	Toxic	TNTC	Toxic	0.71	1.74
	100	0.83	0.84	0.76	1.07	1.27	0.82	6.48‡	11.42‡	1.29	0.61
	10	0.57	1.06	0.90	0.93	1.23	0.96	0.67	1.00	0.67	1.03
	1	0.38	0.86	0.88	0.87	1.27	0.82	0.67	0.74	0.81	1.03
4NQNO	0.1	0.38	0.83	0.84	0.83	1.00	0.64	0.67	0.89	0.67	0.97
	0.5	13.17‡		17.95‡		ND		ND		23.67‡	
	100	ND		ND		ND		11.80‡		ND	
QHCI	100	ND		ND		ND		11.80‡		ND	
NaN <sub>3</sub>	1	ND		ND		25.06‡		ND		ND	
BP	5		5.96‡		6.72‡		ND		4.22‡		8.89‡
2-anthramine	2.5		67.50‡		28.60‡		2.21‡		26.21‡		63.29‡
	1.0		ND		ND		6.83‡		ND		ND

\*NA, no adaptation

†S9 = 9000 × g supernatant fraction of rat liver homogenates (Aroclor pretreated).

‡Positive mutagenic response.

ND, not determined; TNTC, too numerous to count.

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‡Positive mutagenic response.  
 ND, not determined; TNTC, too numerous to count.

TABLE 7-8. Mutagenicity Tests With Quinacrine

TEST SYSTEM	DRUG	METHOD	RESULTS	REFERENCE
Bacterial tests				
<i>Salmonella typhimurium</i> C207 (frameshift)	Quinacrine 5µg/plate	Ames test	"Weak mutagen" (no data provided)	3
<i>S. typhimurium</i> TA 1537 (frameshift)	Quinacrine 100µg/plate ± S9	Ames test	control 12; control + S9 12 drug 1000; drug + S9 1000	19
<i>S. typhimurium</i> his G46 TA 1530, 1531, 1532, 1534	Quinacrine diHCl	Ames test	positive in TA 1532 only	28
<i>Bacillus subtilis</i> his <sup>-</sup> his B103, B201, B204	Quinacrine HCl crystal or solution spot test	Reversion to his <sup>+</sup>	Positive with his B103	13
<i>Escherichia coli</i> K-12 strain ND 160 lac	Quinacrine HCl as positive control 10µg/ml medium	Score lac <sup>+</sup> revertants	Positive	9
<i>E. coli</i> B/r strain ± deficient repair; trp <sup>-</sup>	Quinacrine HCl 10µg/ml medium 20µg/ml medium	Drug incorporated in postirradiation medium score str <sup>r</sup> mutants, score trp <sup>+</sup> mutants	Positive enhancement of U-V induced str <sup>r</sup> mutational yield in strains with intact repair similar results with trp <sup>+</sup> yield	8
<i>S. typhimurium</i> TA 1535, 1537, 98, 100 ± S9	Quinacrine diHCl 4 conc. but amts not listed	Ames test	Positive, but not listed which strain, concentration, etc.	15
<i>S. typhimurium</i> gal E 503	Quinacrine diHCl	Screen for mutagens that cause deletions	Incapable of generating such deletions	2
<i>S. typhimurium</i> G46 mouse host BP substitution	Quinacrine	<i>In vitro</i> spot test for his <sup>+</sup> revertants <i>In vivo</i> host mediated assay plate peritoneal fluid	Negative in both tests	20

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TABLE 7-8. Mutagenicity Tests With Quinacrine (Continued)

TEST SYSTEM	DRUG	METHOD	RESULTS	REFERENCE
<i>S. typhimurium</i> G46 base pair C3076 frameshift	Quinacrine	Spot test with saturated disc	Positive with frameshift Negative with base pair	6
Mammalian (nonhuman) Rat	Quinacrine diHCl 160 mg/ kg	Dominant lethal test	Equivocal, too few animals	12
Mouse sperm	Quinacrine diHCl	Score sperm head abnormalities	Negative	15
Mouse polychromatic erythrocytes of bone marrow	Quinacrine diHCl	Micronucleus assay for chromosome breakage	Negative?	15
Mouse bone marrow erythrocytes	Quinacrine diHCl 0.28 mg/ kg b wt. 1P	Micronuclei	Positive: increase of micronucleated erythrocytes (up to 65% increase)	17
Mouse bone marrow erythrocytes	Quinacrine diHCl 35, 70, 95, 142 mg/kg	Micronuclei	Negative: disputes reference 17	21
Chinese hamster ovary cells	Quinacrine diHCl 5 $\mu$ g/mi medium	Determining cell survival after preirradiation and postirradiation to drug	Sensitized cells to killing by x-rays: greater effect with preirradiation exposure to drug may inhibit DNA repair	26
Chinese hamster ovary cells	Quinacrine diHCl 0.2 $\mu$ M, 2 $\mu$ M, 20 $\mu$ M	Score chromosome breaks in metaphase Score abnormal anaphases	Frequency of chromosome breakage 3 to 5 times that of control (not considered high)	16

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Mouse leukemic cells L 1210	Quinacrine HCl 0.2µg/ml medium	Score ara C <sup>+</sup> → ara C <sup>-</sup>	Frequency reduced 5- to 16-fold "Antimutagenic"	5
Rat sarcoma cells (Jensen) asparagine	Quinacrine HCl 0.01µg, 0.1µg, 1.0µg, 5.0µg/ml	Score asp <sup>-</sup> → asp <sup>+</sup> cells	Ineffective in increasing or decreasing mutation frequency	24
Miscellaneous systems Silkworm pupae	Quinacrine HCl 0.25 mg/capita 0.50 mg	Score mutations by specific locus method	0.5-mg dose increased number of mosaic type mutations in females only	23
<i>Saccharomyces cerevisiae</i>	Quinacrine	Auxotrophic → prototrophic revertants drug-saturated disc placed in center of plate, score ring of mutants	Negative with both strains	6
Human cell tests				
Human sternal marrow cells (D-98)	Quinacrine HCl 0.06µg/ml	Score azaguanine resistant mutants	Cultures with drug contained 1/2-1/3 the mean of mutants found in control cultures	18
Human peripheral leukocytes	Quinacrine HCl 0.3 g for 14 days	Treated for protozoan infection karyotyped three times pretreatment and posttreatment	No abnormalities prior to treatment After treatment modal no. of 48 chromosomes, abnormal marker chromosomes	1

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FIG. 7-1. Karyotype of *Macaca fascicularis* arranged according to standard nomenclature.

mutagenic activity (in a bacterial system) for two chemically related antimalarial drugs (chloroquine and primaquine) suggests that the tubal occlusive properties of nonmutagenic analogs of quinacrine may be a productive area for further research.

