

## Effect of intrauterine and intravascular quinacrine administration on histopathology, blood chemistry, and hematology in cynomolgus monkeys\*

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*Histopathologic features, blood chemistry, and hematologic features were studied in cynomolgus monkeys following intravascular or intrauterine administration of a 30-mg solution of quinacrine hydrochloride. Intrauterine quinacrine administration resulted in extensive necrosis of the endometrial surface, and lesions were observed at 24 hours after treatment which obliterated the cornual areas of the uterus. Necrosis was also observed on occasion in the ampulla or isthmic portion of the tube. Evidence of repair of the reproductive tract was seen 7 and 28 days following treatment. No lesions were observed in any nonreproductive organ examined, whether quinacrine was administered by the intrauterine or intravascular route. Blood chemistry data revealed moderate and transient increases in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and lactic dehydrogenase (LDH). No other blood chemistry or hematologic changes were noted that could be attributed to quinacrine administration. For the conditions described in these studies, intrauterine administration of quinacrine appears to be a safe procedure. However, the potential toxicity of the drug is discussed.  
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Since the early 1970s intrauterine quinacrine has been used as a chemical occlusion agent of the fallopian tube and thus has served as an **alternative** to surgical sterilization.<sup>1</sup> While the toxicology of quinacrine has been widely studied be-

cause of its use as an antimalarial agent, few toxicologic studies are available for the particular mode of administration used for tubal sterilization procedures; namely, intrauterine instillation. In an accompanying report<sup>2</sup> we demonstrated that when a 30-mg/ml quinacrine solution was instilled into the uterus of cynomolgus monkeys, it quickly entered the circulatory system and was concentrated in almost every organ examined. This study describes the results of blood chemistry and hematologic analyses and the **histopathologic** examination of the organs in these animals. In addition, histofluorescent examination of many of the organs was made in an attempt to

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observe the intracellular location of quinacrine following administration by either the intrauterine or intravascular route.

## MATERIALS AND METHODS

Fifteen monkeys, described in an accompanying report,<sup>7</sup> were studied. Three received an 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 an intrauterine injection of a solution of 30 mg quinacrine in 1 ml sterile water. The dose was determined by the solubility of quinacrine and the volume capacity of the uterus. Three monkeys received intrauterine injections of 1 ml physiologic normal saline. This was to control for any injection stress. Saline was chosen rather than water to eliminate possible effects of a hypotonic solution.

Intrauterine injections were made within 14 days of the 1st day of cyclic vaginal bleeding. The details of the injection procedure and characterization of the quinacrine material were described previously.<sup>7</sup> Blood was obtained from the femoral vein before treatment and on days 1, 2, 3, 7, and 28 after treatment or until autopsy for blood chemistry and hematologic analyses.

Blood chemistry determinations were performed by Vet Path Laboratories, Teterboro, NJ. The analyses included serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (AP), lactic dehydrogenase (LDH), cholesterol, triglyceride, total protein, albumin, globulin, blood urea nitrogen (BUN), 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 total and differential white blood cell (WBC) counts, red blood cell (RBC) count, hematocrit, and hemoglobin.

Three saline-treated monkeys and three of the monkeys receiving intravascular quinacrine were necropsied 24 hours after injection. Three monkeys treated with intrauterine injections of quinacrine were also necropsied 24 hours after injection. Three other monkeys receiving intrauterine quinacrine were necropsied at 7 days and the re-

maining three at 28 days. At these time periods, the monkeys were sedated with ketamine hydrochloride and killed with an overdose of sodium pentobarbital administered intravenously. A complete gross examination was performed, and selected tissues were taken for histologic examination and histofluorescence. At the beginning of the study, tissues for microscopy were fixed in buffered 10% formalin, while those for histofluorescence were quick-frozen in dry ice-isobutane. Because of the small size of portions of the reproductive tract, this procedure was soon modified. Frozen tissues were sectioned for both histologic examination and histofluorescence. The frozen blocks were then fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin, sectioned at 6  $\mu$ , and stained with hematoxylin and eosin (H & E). Selected tissues were also stained by special methods including azure-eosin, Masson's trichrome, or reticulin. For histofluorescence, sections were cut on a cryostat microtome at 8  $\mu$ . They were kept in the dark for a short period at -20° C and were examined in a fluorescent microscope using an excitation wavelength of 490 nm. Positive fluorescence was of a light green color and faded after storage of tissues for more than 4 weeks.

