First and second generational effects of colchicine on C57 black mice
by Janice Kay Greathouse

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
Montana State University
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Abstract:
Colchicine was given to C57 black mice systemically before and during pregnancy. The mice and their resulting offspring were then assessed for changes in fertility, viability, weight, and chromosome damage. A marked decrease in the fertility of the mice treated before pregnancy was evident, as indicated by increased reproductive times, changes in uterine scars, and fewer live offspring. The offspring of animals treated before pregnancy had lower weights and fewer survivors. Treatment with colchicine during pregnancy did not dramatically decrease the fertility, viability, or weight of treated individuals or their offspring. Eleven percent of control mice and their offspring from both experiments had abnormal chromosome counts while thirty percent of treated mice and their offspring show abnormal karyotypes. The systemic route of administration and low dosages over prolonged periods of time replicate the usual human treatment with colchicine. The widely reported teratogenic effects of colchicine were not evident in this study.

FIRST AND SECOND GENERATIONAL EFFECTS

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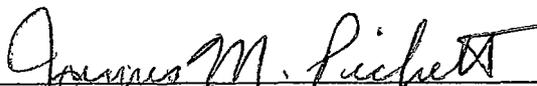of the requirements for the degree

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Graduate Dean

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ABSTRACT

Colchicine was given to C57 black mice systemically before and during pregnancy. The mice and their resulting offspring were then assessed for changes in fertility, viability, weight, and chromosome damage. A marked decrease in the fertility of the mice treated before pregnancy was evident, as indicated by increased reproductive times, changes in uterine scars, and fewer live offspring. The offspring of animals treated before pregnancy had lower weights and fewer survivors. Treatment with colchicine during pregnancy did not dramatically decrease the fertility, viability, or weight of treated individuals or their offspring. Eleven percent of control mice and their offspring from both experiments had abnormal chromosome counts while thirty percent of treated mice and their offspring show abnormal karyotypes. The systemic route of administration and low dosages over prolonged periods of time replicate the usual human treatment with colchicine. The widely reported teratogenic effects of colchicine were not evident in this study.

INTRODUCTION

Colchicine has been used since the sixth century A.D. for the treatment and prophylaxis of acute gouty attacks. Its effectiveness in reducing inflammation of gouty arthritis is well documented (Yu and Gutman, 1961), though the complete mechanism of its action is still unknown.

One proposed mechanism of action is the prevention of lysosomal-phagocytic vacuole formation by colchicine. In vitro and in vivo studies by Malawista (1975) indicate that urate crystals may be coated with a plasma derived material which is degraded when the lysosomal enzymes are released into the phagocytic vacuoles of polymorphonuclear leukocytes (responsible for the uptake of urate). These uncoated crystals are then hypothesized to perforate the vacuole and release digestive enzymes into the cytoplasm with the ensuing death of the cell. This release of digestive enzymes from the polymorphonuclear leukocytes into the synovium then propagates the inflammatory response and theoretically precipitates an acute gouty attack. Colchicine decreases the fusion of lysosomes with the phagocytic vacuoles of the polymorphonuclear leukocytes and this prevents the urate crystal destruction of the lysosomes. It is possible this response of the polymorphonuclear leukocytes is responsible for the relative specificity of colchicine in preventing gouty arthritis

but not other forms of arthritis. This response may be caused by the prevention of microtubule formation through colchicine's spindle arresting properties. Colchicine also inhibits the release of histamine, though whether or not this is pertinent to gouty attacks is debatable.

Wallace (1975), however, points out that tri-methylcolchicinic acid (TMCA), an analog of colchicine that is also effective as a gout prophylactic agent, does not have the microtubular effect of colchicine, and therefore is not a mitotic arrester. This also would argue that colchicine's anti-gout properties may not be related to microtubular inhibition.

In addition to gout, colchicine is now being recommended as a possible treatment for additional diseases. The suggested uses for colchicine include its administration to victims of familial Mediterranean fever (Goldfinger, 1972), phlebitis (Giorgi and Raimondi, 1972), psoriasis (Kaidbey, Petrozzi, and Kligman, 1975), dysmenorrhea (Hebert and Gros, 1960), muscle relaxation during surgery (Pasani, 1968), and embryonic rubella (Katsilambros, 1963). In addition, colchicine has been suggested as an anti-tumor agent due to its anti-fibrotic and anti-mitotic action (Loader and Nathaniel, 1977). Suggestions for treating such a large group of diseases

are no doubt made under the basic assumption that the drug
has been proven safe over centuries of use.

