



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.

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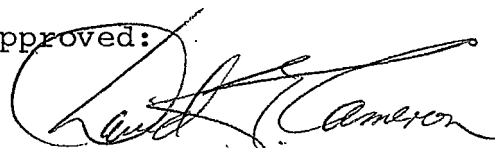
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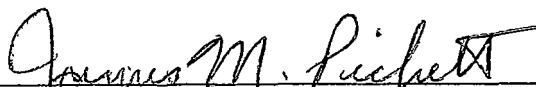
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Approved:



Chairperson, Graduate Committee



Head, Major Department



Graduate Dean

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.

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 trimethylcolchicinic 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 colchicine 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 implantation. 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 intravitreously 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 colchicine 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 (Arasz-kiewicz, 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 animals 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 O^{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 O^2 -demethylcolchicine and O^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 O^2 and O^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 determine if colchicine administration to mice before and during pregnancy affects the fertility or genetic constitution 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 gastrointestinal 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)
- 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 spermatogonia 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 suspended 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 benzylbenzoate 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 then 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 ¹ Time (in days)	Mean Number ² of Offspring	Mean Number ² of Survivors	Percent Survivors	Mean Number ¹ of Uterine Scars
i (C) Control	13	45.9 ± 8.9 ^{c+}	3.5 ± 0.9 ^{d+}	2.6 ± 0.8 ^{b++} _{c*} _{d++}	73.8	8.4 ± 0.9
ii (TM) Treated Males	8	65.4 ± 10.1	5.1 ± 0.9 ^{d*}	1.1 ± 0.5 ^{a++} _{c++} _{d+}	22.0	9.4 ± 0.5
iii (TF) Treated Females	15	65.2 ± 6.0 ^{a+}	5.5 ± 0.7 ^{d++}	3.3 ± 0.6	59.8	11.5 ± 1.5 ^{d*}
iv (TB) Both Males and Females Treated	14	101.4 ± 14.5 ^{a+}	5.0 ± 0.8 ^{a+} _{b*} _{c++}	2.2 ± 0.7 ^{a+} _{b*} _{c*}	44.3	6.3 ± 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 possible) 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 notably 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 survivors 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 abnormal 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 histologically 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

TABLE TWO
 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 ± 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 ± 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 between 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 significant 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 ±0.6 ^{b*}	0.6 ±0.04 ^{d*}
ii (C ₈) Treated Males	23	11	13.0 ±0.4 ^{a+}	0.6 ±0.05
iii (T _{0.5}) Treated Females	18	21	14.4 ±0.6 ^{d+}	0.8 ±0.10 ^{d*}
iv (T _{1.0}) Both Males and Females Treated	18	15	12.6 ±0.3 ^{c+}	0.5 ±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 (C₈), 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

centage of live births per uterine scar was significantly less in groups i (42%), ii (54%) and iii (48%). Thus group iv had fewer possibilities of producing offspring as indicated by the number of uterine scars, yet still gave birth to the average number of offspring. This is possible only if the implanted embryos of group iv were more successful than the implanted embryos of the other groups. Through the loss of non-viable embryos, it is quite plausible that the remaining embryos had an increased chance of being born alive than any of the other groups including the control.

The number of survivors was compared similarly to the number of offspring in a Fischer's exact test to compare the proportion of those surviving rather than the absolute numbers. Thus, while it may appear that group iii (TF) with 3.3 mean survivors produced more survivors than the control with 2.6 new survivors, the percent survival is actually less for group iii. All three treated groups have significantly fewer survivors than the control. It seems somewhat paradoxical that within the treatment groups group iv (in which both males and females were treated) had a better chance of surviving than group ii (where just the males were treated). This could be a reflection of what seems to be the increased viability

of the surviving embryos of group iv (TB), via the hypothesized failure to implant of embryos. The major trend seems to be however, that treatment with colchicine significantly reduces a newborn's chances of survival.

Guerin and Loizzi (1978) report a decrease in the lactose secretion in animals treated with colchicine. It is possible therefore that the decreased survival rate of the treatment groups was caused by a failure in maternal lactose production.

