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Comparison of Teratogenic Effects of Administration by 6-Aminonicotinamide on Days 7 and 9 of Gestation in Mice

(teratogenesity/mice/6-aminonicotinamide)

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Pregnant ICR-JCL mice were given 6-aminonicotinamide (6-AN) in a dose of 8 and 15 mg/kg orally on the days 7 and 9, respectively. The animals were sacrificed on day 18 of gestation and the fetuses examined for external, visceral and skeletal anomalies in cleared specimens stained with alizarin red S.

Weight of all the pregnant mice decreased after treatment with the 6-AN. The fetal mortality rate after administration of 6-AN was significantly high in all groups, as compared with the controls. When two pregnant mice were given 15 mg/kg on day 7, resorptions in the uterus were seen on day 18. The mean body weight of living mice from the administered dams was dependent on the day of administration. There were relationships between the incidence of congenital anomalies and the the day of administration, and the dose level. In the skeleton of the fetuses from dams ingesting 6-AN, many anomalies were concentrated in the lumbosacral region and lower extremities. Congenital hydronephrosis was seen in two fetuses, when 8 mg/kg was given to the dams on day 7.

Antagonists of certain vitamins can produce congenital malformations in experimental animals, when given during pregnancy (1, 2). Since the niacin antimetabolite 6-aminonicotinamide (6-AN) was shown to produce multiple congenital malformations in the rat (3), there have been numerous studies in which the mechanism of teratogenesis was investigated using teratological and biochemical approaches. Recent reports included evidence for cleft lip in mice (4), eye defects in rats (5), impaired development of capillarization in rats (6), cleft palate in mice (7) and in rats (8), limb underdevelopment in several species (9), and energy metabolism in the neural tube in rats (10).

The teratogenic effect of an antimetabolite on the mammalian embryo varies according with the dosage and with developmental stage at the time of administration. According to Wilson (2), the antimetabolites generally have their greatest effects early in pregnancy. Chamberlain and Nelson (11) studied the effects of 6-AN injected into rats on each day of pregnancy from day 9 through day 20.

The present study was undertaken to assess the relationship between the incidence and type of fetal malformations and administration of 6-AN orally

to pregnant mice, a relationship not ascertained in the previously reported studies.

MATERIALS AND METHODS

ICR-JCL mice were purchased from the Central Laboratories for Experimental Animals, Tokyo, Japan. Nulliparous females at 9 to 12 weeks of age were placed in the evening with mature normal males from the same colony and copulation was ascertained the following morning by the presence of a vaginal plug. When a plug was found, the pregnancy was designated as day 0 (zero) and the pregnant mouse was maintained on a stock diet from the Funabashi Farm Company (Japan); distilled water was provided for drinking until day 18 of gestation.

A single oral administration of 0.5% CMC aqueous solution of 6-AN (8 mg/kg and 15 mg/kg body weight) was given to each of 7 mice on days 7 and 9 of gestation. These doses were based on Murphy's data (12). Food and water intake was monitored daily and the mice were killed on the 18th day of gestation. Control mice were given a single administration of 0.5% CMC aqueous solution.

The uterus was examined for resorption sites and the surviving fetuses for external deformities under a stereomicroscope. The living pups were removed, counted, weighed, and measured for crown-rump length. The head, abdomen and thorax were opened and abnormal organs were removed and stored in 10% formalin for subsequent histological study. The pups in each litter were fixed in 95% alcohol for skeletal study, after clearing with potassium hydroxide and staining with alizarin red S, and were then stored in glycerin, by the modification of Dawson's method (13).

RESULTS

The groups given 6-AN are hereafter referred to as 7—8 (8 mg/kg at day 7), 7—15 (15 mg/kg at day 7), 9—8 (8 mg/kg at day 9), and 9—15 (15 mg/kg at day 9).

Dams

The weight dams of given 6-AN decreased with the treatment (Figs. 1 and 2). Four mice out of 14 in 7—15 and 9—15 groups showed an incoordination of the hind limbs after 2 or 3 days of treatment. Recovery was gradual after 6—8 days of treatment. No remarkable findings were noted in the autopsied dams. The 14 control mice showed normal findings at autopsy.