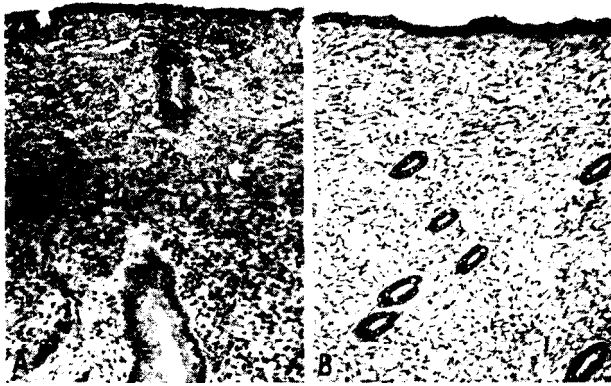
## RESULTS

### GROSS PATHOLOGY

No gross abnormalities were noted in the control animals or in the animals that were administered quinacrine by the intravascular route. In the three monkeys autopsied 24 hours after intrauterine quinacrine administration, there was marked yellow discoloration of the lumen of the reproductive tract, including the vagina, the cervix, the uterus, and the tube. In the uterus, this yellow discoloration extended for approximately 7 mm into the uterine wall. Blood clots were present in the lumen of the uterus in two of the animals, and there was marked edema of the cervix of one of them. The yellow discoloration of the fallopian tubes was of variable extent but in most animals extended about two-thirds of its length.

### MICROSCOPIC EXAMINATION

While some incidental findings were observed, no pathologic change attributable to quinacrine treatment was found in any nonreproductive tissue examined. No significant abnormality in the



**Figure 1**  
Endometrium following intrauterine administration of 30 mg quinacrine. (A), Twenty-four hours after quinacrine administration the entire superficial epithelial lining is necrotic. The necrosis partially extends into the stroma and glands, but underlying tissue remains viable. Necrotic tissue is recognized by clumps of nuclear debris in affected areas. (B), Twenty-eight days after quinacrine administration there is a total regeneration of glands, stroma, and uterine epithelium (original magnification, x 180).

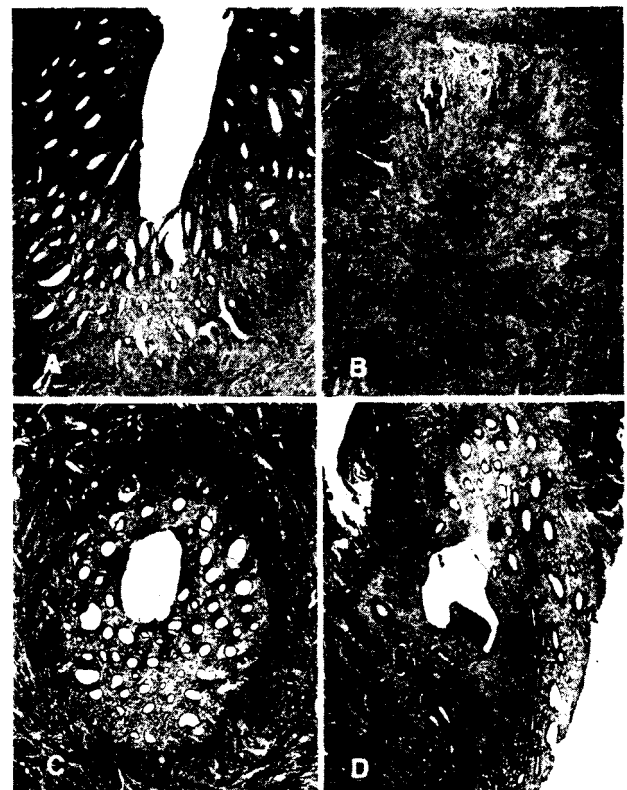
reproductive tract was seen in the animals administered quinacrine via the intravascular route or in those that received intrauterine saline. In contrast, the animals that were examined 24 hours after intrauterine administration showed extensive areas of superficial necrosis of the endometrium. This necrotizing process appeared to affect the entire epithelial layer of the endometrium (Fig. 1). Necrosis extended partially into the stroma, but the underlying tissue remained viable (Fig. 1A). Sections of the cornual regions (Fig. 2) showed the lumen obliterated by necrotic tissue debris (Fig. 2B). One of the monkeys also had marked necrosis of the superficial layers of the endocervix. This was the same animal that, grossly, was seen to have cervical edema. In two monkeys there was focal or individual cell necrosis of the intramural section of the tube. However, the latter changes were not nearly as marked as those occurring in the endometrium. Focal necrosis was observed in the epithelium of the isthmic portion of a tube in one monkey, and necrosis of individual cells was observed in the ampullary portion of another monkey.

