Some Accounts on Teratogenic and Embryocidal Effects of Colchicine on Mouse Embryos[1)2)]

By

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(With 6 Text-figures, 1 Plate and 4 Tables)

Following the notable discovery of colchicine as a mitotic poison by Blakeslee and Avery (1937), the effect of this drug on cell division has been the subjects of repeated investigations by a number of cytogeneticists. Evidence presented by them has uniformly indicated that colchicine inhibits the formation of mitotic spindles, then resulting in accumulation of arrested mitoses in cell population (Levan, 1938). Recently, colchicine has been employed as a chemotherapeutic agent for patients with leukemia or acute gout, because of its pharmacological actions in clinical fields.

Teratogenic effects of growth-inhibiting chemicals like colchicine on mammalian embryos have also attracted attention of recent investigators (Bodenstein, 1947; Haskin, 1948; Nishimura and Takagaki, 1959; Cardinali *et al.*, 1961a, b; Murphy, 1959, 1960, 1962; DeMyer, 1964, 1965; Ohzu and Shoji, 1965): they reported that the multiple malformed fetuses appeared in animals treated in certain gestational stages. Sokal and Lessmann (1960) failed to detect malformed fetuses in pregnant rats and rabbits under treatment with a colchicine derivative, desacetylmethylcholchicine, used as cancer chemotherapeutic agent. In contrast, Ferm (1963) reported several types of abnormal fetuses in hamsters following the colchicine treatment in the gestational period. Some aspects of teratogenic and embryocidal effects on developing mouse embryos after the colchicine administration were preliminarily given by Shoji and Makino (1966). This paper reports additional data derived from further experimental studies, together with the discussion on the possible mechanism of the teratogenic and embyrocidal effects of colchicine based on the results of chromosomal and histological observations.

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Materials and Methods

Mice of dd strain aged 60 to 90 days, and maintained in our laboratory for more than

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30 generations, were used as test animals in the following experiments. Oestrus femals inspected by means of vaginal smears were mated with males of the same strain. After mating they were examined for the presence of vaginal plugs as a sign of successful copulation. The date, on which a vaginal plug was observed on the mated female, was taken as the first day of gestation. Data were collected from the following three experiments.

Observations on malformation and death of the fetus: The females with plugs were devided into 6 groups. Animals of five groups received colchicine (Merck & Co., Inc.) dissolved in a physiological saline solution at dose levels of 0.5 mg/kg, 1.0 mg/kg, 1.25 mg/kg, 1.5 mg/kg and 2.5 mg/kg of body weight, respectively. All of the experimental females received colchicine at the above doses by a single subcutaneous injection during the 2nd to the 14th day of gestation (Table 1). Pregnant mice of the remaining one group served as controls without the administration of colchicine. The treated and untreated mice were sacrificed on the 18th day of gestation and the developmental condition of fetuses *in utero* was examined. The fetuses were recorded as being alive or dead. All living fetuses were examined for the occurrence of gross external malformations under a dissecting microscope at 8X. The resorption sites and placental remnants were regarded as dead fetuses.

Chromosomal observations: Pregnant females were sacrificed at 48 hours after a single injection of colchicine (1.25 mg/kg) on the 9th day of gestation. The whole bodies of embryos were removed from uteri, immediately after minced with sharp scissors in small petridishes. The embryonal shreds were suspended in about 5 ml of culture medium (McCoy's solution) with one-drop of a 0.005% colchicine solution, and incubated at 37°C for one hour. After centrifugation at 1000 r.p.m. for 5 minutes, the supernatant was decanted, and the embryonal shreds were put in 2 ml fresh tap water, mixed gently with pipette, and then allowed at room temperature for 30 minutes. Chromosome slides were made following the air-drying method of Rothfels and Siminovitch (1958) with Giemsa staining. About 100 cells were examined with regard to diploid or polyploid complements in each embryonal specimens under 1500 times.

