Bundesinstitut für Risikobewertung

7th Workshop on the Terminology in Developmental Toxicology
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An introduction into problems of postnatal anomalies regarding their integration into new terminology and classification.

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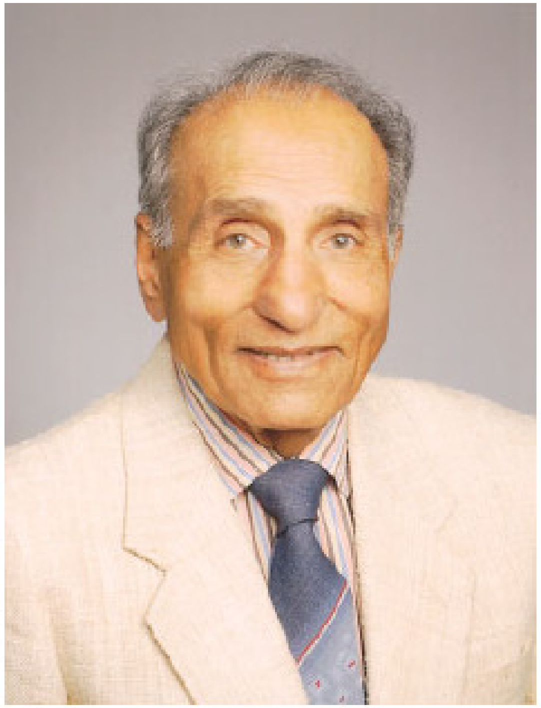
FIOCRUZ



Kundan S. Khera (1922–2003)

FUNDAMENTAL AND APPLIED TOXICOLOGY 1:13-18 (1981)

Common Fetal Aberrations and Their Teratologic Significance: a Review*

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A distinction between aberrations and malformations, based only on observations of anatomical changes of term fetuses may at times become extremely difficult, since the effects of aberrations on postnatal development have not been systematically investigated.

Kundan S. Khera, **1981**

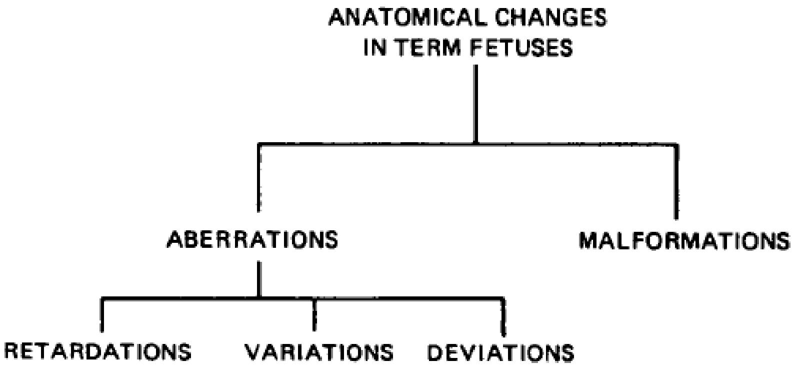
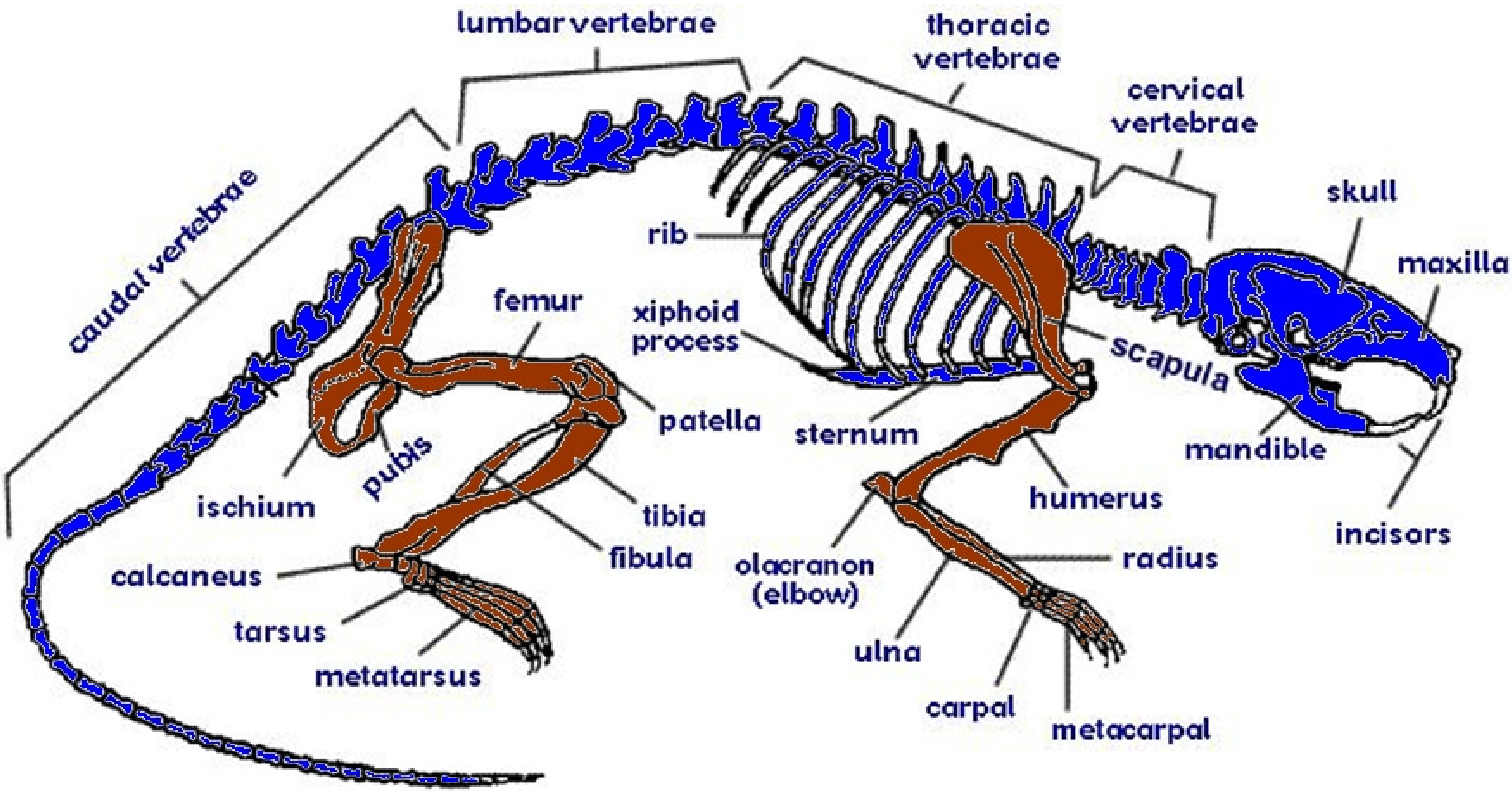


FIG. 1. Types of anatomical changes.

Postnatal fate of some rat axial skeleton anomalies observed at the C-section

RAT SKELETON



Axial skeleton (*it comprises bones located close to or along the central axis of the body, 80 bones in humans*)

Appendicular skeleton (*it refers to bones joined to axial skeleton, ie, bones of limbs and pelvic and pectoral girdles, 126 bones in humans)*

I - Postnatal fate of “*wavy*” ribs and “*bent*” ribs

Wavy ribs→ “undulation(s) along the length of a rib”

Bent ribs → “shaped like an angle” (angulated, bowed)

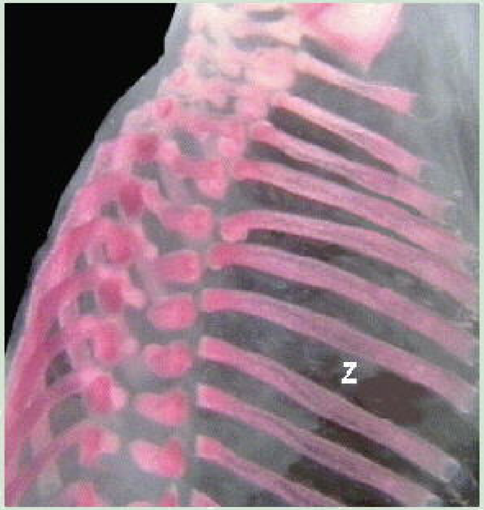
Wavy ribs / bent ribs

Are they distinct anomalies or different grades of severity of a same defect ?

⇒ Distinction between “wavy ribs” and “bent ribs” is based on appearance rather than on etiology and causation

⇒ Kast (1994) suggested that bulbous, knobby, bent, nodulated, undulated, and misshapen ribs are often used as synonyms for “wavy ribs” (*Kast A.*, *Wavy ribs. A reversible pathologic finding in rat fetuses. Exp. Toxicol.Pathol. 46:203-210, 1994*) .

⇒ Variation or a fetal pathologic finding - not a malformation

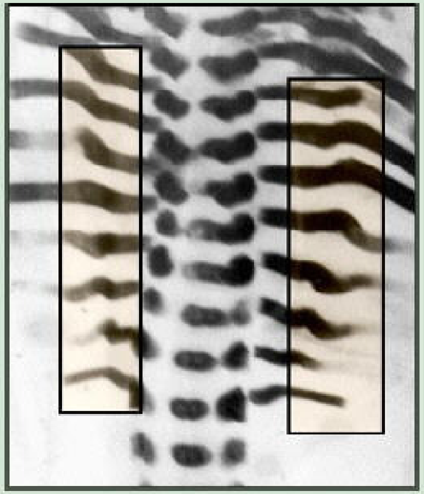


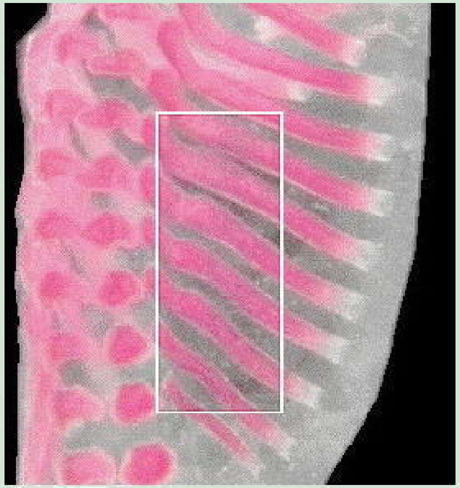
control

Ribs

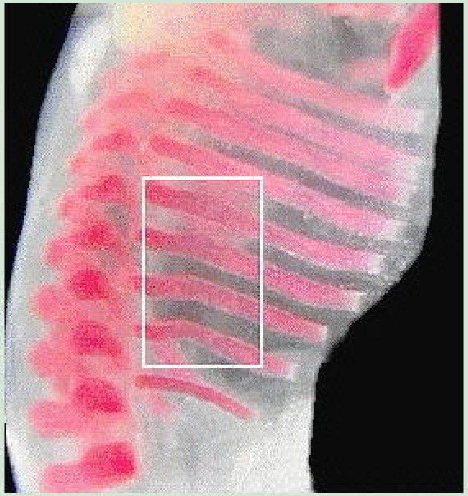
[Rat fetuses on pregnancy day 21 (Alizarin Red S staining)]

Ribs bent



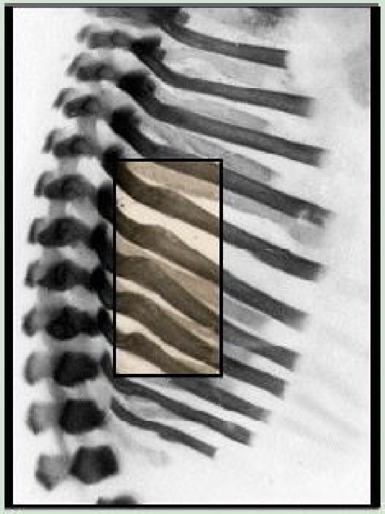


minimal

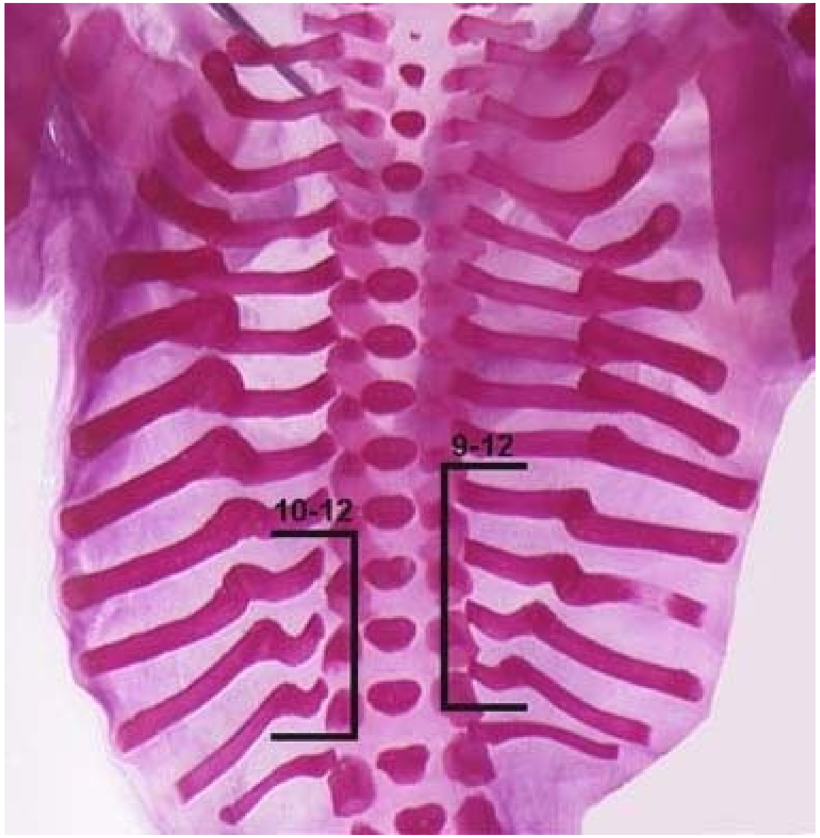


Ribs wavy

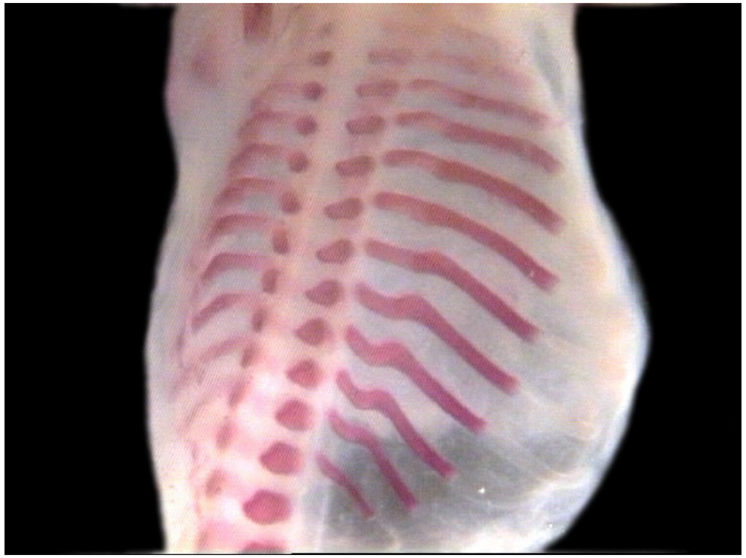
moderate

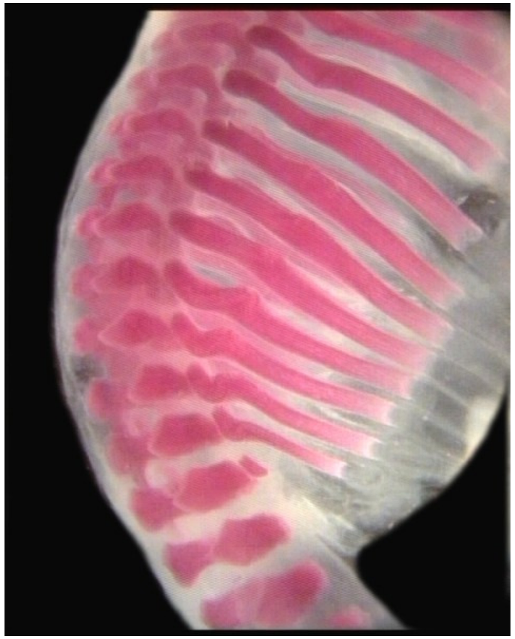


marked



DevTox 2011, bent ribs, rat, Fig.2





Chahoud & Paumgartten 2009, wavy ribs, rat.

