COMPARISON OF TERATOGENIC CHEMICALS IN
THE RAT AND CHICK EMBRYOS

An Exhibit with Additions for Publication

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The chick embryo is used widely in studying drugs for teratogenic activity. Chemicals, which have been injected at the same period of incubation of the chick embryo, may produce separate and characteristic patterns of developmental abnormalities. The effects of many drugs, such as insulin, azaserine, sulfanilamide, 4-aminopteroyl glutamic acid, 8-azaguanine, physostigmine, thallium, lead, boric acid and cortisone, have been reviewed.1-4

The chick embryo in the egg is an isolated and independent system, whereas mammalian embryos are usually intimately involved with the maternal host which may detoxify, excrete or otherwise protect the fetus against noxious chemicals. It was, therefore, of interest to determine the effects of drugs, teratogenic in the chick embryo, on the mammalian fetus. The rat was selected because of its availability and because there is considerable information on rat embryology and teratology.5-8 Because some drugs, apparently inactive in the chick embryo, have produced consistent developmental abnormalities in the rat fetus, the pregnant rat also has been used for the initial study of selected compounds.

The objectives of these studies were: 1) to determine and compare the teratogenic action of drugs on the chick and rat embryos; 2) to determine the consistency and specificity of action of each drug; 3) to determine the relation between the time during gestation when a drug is introduced and the occurrence of specific abnormalities; 4) to determine the relationship of the dose of a drug toxic to the mother, the dose toxic to the embryo, and the dose producing consistent developmental defects, and 5) to detect compounds which protect the embryo from the teratogenic action of a chemical.

Observations on five distinctive compounds are presented in this exhibit.

MATERIALS AND METHODS

White Leghorn eggs were obtained from a commercial source and incubated at 38°C. The test chemicals, at various dose levels, were routinely injected into the yolk sac of the 4-day-old embryos. The embryos surviving the treatment were killed at 18 days of incubation, or 14 days after the injection of the drug.

Previously unmated female rats, Wistar strain, were placed in cages with males and watched for 12 hours. Those observed in copulation were isolated and regarded as zero days pregnant. At various times, ranging from 7 to 16 days of gestation, groups of rats received a single intraperitoneal injection at various dose levels of the test drugs. The pregnant rats were sacrificed on the twenty-first day of gestation, the uteri examined, the implantation sites counted, and the surviving fetuses removed for study.

The chick embryos and rat fetuses were examined grossly, weighed and then cleared and the skeleton stained with alizarin so that it could be examined macroscopically in the whole animal.

The test chemicals were dissolved in saline or suspended in carboxymethylcellulose (CMC) immediately before injection.
FIG. 1.
DEVELOPMENT OF THE CHICK AND RAT EMBRYOS (FIG. I)

Chick Embryos: The incubation period of the chick embryo is 21 days. Growth and differentiation proceed rapidly, and many detailed descriptions are available. The classical monograph on chick embryology is by Lillie and Hamilton. Hamburger and Hamilton have prepared an atlas of the stages of embryonic development, and related them to incubation time. By 18 to 24 hours the primitive streak has appeared, heart beat begins about the end of second day (9 somite stage), the tailbud appears at about 3½ days of incubation, and the initiation of endocrine function and the “end of metamorphosis” begins about the eighth to ninth days. Cartilage appears at the fifth day, and active calcification of bone begins about the eleventh day. When the chick hatches it weighs about 30 gm, has feathers and is able to feed itself.

Rat Embryos: The rat, from the time of fertilization, has a gestation period of about 22 days. Implantation begins about the seventh day, and embryonic development is, in general, about 6 to 8 days behind that of the chick embryo. Thus, the primitive streak appears at 8½ to 9 days, the heart begins to beat about the ninth day, the tailbud appears at 11½ days, and the “end of metamorphosis” appears at 16 to 17 days. Ossification begins on the sixteenth day. Thus, the 4-day-old chick embryo is at about the same stage as the 11 to 12-day-old rat fetus, and the 8-day chick embryo is equivalent in development to the 15 to 16-day rat fetus. These comparative periods are important, because it is noted that teratogenic effects to be described are most commonly produced in the chick embryo treated on the fourth day, and the rat fetus on the tenth to twelfth days. At birth the rat fetus weighs 4 to 5 gm which is equal to the 12-day chick embryo, and its development is far less advanced than the chick.