#### MONKEY CHROMOSOME ANALYSIS

Peripheral blood samples were taken from a total of 15 monkeys before treatment and at varying times thereafter, ranging from 24 hours to 28 days. Nine received intrauterine quinacrine HCl (30 mg), three received intravenous quinacrine HCl (30 mg), and three received intrauterine saline. Chromosomes were prepared from cultured lymphocytes according to standard human cytogenetic procedures,<sup>25</sup> and slides were prepared for fluorescent banding. For each analysis, 20 banded metaphase spreads were examined and a karyotype was prepared. In every case, the modal chromosome number was found to be 42. No apparent aneuploidy or structural rearrangements were observed for any monkey, regardless of treatment. Chromosome breaks and chromatid gaps were not seen. A typical karyotype for the female cynomolgus monkey is included (Fig. 7-1).

#### SUMMARY AND CONCLUSIONS

The purpose of these studies was to evaluate the embryopathic potential of quinacrine in the event that the drug is inadvertently administered to a pregnant patient. Such studies in laboratory animals suffer from two major limitations: (1) uncertain suitability as a model for the human and (2) statistical, particularly type II, error (*i.e.*, concluding that no differences due to treat-

ment exist, when in fact they do). Such an error might easily occur in extrapolation of results from a relatively few animal subjects to a much larger population of human patients. We attempted to address these limitations by including two species, the monkey and the rat. We reasoned that although a statistically significant number of rats could be studied, it would be useful to include teratologic testing of a primate species even though the numbers of treated animals would be statistically insignificant. Moreover, although it was difficult to accomplish, we thought it was necessary to use the intrauterine route of treatment in monkeys as well as in rats in order for the study to be relevant to the human circumstance.

The results of the studies in rats clearly demonstrate the embryotoxic potential of quinacrine, manifested as dose-related resorptions. It is important to note, however, that quinacrine treatment did not cause malformations. Thus, strictly speaking, quinacrine is not a teratogen in rats (*i.e.*, it does not cause malformed fetuses, although it is quite embryo-lethal). It is possible, of course, that a much larger study would have demonstrated a low incidence of quinacrine-induced malformations. The sample size (20 pregnant rats per treatment group) was chosen because it is the conventional size for such studies.

Interpretation of the monkey teratologic results is more difficult, primarily because of the small number of animals (three) per treatment group. This is further complicated by the unanticipated result with the control group, where two pregnancies aborted and one resorbed. Our previous experience with intrauterine injections of 0.9% saline in cynomolgus monkeys at this stage of pregnancy suggested a 70% pregnancy continuation rate. However, two of the three control fetuses were aborted (passed tissue) rather than resorbed. This is in contrast to the quinacrine-treated monkeys, in which fetal resorptions occurred but not abortions. Thus, the resorption effect of quinacrine observed in rats also appeared in monkeys. The grossly malformed fetus appeared to be related to factors other than quinacrine, since the type of malformations suggested that they occurred prior to the treatment.

The mutagenicity studies were performed primarily to corroborate what was already published for quinacrine (*i.e.*, it is a direct-acting frameshift mutagen in bacterial systems). As noted in the review of the literature, quinacrine has much less mutagenic activity in eukaryotic cells and no clear mutagenic activity in mammalian systems. It is particularly noteworthy that no chromosomal abnormalities could be detected in the peripheral lymphocytes of cynomolgus monkeys that had received quinacrine by intrauterine or intravenous injection.

Within the limitations of such extrapolations, from these studies quinacrine would appear to have little potential for inducing fetal malformations when administered by intrauterine injection. The embryo-lethal effect is expected for direct-acting mutagens.

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# 8

## Histologic Changes Following Intrauterine Administration of Quinacrine Hydrochloride

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The histologic changes that occur when quinacrine is placed into the uterus have not been well documented.<sup>1</sup> In addition, the sequential process involved remains unclear.

In this chapter, we will briefly describe a sequence of histopathologic changes taking place in the cornual portion of the fallopian tube subsequent to exposure to quinacrine. This description provides a coherent basis for the two known outcomes of such exposure, tubal closure or tubal patency. Since most of the changes were not observed as part of a prospective timed protocol, the interpretation remains hypothetical.

### METHOD

Two pig uteri were examined approximately 2 weeks after the pigs had been treated with quinacrine. In the operation, the uteri had been opened and 125 mg quinacrine was held forcefully against the cornua for 15 to 20 minutes. We also examined the uteri of nine monkeys removed 24 hours (from three monkeys), 7 days (from three monkeys), and 28 days (from three monkeys) after the intrauterine instillation of 30 mg quinacrine in 1 ml sterile water.<sup>1</sup>

### HYPOTHESIS

The initial reaction of the tissue lining the endometrial cavity and the cornual portion of the tube is autolysis. In most cases, this damage is superficial. For example, in the cornua, the tube was involved no more deeply than the innermost fibers of muscle surrounding the interstitial tube. Despite gross yellow discoloration of the tube beyond the isthmus in monkeys examined

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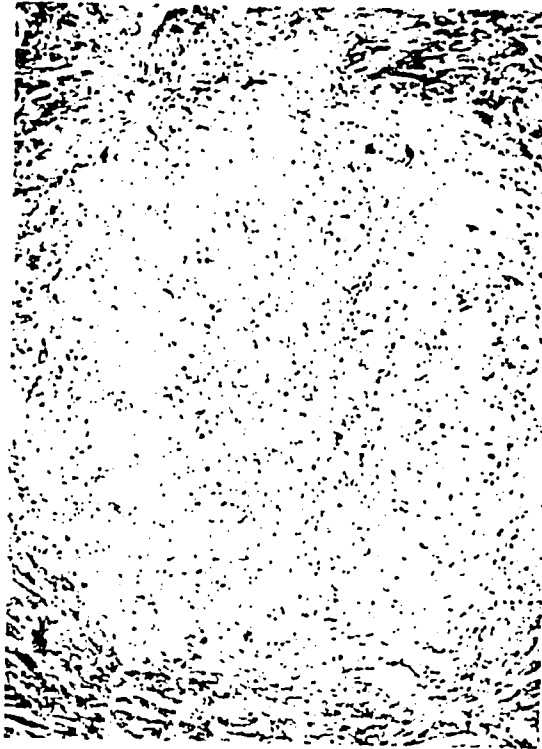
**FIG. 8-1.** An area of central necrosis, subsequent to autolysis, is being invaded by an inflammatory infiltrate. As yet, there is no proliferative repair. Human tissue.

