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Effect of N-Desacetyl Thio Colchicine (TC) and N-Desacetyl-Methyl-Colchicine (MC) on Rat Fetus and Litter *in utero*. (24082)

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TC, when first synthesized by Velluz and Miller(7), had antimitotic activity in non-toxic doses. The single LD-50 for rats, according to Branceni, *et al.*(1) is 175 mg/kg for intraperitoneal doses and 37 mg/kg for intramuscular doses. This is approximately 25 to 100 times less than LD-50 for Colchicine. Rats tolerated daily i.p. doses of 3 mg/kg indefinitely, and 8.5 mg/kg for 21 consecutive days. In preliminary experiments, Jaquier and Feyel-Cabanes (personal communication) found that 10 daily doses of 2.5 mg/kg starting on 2nd, 4th, 8th and 12th day of gestation, had no effect on rat fetuses from 2nd to 12th day, nor when given on 5 consecutive days after 16th day of gestation. TC, however, arrested embryonic development when given beyond 12th day of gestation. Its action appeared directly on the fetus and not on the mothers.

Methods. Experiments were conducted with Long-Evans strain rats bred and reared in our own colony. Only animals which had one satisfactory previous litter were used. Animals were kept on White Breeding diet of Long-Evans, were approximately 6 months old and weighed 250 g each. The compound was given l.p. to groups of rats in different stages of pregnancy. Onset of gestation was determined by massive sperm findings in the vagina. Pregnant animals were kept in single stainless steel cages to avoid urinary or fecal contacts. Mother rats were sacrificed under ether anesthesia on 21st day of gestation. Uteri were removed and contents recorded, weighed and fixed. The fetuses were sectioned or cleared to study skeletal developments. Maternal internal organs were inspected macroscopically, fixed in formalin and studied in H and E paraffin sections.

Results are listed in Tables I and II. TC had no effect on subsequent ovarian cycles or litters when given twice, prior to insemination, to 6 rats (Table II). Doses of 5 mg/kg, given the morning after insemination (day 0) and on day 1 of gestation, were also without effect on litters of 12 rats. Two doses of 7 mg/kg, given on 4th and 5th day of gestation, led to resorption of 23% of fetuses, and complete litter failure in 20% of the experimental group (3 out of 15 rats). Three fetuses were also found dead and 3 others stunted. On 7th and 8th day of gestation, at time of fetal implantation, doses of 5 mg/kg destroyed 34% of all fetuses but only 20% of all litters, whereas 7 mg/kg doses destroyed 65% of all fetuses, and 36% of all litters. On 10th day of gestation a single dose of 1 mg/kg had no effect; 2.5 mg/kg of TC led to resorption of 30% of all fetuses. Only one stunted surviving fetus was found after single 7 mg/kg dose. On 11th day of gestation, a single dose of 5 mg/kg destroyed in one series all litters completely, but in a second series, destroyed only 89% of all litters. However, 2 doses of 5 mg/kg given on 11th and 12th days of gestation destroyed all litters. The 5 mg/kg dose, given on 15th and 16th days of gestation destroyed

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TABLE I. Desacetyl-thio-colchicine, i.p., in Rats.