Histological observations: Embryos for observation were obtained from mothers treated with 1.25 mg/kg dose of colchicine on the 9th day of gestation. Mother animals were sacrificed at 3, 6, 12, 24 and 48 hours after the treatment, and uteri carrying embryos were fixed in Bouin's solution. After being subjected to the routine paraffin method, serial sections were made at 10μ, and stained with Hematoxylin-Eosin. About 100 cells in the course of division were counted in ependymal layer of each embryo.

In untreated control specimens, the chromosomal and histological preparations were made according to the same procedure.

Results

Malformation and death of the fetus: Six hundred and forty-four implanted fetuses including resorption sites and placental remnants were derived from 85 untreated control female mice, and 2214 implanted fetuses derived from 283 colchicine-treated females were examined for external malformations and fetal death. Fetuses of the experimental groups were further divided, according to the gestational age, into the following subgroups: 1) fetuses at the 2nd to the 6th day of gestation; 2) those at the 7th to the 10th day of gestation; 3) those at the 11th to the 14th day of gestation. The results of observations are summarized in Table 1.

Table 1. Summarized data on effects of colchicine on mouse embyros

Dose mg/kg	Day treated after mating	No. of treated dam (a)	No. of implant-ation	No. of living fetuses (b)	No. of dead fetuses (b)	No. of forefoot polydactyly (b)	No. of other malforma-tions (b)
0.5	1) 2- 6	18 (0)	144	133 (92.4)	11 (7.6)	32 (22.2)	1 (0.7)
	2) 7-10	23 (1)	206	169 (82.0)	37 (18.0)	23 (11.2)	5 (2.4)
	3) 11-14	10 (0)	86	65 (75.6)	21 (24.4)	6 (7.0)	2 (2.3)
	Total	51 (1)	436	367 (84.2)	69 (15.9)	61 (14.0)	8 (1.8)
1.0	1) 2- 6	20 (0)	162	138 (85.2)	24 (14.8)	25 (15.4)	3 (1.9)
	2) 7-10	35 (3)	275	209 (76.0)	66*(24.0)	32 (11.6)	6 (2.2)
	3) 11-14	11 (1)	97	73 (75.2)	24*(24.8)	6 (6.2)	6 (6.2)
	Total	66 (4)	534	420 (78.7)	114*(21.3)	63 (11.8)	15 (2.8)
1.25	1) 4- 6	24 (1)	191	119 (62.3)	72*(37.7)	6*(3.1)	28*(14.7)
	2) 7-10	32 (4)	239	79 (33.0)	160*(67.0)	10 (4.2)	17*(7.1)
	3) 11-14	25 (5)	183	129 (70.5)	54*(29.5)	12 (6.6)	10 (5.5)
	Total	81(10)	613	327 (53.3)	286*(46.7)	28*(4.6)	55*(9.0)
1.5	1) 4- 6	17 (4)	127	76 (59.8)	51*(40.2)	5 (3.9)	23*(18.1)
	2) 7-10	28 (7)	207	25 (12.1)	182*(87.9)	4 (1.9)	7*(3.4)
	3) 11-14	24 (3)	172	99 (57.6)	73*(42.4)	5* (2.9)	8 (4.7)
	Total	69(14)	506	200 (39.5)	306*(60.5)	14* (2.8)	38*(7.5)
2.5	1) 2- 6	3 (7)	23	16 (69.6)	7 (30.4)	5 (21.7)	0
	2) 7-10	10(17)	76	8 (10.5)	68*(89.5)	1 (1.3)	1 (1.3)
	3) 11-14	3 (7)	26	5 (19.2)	21*(80.8)	0	1 (3.8)
	Total	16(31)	125	29 (23.2)	96*(76.8)	6 (4.8)	2 (1.6)
Untreated control		85 (0)	644	554 (86.0)	90 (14.0)	78 (12.1)	18 (2.8)

(a) Value in parenthesis represents the number of abortion and/or maternal death.
(b) Values in parentheses represent percentage for number of implantation.
* Difference between control and experimental values was significant at 5 per cent level. according to "t" test.