24 hours after intrauterine quinacrine application, tubal damage did not extend beyond the cornua. On the other hand, in those same monkeys, considerable endometrial damage was found. In most cases, the damage looked superficial with respect to the epithelium, but it extended along this epithelium deep into the glands to the basalis in several spots. In the latter cases, the stroma surrounding the gland might remain relatively intact. There was also one monkey in which there was some endocervical damage, but this damage might have been due to the cervical manipulation required to pass the instillation needle into the uterus.

In several specimens obtained from pigs, however, the damage extended all the way through the myometrium to the serosal surface. It should be noted that the methodology used in these cases was significantly different from that of the rest of the material.

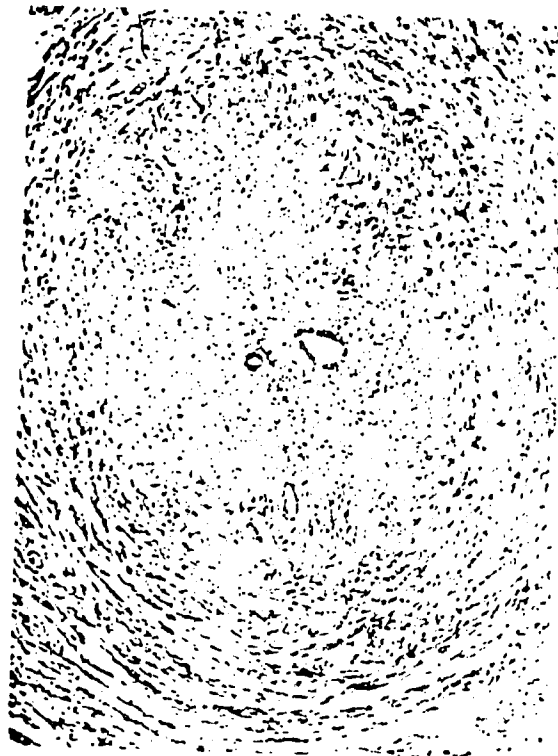
After the initial reaction, the area of autolysis becomes inflamed (Fig. 8-1) and then organized by fibroblastic proliferation and scar (Fig. 8-2). In the cornual portion of the tube, if, as the result of the close apposition of the walls of this narrow portion of the tube, organization occurs in such a manner that the lumen is obliterated, sterilization has been achieved. Occlusion is not always successful, however. If the central or luminal portion of the necrotic area "drops out," presumably because of liquefaction, then this cavity will become reepithelialized before organization occurs (Fig. 8-3). Subsequent or-

**FIG. 8-2** Scar, subsequent to proliferative repair, has almost totally replaced the area of autolysis and necrosis. There is no central epithelium, and the suggestion is that the tubal lumen will be completely scarred across. Human tissue.

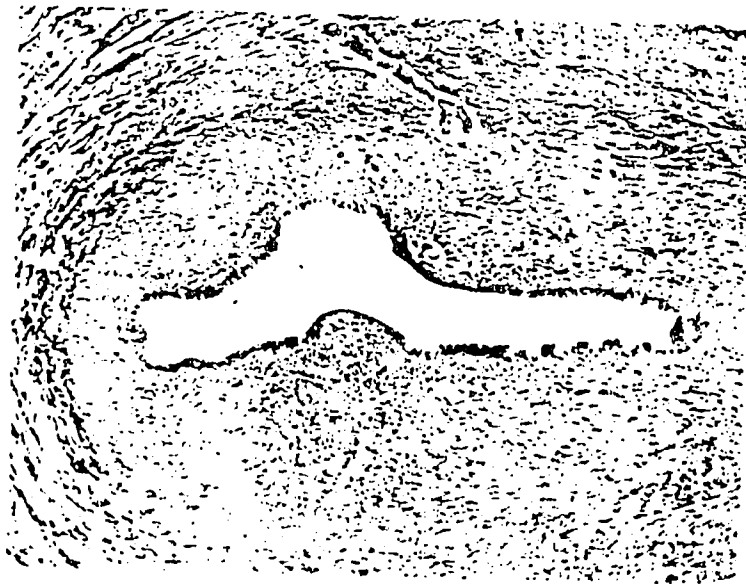


**FIG. 8-3.** Although a central area of necrosis and inflammation is present, there appears to be a lumen, which already has an epithelial lining. This epithelial lining might be expected to remain even after organization takes place. Human tissue.





**FIG. 8-4.** Organization and repair appear to have occurred around two persisting islands of epithelium. Since epithelial surfaces do not adhere, presumably the lumen will not be obliterated in this case. Human tissue.



**FIG. 8-5.** Almost complete reorganization of the tubal lumen has occurred, with only subepithelial inflammation persisting. The flattened tubal epithelium looks metaplastic in places, consistent with its repairing nature. Human tissue.



**FIG. 8-6.** This normal tube has been totally repaired. Human tissue.

ganization does not in this circumstance produce tubal closure (Fig. 8-4) but only the gradual resolution of the inflammatory process (Fig. 8-5) and, eventually, a normal-appearing tube (Fig. 8-6). The frequency with which either outcome occurs is not clear from these data, but it is suggested by clinical efficacy studies reported in Chapters 9, 10, and 12.

In the endometrium of the monkeys examined 1 week and 1 month after the intrauterine of instillation quinacrine, there were two animals in which focal scars were seen. It is not clear that these were related to the quinacrine treatment.

## CONCLUSION

Intrauterine administration of quinacrine effectively occludes the central portion of the fallopian tube in a certain proportion of cases. The factors that determine the frequency with which this occurs are still unclear, but intrauterine chemical sterilization with this or a similar agent seems promising.

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## Quinacrine Hydrochloride: Future Research

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Without doubt, there is a need and a demand for a chemical method of tubal sterilization. Chemicals of various categories, including caustic, sclerosing, granuloma-producing, and cytotoxic agents and tissue adhesives have been tested in both animal models and humans.<sup>2</sup> The most effective agent appears to be quinacrine, and, depending on the mode of delivery, it can cause bilateral tubal closure rates in excess of 90%. Clinically, quinacrine has been used in several developing countries.