No. of rats	Day of dose; mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Mothers with comp. litter destruction	Surviving placentas	Litter wt	Fetus	
					Live	Stunted*	Dead	Gross malf.*	Resorb.				Wt	Length
7	0 & 1	a		45	45									
	5 mg/kg	b	21.7	6.4	6.4					0		41.6	6.5	4.5
		c												
12	10	a		100	100									
	1 mg/kg	b	+25	8.3	8.3					0		44	5.3	5.3
		c			100									
10	10	a		79	55				24		22			
	2.5 mg/kg	b	+24.5	7.9	5.5				2.4	0		30.3	5.5	4.2
		c			69.7				30.3		92			
11	11 & 12	a		96	32		1	1	63	4	30			
	2.5 mg/kg	b	+22.7	8.7	2.9	0	.9	.9	5.7		3.4	17.1	5.9	4.5
		c			33.4		1.0	1.0	65.5	36.4	60.3			
10	7 & 8	a		73	48				25	2	10			
	5 mg/kg	b	+28.2	7.3	4.8				2.5		1	25.9	5.4	4.8
		c			65.8				34.2	20	40			
14	11	a		151	0				151	14	112			
	5 mg/kg	b	+14	10.8					10.8		8.0			
		c			0				100	100	74			
36	11	a			23					32				
	5 mg/kg	b	+19.8									30	5.2	4.5
		c								89				
13	11 & 12	a		114					114	13	100			
	5 mg/kg	b	+16.2	8.8					8.8					
		c							100	100	88			
11	15 & 16	a		94	9	1	10	2	73	4	35			
	5 mg/kg	b	+18.5	8.5	.8	.09	.9	.18	6.6		3.1	7.9	4.3	3.2
		c			9.5	11.1	10.6	22.2	77.4	36.4	48			

a = Total b = Per mother c = Percentage of total * Percentage of live.

TABLE II. Desacetyl-thio-colchicine, 7 mg/kg i.p., in Rats.

No. of rats	Day of dose; mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Mothers with comp. litter destruction	Surviving placentas	Litter wt	Fetus	
					Live	Stunted*	Dead	Gross malf.*	Resorb.				Wt	Length
6	2x7 mg/kg	a		50	50									
	mate avg	b	40.3	8.3	8.3					0		45.6	5.5	4.7
	4.4 days	c												
15	4 & 5	a		106	78	3	3		25	3				
		b	+34.5	7.05	5.2	.2	.2		1.6			29.0	4.87	4.55
		c			73.5	3.8	2.8		23.6	20	34			
25	7 & 8	a		208	71	3			137	9	48			
		b	+23.9	8.3	2.8				5.4		1.9	20.0	4.6	3.84
		c			34.2	4.2			65.8	36	35			
3	10	a		28	1	1			27	2	27			
		b	+ 4.8	9.3					9.0		9.0	1.5	1.5	2.9
		c			3.6	100			96.4	66.6	100			
23	11 & 12	a		198					198	23	135			
		b	+13.3	8.6					8.6		5.9			
		c			0	0	0	0	100	100	68.2			
24	15 & 16	a		204			204			24	204			
		b	+22.3	8.5	0	0	8.5				8.5			
		c					100			100	100			
19	18 & 19	a		156			154		2	19	156			
		b	+22.4	8.2	0	0	8.1		.15		8.2			1.3
		c					98.6		1.4	100	100			dead
9	15 & 16	a		85					85	9	72.7			
	prog./50 mg	b	22.8	9.4					9.4		9.0			
		c							100	100	84.7			

a = Total b = Per mother c = Percentage of total * Percentage of live.

87% of all fetuses, but permitted single fetuses, in litter of 7 rats out of 11 animals, to survive. However, doses of 7 mg/kg, given on 11th and 12th, or 15th and 16th, or 18th and 19th days of gestation, destroyed all fetuses of all litters. Two doses of 50 mg of progesterone, given 1 hour before the 7 mg/kg injection of TC, did not protect litters of experimental rats 15 and 16 days pregnant. It is evident that 1 or 2 i.p. doses of 7 mg/kg of TC is lethal to litters from 11th day of gestation onwards. The same doses have less effect on litters at an earlier gestation age, and no effect when given prior to insemination, or at day 0 and 1 of gestation.

Maternal structures. Macro- and microscopically, no effect of TC on the maternal organism was noted in animals treated up to 12th day of gestation and sacrificed on 21st day. Animals treated later showed a mild depression of their bone marrow. This was most noticeable in rats treated on days 18 and 19 of pregnancy. For peripheral blood and bone marrow studies see below.