NITROGEN MUSTARD (FIG. 2)

Methyl-bis (2-chloroethyl)amine hydrochloride (HN2), a nitrogen mustard, is a crystalline substance, soluble in water. Closely related structurally and pharmacologically to the war-gas, mustard gas, it was intensively studied during World War II as a potential chemical warfare agent. In the past 10 years it has been widely used in the treatment of neoplastic disease in man; principally in tumors of the lymph nodes and blood forming organs. HN2 has wide and clearly defined patterns of biologic effects, which have been reviewed on several occasions. It is carcinogenic, mutagenic, and in mammals toxic doses produce severe depression of the bone marrow, with decrease in the formed elements of the blood, involution of lymphatic tissue, and injury to the intestinal mucosa; these effects are similar to those produced by total body irradiation. HN2 inhibits the growth of transplantable tumors, and produces characteristic histologic changes in mouse tumors, and in mouse and human tumors growing on the chorioallantoic membrane (CAM) of the chick embryo. Teratogenic effects in the rat fetus have been described by Haskins.

Teratogenic Effects

Chick Embryo: The LD₅₀ of HN2 is variable and doses in the range of 0.01 mg/egg may be lethal for the 4-day embryo. Death usually occurs within 1 to 5 days after injection, and those surviving in the dose range of the LD₅₀ are usually normal. An occasional embryo, however, shows weight inhibition, crossed beak, and thin legs.

Rat Embryo: The LD₅₀ of single doses of HN2 given intraperitoneally to the pregnant rat is in the range of 2.0 mg/kg; this is in the same range as the LD₅₀ in the nonpregnant rat (1.9 mg/kg by the subcutaneous route and 1.1 mg/kg intravenously). Pregnant rats were given single injections of HN2 intraperitoneally ranging...
**HN2 (nitrogen mustard)**

\[
\text{CH}_3\text{N<CH}_2\text{CH}_2\text{Cl}
\]

**18 DAY EMBRYO (INJ. 4 DAY)**

**EFFECTIVE DOSE 0.01 MG./EGG**

**ABNORMALITIES INFREQUENT**
- Cross beak
- Thin legs

**MATERNAL LD50 2.0 MG./KG. INJ. 11 DAY**

**TERATOGENIC DOSE 0.5 MG./KG. INJ. 12 DAY**

**ABNORMALITIES INFREQUENT**
- Head flattened
- Mandible short
- Ribs angulated
- Rear extremities rotated
- Lens calcified

**ABNORMALITIES FREQUENT**
- Cranial circular defect
- Parietal, interparietal and supraoccipital
- Ribs angulated
- Vertebral bodies double, T11 and L3 and 4
- Scapulae curved
- Foretoes syndactyly, metacarpals missing

**SACRIFICED 21 DAY**

**Fig. 2.**
from 0.3 to 0.5 mg/kg on the seventh through the fourteenth days of gestation. The rats treated on the seventh day showed no evidence of pregnancy when sacrificed on the twenty-first day. Pregnant rats treated once on given days from the ninth to the fourteenth, while showing only slight increase in the incidence of resorbing implantation sites, had many stunted fetuses with a variety of gross abnormalities. The fetal abnormalities were inconstant in rats treated on the eleventh day of gestation. The fetuses were smaller than normal, the skull was sometimes flattened with a small jaw. The cleared skeleton showed elongated and flattened head, short mandible, angulated ribs, and occasionally calcified lenses and rotated pelvis and rear extremities. The most striking effects were seen in the fetuses of the pregnant rats treated on the twelfth day. Grossly there were encephaloceles at the crown of the head and cleft palates were a rare occurrence. Syndactyly or ectrodactyly of the front as well as the rear toes was sometimes seen. The cleared skeleton, even in specimens without gross evidence of encephalocele, showed a circular cranial defect involving the occipital, frontal, parietal and interparietal bones. The ribs were angulated, with ribs 12 and 13 sometimes missing, the vertebral canal showed widening in the cervical area. The vertebral bodies in the lower thoracic and upper lumbar regions were not fused and gave the appearance of double ossification centers. The scapulae were curved toward the body. Ossification centers in the digits appeared to be delayed although occasional fusion of metacarpals or metatarsals was found.