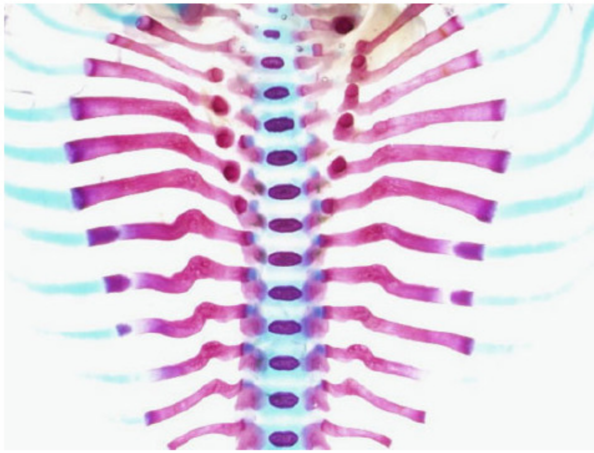
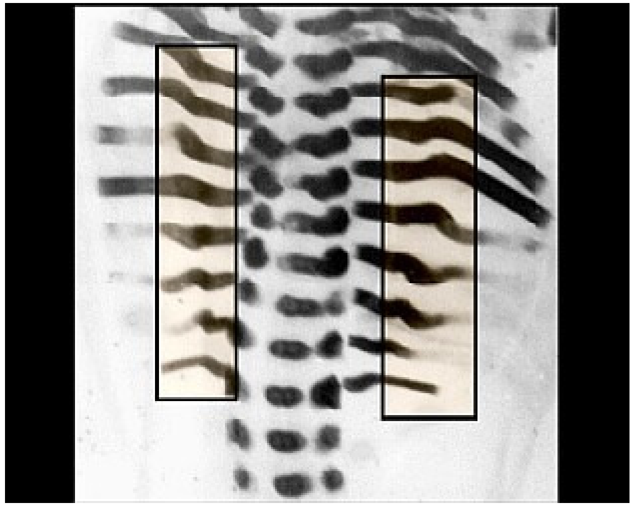


Fig. 2. Wavy ribs in a rat fetus.



“wavy ribs”

Carney & Kimmel, 2007

“bent ribs”

DevTox -2011

Bent ribs → “shaped like an angle” (angulated, bowed)

Wavy ribs→ “undulation(s) along the length of a rib”

Some studies have showed that “wavy ribs” are reversible within a few days or weeks after birth

Little information is available on the postnatal fate of “bent” ribs

Rats

Chondrification of the ribs begins at GD 15 and ossification on GD 15.5 (ribs 3-9), spreading in both directions (slightly faster in the caudal than the rostral direction)

Ossification of ribs is complete by GD 19.

Nishimura et al *(Repairability of drug-induced “wavy ribs” in rat offspring. Arzneimittel-forschung 32: 1518-22, 1982)*

Stages
- 1: delay in ossification in the middle of rib around GD 16-17
- 2: curvature in the unossified middle portion of the rib (GD 18-19)
- 3: ossification of the curved part of the rib (GD 19-20)

Repairibility of “wavy ribs”

Studies on the incidence of wavy ribs over time after treatment

1)- **Nishimura et al (1982)** (fenoterol, β-2 adrenergic agonist, rat, GD 7-17)

GD 21	71 %
PND 1	53.7 %
PND 21	32.4 % (hardly visible)

Clear reduction of incidence and severity (x-rays) over time.

Mortality, however, was high (***Was there a selection of less severe cases ?***)

2)- **Haysaka et al (1985)** (azosemide, loop diuretics, rat, GD 16)

Wavy ribs were not seen in most treated pups on PND 10-14

3)- **Price et al (1996)** (boric acid 0.1-0.2 % in diet, rat, during gestation)

GD 20	2.1 – 9.9 %
PND 21	0 - 0.3 %

In the three studies there was an increase in early post natal mortality.

Occurrence of “wavy” ribs and length of pregnancy

Rats and mice ⟹ spontaneous and treatment-induced

Rabbits ⟹ rare

Ochratoxin A orally on GD 6-18

on GD 30: 16.7% of wavy ribs in treated against 0% in controls

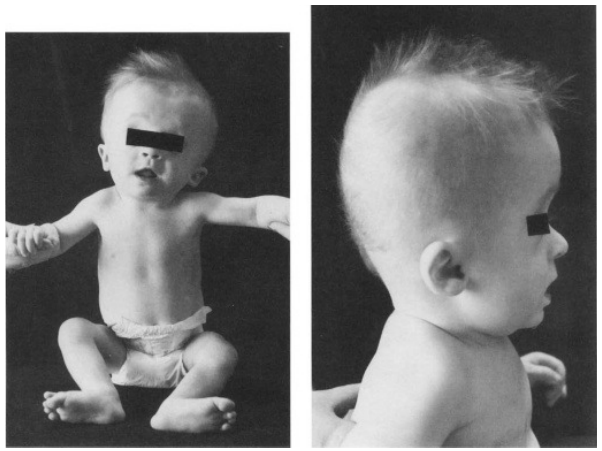
Wangikar et al *(Toxicology 215:37-47, 2005)*

Hypothesis → WR are seldom seen in rabbits because of their longer fetal period during which repair may occur

Nakatsuka and Fujii *(Species specificity in induction of wavy ribs: failure of furosemide to induce the anomaly in rabbit fetuses. Congenit Anom 28:25-32, 1988*)

Humans ⟹ WR not seen (long gestation/fetal period ?)

Report of a case of “thin wavy ribs” in one of three infants prenatally exposed to fluconazole.[fluconazole caused WR in rat segment-II studies too].



Child with fluconazole-induced multiple congenital anomalies. *Left: Note the brachycephaly, trigonocephaly, bitemporal narrowing, and* short ear helices. *Right: Flattening of occiput is evident.*

Pursley TJ et al, Fluconazole-induced congenital anomalies in three infants. Clin Infect Dis 22: 336-40, 1996.

II - Postnatal fate of supernumerary ribs

(Full or short additional ribs found at cervicothoracic or thoracolumbar borders)

Persistence / transience of supernumerary ribs

Wickramaratne GA (1988) Rats. Aspirin (prenatal exposure) doubled the incidence of lumbar rudimentary ribs at birth. On PND 60 the incidence declined to essentially zero while the proportion of pups with a fully developed transverse process on the first lumbar vertebra increased. (Hypothesis: "***Short LR are incorporated into lateral processes of the vertebrae as the skeleton matures postnatally***").

Chernoff et al (1991), Rogers et al (1991) Rats. Bromoxynil (herbicide) induced lumbar ribs rats and mice .

Spontaneous (control) incidence of SNR at term: rats 13%, mice 11%;
on PND 40 it dropped to 0 in both species

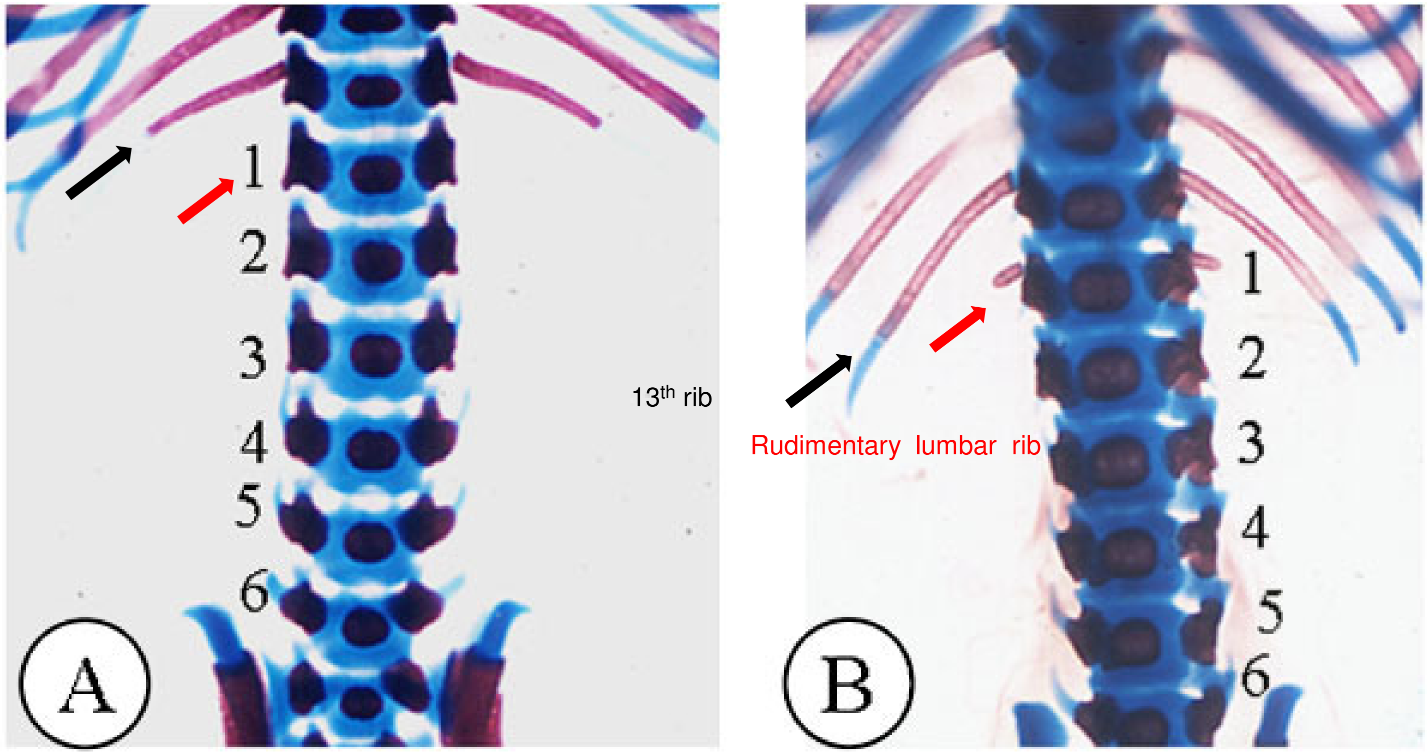
Bromoxynil **Mice** (342 µmol/kg /kg bwt/day po; GD 6-15) Incidence did not decrease postnatally: 45% in term fetuses and 42% on PND 40.

In mice 95% of supernumeray ribs were not rudimentary, i.e., they were extra (length > 50% of the length of the preceding rib)

Rats (54 µmol/kg bwt/day po; GD 6-15) : Incidence declined after birth.

Term fetuses 62%;
PND 20 55%
PND 40 0%

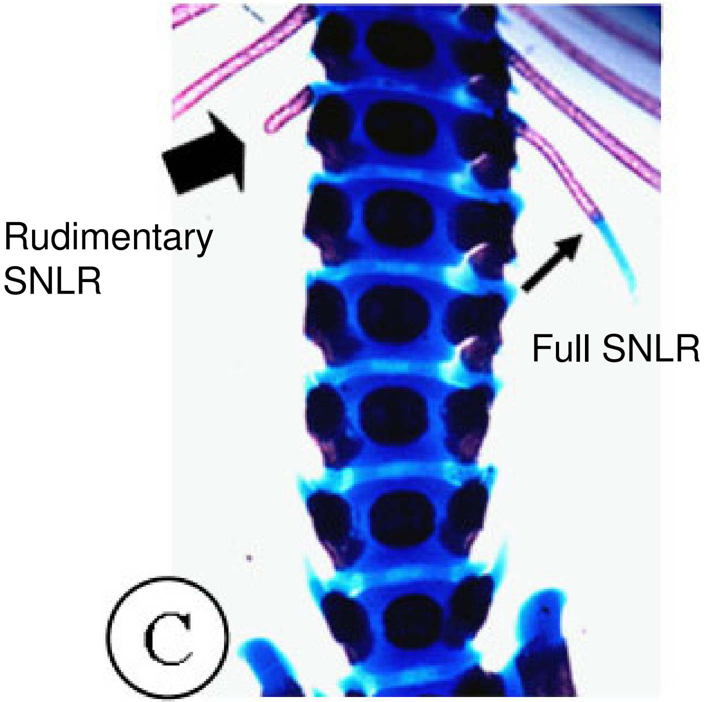
In rats > 90% of SNRs ribs were rudimentary (short) lumbar ribs



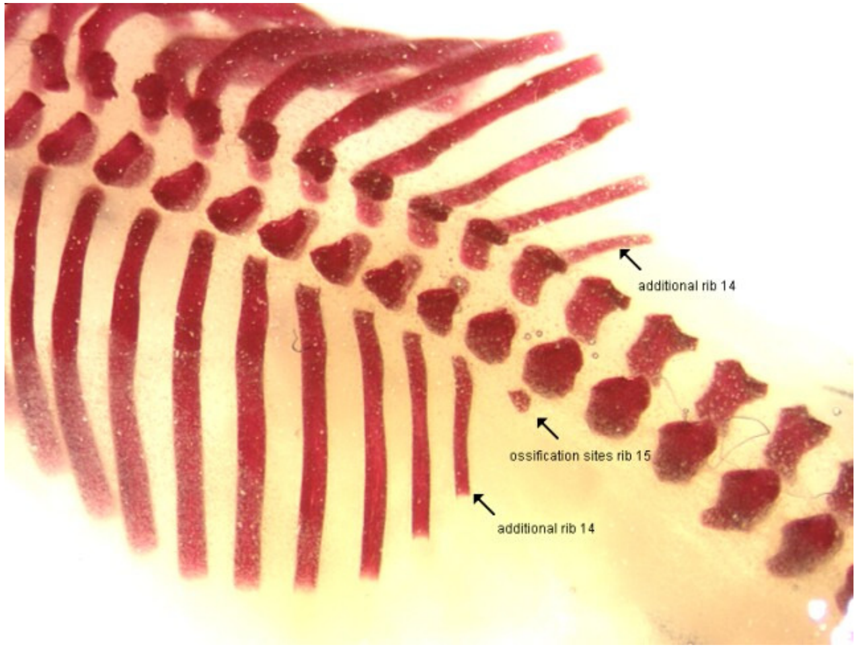
Rat fetuses at term

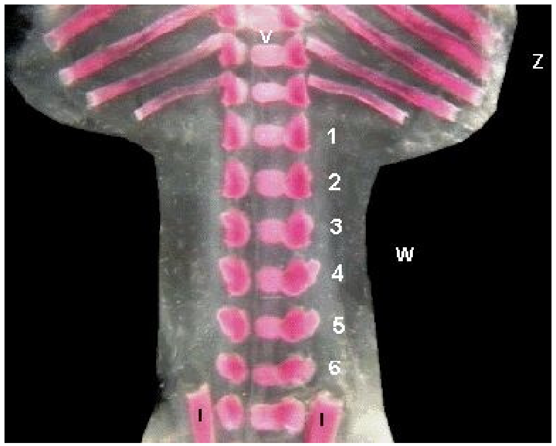
From: *Tyl RW, Chernoff N, Rogers JM, Birth Defects Res (part B) 80: 451-72, 2007.*

Rat fetus at term
Prenatally-treated with bromoxynil



From: *Tyl RW, Chernoff N, Rogers JM,*
Birth Defects Res (part B) 80: 451-72, 2007.