The adverse effects of colchicine, however, are also
well documented. Hair loss, gastro-intestinal disturbances,
and bone marrow disruption are frequent side effects of
colchicine therapy in humans (e.g., Carr, 1965; Dittman,
1959). In addition, there is the possibility that col-
chicine has other less well-known, side effects. Studies
with laboratory animals have shown the possibility of
longer range side effects. Intraperitoneal injections
of colchicine have caused large-scale deformities in
mice (Ingalls, Curly, and Zappasodi, 1968) and hamsters
(Ferm, 1963). Ingall's injections of 0.5 to 2 mg
colchicine/kg body weight into pregnant mice 3.5 to 7.5
days after mating resulted in cranio-facial malformations
in the developing offspring. The period around 6.5-7.5
days was especially toxic to embryos at the time of im-
plantation. Ferm injected colchicine into pregnant
hamsters. Hamsters are resistant to colchicine in the
adult animal and fetal cell cultures, but regardless of
this resistance, the embryos showed a high mortality rate
and numerous abnormalities similar to those found in mice.
Vaccarazza (1973) reports that colchicine injected in-
travitreously can lead to eye disfunctions and blindness.

Additionally, colchicine has been reported to cause various problems with fertility in both humans and laboratory animals. At least one case of human azoospermia from colchicine therapy has been documented (Merlin, 1972). Poffenbarger and Brinkley (1974) found similar results in mice and hamsters upon injecting colchicine subcutaneously. In a study by Handel (1979) histological changes in the spermiogenesis of mice was noted upon treatment with varying concentrations of colchicine. Colchicine affected both seminiferous tubule cells and the mature spermatids adversely. Seminiferous tubule cells showed differential sensitivity. Spermatids have misshapen heads, presumably due to inhibition of the microtubules needed to create the proper configuration. Anovulation in humans has been noted in two studies using healthy volunteers (Board, 1964; Malkinson and Lynfield, 1959).

There is also the possibility that chromosomal aberrations may occur in offspring of gout patients treated with colchicine (Cestari, 1965; Ferreira and Buoniconti, 1968; Ferreira, 1973; Hansteen, 1968). Cestari, et al report of a patient who fathered two abnormal offspring during long-term colchicine therapy. His karyotype during colchicine therapy showed polyploid cells

and endoreduplications, while sperm and karyotypes were
both normal after a three month discontinuation of col-
chicine therapy. Ferreira, et al report a significant
increase in the number of polyploid and aneuploid cells
in three patients on colchicine therapy compared with
matched controls. This work, however, has been highly
disputed among doctors in the United States for lack of
appropriate control groups with respect to the advanced
age of most gout patients (Walker, 1969; Hoefnagel, 1969;
Timson, 1969).

Many of these adverse effects were detected after
colchicine had been in use for hundreds of years. As a
result colchicine has not undergone the kind of intensive
testing to which new drugs are subjected before being
allowed on the market. In light of recent findings on
long range effects of various other agents, further study
of colchicine is warranted. Diethylstilbestrol (DES),
for instance, has since been shown to lead to an increase
in the frequency of vaginal cancer in the daughters of
treated women who were given the drug to prevent miscarriage
(Herbst, et al, 1971). Reimers and Sluss (1978) have
shown a correlation between 6-mercaptopurine use and a
decrease in fertility in the F_1 females of treated mice.

Little research has been done with regard to the

first and second generational effects of colchicine. Yu and Gutman (1961) found seventeen children of colchicine treated fathers to "appear to be normal and healthy." One study in which pregnant women who contracted rubella were given colchicine reported that "the new-borns did not present any congenital malformation, although their weight was far below normal" (Katsilambros, 1963). In addition, in the same study, "a great number of women" were treated with colchicine, ignoring their pregnancy, "without any noxious effect (teratogenic abortion of infants or cardiac malformations)". Considering the extreme teratogenicity of colchicine, as evidenced in the studies of chickens, mice and other rodents (Araszkiewicz, Bartel, and Slawinski, 1973; Ingalls, Curley, and Zappasodi, 1968; Ferm, 1963), this is somewhat surprising. In order to deter rubella embryopathy, the colchicine would have to cross the placental barrier and enter the embryo's bloodstream. This directly contradicts the theory proposed by Hansteen (1969) that the reason few abnormalities in human offspring are seen is because colchicine does not cross the placental barrier.