Decreased birth weights have been reported in human offspring subsequent to treatment with colchicine during pregnancy (Katsilambros, 1963). The mean weight of offspring at the conclusion of the experiment is significantly less in groups iii (TF) and iv (TB) than in the control group. This decreased weight could be attributable to several factors, not the least of which are the age of the offspring at time of weighing (remembering that they were born an average of thirty to sixty days later than the control), the litter size (less nutrients to go around to each), and possibly the nutritional state of the mothers. According to one study of humans (Batt, Cirksena, and Lebherz, 1963), net fetal survival rate in gout-associated pregnancies was only 64%, which was attributed to complications (including low birth weight), anemia, and anoxia of the mother. The same hypothesized conditions could exist

in the mouse receiving colchicine, especially in light of the previous discussion concerning the decrease in circulation of triglycerides, proteins and enzymes (Singh, LeMarchand, Orci and Jeanrenaud, 1975).

In addition to the previous data of Experiment One, a note should be added about the change in routes of administration of the drug to the mice. The initial seven treatments of colchicine were injected subcutaneously following the method of administration and dosage of Poffenbarger and Brinkley (1974). This resulted in extensive sloughing of the skin at the site of injection and minor sloughing elsewhere in the female mice. Since Poffenbarger and Brinkley were studying azoospermia, they did not treat female mice. Neither the males nor the control females had a similar reaction. Gaylorde and Sarkany (1976) report a similar necrosis of the skin of guinea pigs (of unspecified sex) after 5 days treatment with a topical ointment of colchicine. For this reason the subsequent treatments in Experiment One and of the treatments of Experiment Two were given orally. It could be speculated that possibly the males were relatively unaffected with respect to skin irritation due to hormonal influences. A larger scale investigation over a more prolonged period of time could investigate these hormonal influences.

Experiment Two:

Experiment One was designed to show what effect treatment with colchicine before mating would have on the reproductive capacity of treated individuals. Experiment Two was alternately designed to test the effect of low dosages of colchicine on the offspring treated in utero. As such the data should be considered separate but interrelated.

The offspring of group ii (C_8) show significantly better survival than the offspring of groups i (C_{20}) and iv ($T_{1.0}$). It is possible that a real difference in viability is being reflected between the treatment of 1.0 mg/kg of colchicine and its control group (group ii). Worth noting, however, is the fact that the two control groups vary from each other as well. This seems to reflect a measure of the intrinsic variability of the measurements.

Most of the group differences seen in the second experiment seem easily explained by nutritional considerations. The females with larger uteri also weighed more than the other groups. It stands to reason that a bigger than average mouse would have a bigger than average uterus. This theory fails to gain support from the data of Experiment One, however, where those animals which weighed significantly more had smaller uteri (though not signifi-

cantly smaller).

Additionally, the mice in groups which received wheat paste for twenty days (groups i and iii) weighed significantly more than the mice of groups which received the wheat paste for eight days (groups ii and iv), regardless of colchicine treatment. This lack of difference between groups i (C_{20}) and iii ($T_{0.5}$) and between groups ii (C_8) and iv ($T_{1.0}$) fails to support the contention that colchicine treatment during pregnancy had any adverse effect on the weights of the offspring. If indeed the variations seen in the weights and survival of treated groups in Experiment One are due to inhibition of lactose production this is not reflected in the data of Experiment Two. It should be noted, however, that the animals in Experiment Two were not treated as long or with as high a concentration of colchicine as the animals in Experiment One. The only method to determine if this is the case would be to assay milk samples from the lactating mothers.

The chromosome data shows an interesting trend among the treated groups, though not all groups show statistical significance due to small sample sizes. There were approximately three times more abnormal chromosome counts in the colchicine treated groups than in the control groups. Though this does not follow the typical karyo-

typing procedure of counting upwards of 64 cells per animal, it does show an interesting trend. Most of the anomalous chromosome counts fell into the category of aneuploid cells with the most frequent counts between 38-42 chromosomes. Ingalls, Ingenita, and Curley (1963) demonstrated increased aneuploidy and polyploidy in the offspring of mice treated with 6-amino nicotinamide during pregnancy. A similar action may be operating with colchicine. It has also been shown that at the molecular level colchicine interacts by inserting its tropolone ring into the nitrogenous bases of DNA (Buszman, Wilczok, Witman, and Siebert, 1972). In light of the correlation between mutagenesis and carcinogenesis, the chromosomal effects of colchicine warrant further investigation.