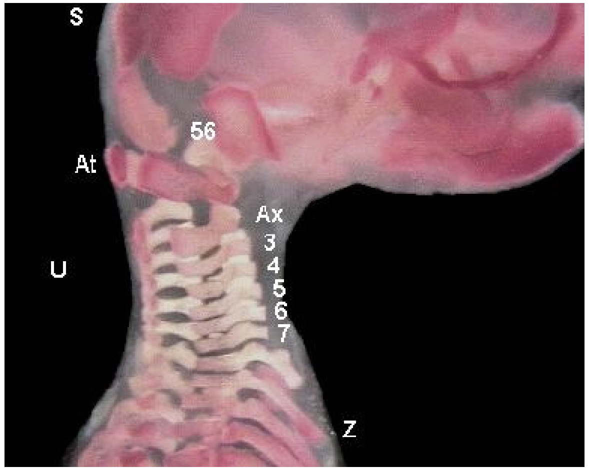




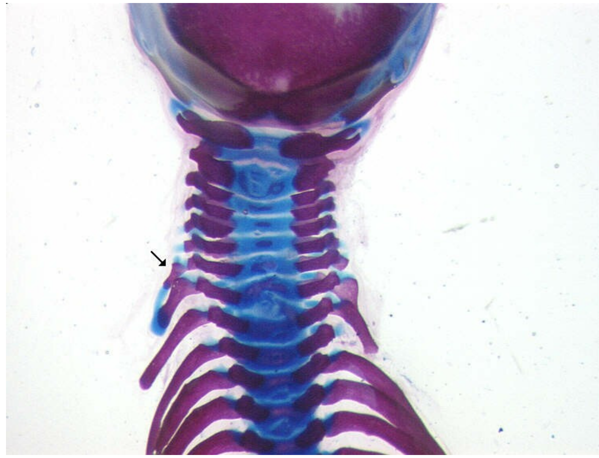
Normal rat at term

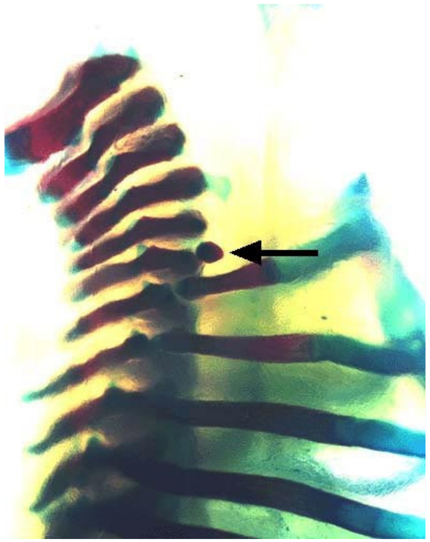
From: DevTox 2011

Rudimentary (short) cervical SNR in rats

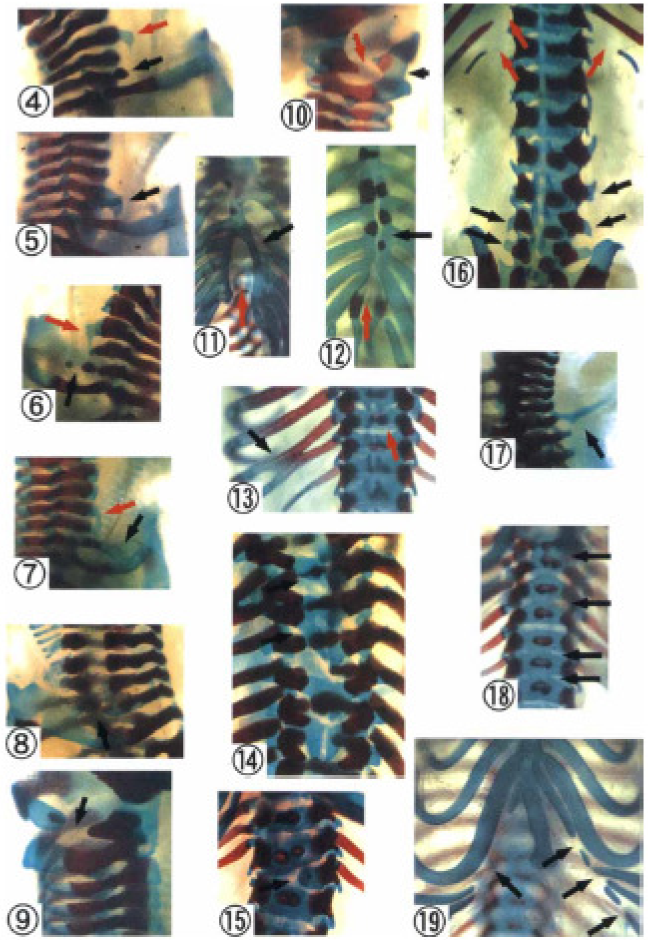


Control rat





Dev Tox 2011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Supernumerary ribs (cervical / lumbar)

Rudimentary /short (length <1/3 ossified portion of the first or the preceding thoracic rib)

↓

Transient abnormality

Extra / full (length >1/3 ossified portion of the first or the preceding thoracic rib)

↓

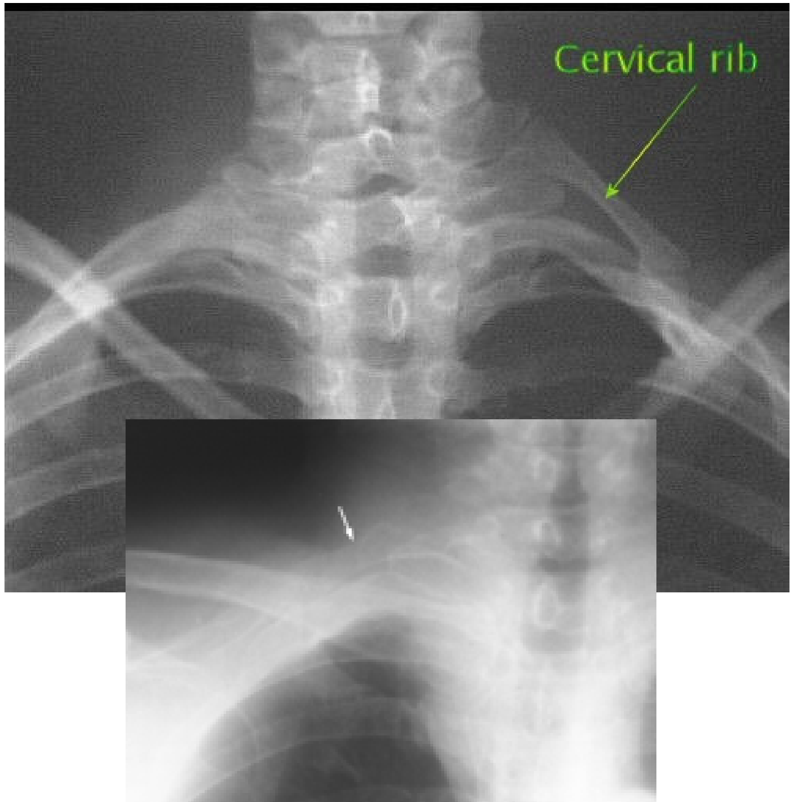
Permanent abnormality

Thoracic Outlet Syndrome (TOS)

Rare condition caused by compression of vessels and nerves in the area of clavicle

Symptoms: pain in the neck and shoulders, numbness in the last three fingers and inner forearm

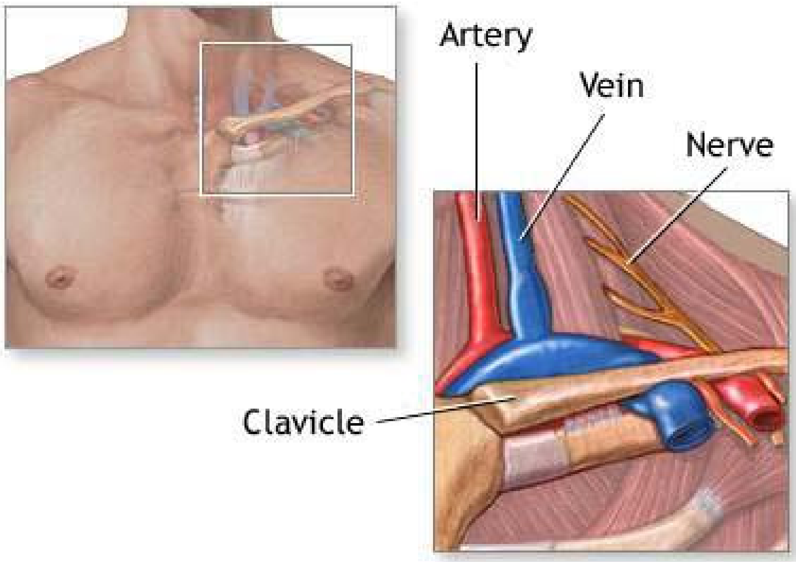
It can happen when there is an **extra cervical rib**

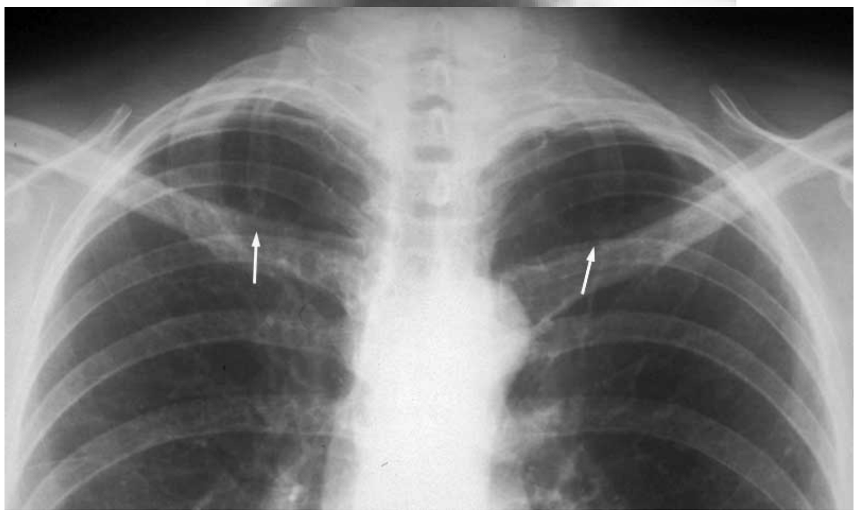


Supernumerary cervical (full) rib

Malformation (permanent and harmful) in **humans**

Variation (permanent and not harmful ?) in **rodents**





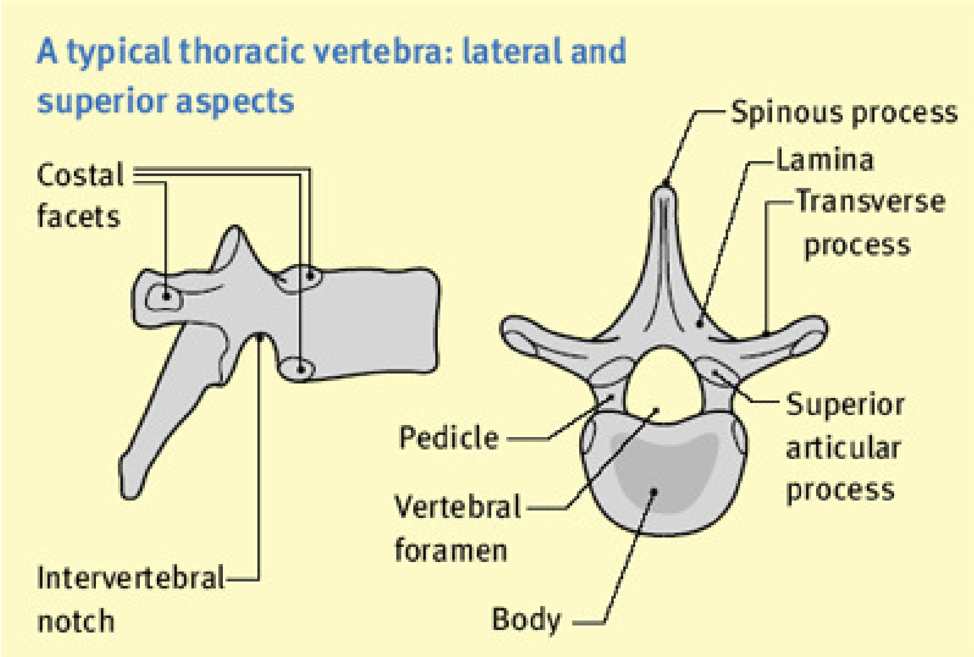
A.D.A.M.