**Discussion**

HN2 has an inconstant effect on the chick embryo injected on the fourth day. It apparently is acutely toxic to the embryo and the surviving embryos show rare and inconstant abnormalities. In this respect HN2 differs from radiation, which produces consistent developmental defects in chick embryos treated on the fourth day. This difference may be related to the fact that tissue recovery after nitrogen mustard seems to be considerably more rapid than after irradiation. The rat fetus is apparently susceptible to the action of HN2 at about one-fourth or one-sixth the dose toxic to the mother. The effects are most striking when the embryo is treated on the twelfth day, but abnormalities can also be produced at higher doses when the embryos are treated on the fourteenth to sixteenth day. The skeletal effects produced have not yet been compared with those produced by irradiating the pregnant rat.

**3,3-DIMETHYL-1-PHENYLTRIAZENE (TRIAZENE) (FIG. 3)**

This triazene is an oily substance, which is prepared for injection by suspending it in 0.5% CMC or by dissolving it in peanut oil. Clarke et al. demonstrated that it inhibited the growth of transplantable Sarcoma-180 in mice; in subsequent studies it prolonged survival in mice with transplantable leukemia, and produced histologic changes and loss of viability in human and mouse tumors growing on the CAM of the chick embryo. In the mouse and rabbit this triazene produced depression of the bone marrow and a decrease in the formed elements of the blood. The histologic changes in the blood-forming organs and intestinal tract of the rabbit, and in tumors growing on the CAM of the chick embryo were similar to those produced by nitrogen mustard. Other distinctive actions of the triazene have been described: micromelia in the chick embryo, prevented by nicotinamide; and a “waltzing” syndrome in Swiss mice, prevented by nicotinic acid.

**Teratogenic Effects**

Chick Embryo: The yolk sac of 4-day embryos was injected with the triazene suspended in CMC; the LD₅₀ was in the range of 0.5 to 1 mg/egg, and embryos surviving these doses showed consistent developmental abnormalities. The general effects were weight inhibition, edema and occasionally short, clubbed feathers. Specific effects occurred in the appendicular skele-
TRIAZENE

CH₃

CH₃

18 DAY EMBRYO (INJ. 4 DAY)
EFFECTIVE DOSE 0.5 MG./EGG
ABNORMALITIES FREQUENT
Parrot beak
Micromelia
Tibiotarsus bent
Tarsometatarsus bent

MATERNAL LD₅₀ 180 MG./KG. 
TERATOGENIC DOSE 30 MG./KG.
SACRIFICED 21 DAY

ABNORMALITIES FREQUENT
Scapula small, narrow, curved
Humerus and radius small
Ulna, tibia and femur missing

ABNORMALITIES FREQUENT
Maxilla and mandible short
Zygoma missing
Toes syndactyous
Ribs small or missing

FIG. 3.
ton, with micromelia, bending of the tibiotalus and occasionally the tarsometatarsus. Beak abnormalities ranged from parrot-beak to shortening of the lower beak or both upper and lower beaks. Nicotinamide, 5 mg/egg, given at the same time as the triazene protected against the skeletal defects, but did not prevent stunting or mortality.

**Rat Embryos:** The LD$_{50}$ of single doses of the triazene (in peanut oil) given intraperitoneally in the pregnant rat was in the range of 180 to 200 mg/kg; death often occurred several days after an LD$_{50}$ dose. Pregnant rats were given single doses of 30 mg/kg of the triazene on days 6, 9, 11, 12, 14, or 16 of gestation. Rats treated on the sixth day showed no abnormal fetuses, and only a slight increase in fetal resorptions; there were few fetal abnormalities in rats treated on the ninth, and the fourteenth and sixteenth day. The fetuses of rats treated on the eleventh or twelfth days showed consistent and severe gross and skeletal abnormalities; few resorptions occurred, however, and the abnormal fetuses survived to 21 days.

The embryos treated at 11 days were stunted and occasionally edematous, and the extremities were shortened. The front paws did not rotate normally and resembled seal flippers. The cleared skeleton typically had normal digits. The front extremities showed a small curved or absent humerus and the hind legs showed a small or absent femur, absent tibia and small pelvic bones. The scapula was small, curved or the spinous process rotated and elongated laterally and downward to resemble a double scapula. The vertebral bodies appeared double from T1 to L3; the sternebrae were double or fused cephalocaudal.

The 12-day embryos were strikingly different. The paws appeared short and pointed, the nose was pointed, the lower jaw often appeared to be absent, the eyes were open and cleft palates were a consistent finding. In the cleared skeletons, the skull had a normal premaxilla, but the zygomatic processes of the maxilla and squamosal bones were absent giving no infraorbital bony framework. The mandible was small with little, if any, angle or temporal portion. The vertebrae were usually normal but the ribs varied from being fused to form a continuous bony plate like a vest of armor, to complete absence of ribs. The bones of the arms and legs and of the scapula and pelvis were normal but the metatarsals, metacarpals and phalanges showed absence or fusion.