Teratogenic effects: Classification of external abnormalities observed in the present experiments is shown in Table 2. Among the malformed types, forefoot polydactyly was detected in both control and treated groups: they are characterized by a finger-like appendage lateral to the fifth toe. The incidence of this anomaly seems to be decreased with colchicine-treatment (Table 1). The difference in total number of the fetus with this anomaly was statistically significant between the control and the experimental groups at dose levels of 1.25 mg/kg and 1.5 mg/kg. Some other malformed specimens were shown in Figs. 4–6. Comparison on the basis of the number of deformed animals except those with forefoot polydactyly revealed that the difference between the control and the experimental mice was statistically significant only in subgroups 1) and 2) at dose levels of 1.25 mg/kg and

Table 2. Classification of abnormalities inspected in dd mouse fetuses treated with colchicine during gestation

Types of malformations	Day treated after mating											Total (%)*	Untreated control (%)*
	4	5	6	7	8	9	10	11	12	13	14		
Exencephaly	4	7	2	5	2	1			2			23(19.7)	
Brain hernia		1						1	1		2	5(4.3)	
Spina bifida	4			4								8(6.8)	
Open eye	10	3	9	3	1							26(22.2)	
Microphthalmia	1			4								5(4.3)	
Anophthalmia		1							1			2(1.7)	
Exophthalmia	2	1		1								4(3.4)	
Incomplate mandible		1		2								3(2.6)	1(5.5)
Agnathia	1		2	1	1	1					1	7(6.0)	1(5.5)
Hare lip											1	1(0.9)	
Cleft palate	6	2	2	5	6	1	1		3	2	8	36(30.8)	10(55.6)
Macroglossia	1	4		4					2			11(9.4)	
Abnormal auricula		1			2						1	4(3.4)	
Sexual defect			1		1						2	4(3.4)	
Anal atresia			1		1						1	3(2.6)	
Umbilical hernia		1	1	1	2					1		6(5.1)	
Short trunk				2								2(1.7)	
Tailless			1									1(0.9)	
Short tail											1	1(0.9)	1(5.5)
Curvature tail			1	2	1					1		5(4.3)	1(5.5)
Hindfoot defect :													
Cloob foot				1								1(0.9)	1(5.5)
Ectrodactyly					1							1(0.9)	
Syndactyly					1							1(0.9)	
Macrodactyly			1		1							2(1.7)	
Polydactyly	1	3	2	2	4			1	1	2	1	17(14.5)	5(27.8)
Total	22	15	18	14	18	2	1	2	6	6	13	117	18

* Values in parentheses represent percentage for the total number of malformed fetuses.

1.5 mg/kg. As shown in Table 2, the frequency of each malformation was low. In contrast, abnormalities of brain and eye were detected only in colchicine-treated animals. Especially, exencephaly and open eye appeared at a comparatively high frequency: they were associated with some other defects in almost all cases (Figs. 4–6). Fig. 1 shows the incidence of malformed fetuses, except those with forefoot polydactyly, in relation to the gestational age when the colchicine treatment was made. Those fetuses were derived mostly from mother mice treated on 4 to 8 days of gestation at dose levels of 1.25 mg/kg and 1.5 mg/kg. Evidence presented suggests that the teratogenic effect of colchicine is associated with early stages of embryonal development.

Lethal effects: The incidence of fetal death including embryonal death was 14 per cent of implanted fetuses in control mice. The difference in number of dead fetuses between the control and the experimental mice was statistically insignificant

at a dose of 0.5 mg/kg, while it was significant in almost all subgroups at dose levels of 1.0 mg/kg or more. The highest fetal mortality was 89.5 per cent of implanted fetuses in subgroup 2) at a dose of 2.5 mg/kg, while it was 87.9 per cent at a 1.5 mg/kg dose. Further, the incidence of abortions and maternal death increased with the increase of dosage (Table 1). Fig. 2 shows the mortality at different developmental ages of embryos after colchicine treatment. The fetal mortality was remarkable in animals administered colchicine on the 7th to the 10th day of gestation, with a dose response to the embryonal ages.