The systemic mode of administration and prolonged administration of colchicine has yielded somewhat different results than other laboratory animal studies. The fertility of mice injected prior to pregnancy was adversely affected, as seen by increased reproductive times and changes in the numbers of uterine scars. This was not a permanent effect however, since the ovaries and testes of treated animals failed to show any histological abnormalities at the termination of the experiments.

The weights and viability of the offspring born to mice treated before pregnancy were also adversely affected, though treatment during pregnancy did not seem to affect these parameters. The fertility of the offspring, as measured by the normality of gonads and uterine size, did not seem to be affected by treatment before or during pregnancy. Damage to the genetic component in the treated mice or their offspring is somewhat difficult to assess due to difficulties with the chromosome technique, but seems to imply a trend toward some damage to the normal cell's chromosome complement in both experiments. These conclusions coincide more closely with the human studies (decreased weight and viability in the offspring and diminished reproductive success in treated individuals) than they do with the laboratory animal studies.

SUMMARY

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.

LITERATURE CITED

LITERATURE CITED

- Araszkiewicz, H., Bartel, H., and Slawinski, W. Teratogenic effect of colcemid on embryogenesis of chick embryos. Folia Morphol., 31: 409-19, 1972.
- Batt, R. E., Cirkseva, W. J., and Lebherz, T. B. Gout and salt-wasting renal disease during pregnancy. American Medical Association Journal, 186: 835-8, 1963.
- Board, J. Effect of colchicine on human ovulation. American Journal of Obstetrics and Gynecology, 89: 830-1, 1964.
- Bremner, W. J. and Paulsen, C. A. Colchicine and testicular function in man. New England Journal Medicine, 294(25): 1384-5, 1976.
- Buszman, E., Wilczok, T., Witman, B., and Siebert, G. Interaction of colchicine with DNA molecules. Hoppe Seylers Physiol. Chem., 358(7): 819-24, 1977.
- Carr, A. Colchicine toxicity. Archives of Internal Medicine, 115: 29-33, 1965.
- Cestari, A., Botelho Vieira Filho, J. P., Yonenaga, Y., Magnelli, N., and Imada, J. A case of human reproductive abnormalities possibly induced by colchicine treatment. Rev. Brasil Biol., 25: 253-6, 1965.
- Cooper, P. Genetic effects of formaldehyde. Food and Cosmetic Toxicology, 17(3): 300-1, 1979.
- Dittman, W. and Ward, J. Demecolcine toxicity. American Journal of Medicine, 27: 519-24, 1959.
- Ferm. V. H. Colchicine teratogenesis in hamster embryos. Proceedings of Society for Experimental Biology and Medicine, 112:775-8, 1963.
- Ferreira, N. R. Colchicine therapy and aneuploid cells. Rev. Bras de Pesquisas Med. e Biol., 6: 141-8, 1973.
- Ferreira, N. and Buoniconti, A. Trisomy after colchicine therapy. Lancet, 2: 1304, 1968.