In monkeys examined 1 week after receiving intrauterine quinacrine there had been extensive regeneration of the endometrial epithelium. One animal showed papillary hyperplasia of the isthmus with cystic dilatation of the ampullary portion of the tube above it. The uterine glands were dilated and contained eosinophilic debris, but these changes were relatively mild. The cornual

area of the endometrium showed evidence of mild scarring and dilatation of glands as well (Fig. 2C). One animal showed evidence of resolving peritonitis around the ovary and ampulla. There were no necrotic lesions seen in any of the animals examined at this time.

In monkeys examined 4 weeks after receiving the intrauterine quinacrine, repair of endometrium was evident (Fig. 1B). Foci of scarring were seen in the uterus in two animals. There was mild submucosal scarring around the cornual area in two of these monkeys as well (Fig. 2D).

In some sections, histofluorescence could be identified, but the technique was inconsistent,



**Figure 2**  
Effect of intrauterine quinacrine on uterus near the cornual region. (A), Control, epithelial lining is simple cuboidal. Tubular glands radiate from the lumen. (B), Twenty-four hours after administration of quinacrine there is total obliteration of the uterine lumen, which is filled with RBCs and necrotic debris. (C), One week after administration of quinacrine there is a thin layer of epithelial cells. The glands are slightly dilated with a thin layer of epithelial cells. There are small foci of inflammatory cells (arrows) within the stroma. There is no evidence of necrotic tissue at this time. (D), Twenty-eight days after administration of quinacrine there is extensive regeneration of the uterine glands with higher columnar epithelium. One area of the uterine submucosa has interstitial fibrosis, indicated by a relative lack of cellularity (arrow) (original magnification, x 50).



**Figure 3**  
Histofluorescence in the uterus 24 hours after intrauterine administration of quinacrine. Fluorescence is localized in the epithelium and stroma, predominantly in nuclei.

and in some tissues where quinacrine was detected biochemically, histofluorescence was not apparent. However, when histofluorescence was observed in the endometrium, it was concentrated in the nuclei of epithelial and stroma cells (Fig. 3). Where there was obvious tissue necrosis, there was no nuclear stain.

Histofluorescence was also localized in the epithelium of the intramural section of the tube. While monkeys receiving intravascular injections of quinacrine and examined 1 day later demonstrated no lesions in the reproductive organs, histofluorescence could be observed in the epithelium and stroma of the endometrium and in the tubal epithelium. Histofluorescence in nonreproductive tissue also could be observed; most notable were the liver and spleen, 24 hours after intravascular administration.