In many instances, *placentas* survived the resorbed fetuses, especially when rats were treated after midterm. Surviving placentas of fetuses destroyed at time of implantation were often small, measuring only 2-4 mm in diameter, and were partly degenerated. Organs of rats treated at midterm or later corresponded to placentas of control animals of that gestation age. Fetal placental development did not occur in placentas which lost their fetus at midterm. The mammary glands of all experimental rats, who had their entire litters destroyed, even at time of implantation, showed secretory activity comparable to that of normal pregnant controls.

Repeated litter destruction in the same animals with TC. 15 experimental rats, 6 months old and weighing approximately 250 g, who had one satisfactory litter, were mated and on sperm findings in the vagina separated in single cages. On day 7 and 8 of gestation, the rats received, during their first experimental pregnancy, .5 mg/kg TC i.p. and were then remated on day 22, the day of expected delivery and ovulation. Again daily checks for vaginal sperm were made and the animals were injected on days 7 and 8 of the new gestation period, now with 7 mg/kg TC. The dosage was increased because one experimental rat, not contained in this group, produced 2 fetuses after 5 mg/kg doses on days 7 and 8 of her pregnancy. This group of rats were aborted 4 successive times and then remated and permitted to litter. The total average time, in which 4 abortions and post-abortive litters were achieved, was 131 days or 26 days per "pregnancy." The average body weight gain during the period of 4 abortions, *i.e.*, from day 0 of first aborted pregnancy to day 0 of last pregnancy which was not interfered with, was 41%. The breeding performance of rats in their sixth pregnancy (1 pre-experimental litter, 4 abortions and 1 post-abortive litter) was good. The 15 rats had a total of 134 fetuses, average of 8.9 fetuses/mother. Litters had an average weight of 53 g. The average fetus had a weight of 5.9 g, and measured 5 cm in length. Litters were reduced to 3 siblings each and permitted to nurse. After 22 days, the young rats were weaned, put on Long-Evans breeding diet and cross bred. Growth of these rats was normal, as was their breeding performance. No abnormalities were seen by roentgenography on their skeletons or in litters produced.

Experiments with desacetyl-methyl-colchicine (MC). MC, first isolated by Santavy and Reichstein(4) and later synthesized by Uffer, *et al.*(6), inhibited mitosis with less toxicity than colchicine/Cernoch, *et al.*(2). The single LD-100 for the rat was 35 mg/kg, which was 7 times less than the LD-100 for colchicine. Didcock, Jackson and Robson (3) treated pregnant mice and rabbits with varying doses of MC orally and s.c. It interrupted the pregnancy of mice 11 to 13 days pregnant, in doses of 1 to 8 mg/kg s.c. without maternal mortality, but was less effective orally. Rabbits 13 to 16 days pregnant showed interruption of their pregnancy in s.c. doses of 2 to 8 mg/kg, but showed no response to a 2 mg/kg oral dose. The effect of MC was manifested in 1 to 2 days after injection. Our experiments were conducted to study effect of MC on rat litter *in utero* and to compare its action with that of other compounds.

Methods. Groups of pregnant rats, as

TABLE III. Desacetyl-Methyl Colchicine, i.p., in Rats.