- Fleischmann, W., Russell, O. Q., Fleischmann, F. K. LD₅₀ and minimal effective antimitotic dose of colchicine in various rodents. Med. Exp. 6:101-104, 1962.
- Gaylorde, P. M. and Sarkany, I. Colchicine therapy for psoriasis. Arch. Dermatol., 112: 556-7, 1976.
- Giorgi, G. and Raimondi, M. Use of a new drug combination in the therapy of phlebitis. Minerva Cardioangiol., 20: 152-6, 1972.
- Goldfinger, S. Colchicine for familial Mediterranean fever. New England Journal of Medicine, 287: 1302, 1972.
- Goldstein, A. Biostatistics - An Introductory Text. The Macmillan Limited, New York, 1964, 272 pp.
- Guerin, M. A. and Loizzi, R. F. Inhibition of mammary gland lactose secretion by colchicine and vincristine. Am. J. Physiol., 234(5): C177-80, 1978.
- Handel, M. A. Effects of colchicine on spermiogenesis in the mouse. J. Embryol. Exp. Morphol. 51: 73-83, 1979.
- Hansteen, I. L. Colchicine and chromosome aberrations. Lancet, 2: 744-5, 1969.
- Hebert, J. and Gros, H. Preventive treatment of dysmenorrhea with orally administered thiocolchicoside. Bull. Fed. Gynec. Obstet. Franc., 12: 257-9, 1960.
- Herbst, A., Ulfelder, H., and Poskanzer, D. Adenocarcinoma of the vagina. New England Journal of Medicine, 284: 878, 1971.
- Hoefnagel, D. Trisomy after colchicine therapy. Lancet, 1: 1160, 1969.
- Ingalls, I. M., Ingenita, E. F., and Curley, J. Acquired chromosomal anomalies induced in mice by injections of a teratogen in pregnancy. Science 141: 810, 1963.

- Ingalls, T., Curley, F., and Zappasodi, P. Colchicine induced cranio-facial defects in the mouse embryo. Archives of Environmental Health (Chicago), 16:326-32, 1968.
- Kaidbey, K. H.; Petrozzi, J. W., Kligman, A. M. Topical colchicine therapy for recalcitrant psoriasis. Arch. Dermatol. 111:33-36, 1975.
- Katsilambros, L. Colchicine in the prophylactic treatment of rubella embryopathy. Arch. Inst. Pasteur Hellen., 9: 97-9, 1963.
- Kleiber, M. The Fire of Life - An Introduction to Animal Energetics. John Wiley and Sons, Inc., New York, 1961.
- Lancet. Formaldehyde toxicity [letter]. 2(8143): 620-1, 1979.
- Loader, K. R. and Nathaniel, E. J. H. Cytologic features of Harding-Passey melanoma following different regimes of colchicine therapy. Am. J. Path., 89: 297-312, 1977.
- Malawista, S. E. The action of colchicine in acute gouty arthritis. Arthritis Rheum. 18(6):835-46, 1975.
- Malkinson, F. D. and Lynfield, Y. L. Colchicine alopecia. Arch. Dermatol. 33:371-84, 1959.
- Merlin, H. E. Axospermia caused by colchicine: a case report. Fertil. Steril. 23:180-1, 1972.
- Orsini, M. W. Technique of preparation, study and photography of benzyl-benzoate cleared material for embryological studies. J. Reprod. Fertil. 3: 283-7, 1962.
- Pasani, E. Muscle decontraction with thiocolchocose in bloodless operations in skeletal traumatology. Minerva Med. 59: 4688-92, 1968.

- Patton, J. L. Chromosome studies of certain pocket mice, genus Perognathus (Rodentia: Heteromyidae). J. Mammol. 48(1): 27-37, 1967.
- Poffenbarger, P. and Brinkley, B. Letter: colchicine for familial Mediterranean fever: possible adverse effects. New England Journal of Medicine, 290: 56, 1974.
- Reimers, T. and Sluss, P. 6-Mercaptopurine treatment of pregnant mice: effects on second and third generations. Science, 201: 65, 1978.
- Schonharting, M., Mende, G. and Siebert, G. Metabolic transformation of colchicine. II [1]. The metabolism of colchicine by mammalian liver microsomes. Hoppe Seyler's Z. Physiol. Chem., 355: 1391-99, 1974.
- Singh, A., LeMarchand, Y, Orci, L. and Jeanrenaud, B. Colchicine administration to mice: a metabolic and ultrastructural study. Eur. J. Clin. Invest. 5(6): 495-505, 1975.
- Timson, J. Trisomy after colchicine therapy. Lancet, 1: 370, 1969.
- Vaccarezza, O. Retinal alterations induced by intra-vitreous colchicine. Virchows Arch. (Zellpathol.), 12: 159-67, 1973.
- Walker, F. A. Trisomy after colchicine therapy. Lancet, 1: 257-8, 1969.
- Wallace, S. L. Colchicine and new antiinflammatory drugs for the treatment of acute gout. Arthritis and Rheumatism 18(6):847-51, 1975.
- Yu, T. and Gutman, A. Efficacy of colchicine prophylaxis in gout. Ann. Int. Med., 55: 179, 1961.