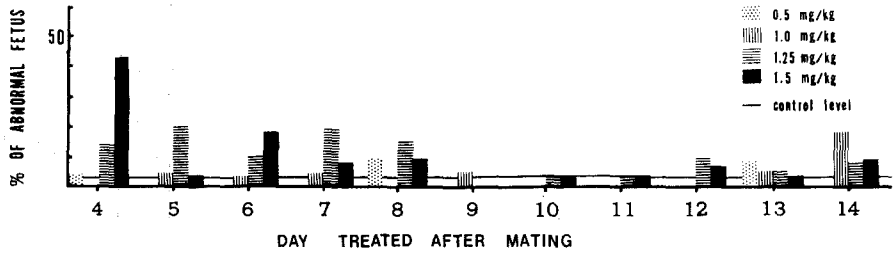


Fig. 1. Incidence of malformed fetuses in mice in relation to gestational ages when the colchicine treatment was made.

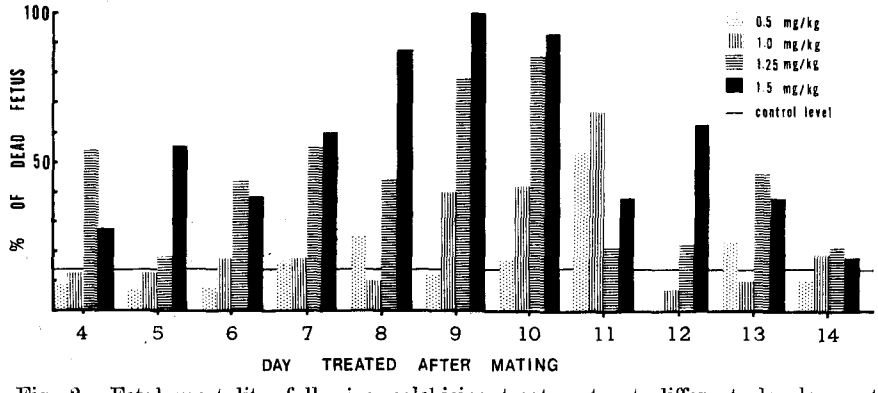
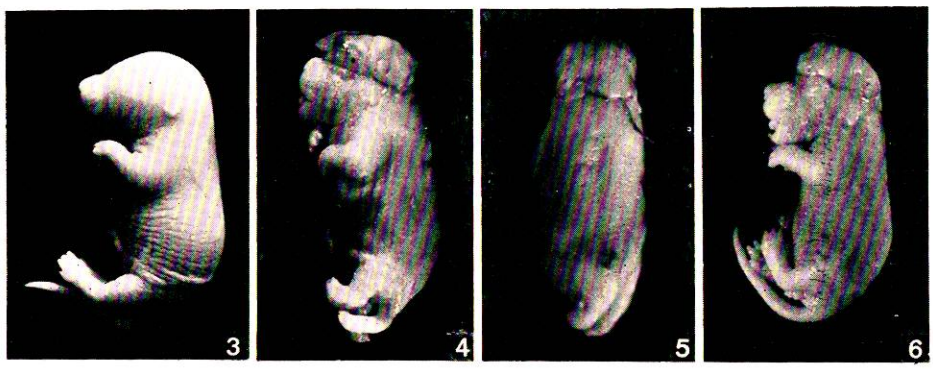


Fig. 2. Fetal mortality following colchicine treatment at different developmental ages of mouse embryos.

Chromosomal observations: As given in Table 3, polyploid cells were found to occur in both control and treated animals at a low frequency. The frequency of polyploid cells in control specimens was 0.7 per cent of metaphase cells observed in 9 embryos. In treated mice, it was 1.3 per cent of the metaphase cells examined in 10 embryos. The incidence of polyploid cells was not remarkably different between those two groups. So far as the present experiments are concerned, there was no morphological change of the chromosomes in the embryos (Plate 1, Figs. A and B).

Mitosis-inhibiting effects: Mitosis-inhibiting data based on ependymal cells of embryos following colchicine treatment are summarized in Table 4. The first sign of histological features was represented by the metaphase arrest in the ependymal cells of the embryos as a characteristic action of colchicine on dividing cells(Plate 1, Fig. D). The incidence of the metaphase cells showed approximately 100 per cent of dividing cells when observed three hours after treatment, whereas it showed the control level at about 75 per cent by 48 hours after treatment. In the ependymal layers of the embryos 6 hours after treatment, cell damage and tissue necrosis were straggling. Such a degenerative change showed a striking increase with the lapse of time (Table 4 and Plate X, Figs. E and F).