**Discussion**

The micromelia produced by the triazene in the chick embryo is similar to that caused by many other drugs; insulin, sulfanilamide, physostigmine, and 6-aminonicotinamide, the micromelic action of these drugs is also prevented by nicotinamide.

The rat fetus is susceptible to the action of the triazene at about one-sixth of the dose toxic to the mother; the effects varying with the duration of pregnancy at the time of injection. While the triazene has some of the biologic activities of an alkylating agent, HN2 did not produce the specific effects on the extremities or the mandible of the rat fetus seen after this triazene. The specificity of the teratogenic action of various alkylating agents in the rat requires more detailed exploration.

**2-ETHYLAMINO-1,3,4-THIADIAZOLE (THIADIAZOLE) (FIG. 4)**

2-Ethylamino-1,3,4-thiadiazole is a water soluble chemical. Olesen et al.$^{25}$ demonstrated that the thiadiazole inhibited the growth of transplantable Sarcoma-180. This observation has been confirmed in other transplantable mouse tumors, and including leukemia.$^{50}$ The mechanism of action of this drug is not known. It is of considerable interest, however, that it produces a marked rise in uric acid excretion in man, presumably due to an increase in de novo purine synthesis since there is no evidence of tissue destruction.$^{50}$

**Teratogenic Effects**

**Chick Embryo:** The yolk sac of the 4-day embryo was injected with the thia-
THIADIAZOLE

\[
\text{H}^-'\text{NHC}_{2}\text{H}_5
\]

18 DAY EMBRYO (INJ. 4 DAY)
EFFECTIVE DOSE 13.5 MG./EGG
ABNORMALITIES INFREQUENT
Upper beak shortened

MATERNAL LD_{50} 200 MG./KG.
INJ. 9 DAY
TERATOGENIC DOSE 100 MG./KG.
INJ. 11 DAY
SACRIFICED 21 DAY

ABNORMALITIES FREQUENT
Vertebral arches and bodies:
Cephalocaudal fusion and fragmentation involving C4 thru L4
Ribs fused and branched 1 thru 13

ABNORMALITIES FREQUENT
Vertebral arches and bodies:
Cephalocaudal fusion T8 to end of vertebral column at level of sacrum
Sacral and caudal vertebrae missing
Ribs fused and branched 8 thru 13
Sternabrae fused or bifurcated

Fig. 4.
diazole; the LD50 is in the range of 13.5 mg/egg. Generally the embryos surviving this dose showed no developmental abnormalities, although occasional embryos surviving to the eighteenth day showed a shortening of the upper beak.

Rat Embryos: The LD50 of single doses of the thiadiazole in the pregnant rat is in the range of 200 mg/kg. Pregnant rats received single doses of 50 to 100 mg/kg of the thiadiazole on days 9, 11 or 12 of gestation. Mothers treated on the ninth day showed no increase in the resorbing implantation sites and gross fetal abnormalities were infrequent. Those affected, however, were reduced in weight, had pointed lower jaws and lordosis of the spine. In the cleared skeleton, the cervical, thoracic and first three lumbar vertebral bodies were distorted by double ossification centers of the bodies or cephalocaldual fusion of the arches. The remaining lumbar, sacral, and caudal vertebral bones were normal. The ribs were branched and fused and the total number appeared to be reduced.

Rats treated on the eleventh day of gestation showed about 50% resorbing fetuses, and the surviving ones were small and had gross skeletal abnormalities. The fetuses had absent or short tails and syndactyly or ectrodactyly of the front and hind paws. The cleared skeletons showed branching and fusion of the eighth through the thirteenth ribs; the lower thoracic and lumbar vertebral arches and bodies showed hemivertebrae, cephalocaldual fusion and fragmentation and usually complete absence of the sacral and caudal vertebrae.

In rats treated on the twelfth day of gestation with doses of 50, 100 or 200 mg/kg of the thiadiazole, fetal resorption was noted only in the group receiving 200 mg/kg. Abnormalities were infrequent in the 50 mg/kg group but were usual at doses of 100 and 200 mg/kg. The affected fetuses showed cleft palate, harelip, encephalocele, syndactyly and ectrodactyly, small pelvis and shortening of the tails. This latter occurred particularly in the group receiving 200 mg/kg. The cleared skeletons showed circular defects of the frontal, parietal, interparietal and occipital bones. The clavicles were often minute and the spinous process of the scapula was rotated from its normal position. The extremities showed syndactyly and shortened or absent fibulae and ulnae. Those receiving the higher dosage also showed retarded ossification of the pelvic bones and less than four caudal vertebral bodies.