APPENDIX

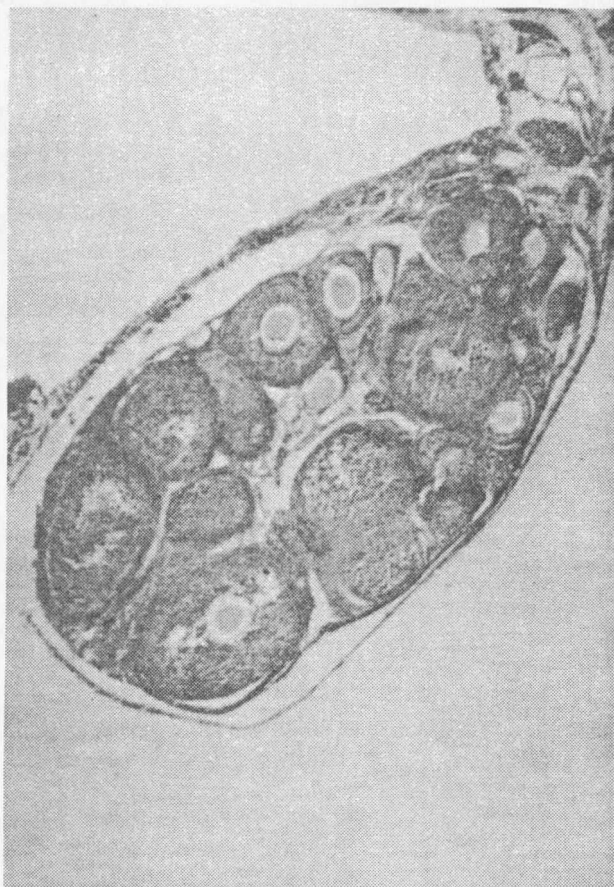
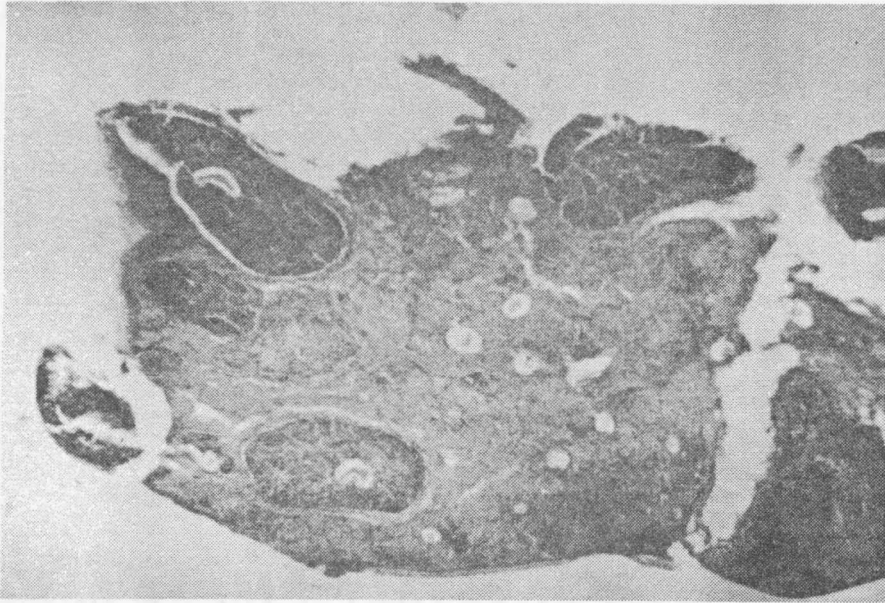
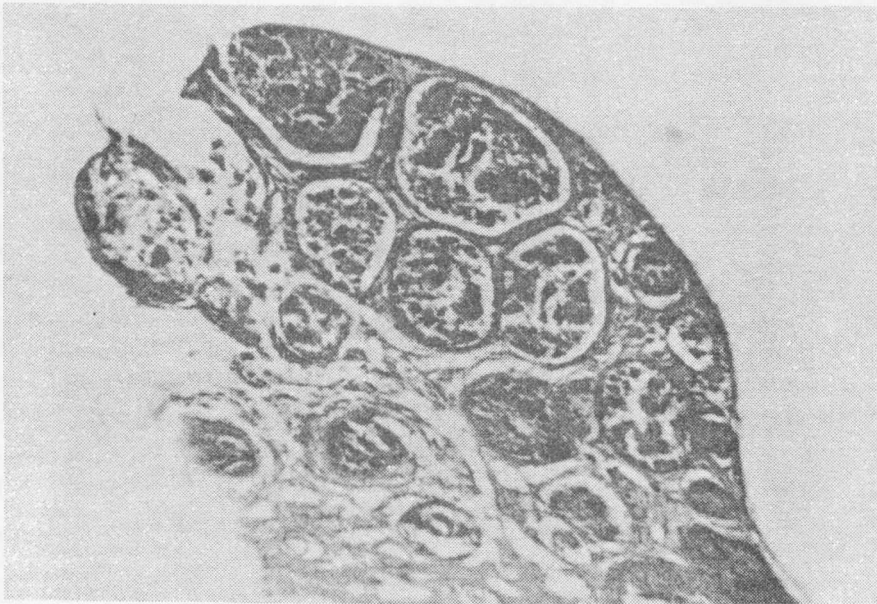