No. of rats	Day of dose; mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Mothers with comp. litter destruction	Surviving placentas	Litter wt	Fetus	
					Live	Stunted*	Dead	Gross malf.*	Resorb.				Wt	Length
14	7 & 8	a		123	43	16	2		78	5	50			
	1 mg	b	+24.6	8.8	3.1	1.8			5.7			15	3.1	3.6
		c			34.9	60.0	1.6		63.5	35.7	63.5			
14	7 & 8	a		134					134	14	134			
	2.5 mg	b	+10.6	9.6					9.6		9.6			
		c							100	100	100			
13	7 & 8	a		115					115	13	95			
	5 mg	b	+ 4.2	8.9					8.9		7.3			
		c							100	100	82			
15	11 & 12	a		126	13				113	12	26			
	1 mg	b	+ 8.4	8.4	8.7				7.5			18.5	4.3	3.8
		c			10.3				89.7	80	20.6			
17	11 & 12	a		177					177	17	171			
	2.5 mg	b	+12.7	10.4					10.4		10			
		c							100	100	96.6			
12	11	a		94			94			12	94			
	5 mg	b	+11.2	7.8			7.8				7.8			
		c					100			100	100			
17	15 & 16	a		166			115		51	17	165			
	2.5 mg	b	+ 7.8	9.8										
		c					69.3		30.7	100	99.4			
10	15 & 16	a		93			93			10	93			
	5.0 mg	b	+13.3	9.3			9.3							
		c					100			100	100			
15	18 & 19	a		118	17		97		4	10	101			
	1.0 mg	b	+28.8	7.9	1.1		6.4				6.7	18.0	4.5	4.5
		c			14.4		82.2		3.4	66.6	100	live	0	
25	18 & 19	a		224			224			25	224			
	2.5 mg	b	+11.7	8.95			8.95				8.95	17.1	2.1	2.8
		c					100			100	100	dead		
14	18 & 19	a		113			113			14	113			
	7.5 mg	b	+24	8.1			8.1			8.1	8.1	17.4	2.15	2.9
		c					100			100	100	dead		

a = Total b = Per mother c = Percentage of total * Percentage of live.

described above, received the compound in mg/kg doses on 2 consecutive days i.p., at different periods of gestation. The results are listed in Table III. 1 mg/kg doses led to resorption of 63% of all fetuses, with stunting of 60% of survivors, when given at time of implantation (7th and 8th days). At midterm (11th and 12th days) 90% of all fetuses and 80% of all litters were destroyed with this dosage. The 2.5 mg/kg doses, as well as 5 mg/kg doses, destroyed all fetuses and all litters when given twice after implantation on days 11 and 12, or 15 and 16, or 18 and 19 of gestation. Autopsies of experimental animals treated on gestation days 15 and 16, or 18 and 19 showed, 6 to 12 hours after dose application, fetuses with marked edema, ascites, and hemorrhagic staining of internal organs and outer skin. Microscopically, the organs of mother animals were normal, except bone marrows and intestinal epithelium of rats treated on days 15 and 16, or 18 and 19 and sacrificed within 48 hours. Here 30% depletion of cellular contents of marrow and increased number of mitosis in the crypts of intestinal mucosa was noticeable. No gross abnormalities were seen, but stunting was noted in the few surviving fetuses of rats treated with the 1 mg/kg doses on days 7 and 8 or 11 and 12 of pregnancy. The skeletal ossification centers corresponded to embryos of a younger gestation age and no special bone was singled out in its inhibition.

Bone marrow and peripheral blood studies. Six rats, 200 g in weight, received 7.5 mg/kg TC and another 6 rats received 2.5 mg/kg MC on 2 consecutive days. Two rats of each group were sacrificed under ether and peripheral blood and bone marrow examined at 24, 48 and 72 hours. Hematocrit, hemoglobin, white blood count, differential count, and prothrombin estimation were carried out and bone marrow of sternum and femur was examined. Within 24 hours, the bone marrow of rats treated with MC was fluid, instead of solid and a marked depletion of myeloid elements was noted, with abnormal forms in the myeloid series involving all elements, from polymorphonuclears to myeloblasts. This persisted to 48th hour and was partly repaired at 72 hours. The hematocrit did not fall more than 3 mm, hemoglobin was reduced by 1.2 g and white blood count fell to 5,000, with a depression of polymorphonuclear leukocytes to 1%. TC was found to be less toxic. The marrow depression was less severe and neither hematocrit nor hemoglobin changed. The white blood count showed its greatest depression within 24 hours. The polymorphonuclear leukocytes were much decreased but began to rise after 24 hours. Prothrombin time (Quick's one stage method) of all 12 rats treated varied from 15 to 18 seconds, or from 29% to 48% of normal.