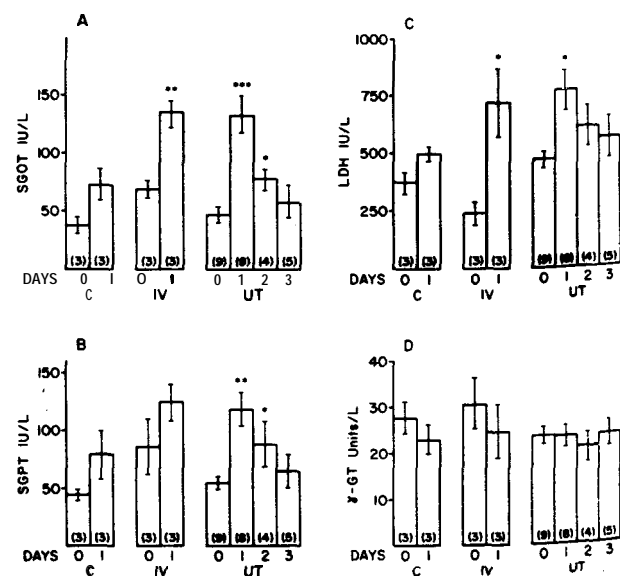
#### BLOOD CHEMISTRY AND HEMATOLOGY

SGOT was elevated at 24 hours (Fig. 4A) in monkeys receiving intrauterine quinacrine ( $133.4 \pm 16.7$  IU/l versus  $47.7 \pm 6.4$  IU/l;  $P < 0.001$ ). Levels were still elevated at 48 hours ( $77.5 \pm 10.2$  IU/l;  $P < 0.05$ ), but were normal by 72 hours. SGOT was also elevated in animals receiv-

ing intravascular quinacrine at 24 hours ( $133.7 \pm 10.2$  IU/l versus  $70.3 \pm 7.5$  IU/l;  $P < 0.01$ ). There was a tendency toward elevated levels of SGOT in saline-treated monkeys as well, but the changes were not significant ( $73.3 \pm 14.1$  IU/l versus  $38.3 \pm 7.8$  IU/l).

SGPT was also elevated significantly (Fig. 4B) in animals receiving intrauterine injections 1 day after treatment, compared with the pretreatment level ( $118.4 \pm 15$  IU/l versus  $55.3 \pm 4.1$  IU/l;  $P < 0.01$ ). At 48 hours, the concentration was  $87.5 \pm 18.1$  IU/l, still significantly elevated over pretreatment values. Within 72 hours, the concentration had returned to normal. Elevated concentrations at 24 hours were also seen in monkeys that received intravascular injections of quinacrine ( $124.0 \pm 15.9$  IU/l versus  $86.0 \pm 24.8$  IU/l) and also in animals receiving intrauterine saline ( $80.0 \pm 21.0$  IU/l versus  $45.6 \pm 4.9$  IU/l); however, neither of these differences were significant. The lack of significance in these latter groups may be due to the smaller number of observations in these treatment groups, compared with the group treated with intrauterine quinacrine.

LDH was elevated at 24 hours (Fig. 4C) in monkeys that received intrauterine quinacrine ( $767.9 \pm 96.6$  IU/l versus  $474.0 \pm 34.5$  IU/l;  $P < 0.05$ ) and intravascular quinacrine ( $737.0 \pm 151$



**Figure 4**  
Effect of treatment on various serum enzymes: (A) SGOT, (B) SGPT, (C) LDH, (D) GGT. Day 0 is the pretreatment level. Treatment groups: C, saline-treated control; IV, intravascular injection of quinacrine; UT, intrauterine administration of quinacrine. \* $P < 0.05$  versus pretreatment level. \*\* $P < 0.01$ . \*\*\* $P < 0.001$ .