Fig. 1. Histological sections of normal ovary from offspring of Experiment 2, group iii ($T_{0.5}$). (Magnification = approximately 100x)



A



B

Fig. 2. Histological section of abnormal ovaries from offspring of (A) Experiment 1, group ii (TM) (magnification = approximately 100x) and (B) Experiment 2, group ii (C₈) (magnification = approximately 120x).

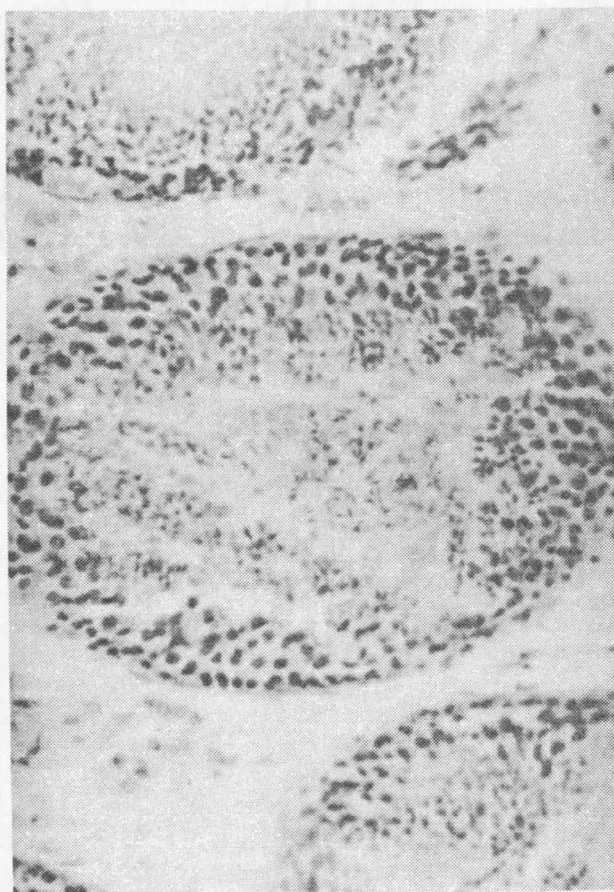


Fig. 3. Histological section of a normal testis from an untreated male of Experiment 1. (Magnification = approximately 320x).