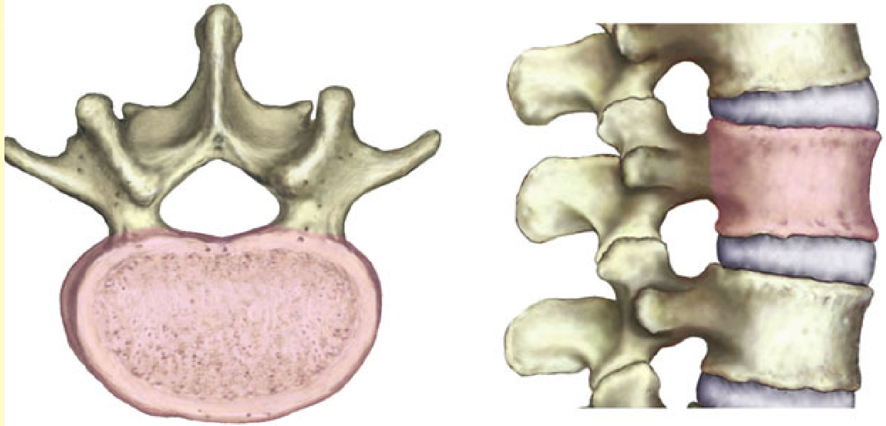
III - Postnatal fate of vertebral body anomalies

Two studies on the postnatal fate of vertebral anomalies

I)- Thiel R, Dillman I, Schimmel A, Bochert G, Chahoud I, Neubert D. (1989) Aspects of designing postnatal studies: III Persistence of skeletal anomalies induced prenatally. Teratology 40: 300 (Abstract, Conference proceedings)

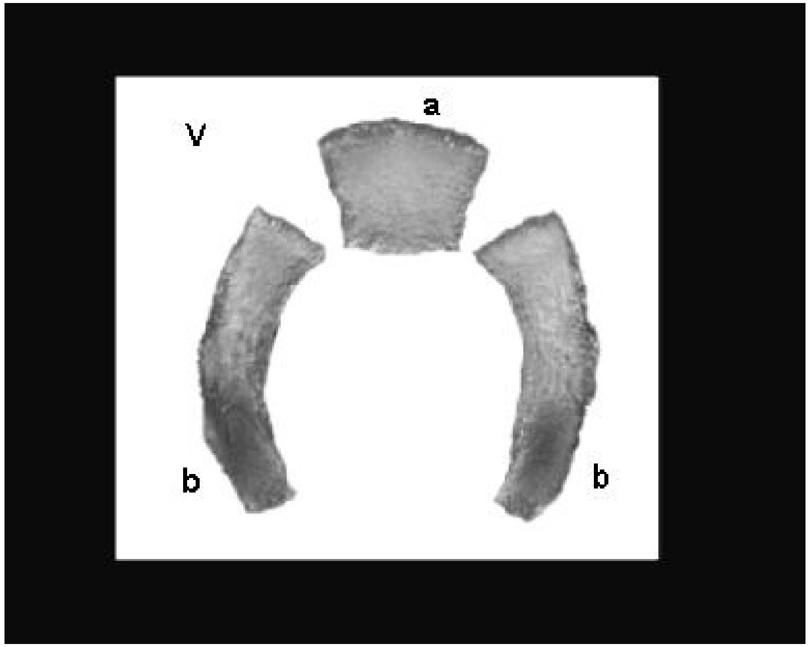
II)- Marr MC, Price CJ, Myers CB, Morrissey RE (1992) Developmental stages of the CD (Sprague-Dawley) rat skeleton after maternal exposure to ethylene glycol. Teratology 46: 169-181.

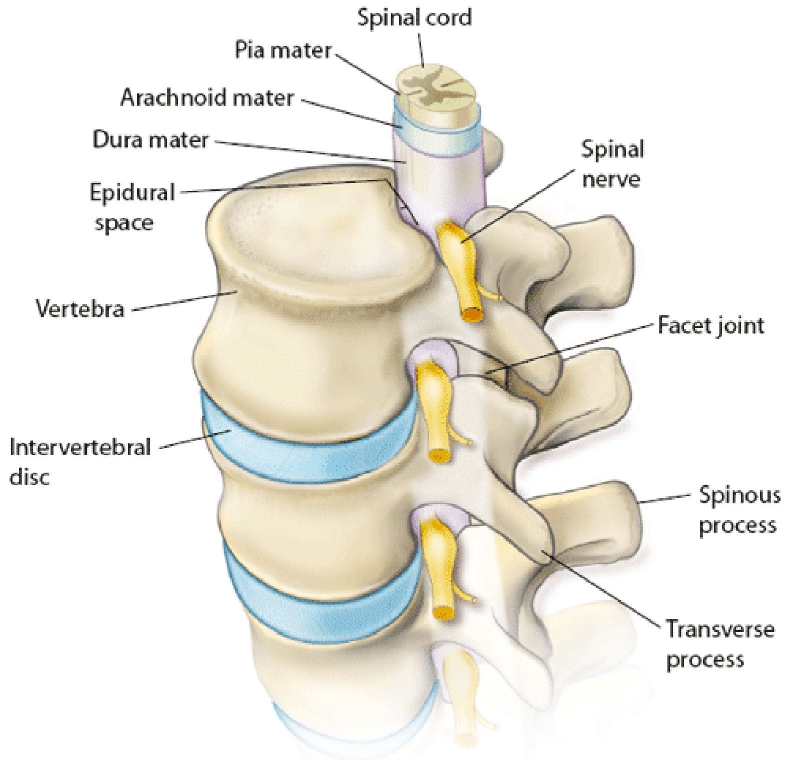




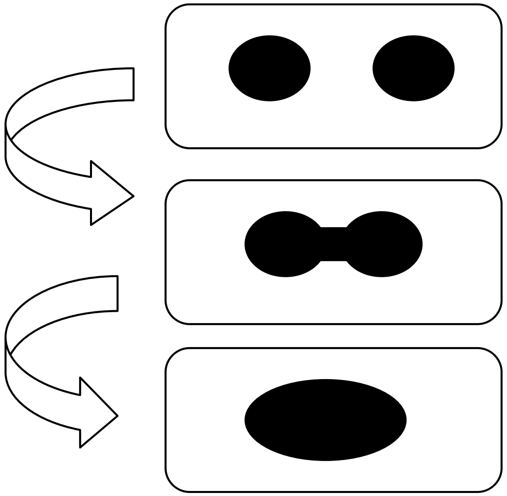
Vertebral Body

Rat thoracic vertebra (GD 21)





Progress of ossification of vertebral centra in the rat



1. Begins with two separate oc (bipartite)
2. The two oc subsequenty fuse to form a single dumbbell shaped oc
3. The dumbbell shaped oc becomes more ovoid in shape

Thoracic and lumbar vertebral centra ossify rapidly during late gestation

Thoracic, lumbar and sacral vertebral bodies ossify first (cervical and caudal later)

Fritz H, Hess R, 1970, Ossification of rat and mouse skeleton in the perinatal period. Teratology 3, 331-338.

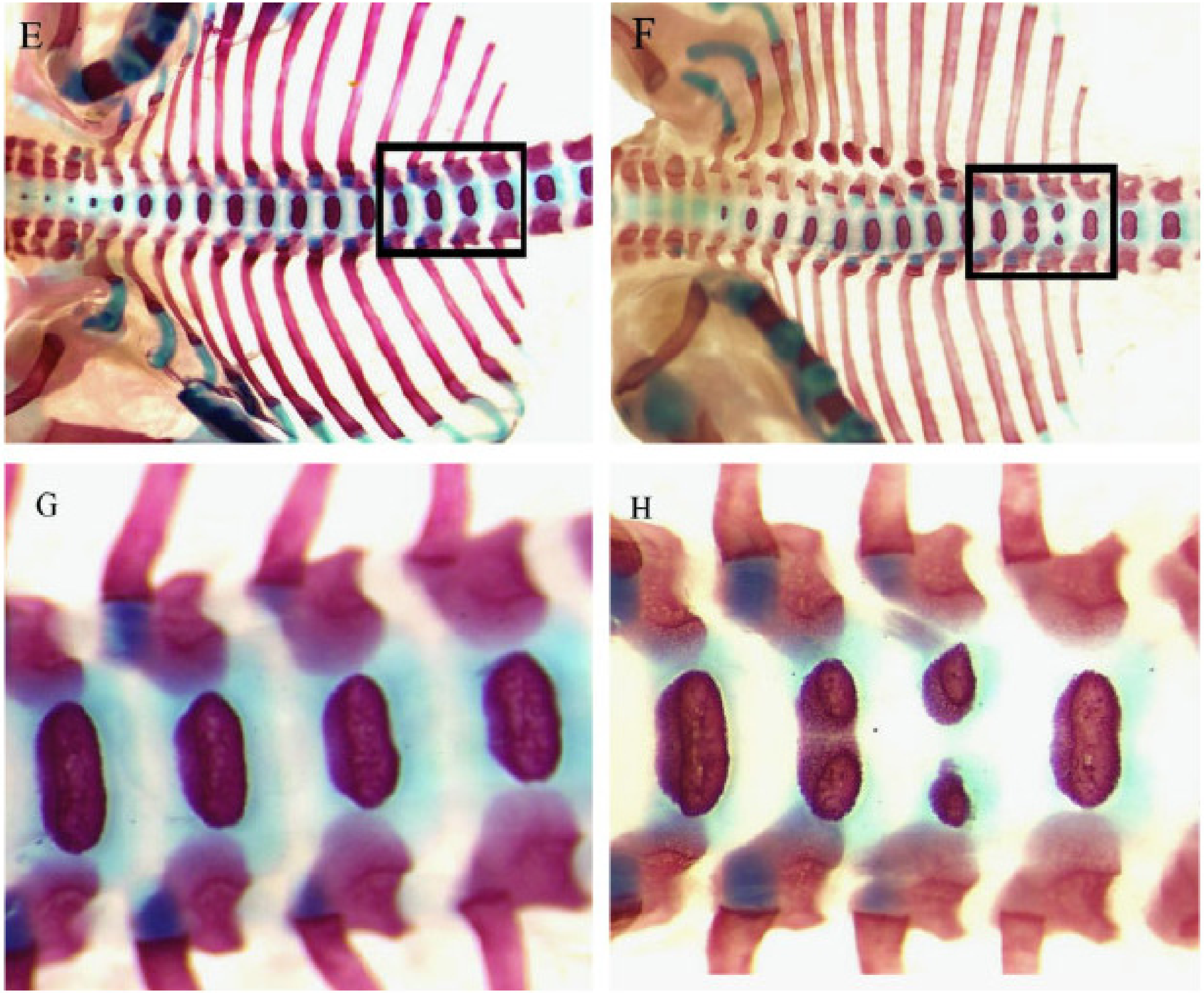
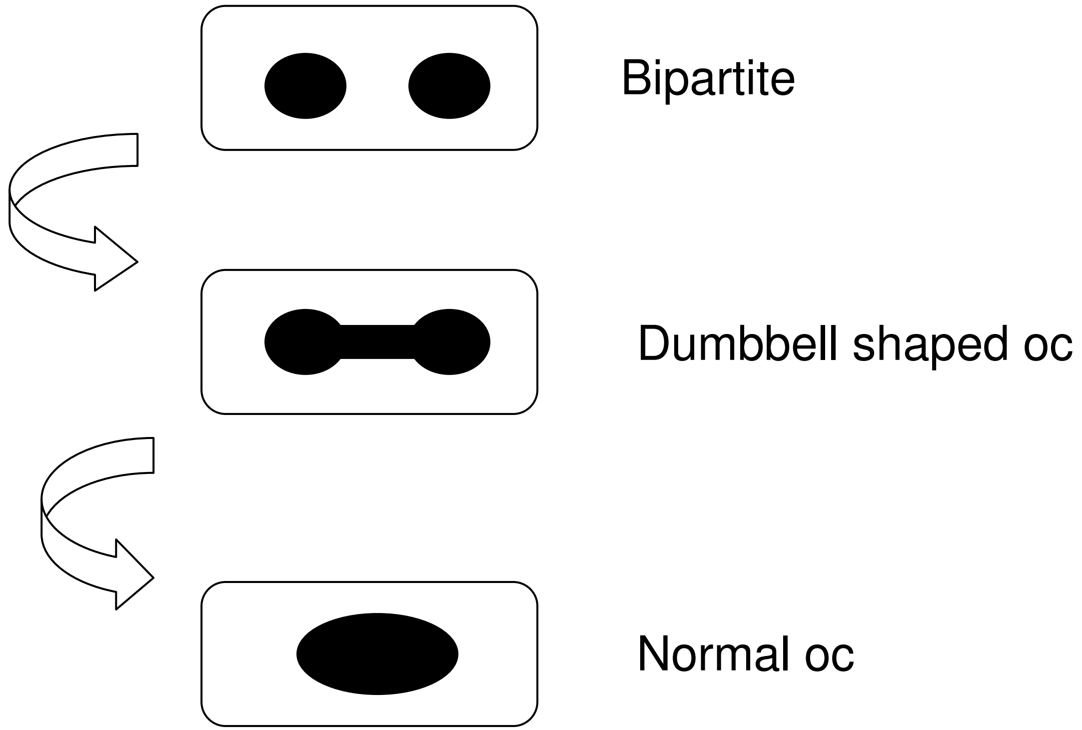
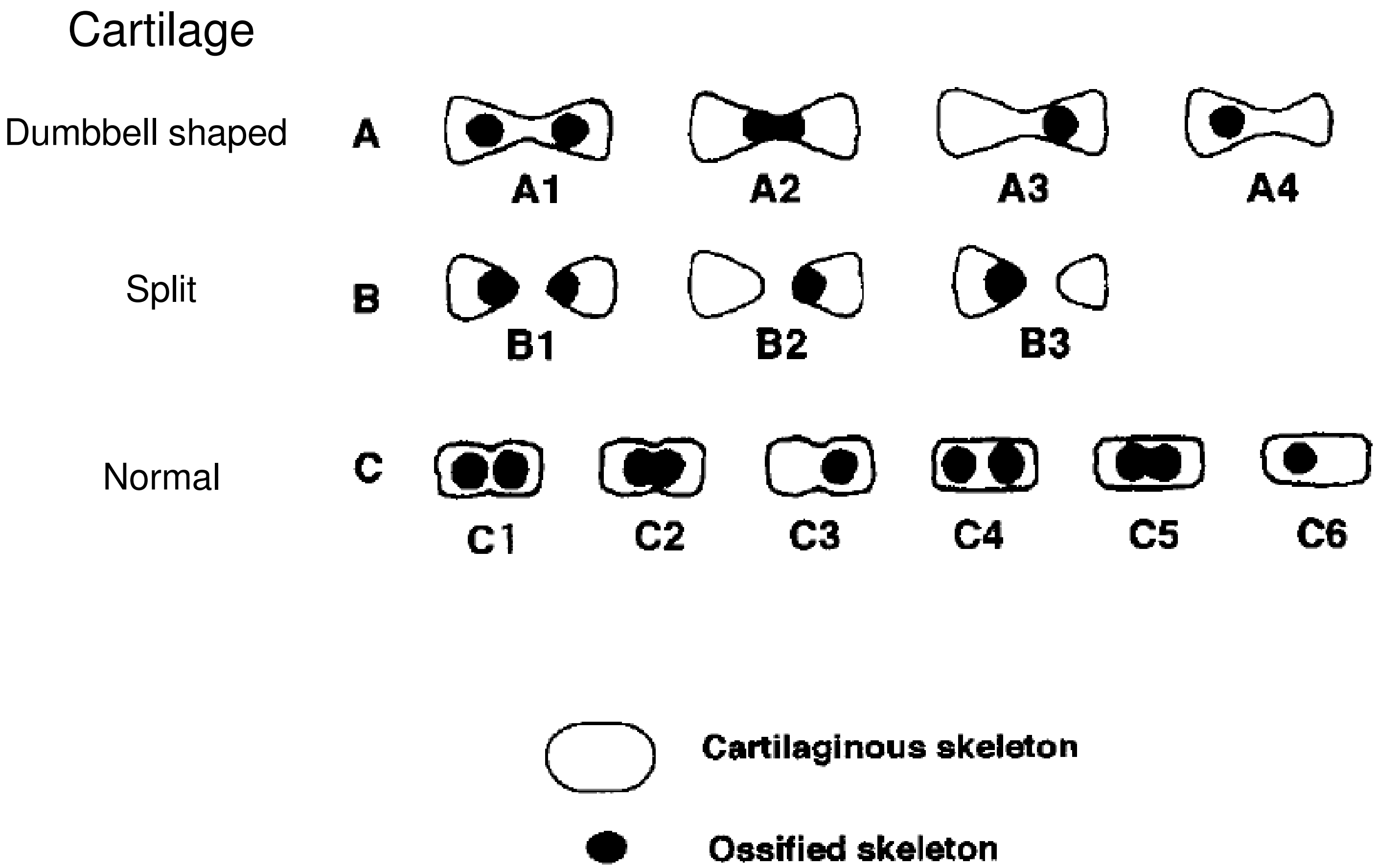


