

## BIOLOGIC CHANGES INDUCED BY UNILATERAL INTRAUTERINE INSTILLATION OF QUINACRINE IN THE RAT AND THEIR REVERSAL BY EITHER ESTRADIOL OR PROGESTERONE\*

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In previous studies<sup>1</sup> we have instilled cytotoxic agents and metabolic depressants into the endometrial cavity of rats, and have thereby induced changes in the endometrium which have resulted in reduced fertility. It was suggested that this reduction of fertility was due to an endometrial environment which was inadequate for implantation.

Two different types of histologic changes have been observed. (1) Some chemical agents, such as cadmium and thio-TEPA (N, N', N''-triethylene thiophosphoramide) have a marked capacity for inhibiting implantation, although they induce only *slight* morphologic alterations of the endometrium. Similar results were produced by physical noxia, such as freezing of the endometrium.<sup>2</sup> In the latter instance, depression of the capacity for implantation could be reversed by local application of histamine.<sup>3</sup> (2) Other cytotoxic substances such as monoiodacetate and ethanol produce *intense* morphologic changes which result in complete obliteration of the uterine lumen. The reduction of fertility in these animals was attributed primarily to mechanical obstruction.<sup>1</sup> The effects of cytotoxics as measured by implantation rates were reversible, except when extensive cicatricial lesions totally blocked the treated horn. Most of the drugs employed were inadequate for use in humans because of their potential toxicity.

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In this series of experiments, we used only those cytotoxic drugs which might be safely employed in humans and which induced alterations in the endometrium of the rat when introduced by the specific technic employed. The effect(s) of these drugs upon the tubal mucosa will be reported in another communication.

Quinacrine, a derivative of acridine, was chosen for this present study because there is clinical evidence which indicates that this substance is harmless in humans when injected directly into the peritoneal or pleural cavities for the purpose of controlling effusions caused by ovarian or endometrial metastasis.<sup>4</sup> Its carcinostatic properties have also been demonstrated *in vitro*<sup>5</sup> and *in vivo*.<sup>6</sup> The present paper describes the changes produced by a single instillation of a suspension of this drug into the uterus of the rat.

### MATERIALS AND METHODS

Albino rats of a nonisogenic strain, weighing about 200 gm. each, were used. Under tribromethanol (Avertin) anesthesia, 0.2 ml. of an aqueous suspension of quinacrine was instilled into the right uterine horn following a technic reported previously.<sup>1</sup> This suspension represents a combination of a concentrated solution and a suspension of quinacrine in water. This volume of suspensions remained the same in all the experiments.

*Experiment A. Search for an Effective Dose.* The drug was tested in dosages<sup>5</sup> which ranged between 5 and 40 mg. of quinacrine. After a single instillation, the

animals of each group were mated with males (4-5 females per male) at different intervals after treatment. Vaginal smears were obtained daily to determine the cycle phase and to detect the presence of sperm. Implantation sites were recorded and corpora lutea were counted on the 9th or 10th day post coitus.

**Experiment B. Search for Blastocysts.** In another group of rats, injected with either 20 or 40 mg. of quinacrine, each uterine horn was flushed twice with saline on the 5th day after coitus and the washings were searched for blastocysts. In addition, corpora lutea were counted.

**Experiment C. Effect of Exogenous Ovarian Hormones on the Changes Induced by Quinacrine.** The right uterine horn of each rat was instilled with 20 mg. of quinacrine. Either an estrogen or a progestogen was administered in varying dosages at different times subsequent to the instillation of quinacrine. Each estrogen injection contained 50  $\mu$ g. of estradiol benzoate, and each injection of progestogen contained 500  $\mu$ g. of progesterone. All the animals were sacrificed 10 days after the last hormone injection. Four groups of animals were studied. The design of the four groups is shown in Table 1.

**Experiment D. Histologic Studies.** Transverse sections of the uterine horns

were stained with hematoxylin and eosin, and were studied microscopically.

#### RESULTS

Tables 2, 3, and 4 summarize the effects of different doses of quinacrine upon implantation and give some indication of the capacity for spontaneous recovery. Doses below 20 mg. did not alter significantly the fertility of the rat (Table 2).

In Table 3 it can be seen that after instillation of quinacrine at a dosage of 20 mg., there is a gradual time-related recovery with regard to the number of implantations. The rate of recovery closely parallels the restoration of uterine patency. The effect obtained with 40 mg. is more marked and persistent (Table 4).