Fetuses

Effects of a single administration of 6-AN on the fetuses are summarized in Tables I-IV, and findings in the control are shown in Tables V and VI. In the 6-AN administered groups and in the controls (Fig. 3), there were

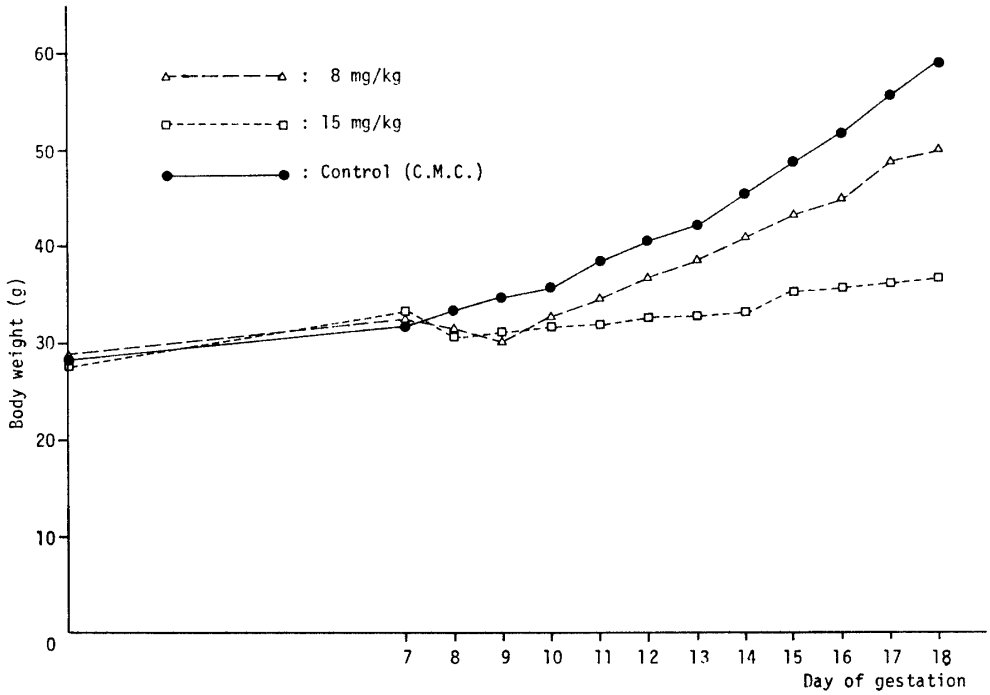

Fig. 1. Mean body weight changes of pregnant mice treated with 6-AN orally on 7th day of gestation. (group sacrificed before delivery)

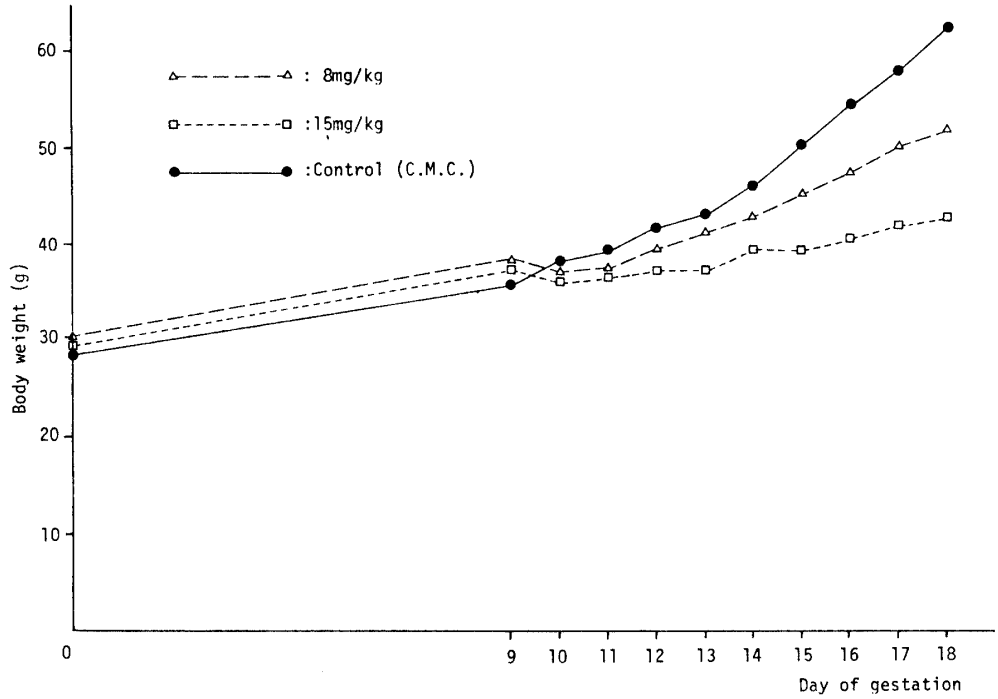

Fig. 2. Mean body weight changes of pregnant mice treated with 6-AN orally on 9th day of gestation. (group sacrificed before delivery)

TABLE I. *Effect of 6-AN (8 mg/kg) on Mouse Fetuses Following Oral Administration to Dams on Day 7 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.		External and visceral anomalies type and No. of each anomaly	
				M	F	M	F	M	F		
1	9	2	7	4	3	1.30	1.31	25.3	26.0	Club foot	1
2	16	3	13	7	6	1.35	1.35	25.4	26.1		
3	13	2	11	6	5	1.50	1.48	27.7	27.8	Cleft palate	1
4	17	9	8	4	4	1.10	1.26	24.0	24.5	Hydronephrosis Club foot	1 1
5	16	15	1	1	0	1.57	—	28.0	—		
6	14	3	11	4	7	1.46	1.44	27.5	27.6		
7	16	13	3	1	2	0.86	1.05	22.0	23.0	Hydronephrosis	1
Total	101	47	54	27	27						5
Mean	14.4	6.7	7.7			1.31	1.32	25.7	25.8		
S.E.	±1.04	±2.10	±1.67			±0.095	±0.063	±0.84	±0.75		