Fig. 1. Examples of normal and delayed ossification in the GD 21 CD rat fetus. **A:** Normal skull (F, frontal bones; P, parietals; IP, interparietal). **B,C:** Delayed ossification of the parietal and interparietal. **D:** Normal sternebrae 1–6 (bottom) and delayed ossification of sternebrae 5 (top, arrow), signified by normal cartilage, but lack of bone staining. **E:** Normal thoracic vertebrae. **F:** Thoracic vertebrae with delayed ossification of centrum 12. **G,H:** High power view of area marked by inset of (E) and (F).

Are bipartite / dumbbel shaped oc in term fetuses signs of delayed ossification ?



Vertebral body ossification



Igarashi, Cong Anom. 38: 39-55, 1998

Postnatal fate of vertebral anomalies

Study performed by Chahoud, Thiel and coworkers

Double stained rat skeleton

FuDR treated (single dose, 40-75 mg/kg sc) on GD 11

Evaluation: GD 21, PND 7, 14, 21.

Preliminary results reported by Thiel et al, 1989

Fetuses/pups were re-evaluated by Chahoud and co-workers 2009-2010

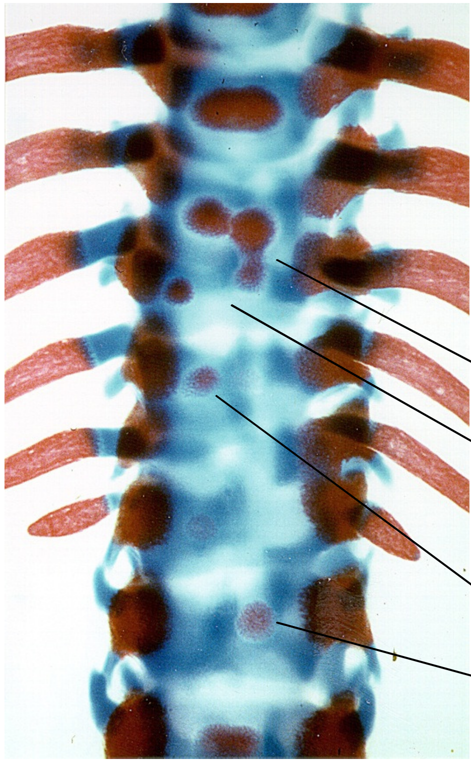
Data on postnatal mortality is missing

Vertebra oc dumbbell shaped

Vertebra oc bipartite

The two anomalies are generally classified as variations .

Therefore, it is believed that they are transient changes *´that are unlikely to adversely affect survival or health*`**.**



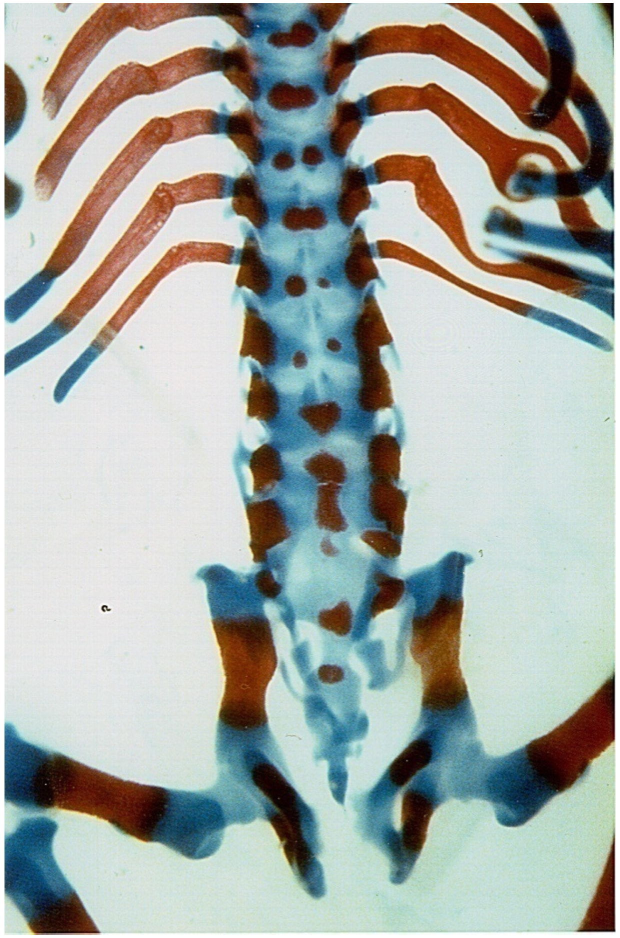
Rat fetuses

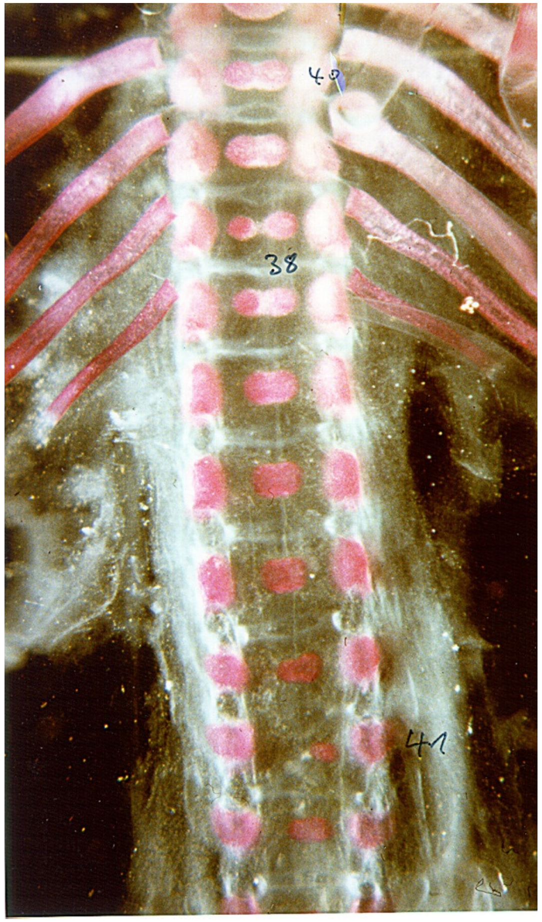
Treatment: FuDR 45 mg/kg *sc*

<u>C-section GD 21</u>

Thoracic vertebra oc dumbbell shaped

Thoracic vertebra oc bipartite





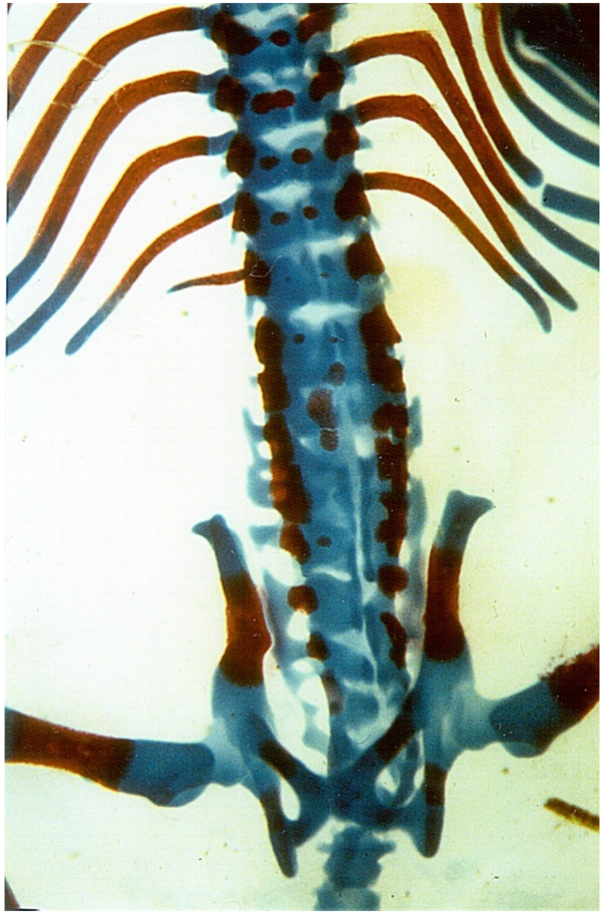
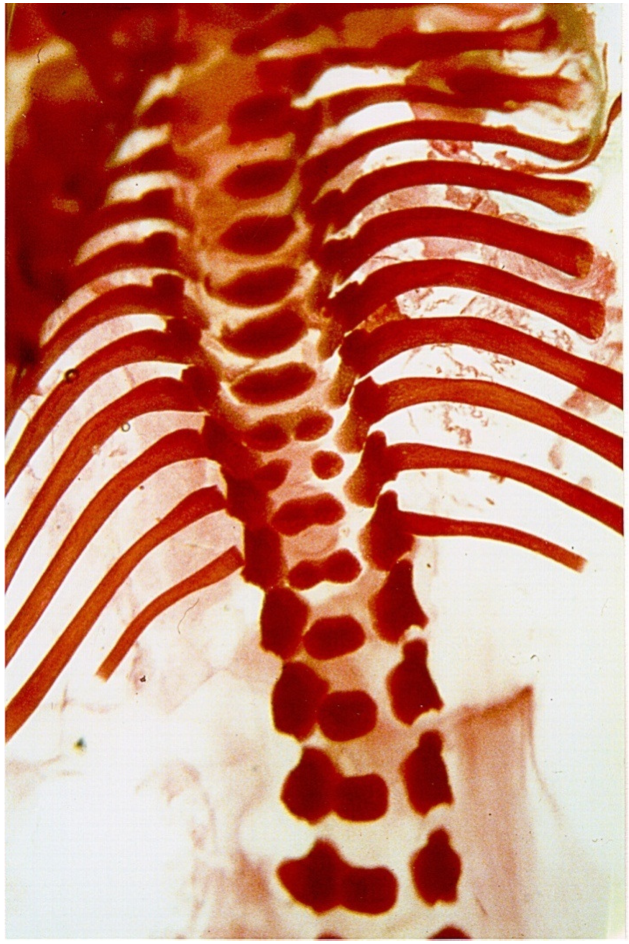
Rat fetuses