There is, then, a discrepancy between studies using laboratory ainmals and those using humans. It is not immediately apparent what mechanisms may be involved in this

differential response; thus these discrepancies are worthy of investigation. It is possible that the routes of administration or the subsequent metabolism of colchicine have some effect on its teratogenicity in rodents. Furthermore, the animal studies have consisted of a single large dose injected intraperitoneally or intravenously, whereas gout patients classically receive a five mg tablet daily. An oral dose given daily could result in lower circulating concentrations of the drug with a longer time of action.

The metabolism of colchicine may also play a part in the teratogenicity of the drug. Species more sensitive to colchicine are known to metabolize the drug differently forming 0^{10}-demethylcolchicine (also known as colchiceine). It would also be interesting to see if humans form colchiceine, considering the apparent resistance of human embryos to colchicine teratogenesis. According to Schonharting, Mende, and Siebert (1974), "limited data. . .suggest that deacetylation (occurs) in man, but no metabolite has been described." Other metabolites of colchicine include 0^2-demethylcolchicine and 0^3-demethylcolchicine, the predominant metabolites of less sensitive species. Schonharting, et al have hypothesized that colchicine may have a formaldehyde molecule driven off during formation of 0^2 and 0^3-demethyl-

colchicine. Considering recent information on the mutagenicity and toxicity of formaldehyde (Cooper, 1979; Lancet, 1979), this could well be worth investigating.

Colchicine also changes the activity of numerous metabolic pathways (Singh, LeMarchand, Orci, and Jeanrenaud, 1975). The amount of circulating proteins, glucose, and triglycerides in mice were all decreased by a single injection of colchicine. The decrease of circulating proteins, glucose, and triglycerides prompted a response of increased liberation of free fatty acids from adipose tissue. Some of the degradation products of fatty acids are ketones, which have known embryotoxic effects. If indeed the levels of ketones increased, this could be a possible mechanism for induction of colchicine teratogenicity in mice. To test this hypothesis in humans the circulating ketone levels of gout patients or colchicine therapy versus those not on colchicine therapy could be compared.

Fleischmann, Russell, and Fleischmann (1962) have succinctly stated the problem of extrapolating between laboratory animals and humans. They observed: "Species differences in the toxicity of drugs emphasize the necessity to employ more than one species of experimental

animals in toxicity studies on new drugs before starting

clinical trials."

The objectives of this study therefore are to de-

termine if colchicine administration to mice before and

during pregnancy affects the fertility or genetic consti-

tution of the adults or offspring, or the viability

and weight of the offspring. To simulate the human

condition as much as possible dosages adjusted to the

metabolic rate and weight of a mouse (Kleiber, 1961) will

be given systemically for a prolonged period of time.

MATERIALS AND METHODS

This research was divided into two parts to analyze the effects of colchicine given both before and after fertilization on the offspring of C57 black mice. The first experimental groups were treated prior to mating, while in the second experiment the animals were treated after mating. Food and water were given ad libitum.

Experiment One:

C57 black mice, aged 4 to 6 months, were treated for 36 days with a 0.03% solution of colchicine in saline. The drug was administered in a dosage of 1.2 mg/kg body weight. The first nine doses were injected subcutaneously, but due to skin sloughing at the injection sites had to be discontinued. The following twenty-one doses were given orally in a paste of 100% whole wheat bread and reconstituted non-fat dry milk. Every fifth day the treated mice received the paste, without colchicine, to prevent weight loss, which initially occurred due to the gastro-intestinal effects of colchicine, i.e., diarrhea.