Approximately 50% of the blastocysts were recovered in the untreated horns of rats instilled with 20 or 40 mg. of quinacrine. This incidence of recovery is based upon the number of corpora lutea found on the ovary of the ipsilateral horn. There was no significant difference between the number of corpora lutea present on either ovary. The recovery of blastocysts in the treated horn was impractical while mechanical obstruction persisted. Recovery

TABLE 2. Rate of Implantations in the Right Horn\* of Rats Instilled with 5 and 10 mg. of Quinacrine, followed for 60 Days

	5 mg.		10 mg.	
	Right horn	Left horn	Right horn	Left horn
	7	1	2	4
	2	5	5	2
	4	3	6	2
	0	8	0	6
	7	3	4	6
	4	2	4	4
	6	6	0	3
	5	4	0	4
	2	5	0	3
	8	3		
Average	4.5	4.0	2.3	4.0

\* Left uterine horn as control.

TABLE 1. Experimental Design to Study the Effect of Exogenous Ovarian Hormones on Endometrial Changes Produced by Quinacrine

Group	no. of rats	Exogenous hormone	Dose	Interval between quinacrine and hormone administrations
1	7	Estradiol benzoate	50 $\mu$ g.	24 hr.
			50 $\mu$ g.	96 hr.
2	5	Estradiol benzoate	50 $\mu$ g.	25 days
			50 $\mu$ g.	28 days
3	6	Progesterone	500 $\mu$ g.	24 hr.
			500 $\mu$ g.	96 hr.
4	5	Progesterone	500 $\mu$ g.	25 days
			500 $\mu$ g.	28 days

TABLE 3. Rate of Implantations in the Right Horn\* of Rats Instilled with 20 mg. of Quinacrine, followed for 104 days

	1st month		2nd month		3rd month		4th month	
	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn
	0	0	0	9	3	9	4	5
	0	6	1	1	0	5	6	5
	0	0	0	3	0	3	1	0
	4		2	7	1	7		
	0	7	0	6	4	5		
	0	3	2	6	0	6		
	2	2	3	2		6		
	0	5	0	2	4	5		
	0	0	0	0	0	5		
	0	0	5	4				
	0	4						
	3	4						
	0	2						
	0	7						
	0	4						
	3	4						
	0	3						
	0	3						
	0	2						
	0	4						
	0	3						
	0	5						
	0	4						
	1	3						
	0	5						
Average	0.5	3.2	1.3	4.0	1.4	5.6	3.6	3.3

\* Left uterine horn as control.

of eggs from the fallopian tubes was not attempted.

Figure 1 illustrates the histologic changes present at different intervals after injection of 20 mg. of quinacrine. Quinacrine induced slight morphologic changes of the endometrium when given in a dosage of 10 mg. The histologic response produced by 20 mg. was that of complete obliteration of the lumen of the uterus, and consisted of a very exuberant foreign body giant cell reaction interspersed with stromal cells. These large foreign body giant cells contained clumps of crystalline and amorphous quinacrine. This particulate material persisted for long periods of time in chronic cases. Slight fibrosis and moderate infiltration by eosinophils and neutrophils were observed in the deep layers of

the endometrium. The infiltration by neutrophils extended into the superficial myometrium. The histologic alterations present in the group of animals which received 20 mg. reverse spontaneously in most cases after a period of about 3 months, whereas changes produced by 40 mg. persist.

When estradiol or progesterone was administered within the 1st week after treatment with 20 mg. of quinacrine (Table 2, Groups 1 and 2) and the animals were sacrificed in the 2nd week after instillation the uterine epithelium showed the typical changes induced by either estrogen or progestogen, and the uterine lumina were patent (Fig. 2).

When one or the other of these hormones was administered at the end of the 1st

TABLE 4. Rate of Implantations in the Right Horn\* of Rats Instilled with 40 mg. of Quinacrine, followed for 130 days

1st month		2nd month		3rd month		More than 3 months		
Right horn	Left horn	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn	
0	1	0	2	0	3	0	7	
0	4	0	5	0	0	0	4	
0	3	0	7	0	0	5	5	
0	10	0	2	0	0	0	4	
0	3	0	5	0	4	0	3	
0	0	0	5	0	4	0	2	
0	4	5	3	2	5	0	2	
0	3	0	4	0	6	0	3	
0	3	4	4	0	4	0	1	
0	6	0	5	0	7	0	5	
0	6	0	3			0	4	
0	5	0	2					
0	4	0	4					
0	4	0	6					
0	0	0	2					
0	4	0	4					
0	4							
0	4							
Average	0	3.7	0.5	3.9	0.2	3.3	0.4	3.6

\* Left uterine horn as control.