Efforts have been directed toward improving the efficacy of quinacrine by manipulating the mode of delivery. One approach has been a change from its administration as a slurry to a compacted pellet. V- or T-shaped IUD vectors, with quinacrine located at the tips, have been especially designed to ensure delivery of the drug to the cornual region of the uterus near the uterotubal junction.

As efficacy of quinacrine sterilization improves, safety comes to the fore. In evaluating the elusive risk/benefit ratio of a drug treatment, the therapeutic index is an important consideration. The therapeutic index is the relationship between the dose of a drug that produces an undesirable effect, compared with the dose that produces the desired effect. That index represents, in essence, the "margin of safety" of the drug. A low index, that is, one in which the toxic dose is very close to the effective dose, may be acceptable in the treatment of a life-threatening disease. Thus, digitalis, which has a very low therapeutic index, is nevertheless an acceptable agent for the treatment of congestive heart failure.

In regard to quinacrine, there are numerous known toxic effects; yet, these were of minor importance when this agent was used to treat patients with malaria. However, the risk/benefit assessment of a drug for the treatment or prevention of a debilitating disease like malaria may not be the same as when the agent is used for voluntary tubal sterilization. As an agent for chemical sterilization, it must be assessed against the risks of current methods of surgical contraception, as well as against the risks of other methods of contraception, and of pregnancy itself.

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Most of our experience with quinacrine toxicity was gained through its widespread use as an antimalarial during World War II. More pertinent, however, is consideration of potential effects that may occur following intrauterine administration of the drug. Solutions of the drug administered into the uterus are known to be quickly absorbed into the blood and distributed throughout the body. This appears not to cause any toxicity as long as the plasma levels do not exceed some critical level.

That some of the more serious toxic effects of quinacrine are related to elevated plasma levels is shown by the following findings:

Accelerations in electroencephalograms and physiological symptoms occurred in quinacrine-treated human subjects when plasma levels of the drug exceeded 30 ng/ml.

In monkey studies, death or convulsions occurred after quinacrine administration, at the time when plasma levels were at peak concentrations.

In rat studies, a decrease in blood pressure occurred immediately after intravenous infusion of quinacrine, with recovery occurring a short time later, unless the decrease was so severe as to result in death (see Chapter 6).

Central nervous system (CNS) excitation syndromes have been reported following intrauterine administration of quinacrine solutions in 2% of treated women.<sup>1</sup> Studies using pellets suggest a lower incidence of this CNS effect.<sup>3</sup> Possibly, the plasma level achieved in women receiving pellets is less than in those women receiving solutions, but this has not been documented. High plasma levels of quinacrine could also result from inadvertent administration of the drug to pregnant women. Increased plasma levels were observed when the same dose of quinacrine was administered to pregnant as compared with nonpregnant monkeys.

Another consideration is that perforation of the uterus is an inevitable complication, if an intrauterine instrument is used for drug delivery. The subsequent administration of the drug directly into the peritoneal cavity might be expected to result in more rapid absorption of the drug into the blood, and hence higher plasma levels and greater likelihood of toxic reaction (see Chapter 6).

A separate issue is that of potential carcinogenicity of the drug. It is known that quinacrine binds to deoxyribonucleic acid (DNA) and that it acts as a frame shift mutagen in bacterial mutagenicity tests (see Chapter 7). However, no association of quinacrine with mutagenicity or cancer has been demonstrated in mammals, and, theoretically, a single exposure would cause less concern than would a long-term exposure. However, such potential hazards must be considered. It is notable that other analogues of quinacrine were found not to be mutagenic in bacterial test systems (see Chapter 7). However, the efficacy of these drugs as tubal occluding agents has not been documented.

By what mechanism quinacrine closes the tube is still not known. It has been suggested that quinacrine action is related to its ability to intercalate with DNA, and its failure to work in some species, such as the rabbit, is related to the concentration of zinc in the tissue, which inhibits binding with DNA. It is possible, however, that the species differences in response to quinacrine

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may involve anatomical rather than biochemical factors. For instance, it may be that the close apposition of the luminal surfaces of the uterotubal junction of some species may be more conducive to fibrotic connections between surfaces, rather than to reepithelialization (see Chapter 8).

Furthermore, tubal occlusion may not be the sole *modus operandi* in the antifertility property of quinacrine. It is conceivable that quinacrine affects the reproductive tract in some as yet unknown way to impair the function of the tube, endometrium, or uterine environment.

Further research on quinacrine is needed. Such research might include the following:

Development of a timed release system for quinacrine to give a rate of release that would ensure low plasma levels of this agent, even if it is accidentally placed in the peritoneal cavity, while at the same time yielding high efficacy in causing tubal occlusion

Determination of the tubal occlusion efficacy of nonmutagenic drugs chemically related to quinacrine

Continued testing of alternative drugs with potentially better therapeutic indices for their ability to cause tubal occlusion

More precise definition of the process by which quinacrine results in decreased fertility

Determination of the mechanism by which quinacrine causes tubal closure

On development of improved drug and/or delivery systems, institution of clinical trials, so that such a sterilization technique would ultimately become available.

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## Short Papers

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J. Med. Primatol. 12:41-48 (1983)

### Hysterosalpingography in Cynomolgus Monkeys

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**Key words:** Cynomolgus · Cervix · Hysterosalpingogram

This paper describes recent success in performing hysterosalpingography in cynomolgus monkeys. The animals were anesthetized, and a needle was manipulated through the cervical canal into the endometrial cavity for injection of contrast material. Difficulties in threading the needle through the canal were due to several blind pouches formed by folds in the cervical mucosa. The uterine lumen and both tubes could be visualized in over 75% of the cases. The study demonstrates that the hysterosalpingography technique can be performed satisfactorily in cynomolgus monkeys.

#### INTRODUCTION

Nonhuman primates have been used for studies in reproductive physiology because of their anatomic and endocrinologic similarity to man. An often-noted anatomical difference between human subjects and monkeys is the tortuosity of the cervix [4]. Recent interest in development of surgical [5] and chemical [8] techniques for tubal closure or reversal [6] requires animal models for these procedures. It would be advantageous to obtain hysterosalpingograms on such animals before and after each manipulation. The present study describes recent success in performing hysterosalpingography in cynomolgus monkeys.

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## MATERIALS AND METHODS

### Anatomical Observation of the Endocervical Canal

Three cervixes were obtained from postmortem necropsies of female cynomolgus monkeys and placed in Bouin's fixative for one week. Cross-sectional slices were made at approximately 2-mm intervals with a single-edge razor blade in order to observe the pathway of the endocervical canal.