Discussion
The thiadiazole does not appear to have a consistent effect on development of the chick embryo. The rat fetus, however, was susceptible to its action in doses in the range of one-fourth to one-half of that lethal to the mother. Some of the gross skeletal abnormalities were similar to those seen with nitrogen mustard and the triazene. The most distinctive feature, however, was the absence of the tail in the fetuses of the rats treated on the eleventh day of gestation.

6-AMINONICOTINAMIDE
6-Aminonicotinamide is a crystalline material which is insoluble in water. It is prepared for injection by suspending it in 0.5% CMC in saline. It appears to be a highly effective antagonist of nicotinamide and approximately 10 times more active than 3-acetylpyridine in mice. Simultaneous administration of nicotinamide will protect mice against eight times the LD50 dose of 6-aminonicotinamide. In Sarcoma-180 in mice 30 mg/kg was lethal while 8 mg/kg gave some toxicity without any evidence of tumor regression.

Teratogenic Effects
Chick Embryo: The yolk sac of the 4-day embryo was injected with 6-aminonicotinamide and the LD50 was in the range of 0.01 mg/egg. Gross skeletal abnormalities occurred regularly with a characteristic syndrome including parrot beak, micromelia, bending of tibiotarsus and tarsometatarsus. These effects could be prevented by the simultaneous administration of nicotinamide.
6-AMINONICOTINAMIDE

\[ \text{H}_2\text{N} \begin{array}{c} \text{N} \\ \text{C} \end{array} \begin{array}{c} \text{C} \\ \text{NH}_2 \end{array} \text{O} \]

**Fig. 5.**

18 DAY EMBRYO (INJ. 4 DAY)
EFFECTIVE DOSE 0.01 MG./EGG
ABNORMALITIES FREQUENT
- Parrot beak
- Micromelia
- Tibiotarsus bent
- Tarsometatarsus bent

MATERNAL LD\(_{50}\)
INJ. 11 DAY
ABNORMALITIES FREQUENT
- Size retarded
- Skull—wide suture lines
- Ossification retarded
- Vertebral canal widened
- Vertebral bodies double
- Kyphosis and scoliosis
- Fibula missing

TERATOGENIC DOSE 8 MG./KG.
INJ. 12 DAY
SACRIFICED 21 DAY
ABNORMALITIES FREQUENT
- Size retarded
- Cleft palate: palatine missing
There appears to be some differences in the LD₅₀ of 6-aminonicotinamide at different stages of gestation for the rat. The LD₅₀ dose is in the range of 8 to 15 mg/kg, intraperitoneally. Rats treated on the tenth day of gestation with 15 mg/kg showed resorbing implantation sites with no viable fetuses at 21 days. With 8 mg/kg on the eleventh or twelfth day of gestation, maternal survival occurred regularly with about 50% fetal resorption. The remaining fetuses were stunted and showed gross abnormalities. Fetuses of mothers treated with 8 mg/kg on the eleventh or twelfth day of gestation showed syndactyly, club feet, harelip and cleft palate. The cleared skeleton showed marked retardation of ossification, the ribs were fused and branched, the vertebral columns curved, the vertebral canals widened and ossification centers of the digits were not visible.

The fetuses treated on the eleventh day of gestation showed an inhibition in size and weight, bilateral harelip and cleft palate or absent palatal structures, syndactyly and often lumbar lordosis. Skeletal preparations showed retarded ossification of all bony structures, wide cranial sutures, and rotated vertebral column with occasional fused or branched ribs and syndactyly.

The fetuses of mothers treated on the twelfth day showed weight and size inhibition, syndactyly, and harelips were more complete on the right with the cleft palates often involving only the posterior portion of the palate. Cleared specimens showed delayed ossification.

The toxic and teratogenic effects of 6-aminonicotinamide on the mother and fetus can be prevented by the administration of 100 mg/kg of nicotinamide just prior to the injection of 6-aminonicotinamide.