Figs. 3–6. Mouse fetuses with remarkable anomalies after colchicine treatment.

Fig. 3. Lateral view of a normal fetus, 18 days of gestation, from a untreated pregnant mouse.

Fig. 4. Lateral view of malformed fetus with exencephaly, microphthalmia, short snout, abnormal auricula, cloob foot, short trank and curvature tail; from a mouse treated with 1.25 mg/kg of colchicine on the 7th day of gestation.

Fig. 5. Dorsal view of mouse fetus as shown in fig. 4 showing spina bifida with a midline furrow along the exposed neural plate.

Fig. 6. Open eye, exophthalmia and exencephaly in a fetus; at dose of 1.25 mg/kg on the 5th day of gestation.

Discussion

Ferm (1963), working on the teratogenic effect of colchicine on hamster embryos at the 8th day of gestation through intravenous injection at varying doses, reported that gross congenital malformations, consisting of microphthalmia, anophthalmia, umbilical hernia, exencephaly and skeletal anomalies, were found in a significant number of survivors, and that increasing the amount of colchicine increased fetal mortality precipitously. The results of the present experiments indicated that there were statistically significant teratogenic effects in the 1.25 mg/kg and 1.5 mg/kg groups (Table 1). The highest frequency of malformed fetuses

Table 3. Frequency distributions of diploid and polyploid cells in mouse embryos at 48 hours after colchicine-treatment on the 9th day of gestation

	Fetus no.	Diploid cell (%)	Polyploid cell (%)	No. of cells observed
Control	1	100	0	110
	2	99.5	0.5	215
	3	99.1	0.9	109
	4	100	0	114
	5	100	0	115
	6	100	0	164
	7	97.9	2.1	141
	8	98.4	1.6	129
	9	98.9	1.1	176
	Total	99.3	0.7	1273
Colchicine-treatment	1	98.2	1.8	110
	2	99.5	0.5	215
	3	99.3	0.8	134
	4	100	0	106
	5	96.3	3.7	107
	6	99.3	0.8	134
	7	98.2	1.8	114
	8	100	0	125
	9	96.9	3.2	127
	10	98.3	1.7	115
	Total	98.7	1.3	1287

Table 4. Effect of colchicine on mitotic inhibition in mouse embryos on the 9th day of gestation

	Hours after treatment	No. of dams	No. of fetuses	No. of metaphase cells (%)*	No. of dividing cells observed
Control	3	3	10	854 (78.6)	1086
	6	3	10	882 (82.7)	1066
	12	2	8	649 (77.8)	834
	24	3	12	854 (66.5)	1285
	48	4	11	836 (73.5)	1138
Colchicine-treatment	3	3	10	1191 (98.9)	1204
	6	3	12	1267 (93.4)	1356
	12	3	10	924 (84.2)	1098
	24	3	10	962 (89.0)	1081
	48	4	10	791 (75.3)	1051

* Value in parenthesis represents percentage for number of dividing cells observed.

was obtained from animals treated on the 4th day of gestation (Fig. 1). This finding is of special interest, because of a general belief among teratologists that malformations are produced particularly during the organogenetic period. A further experimental series has been in progress in order to emphasize the above features. External malformations were chiefly brain and eye defects, which were associated with some other types of malformations (Figs. 4–6). On the basis of the above fact, it may be stated that those defects induced by the colchicine treatment are concentrated in the nervous system, and that severe monsters produced are those associated with several malformations in most cases. Another subject for future study is to examine that the incidence of forefoot polydactyly decreased significantly after colchicine treatment.

Fetal mortality was significantly different in almost all subgroups at dose levels of 1.0 mg/kg or more between the control and the experimental groups, and increased with the increasing colchicine dose (Table 1). The relative frequency of fetal death associated with gestational ages and dose levels showed a remarkable increase in embryos during the early organogenetic period (Fig. 2). In striking contrast, Sokal and Lessmann (1960), on the basis of summarized data so far reported, stated that no malformed fetuses were produced in pregnant rats and rabbits treated with desacetylmethylcolchicine (Colcemid), as well as in pregnant women who received cancer chemotherapy with this drug.