### Performance of Hysterosalpingography

Female cynomolgus monkeys were given intramuscular ketamine hydrochloride (4.4 mg/kg) and placed in a prone position on an inclined surface so that the hips were elevated and the hind limbs hung off the edge of the table. A nasal speculum was used to open the vagina and visualize the cervix. An 18-gauge, blunt-tip needle, approximately 12 cm long and with a slightly bent tip (Fig. 1) was placed into the endocervical canal and manipulated so that it passed into the endometrial cavity. The needle was inserted into the cervical opening with the curve directed ventrally. When resistance was encountered, the needle was withdrawn several millimeters and rotated so that the curve of the needle was in a dorsal position. Gentle probing with the needle was performed until the main canal was engaged and entered. On some occasions the posterior lip of the cervix was grasped with a Halsted clamp to facilitate entry into the cervix by the needle.

After placement of the needle, the animal was placed in a prone position on a photographic plate. A syringe containing 4 ml of contrast material (Salpix, Ortho Pharmaceutical Co., or Sinografin, Squibb & Sons, Inc.) was attached to the needle and 1 ml was injected; an x-ray was taken. To prevent back-flow through the cervix, a flange attached to a sleeve over the needle was used (Fig. 1). This sleeve could be fixed in place with a set-screw. While helpful, this did not completely prevent back-flow.

## RESULTS

The endocervical canal of the cynomolgus monkey is approximately 3–4 cm long and consists of complex mucosal folds which begin immediately upon entrance from the vagina (Fig. 2A). The folds form several branches

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**Fig. 1.** A) Needle used for penetration of cervix of cynomolgus monkeys. This needle has sleeve equipped with a plastic flange (F) (seen separately in C) which fits over needle (B). When needle is in place in the uterus, the sleeve is pushed as close to the cervix as possible to retard back-flow. The set-screw (S) secures the sleeve in place at the time of injection.

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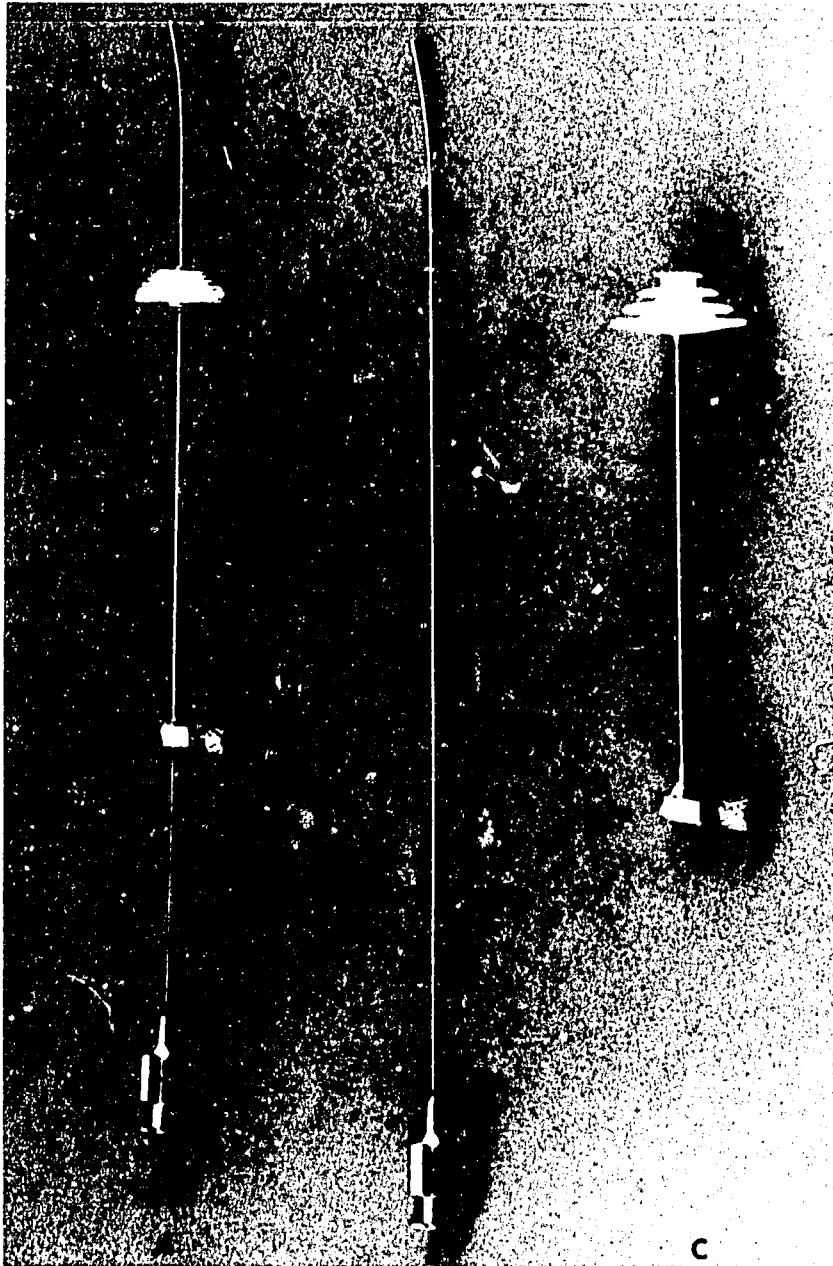
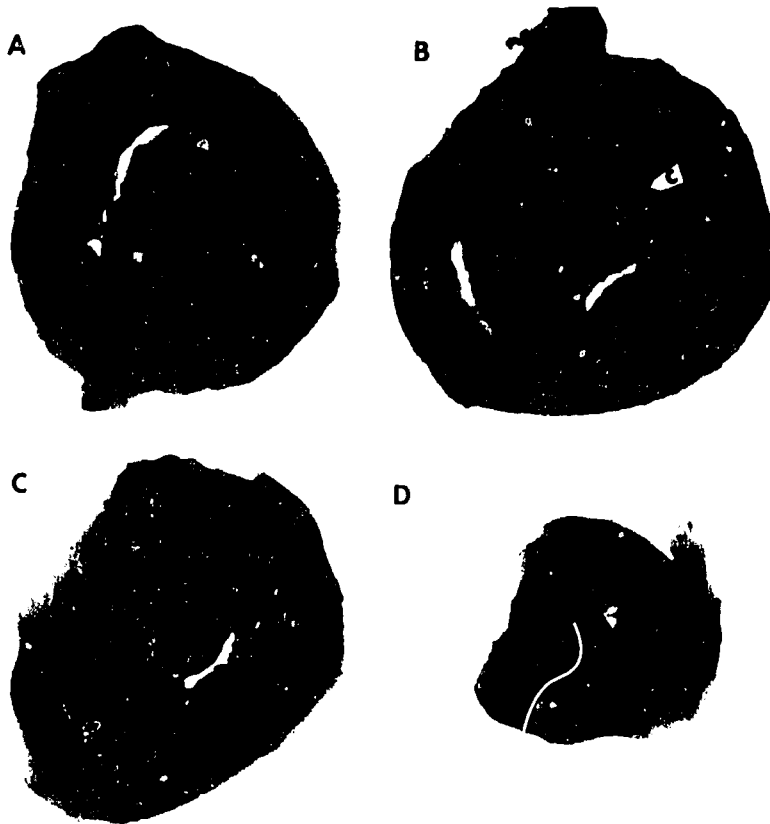