The groups were divided as follows with 25 males and 25 females in each group:

    i.   untreated female x untreated male (C)
   ii.   untreated female x treated male (TM)
  iii.   treated female x untreated male (TF)
   iv.   treated female x treated male (TB)

After treatments were accomplished, the animals were assigned randomly to mating groups. Each male was mated with four females from the appropriate groups. The females were removed as soon as pregnancy was detected by visual observation. Half of the females in groups iii and iv received an additional seven days of treatment, ten days after being placed with the males, in the hope of additionally treating the embryos in utero. This group will be subclassed "a", with those not additionally treated called "b". In experiment one, the reproductive time was defined as the length of time from initial pairing to parturition of the first litter.

Experiment Two:

The females in this portion of the experiment were ten weeks of age. The males were eight months of age and taken from the "proven" matings of the untreated groups above. Fifteen males were each mated randomly with three females in estrus cycle, determined by vaginal smears. Lettuce was given daily in addition to the standard diet as a vitamin supplement. Females were checked daily and assumed to be pregnant when a vaginal plug appeared.

Forty females were randomly assigned to control or treatment groups as follows:

    i.   control, wheat paste for 20 days (C_{20})
   ii.   control, wheat paste last 8 days (C_8^{20})
  iii.   treatment, wheat paste for 5 days,
             wheat paste and colchicine 0.5 mg/kg
             body weight for 15 days ($T_{0.5}$)
   iv.   treatment, wheat paste and colchicine
             1.0 mg/kg body weight for last 8 days
             gestation ($T_{1.0}$)

The wheat paste was prepared as in Experiment One. Since
the gonads start to differentiate oogonia and spermato-
gonia twelve to fourteen days after conception, the last
group was intended to overlap specifically this time
period.

In both experiments the offspring were counted and
any abnormalities noted on the day of littering. At one
month of age the offspring were again counted, sex was
noted, and mean viability determined.

Chromosome Preparation:

The chromosome technique employed follows that of
Patton (1967) with modifications. Prior to sacrificing,
0.01 ml/gm body weight of 0.05% colchicine solution was
injected into the animals intraperitoneally. At four hours
the animals were sacrificed by cervical dislocation and
bone marrow flushed from the femur with a 1.0% aqueous
solution of sodium citrate. The cells were then sus-
pended by aspirating vigorously and incubated at room
temperature for at least 10 minutes. The suspension was
centrifuged at 800 rpm for six minutes. The supernatant

was drawn down to 1/4 ml and the remaining button layered with 1.5 ml 0.075M potassium chloride and 1.5 ml distilled water (37°C). After gently resuspending the cells, they were incubated for 10 minutes at 37°C and then centrifuged for six minutes at 800 rpm. The supernatant was drawn down to 1/4 ml and 3 ml of fresh fixative (three parts methanol to one part acetic acid) was added. The cells were incubated at room temperature for 20 minutes before resuspending and centrifuging at 800 rpm for six minutes. The last wash was then repeated two more times. After the final wash the supernatant was discarded and the button resuspended in 1 ml of fixative. Four or five drops of cell suspension were dropped on chilled slides from a height of 24 inches. The slides were dried and stained in Giemsa for seven minutes, rinsed, dried and coverslipped.

Ovaries and uteri were fixed and cleared in benzyl-benzoate and implantation scars counted following the method of Orsini (1962). Briefly, counting the number of implantation scars tells one how many embryos implanted into the uterine wall. The number of uterine scars may be viewed as an indicator of the fertility available to a given animal. If the number of live births was greatly fewer than the number of implantation scars, then the dif-

ferences were likely to be non-viable offspring which were resorbed in utero or cannabilized at birth. It should be noted that this technique does not inform the investigator as to how many embryos were conceived but failed to implant.

Uteri were measured in mm. to determine if any general action had affected the reproductive tract. Testes were fixed in Bouin's solution. Ovaries and testes were then embedded in paraffin, sectioned, and viewed for histological normality, such as the number, size, and shape of oogonia, ova, spermatogonia, and presence of spermatids in seminiferous tubules.

Analysis of Data:

Data of a continuous nature (birth weights, size of uteri, number of offspring, uterine scars, and reproductive time) from both experiments were first analyzed using the Mann-Whitney U-test (also called the two sample rank test) as described by Goldstein (1967) due to the non-parametric nature of the data. The numbers of offspring and survivors were than analyzed using the Fischer's exact test. By using the Fischer's exact test the relationships between the offspring surviving and those not surviving and the embryos born and not born are determined. The Fischer's exact test was also used for analysis of

the non-continuous data (numbers of abnormal ovaries,
testes, and chromosomes).