B. W. : Body weight, C. R. L. : Crown-rump length, M : Male, F : Female

TABLE II. *Effect of 6-AN (15 mg/kg) on Mouse Fetuses Following Oral Administration to Dams on Day 7 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.		External and visceral anomalies type and No. of each anomaly	
				M	F	M	F	M	F		
1	16	12	4	1	3	1.15	1.26	24.0	26.7	Cleft palate	2
2	20	17	3	2	1	0.68	0.56	19.5	19.0	Club foot	1
3	16	14	2	1	1	1.38	1.47	28.0	27.0	Cleft palate	1
4	16	16	0	0	0	—	—	—	—		
5	16	16	0	0	0	—	—	—	—		
6	12	4	8	3	5	1.05	1.11	24.7	25.4	Hydrocephalus	1
7	14	13	1	1	0	1.12	—	25.0	—		
Total	110	92	18	8	10						5
Mean	15.7	13.1	2.6			1.08	1.10	24.2	24.5		
S.E.	±0.92	±1.67	±1.07			±0.114	±0.194	±1.37	±1.88		

B. W. : Body weight, C. R. L. : Crown-rump length, M : Male, F : Female

TABLE III. *Effect of 6-AN (8 mg/kg) on Mouse Fetuses Following Oral Administration to Dams on Day 9 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.		External and visceral anomalies type and No. of each anomaly	
				M	F	M	F	M	F		
1	11	1	10	5	5	1.51	1.48	26.6	26.2	Club foot	1
2	15	5	10	6	4	1.12	0.97	24.3	22.8	Cleft palate	1
3	12	4	8	4	4	1.02	0.98	23.3	24.0	Cleft palate Syndactly Oligodactly	2 1 1
4	18	9	9	5	4	1.35	1.29	26.0	25.3		
5	13	1	12	5	7	1.22	1.17	25.6	25.6		
6	11	2	9	4	5	1.36	1.31	27.5	27.2		
7	12	6	6	1	5	0.82	1.24	22.0	26.0	Cleft foot	2
Total	92	28	64	30	34						8
Mean	13.1	4.0	9.1			1.20	1.21	25.0	25.3		
S.E.	±0.96	±1.11	±0.70			±0.089	±0.070	±0.73	±0.56		

B. W. : Body weight, C. R. L. : Crown-rump length, M : Male, F : Female

TABLE IV. *Effect of 6-AN (15 mg/kg) on Mouse Fetuses Following Oral Administration to Dams on Day 9 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.		External and visceral anomalies type and No. of each anomaly	
				M	F	M	F	M	F		
1	17	7	10	3	7	1.18	1.14	26.7	25.6		
2	16	12	4	2	2	1.09	1.00	24.0	24.5	Cleft palate	3
3	12	5	7	3	4	1.27	1.26	26.3	25.0		
4	14	12	2	2	0	0.72	—	20.5	—	Oligodactly	1
5	13	10	3	1	2	0.98	0.97	23.5	23.0	Coiled tail	1
6	11	5	6	5	1	0.97	0.89	21.6	24.0	Cleft palate Oligodactyly Club foot Syndactyly	2 1 1 1
7	15	11	4	2	2	0.86	0.99	21.5	23.5	Cleft palate Harelip Club foot	1 1 4
Total	98	62	36	18	18						
Mean	14.0	8.9	5.14			1.01	1.04	23.4	24.3		
S.E.	±0.82	±1.03				±0.074	±0.055	±0.91	±0.40		

B. W. : Body weight, C. R. L. : Crown-rump length, M : Male, F : Female

TABLE V. *Effect of Control (0.5% C. M. C.) on Mouse Fetuses Following Oral Administration to Dams on Day 7 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.	
				M	F	M	F	M	F
1	14	4	10	6	4	1.42	1.32	25.7	25.8
2	15	2	13	7	6	1.36	1.37	26.1	26.1
3	13	1	12	8	4	1.32	1.27	26.5	26.5
4	13	0	13	6	7	1.48	1.42	27.8	27.2
5	11	2	9	2	7	1.51	1.36	28.5	26.1
6	16	1	15	8	7	1.36	1.30	27.1	26.1
7	13	2	11	5	6	1.46	1.40	27.2	26.8
Total	95	12	83	42	41				
Mean	13.6	1.7	11.9			1.42	1.35	27.0	26.4
S.E.	±0.61	±0.47	±0.77			±0.032	±0.001	±0.37	±0.18