It has been shown by recent investigators that the natural resistance to colchicine differs in different species of experimental animals, *in vivo* or *in vitro* (Orsini and Pansky, 1952; Turbyfill and Soderwall, 1957; Midgley *et al.*, 1959; Cardinali *et al.*, 1961b). Orsini and Pansky (1952)described that rats, mice and rabbits killed by the lethal effect of colchicine displayed a classical symptom of colchicine poisoning, while hamsters showed no effect whatever. Data obtained in the present experiments with the mouse embryos seem to support the result of Ferm (1963). Thus, the teratogenic action of colchicine on rats, rabbits and human beings seems to require further studies to make any definite statement.

Chromosomal data in the present experiments indicate that no significantly different incidences of polyploid cells occurred between the control and treated embryos (Table 3). Chang (1944) reported the occurrence of monsters in the rabbit inseminated with the spermatozoa of one male suspended in a solution of colchicine, and mentioned that the effect was likely due to polyploidy or some disturbance on the nuclear mechanism or organizers in the tissue affected. The present observation showed that an effective dose of colchicine on the developing mouse embryos did not induce polyploidization on the embryonal cells. Further detailed observations are in progress in order to answer this question.

Numerous dividing cells were observed in ependymal layers of developing mouse embryos before or after the 10th day of gestation (Plate X, Fig. C). The results of some histological observations after the transplacental colchicine treatment in this study indicated a remarkable metaphase accumulation of dividing ependymal cells (Table 4). Generally, in plant cell population, colchicine in-

hibited the formation of mitotic spindles, resulting in an accumulation of arrested mitoses (Levan 1938).

Ferm (1964) investigated effects of transplacental mitotic inhibitors including colchicine, vincaleukoblastine (Velban) and vincristine, on the fetal hamster eye, and reported that colchicine had the most marked mitosis-inhibiting activity amongst those three drugs, being represented by nuclear pyknosis, cell rupture and severe necrotic tissue. Furthermore, the most effective mitosis-inhibiting dosage of colchicine also correlated with its most effective teratogenic dose. Murphy (1960), working on teratogenic effects of growth-inhibiting chemicals on rat embryos in various doses, stated that at the cellular level a given growth inhibitor probably behaved in uniform manner in different systems, and that in embryos the outcome of drug effect depended also on the specific drug as well as the quality and denisty of events taking place within embryos.

On the basis of the above situation, the findings in the present experiments seem to agree with statements of Murphy (1960) and Ferm (1964). The possible conclusion is drawn that the placenta of the mouse permits the permeation of colchicine, and that the most effective teratogenic and embryocidal action of colchicine may be associated with cell degeneration of developing mouse embryos.

Summary

1. Effects of colchicine on mouse embryos were investigated with special regard to the frequency of fetal malformation and death. Pregnant females at different gestational stages, ranging from 2 to 4 days after mating, received colchicine by a single subcutaneous injection at dose levels of 0.5 mg/kg to 2.5 mg/kg of body weight.

2. Fetal abnormalities observed were exencephaly, open eye, cleft palate and others. The difference in number of those anomalies was statistically signfiicant only in mice treated before 10 days of gestational age at doses of 1.25 mg/kg and 1.5 mg/kg. A high frequency of malformed fetuses was obtained from mice treated with colchicine during the early stages of gestation.

3. Fetal mortality increased with the increase of colchicine dose. Statistically the difference in number of dead fetuses between the control and the experimental mice was significant at doses of 1.0 mg/kg or more, being nearly 100 per cent at 1.5 mg/kg in the 9th day of gestation.

4. Some chromosomal data obtained 48 hours after the treatment at 1.25 mg/kg on the 9th day of gestational age indicated that most cells were diploid, seldom polyploid.

5. Ependymal cells showed a metaphase accumulation in embryonal specimens after the 3-hour-treatment. The placenta of the mouse seems to permit permeation of colchicine.