Treatment: FuDR 50 mg/kg *sc*

<u>C-section GD 21</u>

Thoracic vertebra oc dumbbell shaped

Thoracic vertebra oc bipartite

Rat fetuses

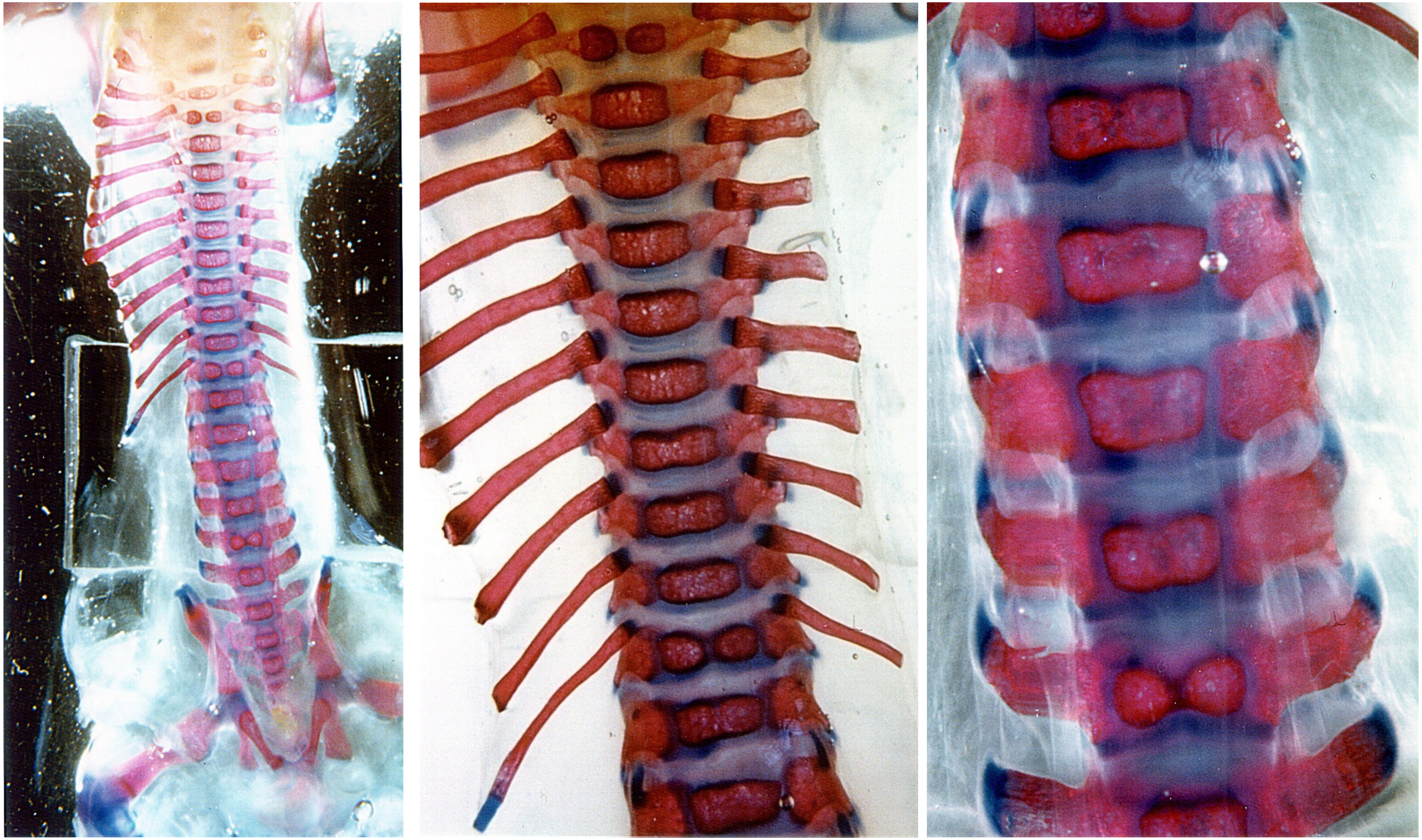
Treatment: FuDR 75 mg/kg *sc*

C-section GD 21

Thoracic vertebra oc dumbbell shaped

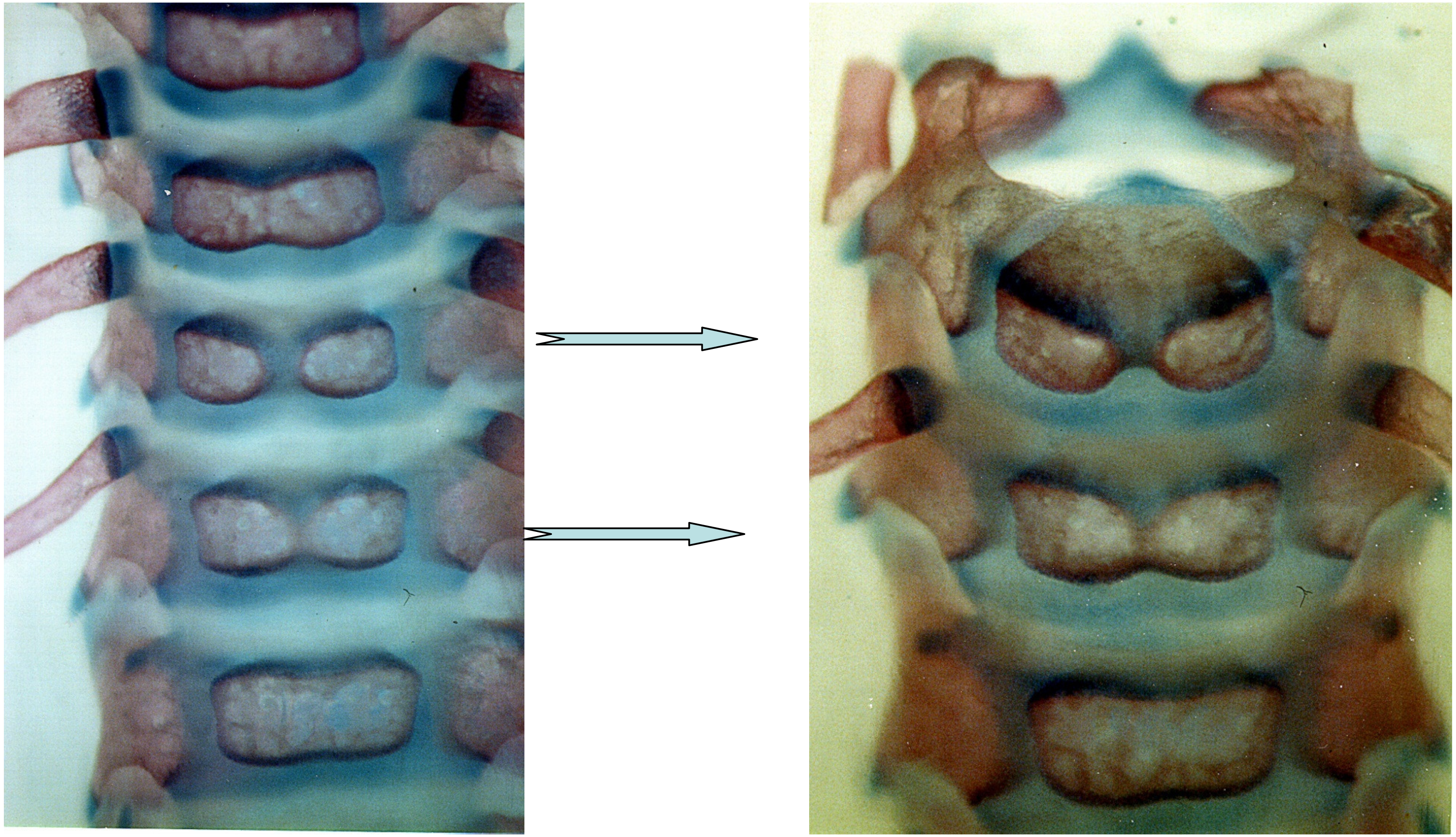
Thoracic vertebra oc bipartite

Rat pup, FuDR (40 mg/kg *sc* on GD 11), **Post natal day 7**



Vertebra oc dumbbell shaped

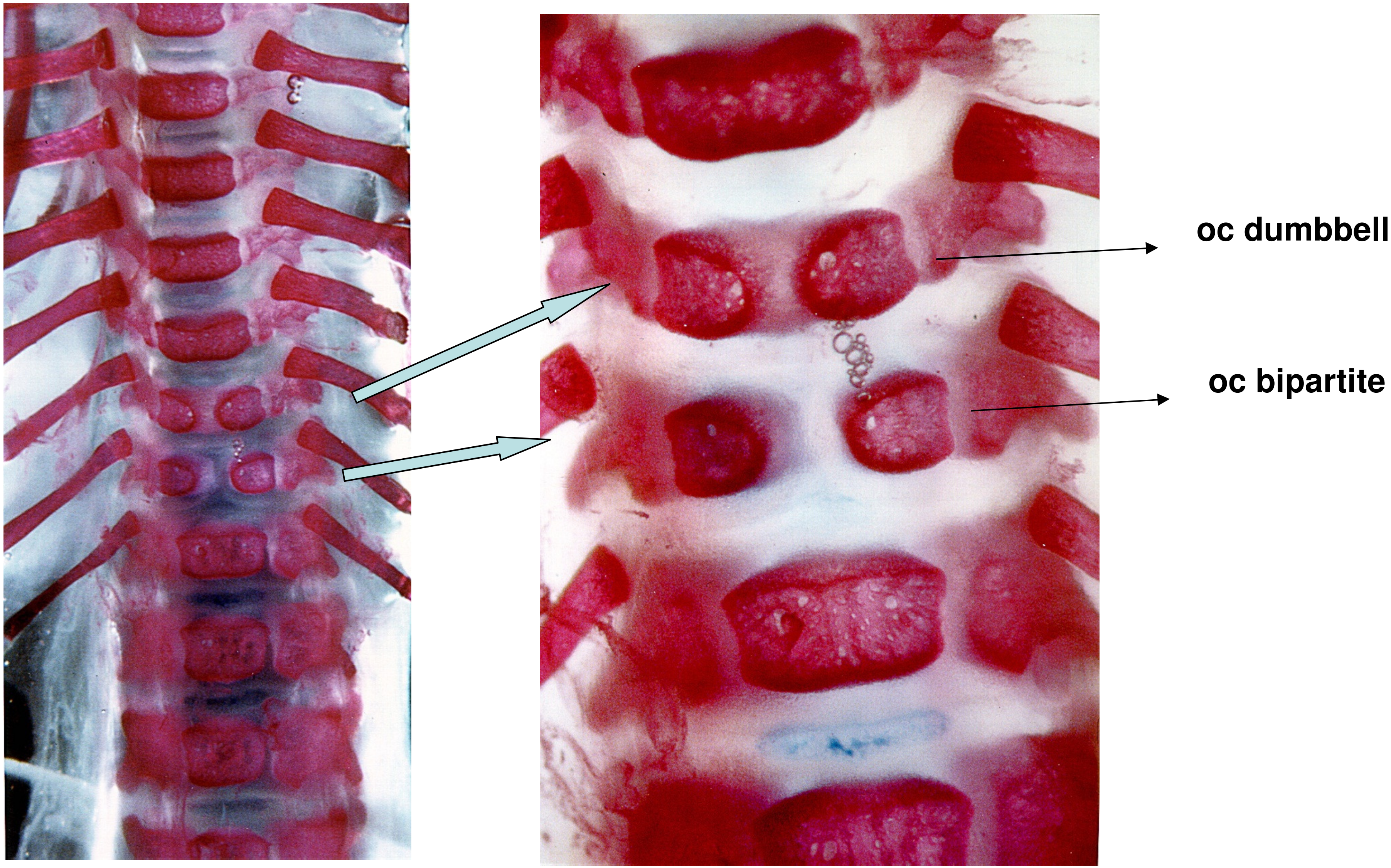
Rat pup, FuDR (40 mg/kg *sc* on GD 11), **Post natal day 7**



Thoracic vertebra oc dumbbell shaped

[*By changing the position (angle) from which the photo was taken, it is possible to see that the abnormality (a cleft) is rather in the ventral part of the oc*.]

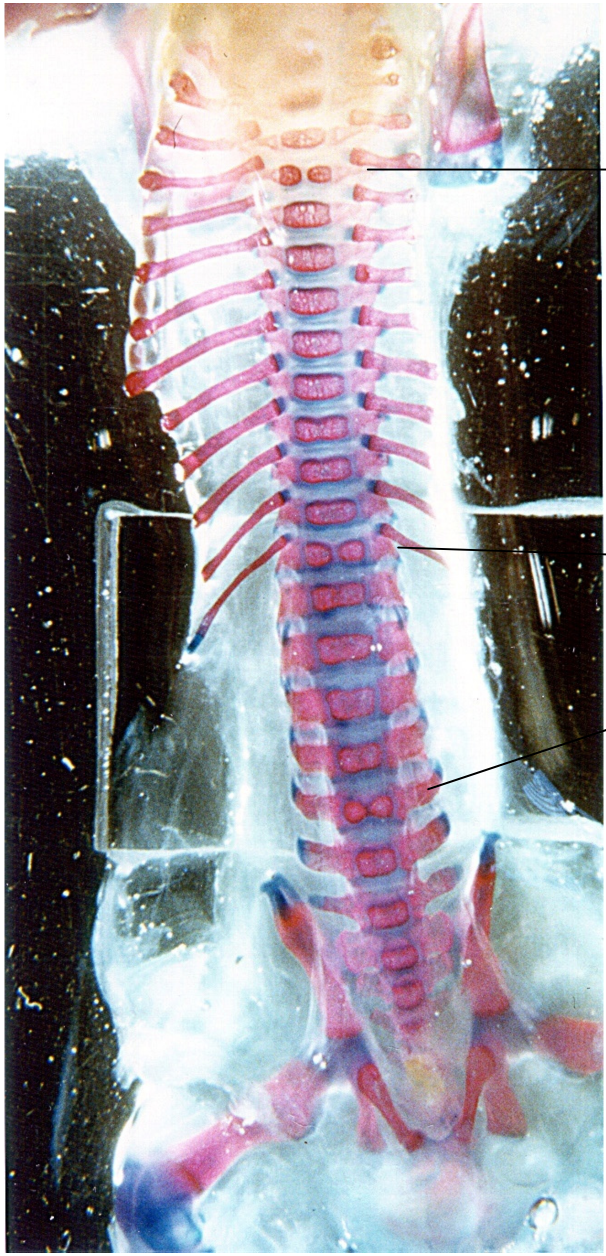
Rat pup, FuDR (45 mg/kg *sc* on GD 11), **Post natal day 7**



Rat pup, FuDR (45 mg/kg *sc* on GD 11), **Post natal day 7**

Vert oc dumbbell shaped

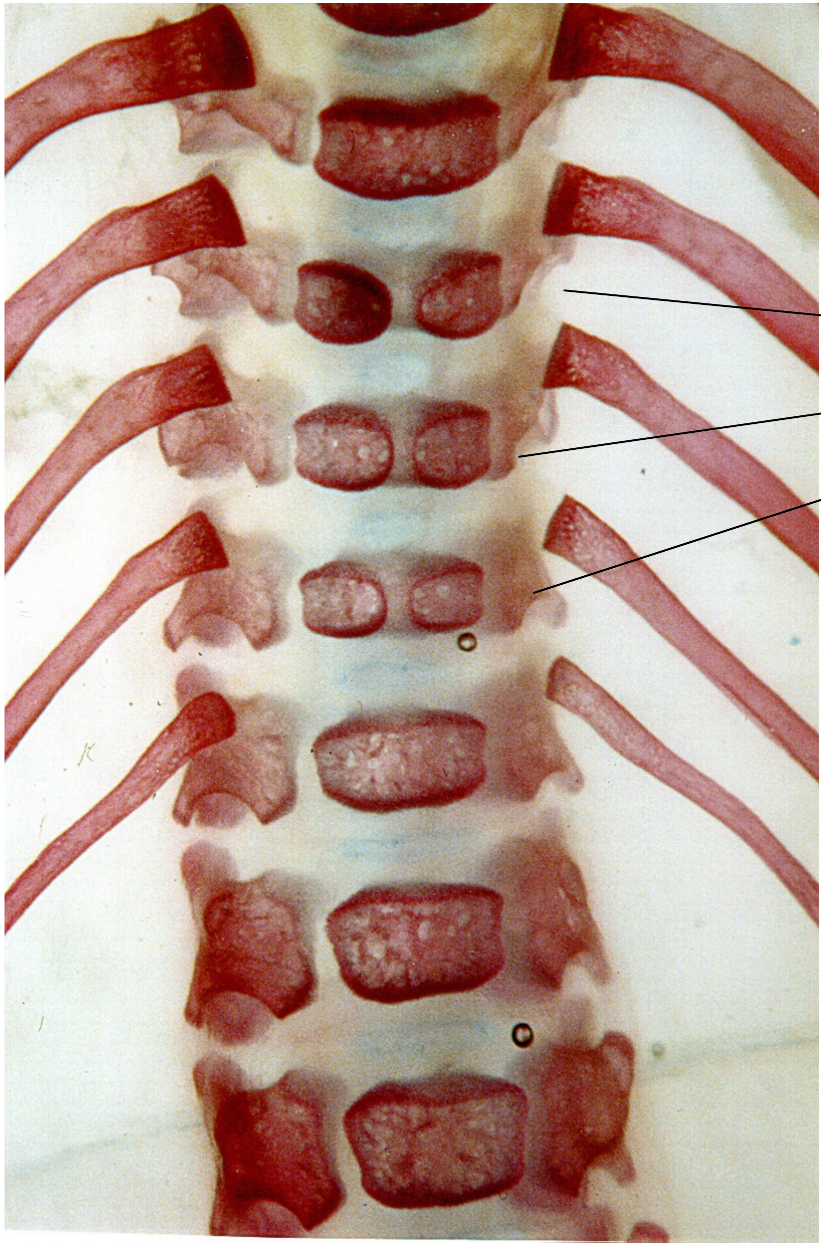
Rat pup, FuDR (65 mg/kg *sc* on GD 11), <u>**Post natal day 7**</u>