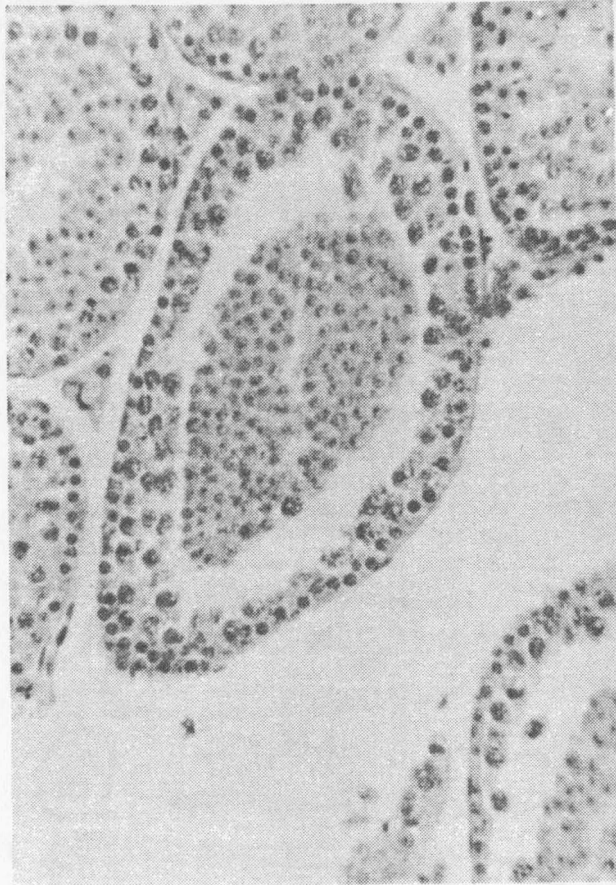
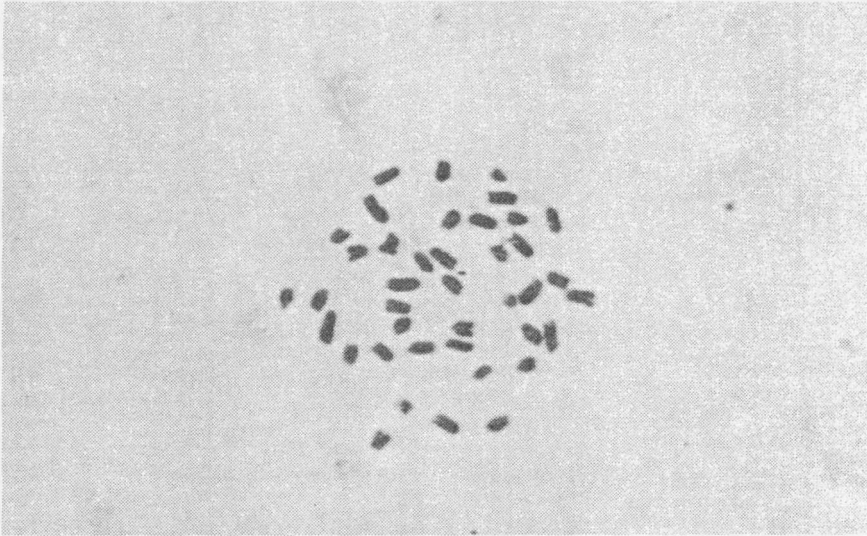
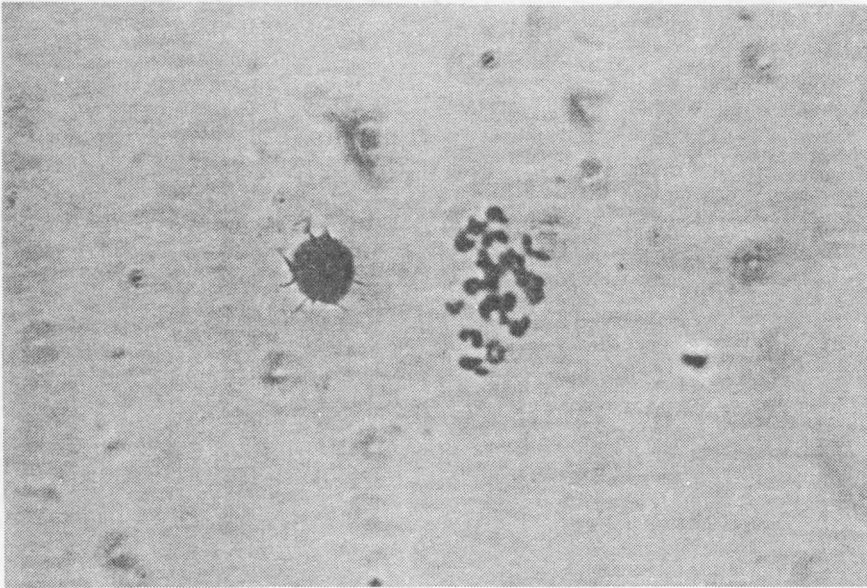


Fig. 4. Histological section of abnormal testis from an offspring of Experiment 2, group iv ($T_{1.0}$). (Magnification = approximately 320x).