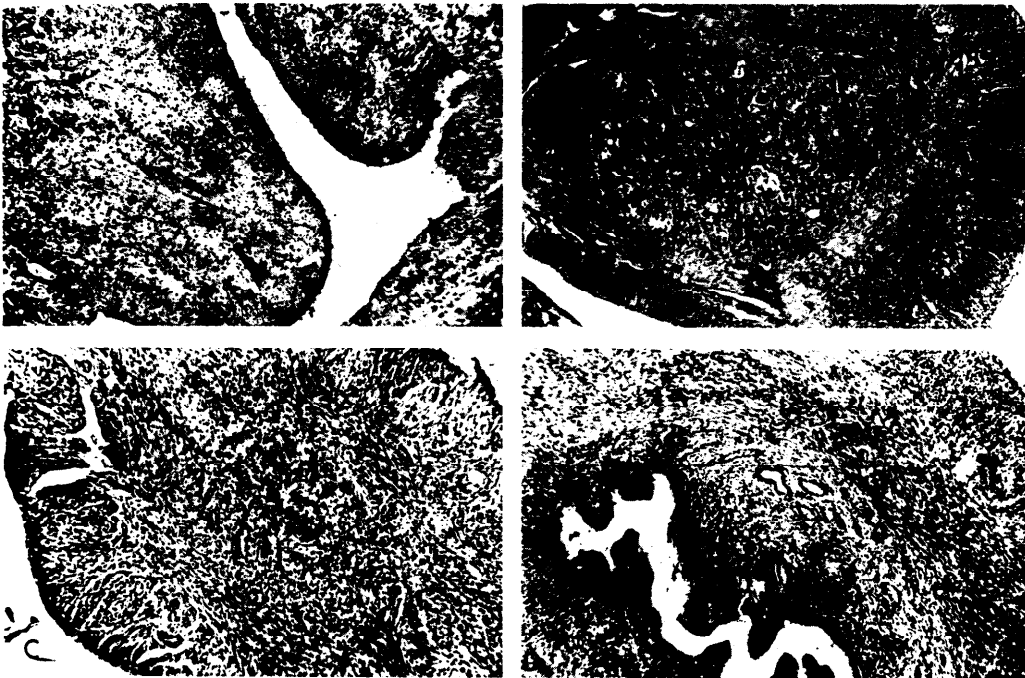


FIG. 1. Sections of rat uteri instilled with 20 mg. of quinacrine. A, 24 hr. after instillation ( $\times 100$ ); B, 2nd week after instillation ( $\times 70$ ); C, 2nd month after instillation ( $\times 70$ ); D, 4th month after instillation ( $\times 70$ ). Note spontaneous recovery of the lumen.