External and visceral anomalies were nil.
B. W. : Body weight, C. R. L. : Crown-rump length, M : Male, F : Female

TABLE VI. *Effect of Control (0.5% C. M. C) on Mouse fetuses Following Oral Administration to Dams on Day 7 of Gestation*

Dam	No. of implants	Resorbed or dead	Alive	Sex		Mean B. W.		C. R. L.	
				M	F	M	F	M	F
1	15	0	15	10	5	1.40	1.36	27.1	27.0
2	16	0	16	8	8	1.17	1.19	25.5	25.1
3	13	3	10	7	3	1.43	1.32	26.6	25.3
4	15	1	14	7	7	1.45	1.51	27.7	26.7
5	11	0	11	7	4	1.55	1.48	28.1	27.5
6	12	1	11	4	7	1.82	1.65	29.0	28.0
7	14	3	11	5	6	1.39	1.31	26.8	26.7
Total	96	8	88	48	40				
Mean	13.7	1.1	12.6			1.46	1.40	27.3	26.6
S.E.	±0.46	±0.51	±0.0			±0.074	±0.057	±0.43	±0.41

External and visceral anomalies were nil.
B. W. : weight, C. R. L. : Crown-rump length, M : Male, F : Female

no significant differences in the number of implantations and sex ratio of the living fetuses, regardless of the day of treatment and dosage levels of 6-AN. The incidences of dead fetuses and resorptions were 46.5% (7—8), 83.6% (7—15), 30.4% (9—8), and 63.3% (9—15), respectively, while that of the control groups was about 10%. The mean body weight of the 6-AN group was related to the day of administration. The mean body weight was light in the male fetuses in the 9—8 and 9—15 groups. Such was significantly different from the control. A trend toward the mean of crown-rump length was similar to that of the body weight.

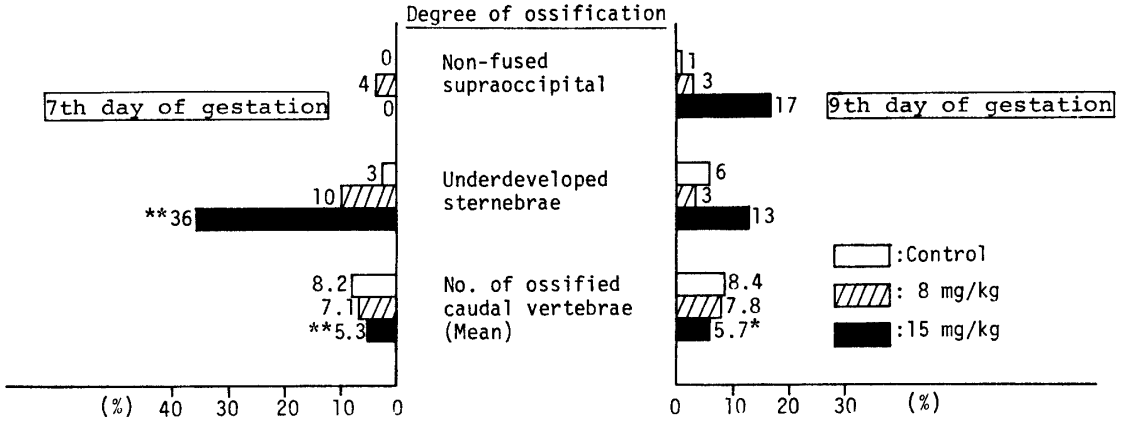

Fig. 12. Comparison of the degree of ossification in mice treated with 6-AN orally on the 7th and 9th days of gestation.

* : Statistical difference from control ($P < 0.05$)
** : Statistical difference from control ($P < 0.05$)