Thor vert oc bipartite

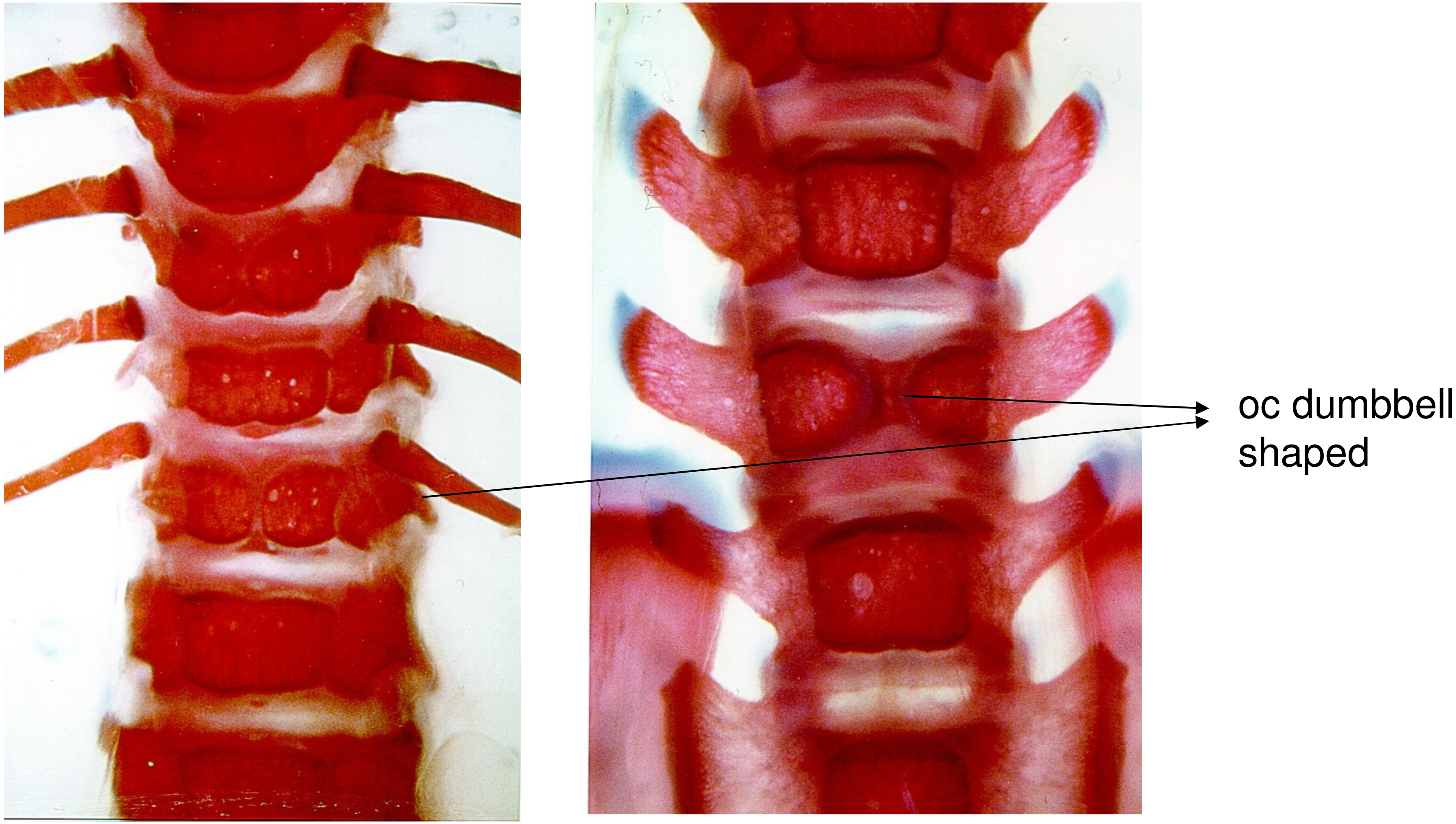
Vert oc dumbbell shaped

Rat pup, FuDR (55 mg/kg *sc* on GD 11), **Post natal day 7**

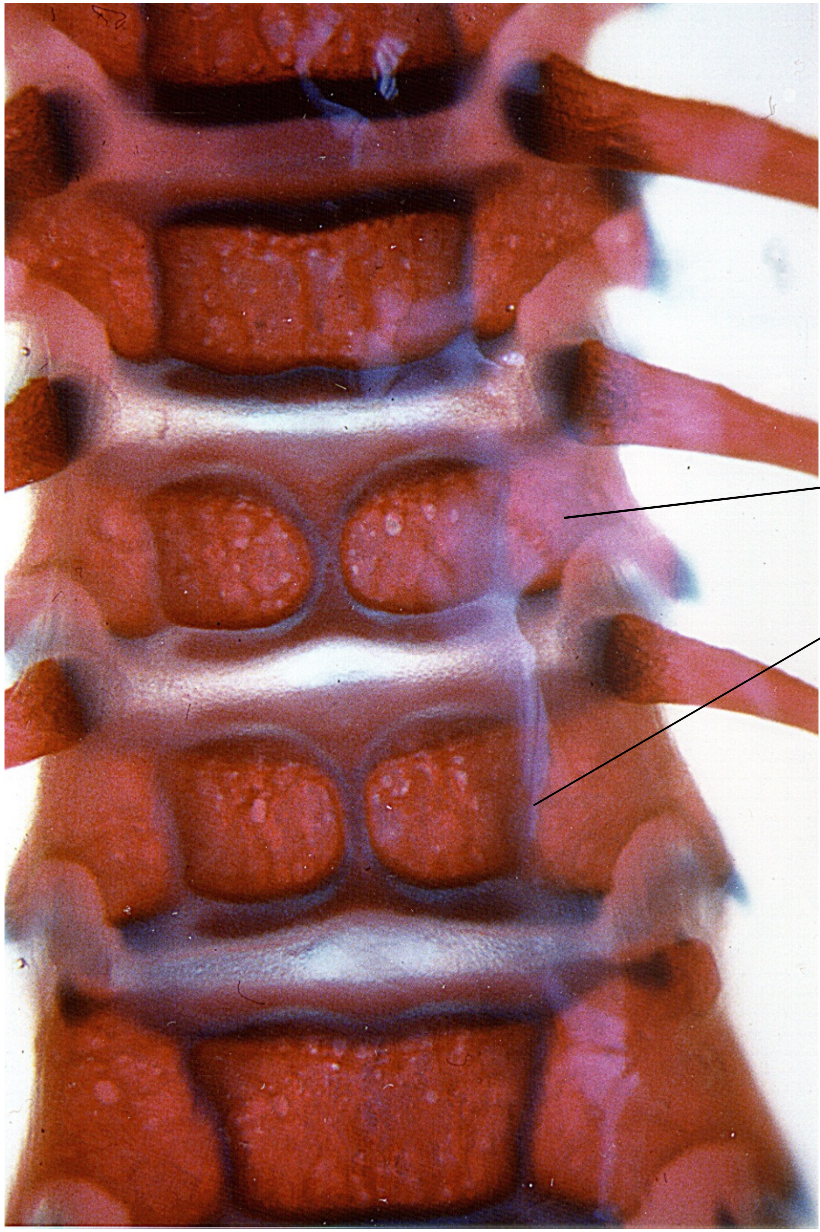


Thor vert oc dumbbell shaped

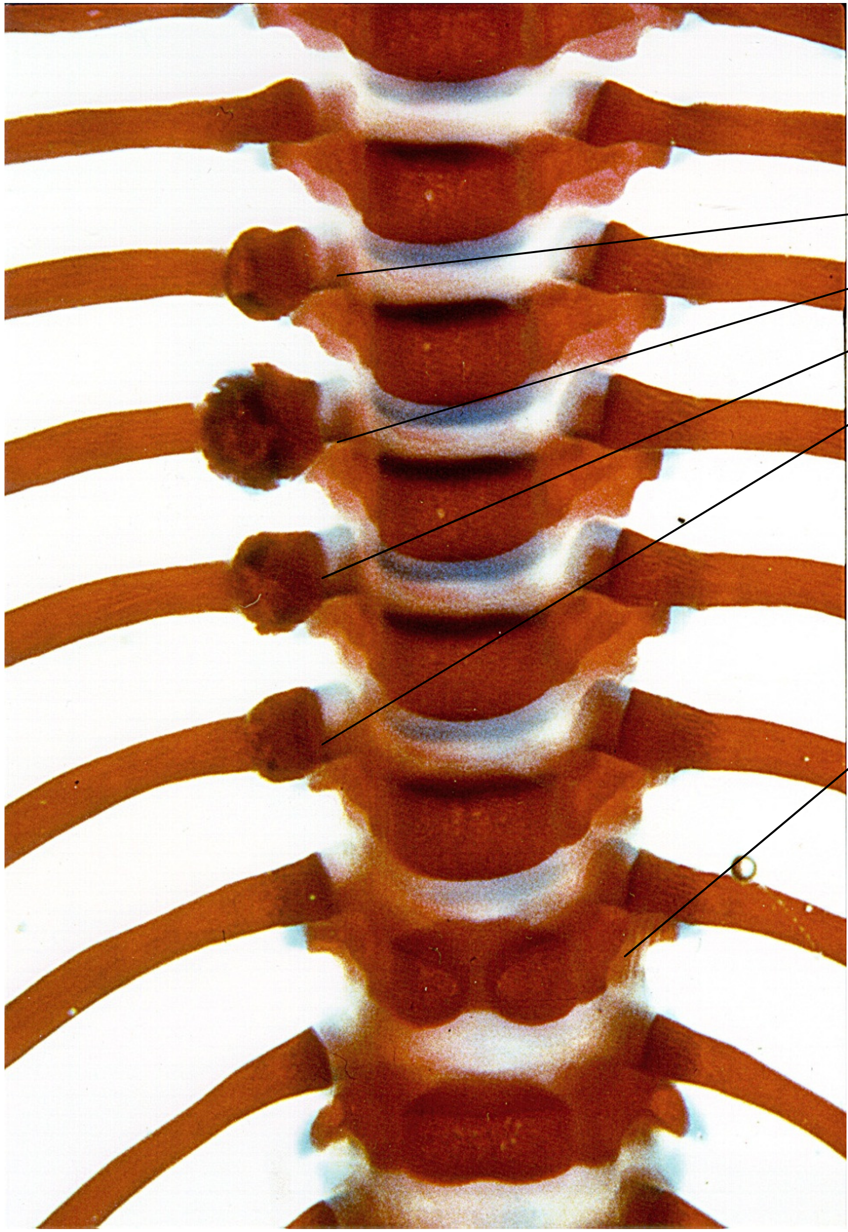
Rat pup, FuDR (40 mg/kg *sc* on GD 11), **Post natal day 21**



Rat pup, FuDR (45 mg/kg *sc* on GD 11), **<u>Post natal day 21</u>**



Rat pup, FuDR (50 mg/kg *sc* on GD 11), **Post natal day 21**



Broken ribs ? Healing calcification ?

Previously bent ribs ?

Thor vert oc dumbbell shaped

Conclusions:

⇒ Some of the vertebral abnormalities (bipartite/dumbbell shaped oc) seen in treated fetuses at term (C-section, GD21) were also found postnatally in pups examined on PND 7 and 21.

⇒ As a rule, abnormalities observed in term fetuses were more severe than those found postnatally. It should be borne in mind, however, that only some of the prenatally-exposed individuals survived postnatally until PND 7 and 21.

⇒ It is likely that pups examined on PND 7 and 21 were representatives of the less severely affected individuals.

⇒ Some (partial) recovery of vertebral oc anomalies may also have occurred.

Conclusions (cont.):

Vertebral *oc* abnormalities (*e.g.* *oc* dumbbell-shaped) found in term fetuses are still present on PND 21 thereby suggesting that either they are **not transient**, or they are **very slowly reversible** changes.

Survival was not evaluated in this study.

Influence of postnatal vertebral body anomalies on survival/health is difficult to be ascertained because FuDR treated rats (GD11) may have had several structural (and functional) defects in addition to the abnormalities evaluated in this study.

The effect of postnatal persistence of vertebral body anomalies on survival and health remains unclear.

Additional studies should be undertaken along this line.

TERATOLOGY 46:169–181 (1992)

Developmental Stages of the CD® (Sprague-Dawley) Rat Skeleton After Maternal Exposure to Ethylene Glycol

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Double stained rat skeleton

Ethylene glycol treated orally (0 and 2500 mg/kg/d) on GD 6-15

Evaluation: GD18, 20, PND 1, 4, 14, 21, 63.

Data on postnatal mortality unclear.

TABLE 2. Combined fetal/pup weights after maternal exposure to gd 6–15 with 0 or 2,500 mg/kg of EG

	Dose (mg/kg/day, po)		
Age	0	2,500	% of control
gd 18	1.26 ± 0.04 (7)[1]	0.93 ± 0.03 (7)*	74
gd 20	3.63 ± 0.09 (6)	2.75 ± 0.10 (7)*	76
pnd 1	6.90 ± 0.11 (29)	6.21 ± 0.12 (24)*	90
pnd 4	10.07 ± 0.30 (23)	9.22 ± 0.30 (18)	92
pnd 7	16.15 ± 0.35 (17)	14.99 ± 0.59 (14)	93
pnd 14	34.78 ± 0.71 (17)	33.20 ± 0.99 (14)	95
pnd 21	53.28 ± 2.29 (12)	51.18 ± 1.93 (9)	96
pnd 63	338.7 ± 11.0 (5)	320.6 ± 7.71 (5)	95

[1]Presented as average fetal or pup body weight (g) per litter (mean ± S.E.M.). The no. of litters appears in parentheses.
*$P < .05$, Student's t-test.