Figure 1

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**Fig. 2.** A) Cross section through the cervix near entrance from vagina. B) A cross section approximately midway through the cervix demonstrating complex branching of the endocervical canal formed by the mucosal folds. a, b, and c represent branches which eventually end blindly. o represents the portion of the canal where the needle must traverse to eventually reach the uterus. C) A cross section 2 mm distal to the previous section showing the formation of the branch (a) which disappears blindly within the next 5 mm. Pouch b from the previous section has terminated. c also eventually forms a branch which ends blindly. D) A cross section where the cervix enters the uterus showing one narrow canal which becomes continuous with the uterine lumen.

(Fig. 2B), the exact configuration of which varied considerably among monkeys. However, all the endocervical canals examined appeared to have three predominant branches which ended blindly (Fig. 2B,C). The endocervical folds converge eventually to a narrow canal at the entrance into the uterus (Fig. 2D).

Of the 21 monkeys in which hysterosalpingography was attempted, there were five cases in which the needle could not be threaded through the cervix.

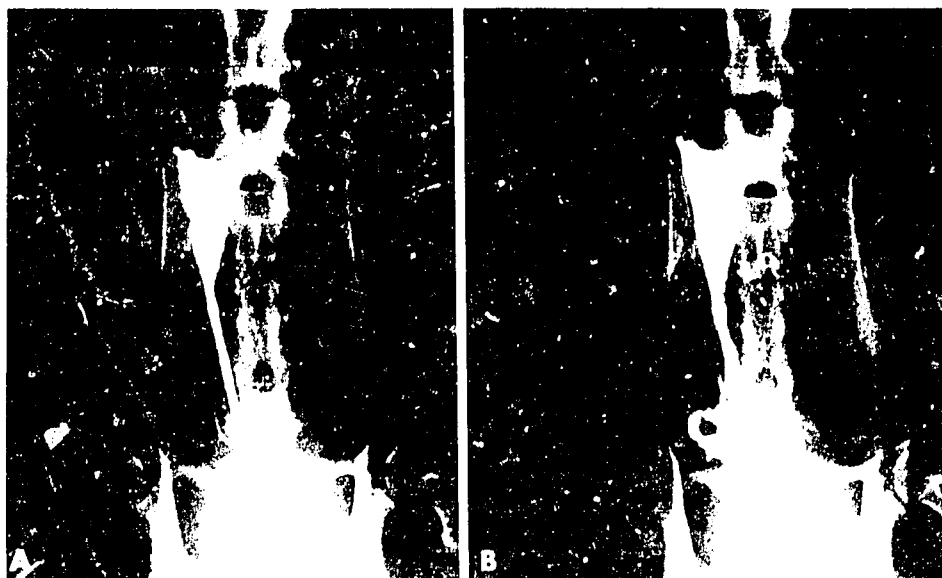
Four of these cases were repeated on another day with apparent success. Upon examination of the x-ray plates of the 20 apparently successful attempts, one plate was overexposed due to a darkroom accident. In two other plates, we realized that the needle was not in the uterus but still in the cervix. In these cases, no radio-opaque material was visualized in the uterine cavity, but there was spillage into the vaginal cavity. Of the 17 plates which revealed successful penetration into the uterine cavity, there were six cases where both tubes were visualized after injection of 1 ml contrast medium (Fig. 3), while 13 pairs of tubes were observed only after 4 ml contrast medium (Fig. 4A,B). With 4 ml, there were three cases in which only one tube was observed and one case in which no tubes were observed.

### DISCUSSION

The primate animal has been used as a model for studies on reproduction because of its anatomical similarity to women. Recent interest in tubal



**Fig 3.** A hysterosalpingogram following administration of 1 ml contrast media into a cynomolgus monkey uterus. The uterine cavity and both tubes are well delineated. A) Needle. B) Uterine cavity. C) Oviducts (tubes) containing contrast media.



**Fig. 4.** Hysterosalpingogram following intrauterine administration of 1 ml contrast media (A) and of 4 ml contrast media (B) to cynomolgus monkey. Tubes visualized after 4 ml contrast media injected. Note also back-spill of contrast media into vagina after 4-ml injection.

function and in chemical occluding agents such as methacrylamide and quinaerine [7] make the monkey model attractive. It would be desirable to test patency of the tube in the experimental animal prior to and/or after treatment. While hysterosalpingography is routinely performed in women, anatomical differences in the cervix of other primates make this procedure more difficult. In many primate animals, the cervical canal is not a straight path into the uterine cavity as it is in women, but of various degrees of tortuosity depending upon species [4]. The *Macaca arctoides* has been previously described [1] as a primate with a straight-bore cervix; this allows direct entry into the uterus, permitting hysterosalpingograms to be obtained on these animals. These primates were compared with *M. fascicularis* (cynomolgus) and *M. mulatta* (rhesus). It has been noted [1] that the cynomolgus has a cervix which lacks the prominent colliculi of the rhesus but also that the cynomolgus monkey has several blind pouches which would make passage difficult. Since we have previously performed intrauterine injections in pregnant cynomolgus monkeys [2,3], this species was chosen for the present study.