## DISCUSSION

The present study indicates that 30 mg intrauterine quinacrine solution administered to cynomolgus monkeys can produce initial morphologic changes similar to those described in humans.<sup>3</sup> Lesions were observed that obliterated the cornual areas of the uterus and thus the openings into the tube. Extensive necrosis of the endometrium and at times the endocervix was also observed, leading to the conclusion that the necrotizing process is not a lesion specifically localized to the cornua. Rather, the prompt regeneration of much of the endometrium restores its normal configuration. It is postulated that the necrotic surfaces of the cornual area, which are apposed to each other, may tend to heal by scarring, with subsequent obliteration. Scarring was observed in the endometrium near the cornua and in the intramural tube, but complete morphologic obliteration was only observed in one instance. Seven days after instillation of quinacrine, obstruction with cystic dilations of the isthmus was found unilaterally in one animal.

Quinacrine was localized in nuclei of epithelial and stromal cells of endometrial tissue and in the epithelial cells of the intramural section of the tube. This is consistent with reports that quinacrine intercalates with deoxyribonucleic acid (DNA).<sup>4</sup> Where necrosis was obvious, there was no histofluorescence observed. The inability to see histofluorescence in a tissue did not necessarily mean it was not present, but only that the technique lacked sensitivity or specificity. In many cases quinacrine was detected by spectrophotofluorometric assay<sup>2</sup> without fluoromicroscopic observation.

When intravascular quinacrine was administered, no histologic damage to any reproductive tissue was observed at 24 hours, even though histofluorescence could be identified in the nuclei of endometrial and tubal epithelium, suggesting that the damage caused by intrauterine quinacrine at 24 hours resulted from effects other than binding with DNA.

Quinacrine was concentrated in all tissues examined, as detected spectrophotofluorometrically,<sup>2</sup> whether the drug was administered via the intravascular or the intrauterine route. At 1 week after intrauterine injection, levels were generally lower than those observed at 24 hours but still detectable in most tissues. By 1 month after injection, all tissue levels of quinacrine were at or near the limit of detection.

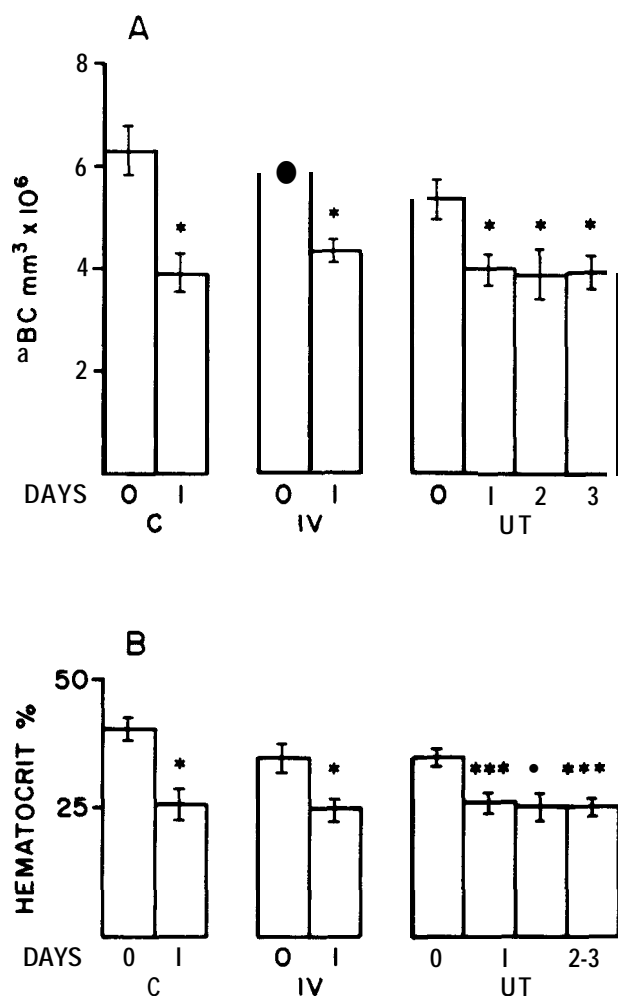


Figure 5  
Effect of treatment on RBC count and hematocrit values. Day 0 is the pretreatment level. C, saline-treated control; IV, intravascular injection of quinacrine; UT, intrauterine administration of quinacrine. \* $P < 0.05$  versus pretreatment level. \*\* $P < 0.01$ . \*\*\* $P < 0.001$ .