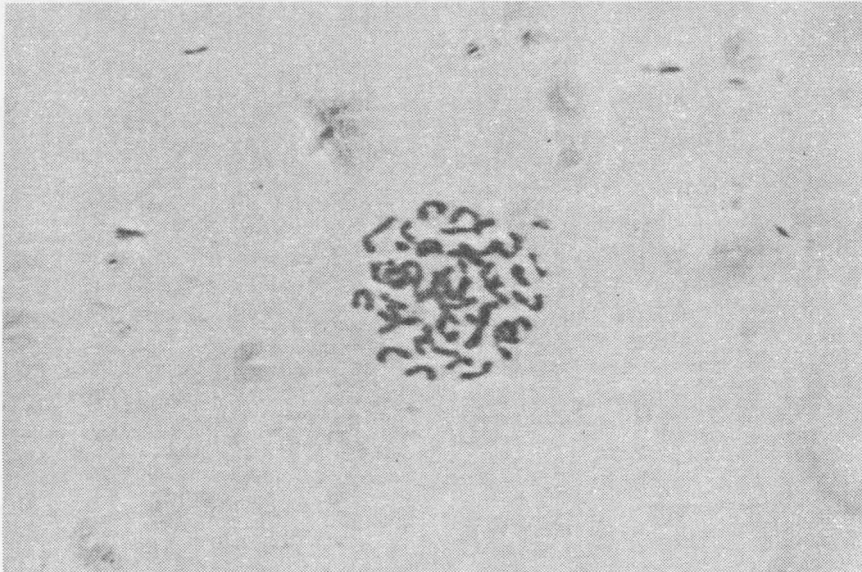


A

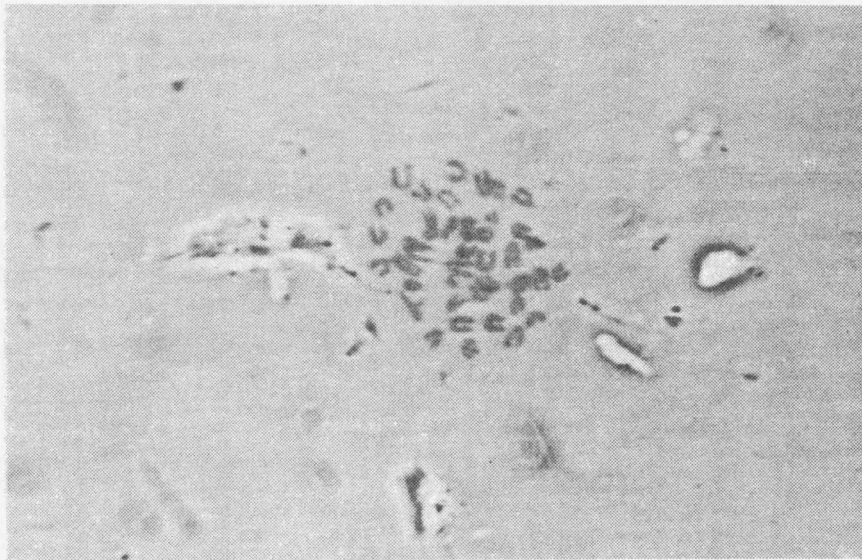


B

Fig. 5. Chromosome preparations from offspring of Experiment 2, group iii ($T_{0.5}$), showing (A) 40 chromosomes and (B) approximately 20 chromosomes (magnification = approximately 1250x).



A



B

Fig. 6. Chromosome preparations from offspring of Experiment 2, group iii ($T_{0,5}$) showing (A) 43 chromosomes and (B) possible translocation chromosome (magnification = approximately 1250x).