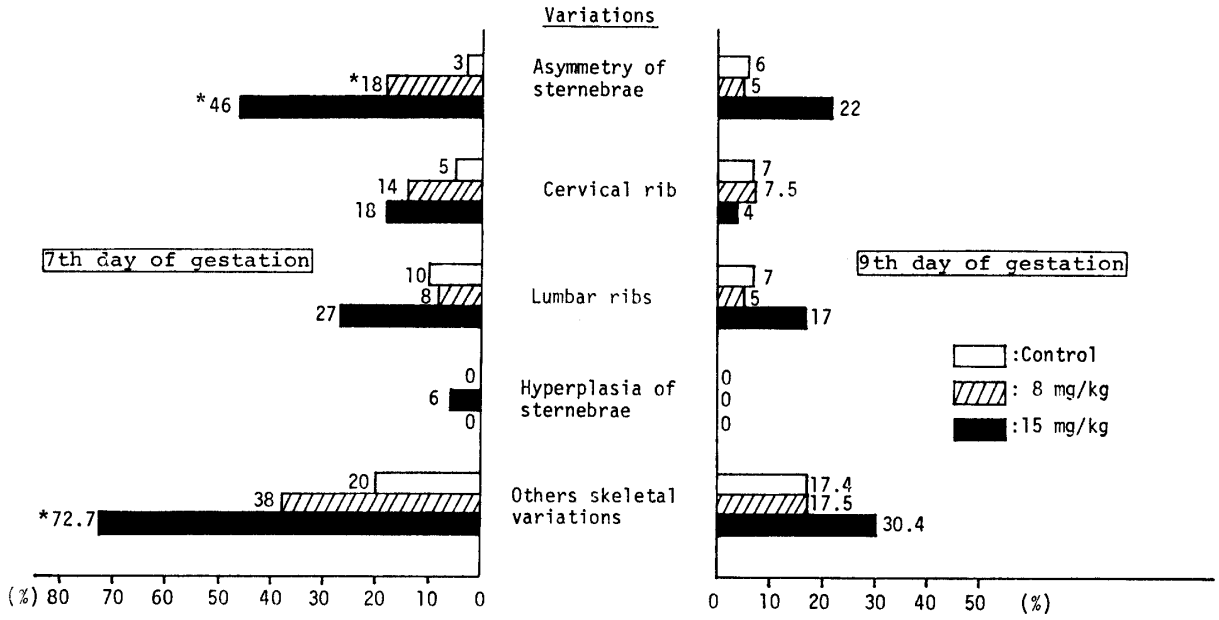

Fig. 13. Comparison of skeletal variants of mice treated with 6-AN orally on the 7th and 9th days of gestation.

* : Statistical difference from control ($P < 0.05$)
** : Statistical difference from control ($P < 0.05$)