6. The possible conclusion is that the fetal malformation and death may be induced by mitosis-arresting activity of colchicine on developing embryonic tissues.

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Explanation of plate X

Figs. A and B. Metaphase chromosomes from mouse embryos, at 48 hours after colchicine-treatment on the 9th day of gestation. Fig. A. A diploid cell. Fig. B. A tetraploid cell.

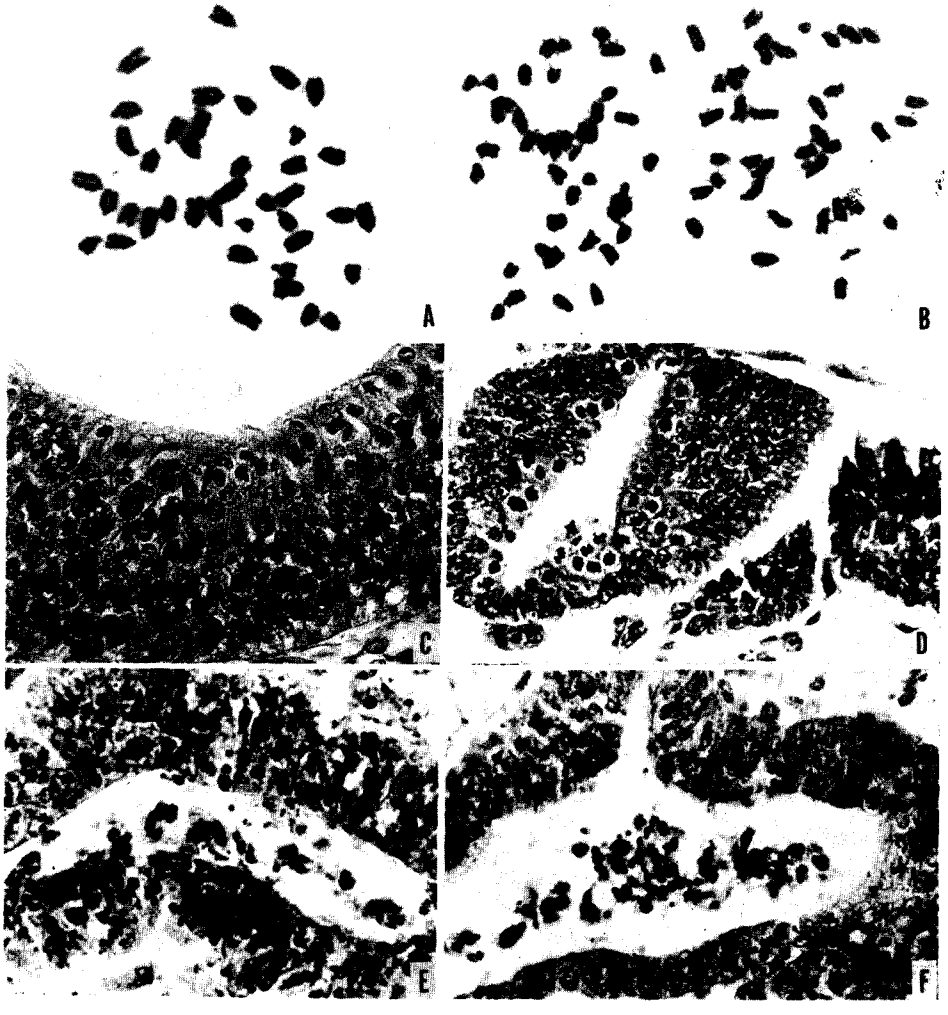
Figs. C-F. Sections through ependymal layer of mouse embryos whose mother treated with colchicine on the 9th day of gestation.

Fig. C. Ependymal cells in the course of mitotic division; from an untreated fetus.

Fig. D. Histological feature showing metaphase-arrest in ependymal layer, at 3 hours after treatment.

Fig. E. Degenerating cells showing pycnosis and rupture, 6 hours after treatment.

Fig. F. Necrotic disintegration of the ependymal layer in mouse embryo, 24 hours after treatment.



R. Shoji: Effects of Cholchicine on Mouse Embryos