RESULTS

Experiment One:

There were no significant differences between the "a" and "b" groups of Experiment One in any tests, therefore the data were pooled and will be treated as one group hereafter. The reproductive time, numbers of offspring, offspring surviving to one month, uterine scars, and percent survival of offspring are recorded on Table One. The reproductive time was shorter in the control group (i) than in groups iii (TF) [p < 0.01] and iv (TB) [p < 0.01]. Group ii (TM) followed the trend of a longer reproductive time but was not significantly different from the controls [p=0.11]. There were no significant differences in reproductive time between the treatment groups.

Significantly more uterine scars were found in group iii (TF) than in group iv (TB) [p=.011], though neither of these groups were statistically different from the controls (group i). It should be noted that this occurred by group iii (TF) having more uterine scars than the control [p<0.10], while group iv (TB) had somewhat fewer uterine scars than the control [p > 0.10].

No differences were detectable in the absolute number of offspring between any of the groups. If, however, the

TABLE ONE

Group	Female N	Mean Reproductive Time (in days)[1]	Mean Number of Offspring[2]	Mean Number of Survivors[2]	Percent Survivors	Mean Number of Uterine Scars[1]
i (C) Control	13	$45.9 \pm 8.9^{c+}_{d+}$	$3.5 \pm 0.9^{d+}$	$2.6 \pm 0.8^{b++}_{c*}{}_{d++}$	73.8	8.4 ± 0.9
ii (TM) Treated Males	8	65.4 ± 10.1	$5.1 \pm 0.9^{d*}$	$1.1 \pm 0.5^{a++}_{c++}{}_{d+}$	22.0	9.4 ± 0.5
iii (TF) Treated Females	15	$65.2 \pm 6.0^{a+}$	$5.5 \pm 0.7^{d++}$	3.3 ± 0.6	59.8	$11.5 \pm 1.5^{d*}$
iv (TB) Both Males and Females Treated	14	$101.4 \pm 14.5^{a+}$	$5.0 \pm 0.8^{a+}_{b*}{}_{c++}$	$2.2 \pm 0.7^{a+}_{b+}{}_{c*}$	44.3	$6.3 \pm 1.1^{c*}$

"a" designates significant difference at the stated p level from group i
"b" designates significant difference at the stated p level from group ii
"c" designates significant difference at the stated p level from group iii
"d" designates significant difference at the stated p level from group iv
"1" two sample
"2" Fischer's exact
 * $p < 0.05$
 + $p < 0.01$
++ $p < 0.001$

number of offspring is analyzed with respect to the number

of uterine scars (i.e., the total number of offspring pos-

sible) then significant differences between groups become

evident. Group iv (TB) had significantly more offspring

in proportion to the number of uterine scars than groups

i (C) [p < 0.01], ii (TM) [p=0.05], and iii (TF) [p< 0.001].

Therefore, the embryos of group iv (TB) were somewhat less

likely to implant than in the other groups (most noteably

group iii), but once implanted were more likely to be

born. This will be more fully discussed below.

Using the two sample rank test, more offspring of

group i (C) survived to one month of age than those in

group ii (TM), but this was not a significant difference

[p=0.11]. However, if the survival rates are compared to

the number of offspring born using a Fischer's exact

test, the groups fall into the following order. All

three treatment groups have proportionally fewer sur-

vivors than the control group. Within the treatment groups,

group iii (TF) had the best survival rate (59.8%), followed

by group iv (TB) (44.3%), with group ii having the poorest

survival (22.0%).

No abnormal testes (i.e., testes without spermatids)

were found in any of the adult mice in Experiment One. In

untreated female mice, four out of sixteen had histologic-

ally abnormal ovaries (with misshapen or absent ova) while
in treated female mice, five out of twenty-six had ab-
normal ovaries (see Appendix for example of abnormalities).
This is not a statistically significant difference.

Twenty-two cells in the control group were countable
with one cell showing an abnormal chromosome count.
Twenty-six cells of treated animals were countable with
seven cells showing abnormal complements. The control
group showed significantly fewer abnormal chromosome
complements than the pooled treatment groups [p< 0.05].