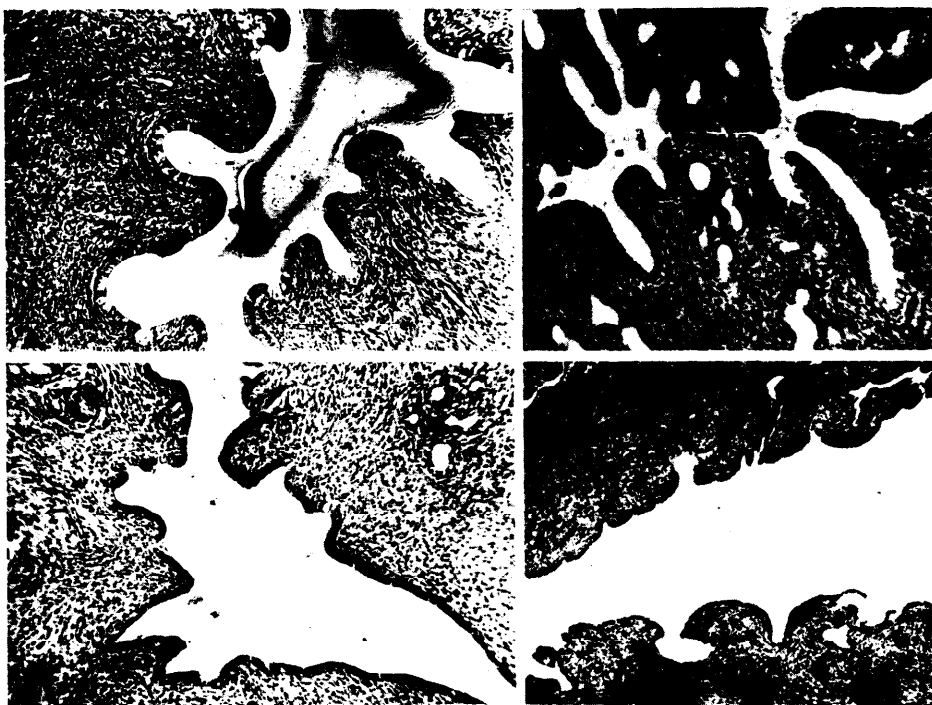


FIG. 2. Effect of hormonal treatment after 20 mg. of quinacrine administration ( $\times 100$ ). A, estrogen 2nd week (Experiment C, Group 1); B, estrogen 2nd month (Experiment C, Group 2); C, progesterone 2nd week (Experiment C, Group 3); D, progesterone 2nd month (Experiment C, Group 4).

month after quinacrine instillation (Table 2, Groups 3 and 4), recovery from the characteristic histologic alterations occurred in, practically all instances (Fig. 2).

#### DISCUSSION

In these experiments, we have been able to show that when quinacrine is instilled into the uterine horn of the rat a proliferative reaction is induced in the endometrium, and a complete obstruction of the lumen often occurs. This pharmacologic effect of quinacrine identifies it among the group of drugs capable of producing a decrease in fertility due to obstruction of the uterine lumen. Since quinacrine in quantities of 100-200 mg./ml. is a suspension, the role of particulate matter in producing the tissue reaction needed to be assessed. In order to study this phenomenon we prepared a suspension of talc in distilled water and instilled 0.2 ml. (200 mg. of talc/ml.) into the rat uterus. Seven

rats were included in this experiment. The animals were mated during the 1st month postinstillation. In each instance there was a normal number of bilateral implantations. Study of the uteri under light microscopy revealed no morphologic changes. It is our impression from this experiment that the morphologic changes induced in the rat uterus by a suspension of quinacrine cannot be due primarily to mechanical factors incident to the presence of particulate matter. A complete report of the role of particulate material in this phenomenon will be presented in another publication.

The action of quinacrine can be prevented by an estrogen or a progestogen when one or the other is injected within a short time after the quinacrine instillations. The luminal patency is restored when these hormones are administered to chronically obstructed uteri. Under similar conditions we have been unable to obtain any effect on the implantation rate of rats when we

used another antimalarial drug, chloroquine.<sup>7</sup> It is possible, therefore, that the obstructive effect of quinacrine may be independent of its antimalarial action.

The effect of quinacrine at the cellular level may be due in part to its interference with nucleic acid metabolism and the formation of well-defined metachromatic complexes with deoxyribonucleic acid and ribonucleic acid.<sup>8</sup> Acridine derivatives induce frame-shift mutants and have been used to demonstrate the triplet nature of the genetic code. The mechanisms of action of this class of chemicals on the endometrial cell in the rat and on the tubal epithelium in humans<sup>7</sup> could be due to molecular binding at the nuclear level in this specific type of cell. Reversal of these effects by estradiol and by progesterone may be attributed to the high competitive affinity of the cells of the target organ for these hormones. The relatively slow capacity of the endometrial cells to recover spontaneously could be due in part to the low level of the endogenous ovarian hormones found normally in the systemic circulation.

When quinacrine has produced obstruction of the uterine lumen, microcrystals and amorphous particles of this substance have been observed within the mass of foreign body giant cells for as long as 90 days after the last intrauterine instillation. It is logical, therefore, to assume that the prolonged effect of quinacrine may be due in part to the persistence or sequestration of the microcrystals within the pro-

liferative tissue.

#### SUMMARY

Intrauterine instillation of a suspension of quinacrine in the rat has been shown to induce a giant cell foreign body reaction in the endometrium and a consequent obstruction of the lumen. There is a long lasting decrease in fertility subsequent to this obstruction.

This effect can be inhibited or reversed by either estradiol benzoate or progesterone.

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