Our own anatomical examination of dissected cervixes from cynomolgus monkeys confirmed the existence of blind passages. Upon penetration of the endocervical canal, a needle traversed a path of least resistance which inevitably ended in a blind pouch, usually in what "felt" like the ventral location (labeled "a" in Fig. 2C). By retracting the needle several millimeters back to the position pictured in Figure 2B and rotating it 180° so that the curve pointed in a dorsal direction, we could engage that portion of the canal (Fig. 2B,C labeled "o") that would lead to the uterus. Engaging this "true" passageway was the major obstacle in the procedure. While the procedure was accomplished in some cases within a matter of minutes, it was often difficult to find this pathway. The manipulation for the needle in the cervix as described above was the initial strategy taken for penetration of the cervical canal, but it was not foolproof; often the procedure required persistence. While we did not accomplish the task in five monkeys, we feel that this was a matter of not enough perseverance. In four of those monkeys, the procedure was attempted a second time with success. It is also possible that the ease of penetration is a function of the stage of the cycle, a variable not considered in this study.

The hysterosalpingograms obtained were of good quality and the presence of the radio-opaque material in both tubes could be easily visualized in about 75% of the cases where the needle was successfully inserted into the uterus. The study demonstrates that the hysterosalpingography technique can be performed satisfactorily in cynomolgus monkeys.

#### ACKNOWLEDGMENTS

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Comparison of the Operating Microscope and Loupe for  
Microsurgical Tubal Anastomosis:  
A Randomized Clinical Trial†

Key Words: Reversal of Sterilization

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SYNOPSIS

Reversal of sterilization was performed by microsurgical tubal anastomosis in 72 women using either loupe (n = 36) or microscope (n = 36). The study design called for the randomization of patients within pairs which were matched for method of sterilization and the site of anastomosis. A significant difference between methods could not be demonstrated at 12 months (p = .39) or 24 months (p = .37) postprocedure.

## INTRODUCTION

There has been some controversy over the efficacy of the loupe in providing adequate magnification for the reversal of sterilization i.e., pregnancy success rates comparable to operating microscope after anastomosis.<sup>1</sup> Although the loupe may provide sufficient magnification to perform an isthmic-isthmic, ampullary-ampullary or an ampullary-isthmic anastomosis, there is some question as to the precision and accuracy of suture placement. There is little debate that a microscope is needed to obtain sufficient magnification to perform an isthmic-cornual anastomosis. This randomized controlled clinical trial was carefully designed to determine the efficacy of the loupe as compared to the microscope in performing a microsurgical anastomosis of tubal segments other than the cornual-isthmic region with respect to a subsequent viable term pregnancy.

## MATERIALS AND METHODS

Three hundred thirty-five women were evaluated for reversal of sterilization at the Johns Hopkins Hospital, Baltimore, Maryland between January 1, 1978 and December 31, 1980. Of these, 72 women met the criteria for entry into this randomized clinical trial, i.e., less than 36 years of age and at least 5 centimeters (cm) of oviduct remaining after sterilization.

The surgeon was informed of the device to deliver magnification (loupe or microscope) just prior to performing the surgical procedure. All anastomoses were performed or supervised by one of two experienced microsurgeons (JAR, CAB). One surgeon (JAR) performed 90% of the surgical procedures. The microsurgical techniques have been described in detail elsewhere.<sup>2,3,4</sup> Magnification (2.0 to 4.5) was obtained with the loupe (Designs for Vision, Inc., New York, NY), (Edroy Products, New York, NY), while 4 to 16X magnification was obtained by using the OPMI-7 Zeiss microscope (Carl Zeiss, Inc., New York, NY).

#### STATISTICAL METHODS

A sequential design, calling for pairs of patients with randomization to the loupe or to the microscope, was chosen originally with tubular patency as the outcome variable. Within pairs, patients were matched for sterilization procedure and segments anastomosed. For any pair, this outcome was known before the next pair was entered. Subsequently, this outcome variable was replaced by term pregnancy, which takes longer to ascertain. The results have been evaluated both in terms of the sequential procedure and by the use of standard fixed sample size methods. It is seen that the sequential method would have permitted conclusions with many fewer pairs if earlier determination of outcome had been possible. This is a general property of sequential designs.

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The Sequential Probability Ratio Test (SPRT)<sup>5</sup> for double dichotomies was the sequential design chosen for this study. This design was characterized by the following definitions:  $P_E$  = probability of term pregnancy for anastomosis with the microscope;  $P_S$  = probability of term pregnancy for anastomosis with the loupe;  $H_1$  = a hypothesis which specifies values for  $P_E$  and  $P_S$  such that  $P_S = P_E$ ;  $H_2$  = a hypothesis which specifies values for  $P_E$  and  $P_S$  such that  $P_E > P_S$ ; alpha = probability of concluding that  $H_2$  is true when in fact  $H_1$  is true; and beta = probability of concluding that  $H_1$  is true when in fact  $H_2$  is true.

The probability of term pregnancy is believed to be in the neighborhood of 0.6. Accordingly, the following values for the trial were chosen:

(Insert Formula 1)

The SPRT provided for detection of a difference between the two surgical procedures of 20% at the 5% level of significance with a power of 80%. Each pair resulted in one of the following outcomes: 1) Term pregnancy in both; 2) No pregnancy in either; 3) Term pregnancy for the patient using the loupe and no pregnancy for the patient using the microscope; and 4) Term pregnancy for the patient using the microscope and no term pregnancy for the patient using the loupe

Results (1) and (2) provide no information about the difference between the techniques. Results (3) and (4) are called favorable pairs, being favorable to one or the other technique.

These latter results are used to plot the trial data, pair by pair, as they accumulate. After the pregnancy for each pair has been determined, one calculates:

(Insert Formula 2)

These points are plotted sequentially, as in Figure 1. The trial terminates when any point falls outside the parallel lines in Figure 1 and the indicated decision is made. The trial terminates with rejection of the microscope technique if  $Y$  falls above the upper line or acceptance of the microscope technique if  $Y$  falls below the lower line.

By the very nature of a sequential clinical trial, the sample size is not known in advance, i.e., it is a random variable. However, the expected sample size can be computed and, for this trial, it was 12 favorable pairs under  $H_1$ . In fact,  $H_1$  was accepted after 10 pairs based on pregnancy within 12 months. Additional pairs were obtained to provide an adequate sample size for standard tests.

The SPRT and the McNemar tests were used to test for significance between the two procedures. Additionally the Chi-square analysis was applied to the comparison of certain variables within the patient groups. A follow-up of two years was obtained on all patients, as well as four years on the first eighteen pairs of women.