Table Two records the weights and size of uteri of
the offspring. The weights of the control group (i)
were greater than in groups iii (TF) [p < 0.05] and
iv (TB) though group iv was not significant at the
0.05 level of confidence [p=0.058]. Two histological-
ly abnormal ovaries (misshapen or absent ova) were found
in each of groups i (C), iii (TF), and iv (TB) and one
abnormal ovary was found in group ii (TM). The only testis
without spermatids found in the offspring of a group from
Experiment One was in the control group (i). One abnormal
karyotype was found in the eight cells scored from the
control group, and five abnormal karyotypes were found in
the 22 cells scored from the treatment groups. There were

Mean Weights and Uterine Size of Offspring, Experiment One

Group	Female N	Male N	Mean weight[1] of offspring (gm)	Mean size[1] of uterus (mm)
i (C) Control	6	16	$23.7 \pm 0.7^{c*}$	0.9 ± 0.04
ii (TM) Treated Males	1	0	18.1 ± 0.0	0.8 ± 0.00
iii (TF) Treated Females	12	13	$21.8 \pm 0.5^{a*}$	1.2 ± 0.05
iv (TB) Both Males and Females Treated	14	12	21.8 ± 1.1	1.1 ± 0.03

"a" designates significant difference at the stated p level from group i
"c" designates significant difference at the stated p level from group iii
"1" two sample
* $p < 0.05$

no significant differences found in the size of uteri,
numbers of abnormal gonads, or abnormal karyotypes be-
tween groups of offspring in Experiment One.

Experiment Two:

Table Three shows the maternal statistics which
include the mean numbers of offspring, offspring survival,
and uterine scars of Experiment Two. Three abnormal
ovaries were found from group ii (C_8) females, and two
abnormal ovaries were found in each of the treated
groups iii ($T_{0.5}$) and iv ($T_{1.0}$). There were no signifi-
cant differences between groups with regard to abnormal
ovaries. Two abnormal chromosome counts were found in
16 cells scored from group ii (C_8), while 10 abnormal
karyotypes presented from the 21 cells scored from group
iii ($T_{0.5}$). The control group showed fewer aberrant
karyotypes than the treated group [$p < 0.05$].

In Table Four is presented the weights and uterine
sizes of the offspring. Uteri were found to be larger
in groups i (C_{20}) and iii ($T_{0.5}$) than in group iv ($T_{1.0}$)
[$p=0.05$ and $p=0.04$, respectively]. The weights of group i
(C_{20}) offspring were greater than those of group ii (C_8)
[$p=0.05$] and weights of group iii ($T_{0.5}$) were significantly
greater than those of group iv ($T_{1.0}$) [$p=0.01$]. Two
abnormal ovaries were noted in group i (C_{20}), twelve in
group ii (C_8), seven in group iii ($T_{0.5}$) and two in group iv

TABLE THREE
Parental Reproductive Performance, Experiment Two

Group	Female N	Mean number[2] of offspring	Mean number[2] of survivors	Percent survival	Mean number of[1] uterine scars
i (C_{20}) Control	10	4.0 ±1.1	3.2 ±0.9[b*]	80.0	5.4 ±1.5
ii (C_8) Treated Males	7	5.0 ±1.3	4.9 ±1.3[a*d+]	97.1	7.0 ±1.2
iii ($T_{0.5}$) Treated Females	9	5.2 ±1.2	4.3 ±1.0	83.0	7.2 ±1.4
iv ($T_{1.0}$) Both Males and Females Treated	9	5.3 ±1.1	4.1 ±1.1[b+]	77.1	7.1 ±1.7

"a" designates significant difference at the stated p level from group i
"b" designates significant difference at the stated p level from group ii
"d" designates significant difference at the stated p level from group iv
"1" two sample
"2" Fischer's exact test
* $p < 0.05$
+ $p < 0.01$