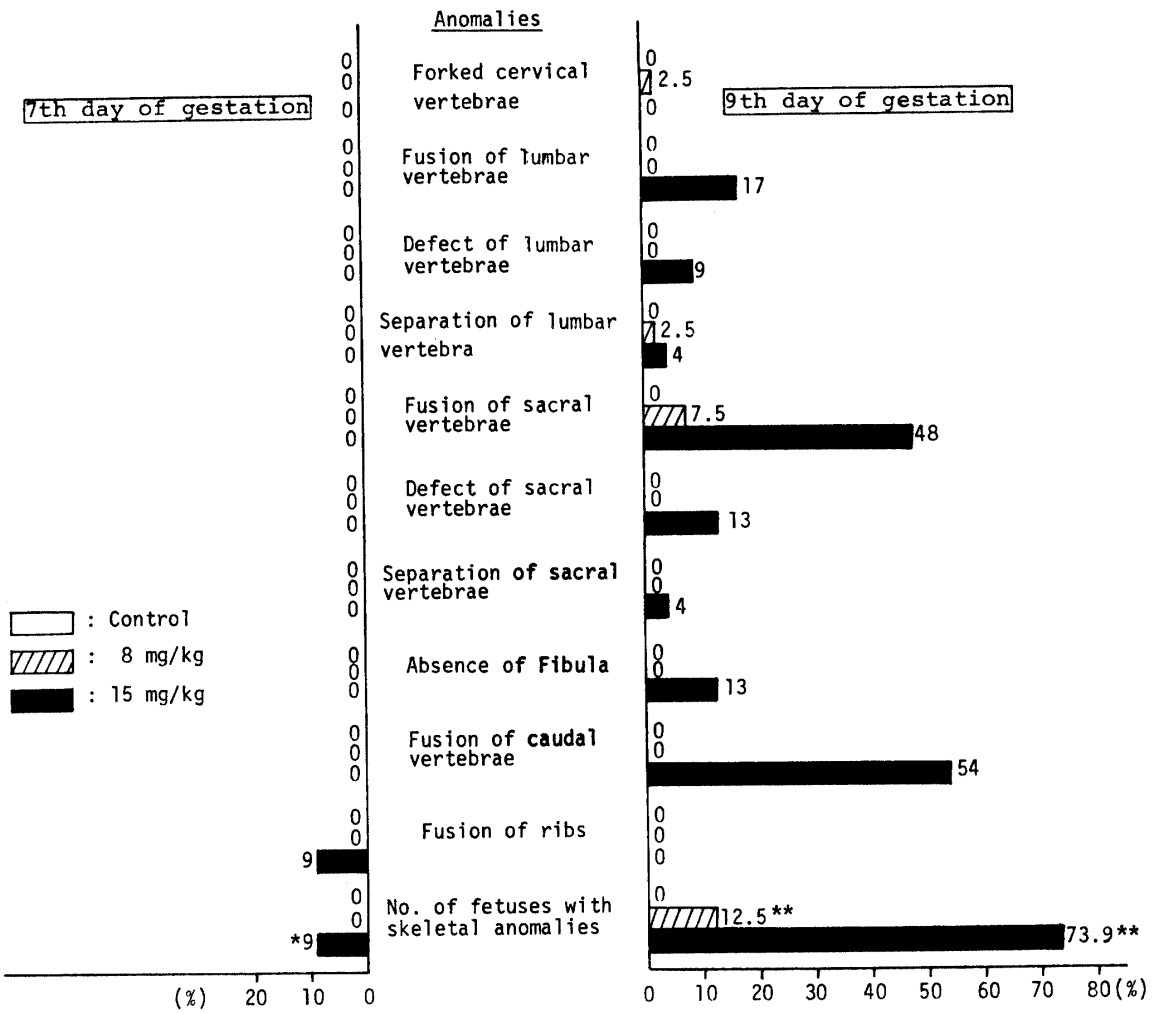

Fig. 14. Comparison of skeletal anomalies of mice treated with 6-AN orally on the 7th and 9th days of gestation.

* : statistical difference from control ($P<0.05$)
** : statistical difference from control ($P<0.01$)

Congenital Abnormalities

External malformation of the group at day 7 was 1 cleft palate (7—8), 2 cleft palates (7—15) (Fig. 4), and 2 club feet (7—8, 7—15). The incidence of these 5 cases was significantly different from that of the control group. In the administered group at day 9, there were 3 cleft palates, 2 cleft feet, 1 syndactyly, 1 club foot, and 1 oligodactyly (9—8) (Fig. 5); 9 cleft palates, 5 club feet, 4 oligodactyly (Fig. 6), cleft lip, absence of the foot (Fig. 7), 1 syndactyly (Fig. 8) and 1 coiled tail (9—15). The incidence of these external malformations showed a highly significant difference from the control group. Four (9—15) and one (7—8) generalized edematoses (Fig. 9) were observed, but these anomalies were not observed in the control groups. Although hydronephrosis were observed in 2 (7—8) and 1 (9—15) out of five edematoses, such was not significantly different from the control group.

Abnormalities of the skeleton were observed following a single administra-

tion of 6-AN in 7—15, 9—8 and 9—15 groups. In particular,73.9% fetuses in the 9—15 group had various skeletal anomalies. Two types out of these anomalies are shown in Figs. 10 and 11. However, this incidence included cases with the combined skeletal anomalies in a fetus. In each group of 7—15, 9—8 and 9—15, the incidence was highly significant from control. The skeletal findings in the present study are summarized in Figs. 12—14. Thus, the skeletal variations and the incidence of occurrence of asymmetry of sternal ossification centers was significantly different from control in 7—8 and 7—15. The numbers of the coccygeal ossification centers were counted as an indicator of the degree of ossification. We found that the ossification was retarded in the administered groups, on days 7 and 9. A non-ossified center of the sternum was evident in the fetuses of both the administered and control group, and was remarkable in the 7—15 group.

The incidence of abnormal living young was 9.2% (7—8), 0.32% (9—8), 33.3% (7—15), and 54.5% (9—15), respectively. There were relationships in the incidence of the skeletal and other abnormalities between the day of administration, and the dose levels.

DISCUSSION

There were more early resorptions than dead fetuses in the mice given 6-AN and the incidence was higher on day 7 than on day 9. Overdoses of vitamin D_2 to pregnant rats resulted in a resorption of the implanted blastocysts (14). Oxazepam given to mice caused a significant number of resorptions, as compared to controls (15). The high percentage of embryonic mortality and abnormalities when 6-AN was injected in a single administration during the third week of pregnancy is noteworthy, as very few procedures have been shown to affect embryonic development when instituted during the latter part of gestation and this may reflect the increased fetal need for niacin during this period (11). External malformations in the group at day 9 were higher than at day 7, regardless of the dose given to the dams. Externally malformations in the day 9 groups were more various than at day 7. There have been numerous studies on teratogenic effects of 6-AN during organogenesis in the chick embryo, mouse, rat, rabbit, hamster, and monkey (16—26). Curley and Zappasodi reported congenital defects of the cleft palate, abnormal head and genital abnormalities of fetal mice following intraperitoneal injection with 20 mg/kg of 6-AN during pregnancy. They also reported vertebral defects, such as lumbosacral vertebral defects, absence of the tibia and fibula, fusion on ribs, and forked rib (27). These malformed types were similar to results of the present study in mice following oral administration of 8 mg/kg and 15 mg/kg. In the present study, skeletal defects such as fusion of ribs and agenesis of the tibia resembled those produced by administration of 6-AN in mice. Many malformations were noted in the lumbosacral region (Fig. 11) and limbs, as seen in the form of defects in the tibia and fibula. The limb defects seen in the present study were similar to the findings

in McLachlan's investigation (9) and others (2, 18, 28). In the present study, it was clear that the degree of teratogenic effects of 6-AN was dependent on the period of time and on the dose of the antimetabolite administered during organogenesis.