**Discussion**

6-Aminonicotinamide produces a micro-melic syndrome in the chick embryo which is prevented by nicotinamide. This syndrome is similar to that produced by a variety of other agents and is similarly prevented in most instances by nicotinamide. In the rat fetus there is little difference between the dose of 6-aminonicotinamide toxic to the mother and to the fetus; dosage which caused consistent injury to the fetuses were also debilitating or lethal to the mother. Fetal development was severely affected with stunting, defects in ossification and skeletal deformities.

**AZASERINE (FIG. 6)**

O-diazoacetyl-l-serine (azaserine) is a white crystalline powder soluble in water. It proved to be the active ingredient of a filtrate of Streptomyces which was found to inhibit the growth of the mouse Sarcoma-180. Azaserine is inhibitory to a wide spectrum of transplantable rat and mouse tumors; it produces mutations in E. coli, and it causes developmental abnormalities of Drosophila and the chick embryo. Azaserine is of particular interest in teratogenesis because of its specific inhibitory action on purine synthesis.

**Teratogenic Effects**

Azaserine was injected into the yolk sac of the 4-day embryo; the LD₅₀ was in the range of 0.15 to 0.2 mg/egg. The majority of the injected embryos died around the twelfth day of development, but some survived to the sixteenth day. The affected embryos showed a characteristic picture of inhibition of growth, paleness, edema and impaired feather formation. There were also gross abnormalities of the head and the extremities. The cleared skeleton showed a well-developed axial skeleton in contrast to defective limbs and beak. The facial defects ranged from a shortened lower beak, to parrot beak to absence of the lateral elements of the maxilla, usually associated with a greatly reduced mandible and cleft palate. The appendicular defects...
AZASERINE

\[
\begin{align*}
&\text{N}_2\text{CHC-O-CH}_2\text{CHCOOH} \\
&\text{NH}_2
\end{align*}
\]

18 DAY EMBRYO (INJ. 4 DAY)
EFFECTIVE DOSE 0.25 MG/EGG
ABNORMALITIES FREQUENT
Facial coloboma with cleft palate
Mandible shortened
Wing: distal defect
Single, small metacarpal
Tarsometatarsals short and bent
Ectrodactyly
Hypophalangy

MATERNAL LD50 75 MG./KG.
INJ. 10 DAY
ABNORMALITIES FREQUENT
Cleft palate
Vertebral bodies double
Vertebral arches and bodies:
Cephalocaudal fusion T4 thru L3
Hemivertebrae
Ribs fused

TERATOGENIC DOSE 2.5 MG./KG.
INJ. 12 DAY
ABNORMALITIES FREQUENT
Vertebral bodies double T6 thru L4
Ischium small
Femora missing
Fibulae missing

FIG. 6.
consisted principally of shortening of the tarsometatarsals with ectrodactyly ranging from the loss of one to all four toes. An analogue of azaserine, 6-diazo-5-oxo-1-norleucine (DON) gave approximately the same effects but is about 50 times more active by weight.

**Rat Embryos:** The LD₅₀ of azaserine in the pregnant rat ranges between 75 and 100 mg/kg by the intraperitoneal route. Pregnant rats received single doses of 2.5 mg/kg of azaserine from the seventh to fourteenth day of gestation. Those treated on the seventh and fourteenth days delivered normally. Pregnant rats treated on the ninth day showed 100% resorption of the implantation sites. Rats treated on days 10, 11 and 12 showed approximately 50% resorption with 80% of the surviving embryos showing abnormalities, although weight inhibition was not marked. Embryos treated on the tenth day occasionally showed cleft palate and harelips. The cleared skeleton revealed fused or branched ribs, abnormalities of the lower thoracic vertebral bodies and arches manifested as cephalocaudal fusions of the bodies or hemivertebrae.

The 11-day-treated embryos showed cleft palate, bifid or fused sternebrae, small or absent radii, and vertebral abnormalities similar to the tenth day, but involving the lumbar instead of the thoracic vertebrae. In those treated at 12 days, the skeletal abnormalities were similar to those at 11 days; but included small or absent pelvic bones, femur or fibula. The extremities showed syndactyly or absent toes.

DON was far more active by weight than azaserine; pregnant rats treated on the tenth day with 0.2 mg/kg intraperitoneally showed complete fetal resorption, and 50% resorption occurred at 0.1 mg/kg. Surviving animals did not show the incidence of developmental abnormalities produced by azaserine.

**Discussion**

Azaserine, a highly specific antimetabolite, produced severe developmental abnormalities in the chick embryo and rat fetus. The abnormalities of the appendicular skeleton in the rat fetus and chick embryo appeared to be related.

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