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RESULTSPATIENT PROFILE

The profile of the patient groups (loupe versus microscope) were compared with respect to the number of patients and pregnancy rate for age, parity and interval from sterilization to reversal (Table 1). A significant difference between the patient groups could not be demonstrated. Seventy-five percent of patients from each group (loupe versus microscope) had an ampullary-isthmic anastomosis. Twenty-two percent (loupe) and 20% (microscope) had an ampullary-ampullary anastomosis. Three percent (loupe) and 6% (microscope) had an isthmic-isthmic anastomosis. Patients within pairs were matched for the segments anastomosed.

PREGNANCY SUCCESS

Patient groups (loupe versus microscope) were compared with respect to total pregnancies, term pregnancies, spontaneous abortions, and ectopic pregnancies. At 12 and 24 months post-procedure (Table 2), differences between patient groups could not be demonstrated. The SPRT revealed no difference between microscope and loupe with respect to pregnancy success (Fig.1) (Tables 3, 4).

The trial could have been terminated at 11 months when a pair fell below the parallel lines (Fig. 1), if the outcome had been ascertained at that point. However, the trial was continued through 24 months, and no difference could be demonstrated between



the magnification methods. All patients had a minimum of 24 months follow-up. Over half of the patients had a four-year follow-up interval. No patients were lost to follow-up.

### DISCUSSION

Tubal reanastomosis after previous sterilization prior to the early 1970's afforded approximately a 40% to 50% chance of a pregnancy.<sup>3</sup> With the application of microsurgical techniques, a pregnancy rate of 60% to 70% has been achieved.<sup>6,7</sup> As a result of this improved pregnancy success, tubal microsurgery has received widespread interest.

Magnification may be provided with an operating microscope or loupe. The maximum magnification needed for optimum results is unknown. The loupe provides adequate magnification to perform a microsurgical tubal anastomosis, however, it provides a variable working distance. That is, if there is surgeon fatigue, some difficulty may be encountered in maintaining proper focus. Furthermore, with higher magnification, loupes may have increased weight which may cause surgeon fatigue. With the newer generation of loupes of lightweight construction, this problem has largely been resolved.

There are no randomized clinical trials which have established the necessity of the operating microscope to supply magnification for a microsurgical tubal anastomosis. Authors to date have preferred to study consecutive series of patients. As innovations in medicine and surgery have occurred, the randomized controlled trial replaced personal experience as a principal source of data on

which to base therapeutic decisions. However, this has been difficult in tubal surgery due to the small patient groups available for study. The SPRT has provided an acceptable design for a randomized trial where sufficient patients may be accrued in a reasonable period of time.

This randomized clinical trial revealed no difference in pregnancy success following tubal anastomosis with loupe as compared to the operating microscope. This does not imply that any surgeon may use loupes with comfort and confidence. The selection of a magnification system is a personal choice. However, this choice should not be influenced by concern over a difference in subsequent pregnancy success.

Finally, this study addresses only tubal anastomosis of the ampullary and isthmic segments. A microscope should be used for isthmic-cornual anastomosis where greater magnification is needed and cannot be obtained with the current loupe magnification systems.

TABLE 1. - PATIENT PROFILE

<u>Age (Years)<sup>a</sup></u>	<u>LOUPE</u>	<u>MICROSCOPE</u>
	<u>Term Pregnancies/Total Patients</u>	<u>Ter Pregnancies/Total Patients</u>
15 - 29	9/19 (47.4%)	11/21 (52.4%)
30 - 35	14/17 (82.4%)	9/15 (60%)
 <u>Parity<sup>a</sup></u>		
0 - 1	2/4 (50%)	1/3 (33.3%)
2	10/10 (55.6%)	7/11 (63/6%)
3	7/8 (87.5%)	8/16 (50%)
4 or more	4/6 (66.7%)	4/6 (66.7%)
 <u>Interval from Sterilization to Reversal (Month)<sup>a</sup></u>		
12 - 23	2/3 (66.7%)	5/8 (62.5%)
24 - 48	6/11 (54.5%)	5/12 (41.7%)
49 - 72	9/13 (69%)	2/4 (50%)
73 - 96	3/4 (75%)	5/8 (75%)
> 96	3/5 (60%)	3/4 (75%)

a = Differences among groups, i.e., Loupe versus Microscope with respect to number of patients within groups and patients pregnant were not significant.

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TABLE 2 - PREGNANCY SUCCESS FOLLOWING TUBAL REVERSAL

<u>Outcome<sup>a</sup></u>	<u>12 Months</u>		<u>24 Months</u>	
	<u>Loupe</u>	<u>Microscope</u>	<u>Loupe</u>	<u>Microscope</u>
Total Pregnancies	26/36 (72.2%)	22/36 (61.1%)	28/36 (77.8%)	26/36 (73.2%)
Term Pregnancies	17/36 (47.2%)	13/36 (36.1%)	23/36 (63.9%)	19/36 (52.8%)
Spontaneous Abortion	10/36 (27.8%)	9/36 (25.0%)	10/36 (27.8%)	14/36 (38.9%)
Ectopic Pregnancy	1/36 ( 2.8%)	1/36 ( 2.8%)	3/36 ( 8.3%)	2/36 ( 5.6%)

a = Differences between patient groups were not significant.

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TABLE 3.

MICROSURGICAL TUBAL ANASTOMOSIS WITH LOUPE VERSUS MICROSCOPEOutcome by Pairs at 12 Months<sup>a</sup>

		<u>LOUPE</u>		
		<u>Success</u>	<u>Failure</u>	<u>Total</u>
	<u>Success</u>	4	9	13
<u>MICROSCOPE</u>	<u>Failure</u>	<u>13</u>	<u>10</u>	<u>23</u>
	Total	17	19	36

a:  $\chi^2 = 0.727$      $p = .39$

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TABLE 4.

MICROSURGICAL TUBAL ANASTOMOSIS WITH LOUPE VERSUS MICROSCOPE

Outcome of Pairs at 24 Months<sup>a</sup>

		<u>LOUPE</u>		
		<u>Success</u>	<u>Failure</u>	<u>Total</u>
<u>MICROSCOPE</u>	<u>Success</u>	11	8	19
	<u>Failure</u>	<u>12</u>	<u>5</u>	<u>17</u>
	<u>Total</u>	23	13	36



a:  $\chi^2 = 0.800$        $p = 0.37$

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LEGENDS

Figure 1.  "squares" represent term pregnancy within twelve months. X represents term pregnancy within twenty-four months.  "solid squares" represent entries which are superimposed.