Explanations of the mechanism of teratogenesis of 6-AN have been reported (10, 24, 25 and 29–34). Ritter *et al.* found a dramatic decrease in whole-embryo ATP following administration of the antimetabolite (34). In other studies, pregnant rats were injected with 2 mg/kg 6-AN on day 9 of gestation, and glucose, glycogen, lactate, ATP, phosphocreatine, and gamma aminobutyric acid levels were measured in the neural tube (10). Two metabolites, ATP and phosphocreatine, were increased under conditions of this administration. The mechanism of these alterations in metabolites is unclear, but may be related to a decrease in metabolic demand.

Malformations can be induced with the niacin analog 6-AN, that is, a specific antagonist to normal dietary factors. The mechanism of teratogenesis of 6-AN remains to be elucidated. Our data provide an insight into the pathological effects of antimetabolites.

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EXPLANATION OF FIGURES

Fig. 3. Lateral view of the normal fetus on day 18 of gestation (control). (× 4.5)

Fig. 4. Ventral view of the palate in day 18. (× 5.7) 6-AN in a dose of 8 mg/kg was given on day 7 of gestation. Note non-fusion of the bilateral maxillary process (arrows).

Fig. 5. Palmar view of the left foot in the fetus in the case of administration of 15 mg/kg of 6-AN on day 9 of gestation. (× 5.7) Note the absence of I, II, and V toes.

Fig. 6. Lateral view of the right foot in the fetus in the case of administration of 15 mg/kg of 6-AN on day 9 of gestation. (× 5.4) Note the absence of II, and fusion of III and IV toes.

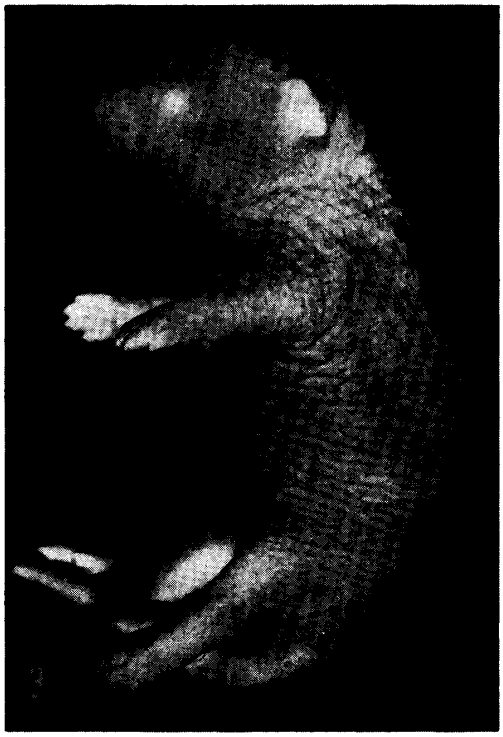
Fig. 7. Lateral view of the left lower limb in a fetus. 15 mg/kg of 6-AN had been given to the dam on day 9 of gestation. (× 5.2) Note the absence of the lower part in the leg.

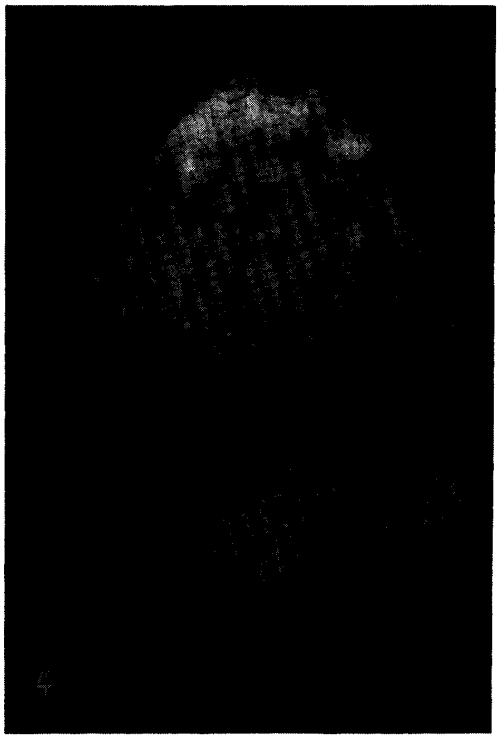
Fig. 8. Lateral view of the left foot in the fetus in the case of administration of 15 mg/kg of 6-AN on day 9 of gestation. (× 5.7) Note the fusion of IV and V toes.