Discussion. TC and MC are used today in chemotherapy of leukemias and gout in man. It is therefore significant that the present data show that the litter of groups of rats have successfully been destroyed with 1 or 2 doses of TC or MC. The price in maternal tissues for the destruction of the litter consisted in a transitory depression of myeloid elements in bone marrow and of polymorphonuclear leukocytes in peripheral blood as well as inhibition of mitosis of epithelial lining of intestinal tract (as already stressed by Branceni, *et al.* (1).) The prothrombin time of our rats was also prolonged indicating a disturbed liver function. Both compounds permitted complete litter destruction without increase in dosage from the time of implantation of fetuses to day before littering. This could not be induced with other antimetabolites without severely intoxicating or sacrificing the mothers (5). Both TC and MC acted on fetuses within hours and induced general edema, ascites and death. In pregnant rats up to midterm, and frequently also at 11th and 12th days of gestation, the fetus would be resorbed with placentas surviving at their developmental stage, corresponding to last living hours of fetuses. In rats pregnant in the second half of their gestation period, the fetuses would stay *in situ* and shrink to a soft gray lifeless and often macerated mass.

The experiment with TC, in which 15 rats were 4 times aborted, was conducted to study the possible permanent effect of TC on fertility, subsequent litters and general intoxication of the mothers. The fact that experimental animals were able to complete 4 gestation periods with 4 abortions and successfully

carry a subsequent normal litter within 131 days is evidence of lack of intoxication. This is supported by an average weight gain of 41.4% of mother animals from onset of first aborted pregnancy to first day of the last carried through litter.

Summary. (1) One or 2 doses of 7 mg/kg of TC or 2.5 mg/kg of MC destroyed the litters of rats when given after 10th day of gestation period. (2) Placentas survived the fetuses. (3) Maternal response to dosage consisted in depression of myeloid tissues of bone marrow, peripheral WBC and prothrombin time. (4) Four consecutive litter destructions *in utero* with TC were well tolerated by a group of rats, did not impair their subsequent fertility or reproduction, nor cause abnormalities in subsequent offspring. (5) The same doses of TC or MC given prior to fertilization, or implantation were less effective in litter destruction.

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Cytochemistry of Male Reproductive Tract in Scurvy and Inanition.* (24083)

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Investigations have related ascorbic acid to the male reproductive tract. Lindsey and Medes(1) found degeneration of the germinal epithelium in acute scurvy. The inanition which accompanied scorbutus produced the same alterations(1). Goettsch(2) noted similar changes in the testes of scorbutic guinea pigs. Mukherjee and Banerjee(3) observed degeneration and fibrosis of germinal epithelium and Leydig cells in chronic scurvy. They related these alterations specifically to ascorbic acid deficiency rather than to the accompanying inanition. Vit. C studies on animals other than the guinea pig indicate that ascorbic acid is necessary to maintain male reproductive function(4). In the present investigation, cytochemical studies were carried out on the effects of avitaminosis C on the reproductive tract of the male guinea pig, and an attempt was made to differentiate these changes from those which result from inanition in the presence of an adequate dietary intake of ascorbic acid.

Materials and methods. A total of 80 male guinea pigs were used in this investigation. Each experimental group consisted of 4 animals which were fed as follows: (a) Vit. C deleted diet, *ad lib.*, (b) Vit. C deleted diet, *ad lib.*, plus 10 mg ascorbic acid daily, (c) Vit. C deleted diet plus 10 mg ascorbic acid daily with food consumption adjusted in order that the animal's weight losses would follow those of the scorbutic guinea pig, and (d) commercially prepared Vit. C fortified diet *ad lib.* The animal on the restricted diet (c) was used to differentiate between changes brought about by caloric restriction and those induced by ascorbic acid deficiency. The Vit. C deleted diet was prepared according to Woodruff *et al.*(5) with the following additions: 10 g $CaCo_3$, 2 g K acetate, 40 g Ca pantothenate, 12 mg folic acid and 200 mg ferric citrate per kg of diet. Groups remained on their respective diets until the scorbutic animal appeared

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