TABLE 5. Morphological abnormalities observed in CD rat fetuses/pups following maternal exposure to ethylene glycol on gestational days 6–15: listing by defect type (continued)[1]

	Day of sacrifice													
	GD 18		GD 20		PND 1		PND 4		PND 14		PND 21		PND 63	
	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500
Dumbbell cartilage, dumbbell ossification center														
Thoracic centrum	1			9		2	2			3				
Lumbar centrum				2										
Dumbbell cartilage, normal ossification center														
Cervical centrum		1		3				1						
Thoracic centrum	1	42		3			4							
Lumbar centrum		8		1										
Normal cartilage, unilateral ossification center														
Cervical centrum								2		1		1		
Thoracic centrum	3			28		3		20		11		1		3
Lumbar centrum				7		1		1						
Normal cartilage, bipartite ossification center														
Cervical centrum				1		3	2	16			9		1	
Thoracic centrum	2		1	38		15	14	47	5	23		16		8
Lumbar centrum	1			19		12				6		2		
Normal cartilage, misshapen ossification center														
Lumbar centrum						1								
Normal cartilage, unossified ossification center														
Lumbar centrum								1						
Incomplete ossification, cartilage present														
Cervical centrum								3						
Lumbar centrum								1						
Extra ossification site														
Cervical centrum VII				1										
Thoracic centrum V													1	
Lumbar centrum I				1										
Lumbar centrum VII				6										

[1]A single fetus/pup may be represented more than once in listing individual defects. A defect may appear at one or more sites in a single fetus/pup.
[2]Only live fetuses/pups were examined for malformations.
[3]Includes only litters with live fetuses/pups.
[4]Fetuses/pups with one or more malformations.
[5]Litters with one or more malformed fetuses/pups.
[6]Fetuses/pups with one or more variations.
[7]Litters with one or more fetuses/pups with variations.

TABLE 5. Morphological abnormalities observed in CD rat fetuses/pups following maternal exposure to ethylene glycol on gestational days 6–15: listing by defect type (continued)[1]

	Day of sacrifice													
	GD 18		GD 20		PND 1		PND 4		PND 14		PND 21		PND 63	
	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500
Dumbbell cartilage, unilateral ossification center														
Thoracic centrum				5										
Lumbar centrum	1			1		1								
Dumbbell cartilage, bipartite ossification center														
Cervical centrum							1							
Thoracic centrum	1			15		13	2	1		1		10		
Lumbar centrum				4		5		1				3		

TABLE 5. Morphological abnormalities observed in CD rat fetuses/pups following maternal exposure to ethylene glycol on gestational days 6–15: listing by defect type[1]

	Day of sacrifice													
	GD 18		GD 20		PND 1		PND 4		PND 14		PND 21		PND 63	
	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500
Bipartite cartilage, unilateral ossification center														
Thoracic centrum				15		3								
Lumbar centrum				2		4								
Bipartite cartilage, bipartite ossificaiton center														
Cervical centrum								3				3		
Thoracic centrum				13		23	3	8		16		21		1
Lumbar centrum				1		9		2				3		
Bipartite cartilage, dumbbell ossification center														
Cervical centrum								1						
Thoracic centrum						1	1							
Bipartite cartilage, normal ossification center														
Cervical centrum		3		22		23		2						
Thoracic centrum		51		20				2						
Lumbar centrum		16		3										

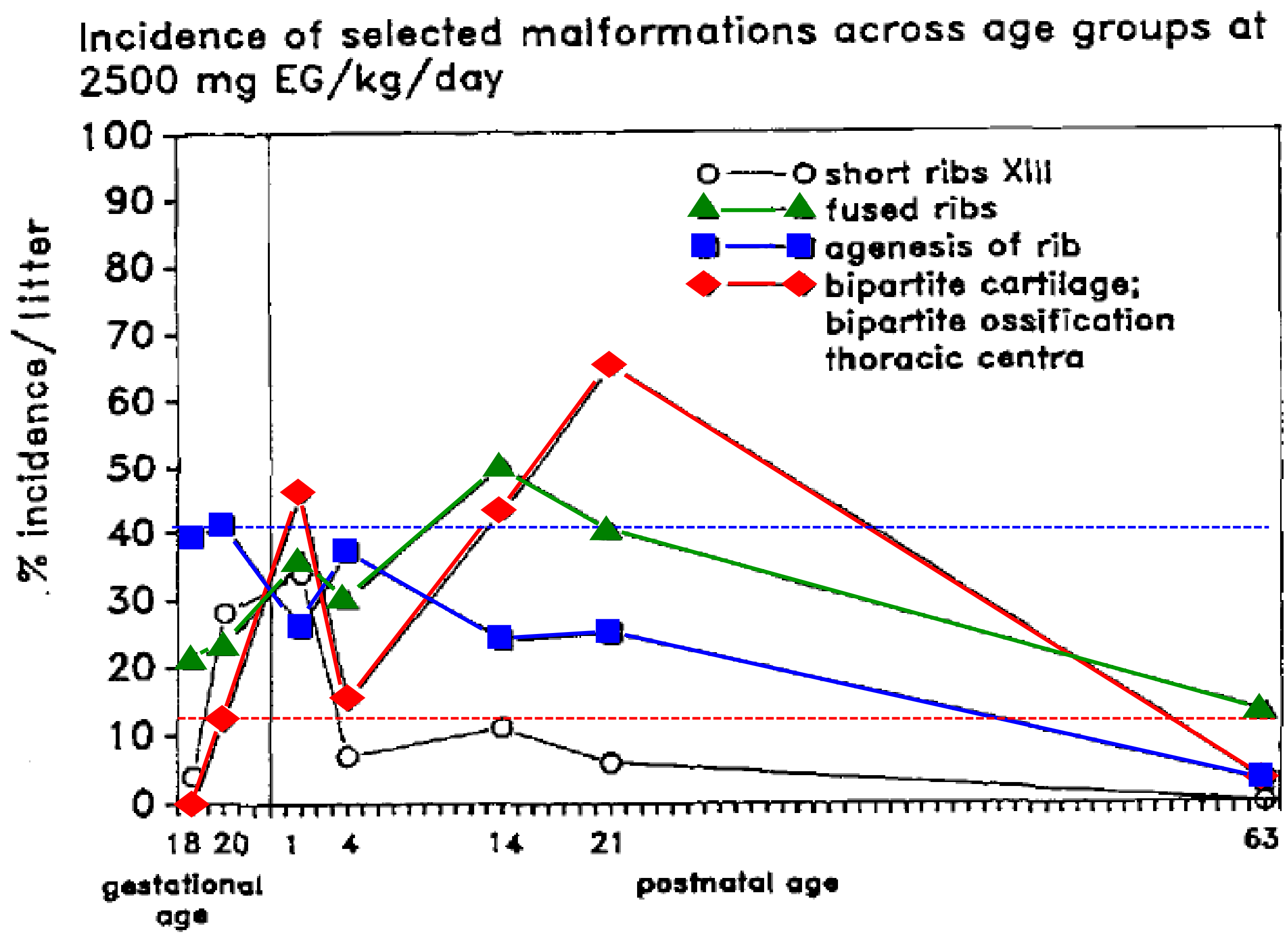
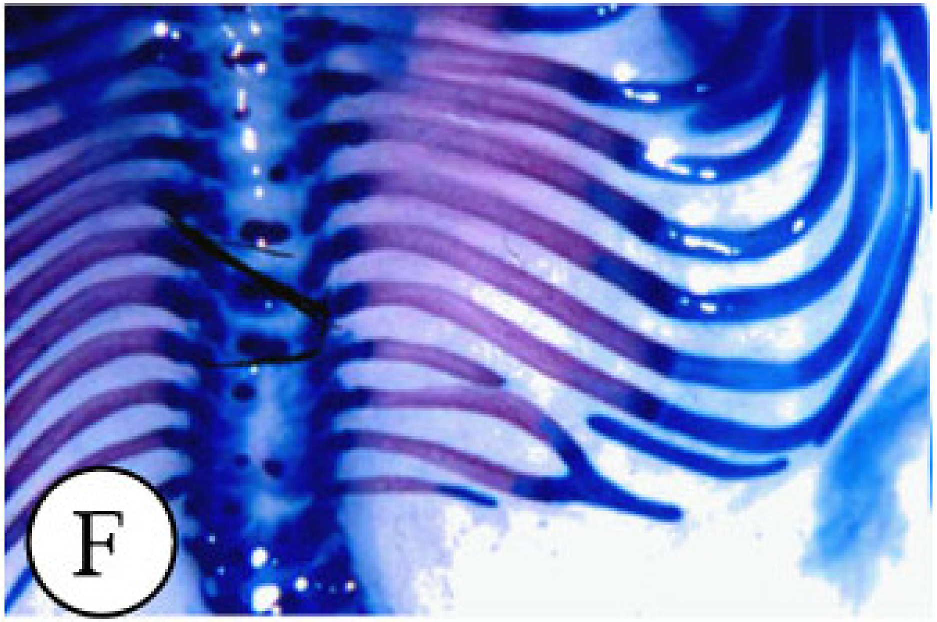


Fig. 1. Incidence (no. malformed/no. fetuses examined) of selected skeletal malformations across age groups at 2,500 mg EG/kg/day.

Table 5
Rib findings

Finding	Index of agreement
Rib, absent	92.3
Rib, bent	−15.4
Rib, branched	61.5
Rib cartilage, branched	−15.4
Ribs cartilage, fused	−7.7
Rib, cervical	−61.5
Rib, detached	80.0
Rib, discontinuous	−41.7
Rib, fused	84.6
Rib, incomplete ossification	−69.2
Rib, intercostal	72.7
Rib, knobby	−66.7
Rib, malpositioned	100
Rib, misaligned	−9.1
Rib, misshapened	61.5
Ribs, short	38.5
Ribs, thickened	−83.3
Ribs, unossified	−30.8
Ribs, wavy	−76.9
Ribs, supernumerary, full	−84.6
Ribs, supernumerary, short	−100



Fused and split ribs and vertebral centrum anomalies in rat fetuses at term. The dam was treated with ethylene glycol during pregnancy.

From: *Tyl RW, Chernoff N, Rogers JM, Birth Defects Res (part B) 80: 451-72, 2007.*

TABLE 5. Morphological abnormalities observed in CD rat fetuses/pups following maternal exposure to ethylene glycol on gestational days 6–15: listing by defect type[1]

	Day of sacrifice													
	GD 18		GD 20		PND 1		PND 4		PND 14		PND 21		PND 63	
	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500	0	2,500
Total fetuses/pups examined[2]	83	96	85	105	87	50	84	54	40	37	54	32	40	39
Total litters examined[3]	7	7	6	7	6	6	6	4	5	5	7	4	5	5
Skeletal malformations														
No. fetuses/pups with malformations[4]	0	72	1	92	0	46	6	45	0	28	0	28	3	11
No. litters with malformations[5]	0	7	1	7	0	6	3	4	0	5	0	4	1	4
Agenesis of the sternebrae						4						1		
Perforated sternum				1										
Fused sternebrae				2		15		9		9		12	3	7
Scrambled sternebrae						2								
Abnormal sternebrae				1		10						4		
Agenesis of a rib		37		43		13		20		9		8		1
Agenesis of a rib cartilage				2										
Fused ribs		20		24		18		16		18		13		5
Fused rib cartilage		5		7		16		1				1		
Branched rib		1		5				3						
Branched rib cartilage		3												
Discontinuous rib cartilage				1		2								
Rib cartilage not attached to sternum: ribs I–VII				4		3		1						
Extra rib: cervical VII attached to rib I				4		5								
Short rib														
I								1						
VI												1		
XI		1												
XII		1												
XIII		4	1	29		17		4		4		2		

Dumbbell and bipartite vertebral oc

weight. Thiel et al. ('89) examined double-stained rat skeletons for the persistence of dumbbell and bicentric ossification centers in 1 week and 3 week old pups. Their conclusion was that both alterations persisted in the 1 and 3 week old skeletons and thus were not retardations but persistent defects. In this investigation using ethylene glycol, our conclusion would have been the same as Thiel et al. ('89), through pnd 21, but our additional data at pnd 63 led us to the opposite conclusion.

Marr et al, 1992:

the incidence dropped dramatically by pnd 63. This was not unexpected in skeletal defects of the vertebral centrum, since post-natal day 21 skeletons exhibiting bipartite centra have surrounding cartilage and growth is still possible.

Marr et al, 1992:

The absence of malformations involving fusion and agenesis of the ribs by pnd 63 may be explained by skeletal remodeling in the postnatal period

Taken together results from both studies suggested that vertebral body anomalies seen at C-section are to some extent repaired after birth. The repair process, however, seems to be rather slow and vertebral centrum anomalies are still observed at weaning on PND 21.

Additionally, data provided by Marr et al's study indicated that a number of rib anomalies generally classified as malformations (fused, absent, branched ribs and others) are not permanent changes as well.

If the aforementioned rib anomalies are in fact repaired ("*through skeletal remodeling*") after birth they should be classified as "variations" and not as "malformations".

Further studies are needed to clarify and confirm these findings.

Summing up

1. There are very few studies on the postnatal persistence of fetal anomalies in laboratory animals.
2. Uncertainties about the postnatal fate (persistence/health effects) of some fetal abnormalities detected at term remain as a major obstacle for classifying them (“grey zone anomalies”) using the proposed two category (variations or malformations) scheme.
3. Results from one study by Marr et al (1992) suggested that some rib anomalies generally classified as malformations are transient changes due to skeleton remodeling.

Summing up

4. Peri and postnatal mortality is a confounding factor (possible selection of less severely affected individuals) in studies on the permanence/transience of fetal abnormalities.
5. Following up a fetal anomaly repair in a same individual using non-invasive imaging methods (x rays, tomography, MRI) is suggested as an alternative approach in further studies.
6. To study the postnatal health consequences of anomalies observed in term fetuses is one of the biggest challenges to be faced.
7. A devtox agent used to increase the incidence of the anomaly may cause structural and functional defects other than that being evaluated. Evidence should be provided that the eventual health effects were due to the anomaly under examination and not to other effects of the teratogen.

Summing up

8. Studies on the postnatal consequences (permanence/transience and health effects) of fetal abnormalities are urgently needed to eventually correct current misclassifications and to reduce the number of grey zone anomalies.

Thank you !!!

Bipartite ossification	Ossification centers not fused. Commonly used for structures arising from two or more primary ossification centers (e.g., sternebrae, vertebral centra). Applies only to the ossification sites and does not imply that the structure, as represented by the bone precursor, is divided (split)
Split	Division of a single structure (usually into two parts) with no intervening structure between the parts. In the case of bone, a structural change involving the bone precursor. Not to be used to describe a bipartite ossification site where there is no evidence that the precursor is split. In endochondral bone, cartilage staining can aid assessment
Dumbbell shaped	Two approximately spherical areas attached at or near the mid-line by a bridge. In the case of bone, a structural change involving the bone precursor (cartilage) and commonly used for structures arising from two primary centers (e.g., sternebrae, vertebral centra). Not to be used to describe a dumbbell ossification site where there is no evidence that the precursor is dumbbell shaped. Cartilage staining is generally necessary for accurate assessment