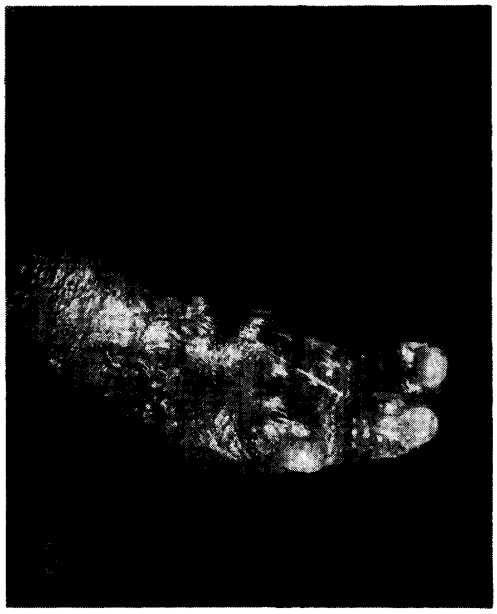
Fig. 9. Lateral view of externally malformed fetus at day 18. (× 4.5). 6-AN administered with 15 mg/kg on day 9 of gestation. Note the stocky feature, short upper limbs and clubfoot.

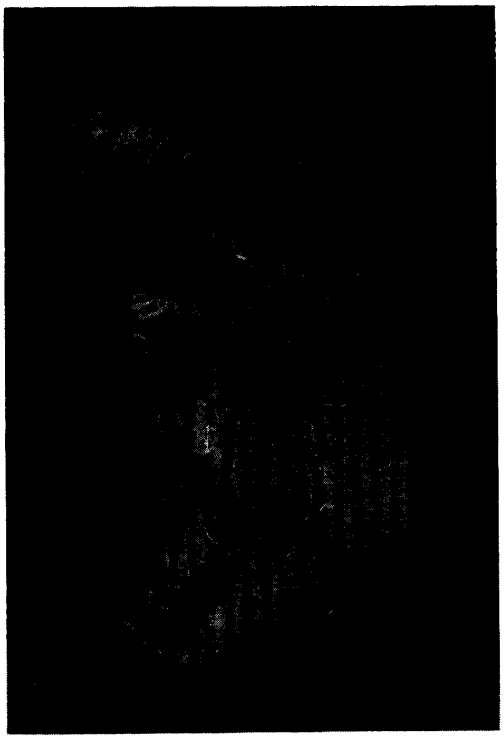
Fig. 10. Frontal view of the cleared specimen stained with alizarin red S. (× 5.2) 6-AN in a dose of 8 mg/kg had been given on day 7 of gestation. Note the asymmetry of the sternal ossification centers (arrow).

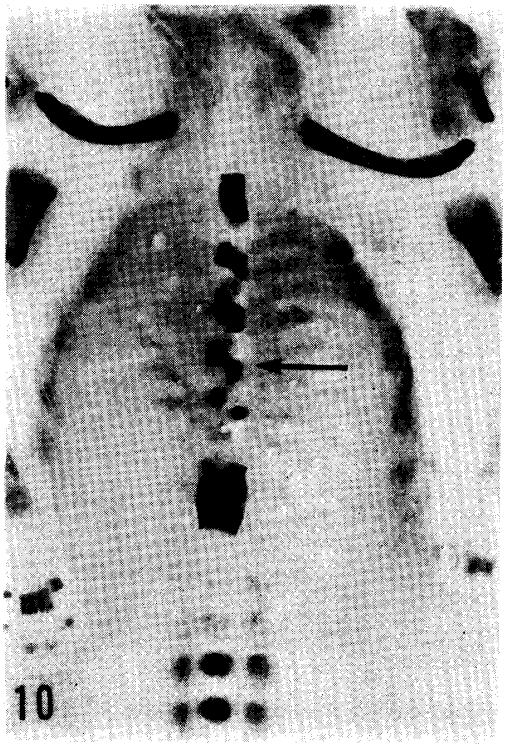
Fig. 11. Frontal view of the cleared specimen stained with alizarin red S. (× 5.2) Note the absence of the sacral vertebra (arrow).











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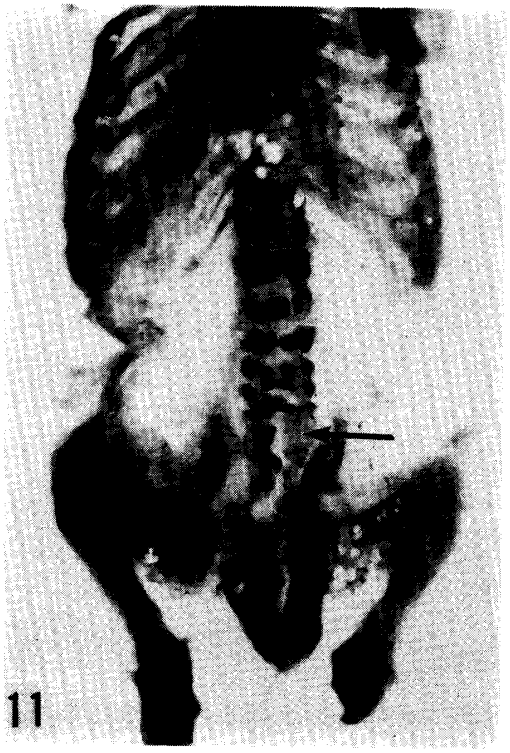

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