IU/l versus  $240.7 \pm 47.4$  IU/l;  $P < 0.05$ ). In the animals that received intrauterine quinacrine, there was no significant elevation after 24 hours. While LDH concentrations were higher in saline-treated monkeys at 24 hours, the difference was not significant ( $506.0 \pm 27.3$  IU/l versus  $381.6 \pm 42$  IU/l).

In contrast to the previously discussed enzymes, GGT showed no changes following the injection of quinacrine (Fig. 4D).

A significant decrease in RBC count and hematocrit occurred 24 hours after all treatments (Fig. 5). There were no other changes in serum chemistry or hematologic measurements that could be attributed to quinacrine administration.

Quinacrine binding could be observed **histofluorometrically** in several nonreproductive tissues 24 hours after administration of quinacrine. However, no histopathologic change attributable to quinacrine was observed in any organ examined up to 28 days after treatment.

It should be noted that the dose of quinacrine (30 mg/ml) administered to the monkey was determined by its solubility in water and the volume the intrauterine cavity could hold. This dose is approximately twice the dose administered at one time to women on a body-weight basis. While pellets have been used in most recent clinical procedures,<sup>5</sup> we chose an aqueous solution in these studies. We thought this would represent a "worst case" condition, in that the maximum amount of drug would be absorbed, and thus would be more suited to toxicity testing of the drug. More profound effects on the reproductive tract might be expected with pellets; and, in fact, greater efficacy rates have been demonstrated with pellets after a 1-year follow-up, compared with slurry administration.

The blood chemistry data suggest that a moderate and transient change of some enzymes thought to reflect liver function (SGOT, SGPT) occurs following the treatment procedure. Since the control animals showed changes in the same direction, the effects observed may, in part, be due to the procedural factors rather than a specific quinacrine effect. Also, these enzymes are not exclusively liver-specific and may reflect changes in other organs. It is notable that GGT, another enzyme with high liver specificity,<sup>6</sup> did not change in any of the treatment groups. LDH showed transient increases in the quinacrine-treated monkeys. No other biochemical change attributable to quinacrine was noted.

The RBC count and hematocrit values decreased in both treated and control groups. These hematologic changes are probably due to the sequential bleeding of the monkeys for the determinations. Although the decrease in RBCs occurred in all groups, including those receiving saline, it should be noted that long-term quinacrine administration has been associated with **aplastic anemia.** In our monkeys, **histopathologic** observation revealed normal bone marrow regardless of treatment. Ketamine hydrochloride has been shown to cause a reduction in **hematocrit** values in rhesus monkeys.<sup>7</sup>

In summary, except for changes in the reproductive tract following intrauterine quinacrine,

up to 1 month after administration of the drug, there was no histopathologic change in other organs that could be attributed to quinacrine. There were some moderate enzyme changes observed in serum that could have been treatment-related, but these were transient.

For the conditions described in these studies, intrauterine administration of quinacrine would appear to be a safe procedure. However, certain findings exist that may preclude its acceptability. A "cerebral excitation syndrome" has been reported following both **oral**<sup>9</sup> and intrauterine administration.<sup>5</sup> There have been no documented reports of death following intrauterine administration of quinacrine to women. However, a recent publication has demonstrated that high doses of quinacrine suspension placed in the peritoneal cavity result in death in rhesus monkeys,<sup>10</sup> although the same doses placed in the uterus were without effect. We have **confirmed** that high doses (250 mg) of quinacrine, in pellet form, when placed directly into the peritoneal cavity of cynomolgus monkeys can also result in death of these **animals.**<sup>11</sup> These studies became relevant when one considers that perforation of the uterus during application of the drug is a possibility and would result in the accidental peritoneal application of the drug.

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