TABLE FOUR
Mean Weights and Uterine Size of Offspring, Experiment Two

Group	Female N	Male N	Mean weight[1] of offspring (gm)	Mean size[1] of uterus (mm)
i (C_{20}) Control	17	15	$14.2 \pm 0.6^{b*}$	$0.6 \pm 0.04^{d*}$
ii (C_8) Treated Males	23	11	$13.0 \pm 0.4^{a+}$	0.6 ± 0.05
iii $(T_{0.5})$ Treated Females	18	21	$14.4 \pm 0.6^{d+}$	$0.8 \pm 0.10^{d*}$
iv $(T_{1.0})$ Both Males and Females Treated	18	15	$12.6 \pm 0.3^{c+}$	$0.5 \pm 0.03^{a*,c*}$

"a" designates significant difference at the stated p level from group i
"b" designates significant difference at the stated p level from group ii
"c" designates significant difference at the stated p level from group iii
"d" designates significant difference at the stated p level from group iv
[1] two sample
* $p < 0.05$
+ $p < 0.01$

$(T_{1.0})$. One testis without sperm was scored in each of groups ii (C_8), iii $(T_{0.5})$ and iv $(T_{1.0})$. Only three cells from the offspring control animals could be scored with no abnormal karyotypes presenting. Sixty-one cells from group iii $(T_{0.5})$ were scored with nine of these showing some sort of chromosomal abnormalities. There was no statistical difference between the number of abnormal ovaries, testes or chromosome counts of the offspring of Experiment Two.

DISCUSSION

The data from Experiment One seem to verify what has been postulated from human case studies. The treatment of mice prior to breeding caused a significant increase in the reproductive time. It is assumed that the control group was successfully bred an average of twenty-six days after mating with offspring arriving twenty days later for a total of forty-six days post-mating required for the first litter. It is doubtful that the gestation period itself was increased though this is a possibility. Both groups in which the females were treated show a marked increase in reproductive time, while the group in which males were treated shows a slight increase in reproductive time. The trend is most obvious with the group in which both males and females were treated, requiring more than twice as long as the control group to reproduce. These results seem to agree well with most of the studies previously cited. To recapitulate, Merlin (1972), Board (1964), and Malkinson and Lynfield (1959) found decreased fecundity in human populations treated with colchicine. Handel (1979) and Poffenbarger and Brinkley (1974) offer supporting evidence from animal studies. Bremner and Paulsen did not find any decrease in sperm counts of seven men treated

with colchicine (1976), but suggest that possibly certain individuals are more sensitive than others to this effect of colchicine. It should be noted, however, that this is a transitory phenomenon, as can be seen by the eventually successful matings seen in group ii (TM). Merlin noted in 1972 that the effects of male sterility were temporary, as evidenced by lack of histological abnormalities in his patient four months after discontinuing colchicine therapy, and the eventual fathering of a child.

The ability of the treated mice to produce offspring seems to be further affected by the ability (or inability) of the embryos to implant. When compared to the controls, the number of uterine scars (i.e., embryos which implanted) was raised slightly by treating the males with colchicine (group ii), raised by almost 50% by treating the females (group iii), but decreased slightly by treating both the males and females (group iv). This change in the direction of the affect of treatment led group iii (TF) to have significantly more scars than group iv (TB), but created no difference between these groups and the control or between other groups. The method by which this could occur seems rather elusive. Treatment with colchicine may have deferred ovulation during the time the animals were treated. After treatment it is plausible that all the

deferred eggs were simultaneously released, similar to the action of some fertility drugs. It is alternately possible that the uterine scars in groups ii (TM) and iii (TF) represent the remnants of resorbed fetuses from an earlier, unsuccessful pregnancy. Substantiating this is the increase in the reproductive time. In addition, Ingalls et al (1968) demonstrated that the frequency of resorptions increases as the concentration of colchicine given to a mouse increases. While group iv (TB) seems to directly contradict both of these theories, it is likely that any developing embryos could have been so severely affected by both parents being treated that the embryos were unable to achieve implantation. Group iv then would have a decreased number of embryos implanting to make uterine scars. Ingalls states that injections administered in dosages of 2.0 mg/kg/day at the time just before implantation as prohibiting the fetuses of nine out of ten mice from implanting.

While absolute number of offspring of the treated mice is not statistically different from the control mice, Fischer's exact test shows group iv (TB) to be more successful at bearing offspring when one considers the number of uterine scars. In group iv (TB) 79% of the uterine scars resulted in offspring born alive. The per