Acta orthop. scand. *50*, 151–159, 1979

EFFECT OF CHLORPROMAZINE ON SKELETOGENESIS

The Result of Maternal Administration of the Drug in Experimental Rats

SHAMER SINGH & R. PADMANABHAN

Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Chlorpromazine hydrochloride (CPZ) is known to induce intrauterine and extrauterine growth retardation. A single dose of CPZ (100 mg/kg) was administered to pregnant CF rats on the 14th day of gestation (sperm positive = day 0). Fetuses were collected from the 16th to the 20th day of gestation and processed for alizarin red-S stain. Ossification was delayed by 1 to 3 days in the long bones of the extremities, by 1 day in the scapulae and by 2 to 3 days in the ilium. Ischium and pubis remained unossified until the 20th day of gestation. Ossification of the skull bones was also delayed as manifested by the presence of wide sutures in the treated cases. The number and range of ossified vertebral bodies and arches in the treated group were always less than those in the control group. The sternebrae were most affected. The ribs also showed a significant delay in maturity.

Key words: chlorpromazine; pregnant rats; fetal skeleton; alizarin red-S; skeletogenesis inhibition

Accepted 24.v.78

Phenothiazine derivatives are classified (Rumeau-Rouquette et al. 1977) into four groups depending on their chemical structure:

1. 3-carbon aliphatic side chain, e.g., chlorpromazine;
2. 2-carbon aliphatic side chain, e.g., promethazine;
3. piperazine side chain, e.g., fluphenazine, prochlorperazine;
4. piperidine side chain, e.g., thioridazine.

Rumeau-Rouquette et al. (1977) found a significant increase in the malformation rate in the human with the intake of phenothiazines with a 3-carbon aliphatic side chain. Horvath et al. (1976) found the teratogenicity to be increased in experimental animals with the length of the N-alkyl side chain especially with the substituted piperazine ring. Previous reports of phenothiazine effects on embryonic development have often been contradictory (Chambon 1955, 1956, Courvoisier & Ducrot 1954, Eyal-Giladi et al. 1962, 1963, 1964, Fratta et al. 1964). It has been shown that chlorpromazine (CPZ), a phenothiazine derivative, prolongs gestation, reduces litter size and birth weight, increases postnatal mortality and causes intrauterine and extrauterine growth retardation (Ordy et al. 1963, Horvath & Druga 1975, Horvath et al. 1976, Singh & Padmanabhan 1978a, 1978b).

In some studies it was also found to be teratogenic (Walker 1969, Horvath & Druga 1975, Horvath et al. 1976, Singh & Padmanabhan 1978c). However, fetal growth retardation was an observation common to most of these investigations. There seems to be no report of the effect of chlorpromazine

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on fetal skeletogenesis, the disturbance of which could be a major cause of such growth retardation. The present investigation was therefore undertaken to study the prenatal development of the skeleton in rat fetuses after maternal administration of CPZ.

MATERIALS AND METHODS

CF female rats of about 200 g body weight were mated with males of the same strain in the evening and pregnancy was confirmed by the presence of sperm in the vaginal smear examined on the following morning, which was designated as day 0 of gestation. A single dose (100 mg/kg body weight) of chlorpromazine (Largactil—May & Baker) was given to the pregnant rats intraperitoneally (i.p.) on the 14th day of gestation and they were caged separately. The controls were injected with a corresponding volume of physiological saline. Food (Hind-Lever diet) and water were provided *ad libitum.* 0.5 ml saline injection was given to the controls as well as the treated animals when dehydration was anticipated in the latter (Walker & Patterson 1974). The fetuses were collected from the 16th to the 20th day of gestation by uterotomy following ether anaesthesia. They were blotted dry, weighed individually and fixed in 95 per cent ethyl alcohol and subsequently processed for alizarin red-S stain. The younger fetuses were processed by the technique of Hurley (1965) and the older ones by that of Staples & Schnell (1964) and stored in 100 per cent glycerine. The specimens were examined under a dissection microscope. Five to eight animals (30–50 fetuses) made up both the control and treated groups on each collection day. Only living fetuses and intact preparations were used for this study.

Figures 1–5 show the effect of maternal administration of CPZ (100 mg/kg) on the fetal skeleton (cleared in KOH and stained with alizarin red-S). C-control; T-treated.

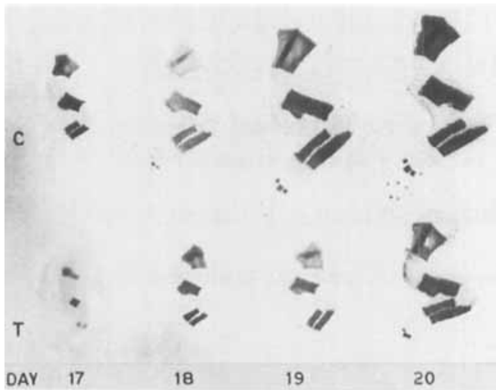

Figure 1. Forelimb skeletons of 17- to 20-day-old rat fetuses. Note the absence of the metacarpals and the reduced size of other bones in 17- to 19-day-old treated fetuses. The centres for the metacarpals have appeared in the 20-day-old treated fetuses but the ossification centres for the phalanges are absent (cf. corresponding controls).

RESULTS

There were cases of scanty ossification to no ossification in the treated groups on the various days of gestation. The term 'ossified' in a treated specimen is used only when the degree of ossification in such a specimen was comparable to that of the corresponding control.

Table 1. Effect of maternal i.p. injection of CPZ (100 mg/kg on the 14th day of gestation) on the fetal skeleton—Bones of the upper limb

	Control—Percentage ossified					Treated—Percentage ossified				
Day	16	17	18	19	20	16	17	18	19	20
Clavicle	100.0	—	—	—	—	46.3	50.0	90.9	100.0	—
Scapula	35.7	100.0	—	—	—	—	6.3	100.0	—	—
Humerus	71.4	100.0	—	—	—	—	53.0	84.8	100.0	—
Radius	21.4	100.0	—	—	—	—	25.0	75.6	100.0	—
Ulna	21.4	100.0	—	—	—	—	21.8	78.8	100.0	—
Carpals	—	—	—	—	—	—	—	—	—	—
Metacarpals	—	—	16.3	100.0	—	—	—	—	70.6	96.8
Phalanges	—	—	—	—	60.0	—	—	—	—	—

Bones of the upper limb (Table 1, Figures 1, 3). Among the bones of the upper limb, the clavicle was the first to ossify in both the control and the treated groups. Ossification was seen in 100 per cent of the clavicles in the control group on the 16th day whereas in the treated groups not all of the clavicles were ossified until the 19th day. In 93.7 per cent of the scapulae of the treated group, ossification was delayed by 1 day. The humerus, radius and ulna of the control group showed ossification in 100 per cent of cases by the 17th day while in the drug-treated group this could not be observed until the 19th day. Metacarpals 2, 3 and 4 had appeared in all cases in the control group by the 19th day, and by the 20th day 32.5 per cent of the cases showed ossification centres for the 5th metacarpal also. However, in the treated group, even on the 19th day, only 70.6 per cent of the cases showed ossification of metacarpals 2, 3 and 4. The ossification centre for metacarpal 5 was not seen in any case. The ossification centres for the phalanges were inconsistent in the controls and none could be observed in the treated group.

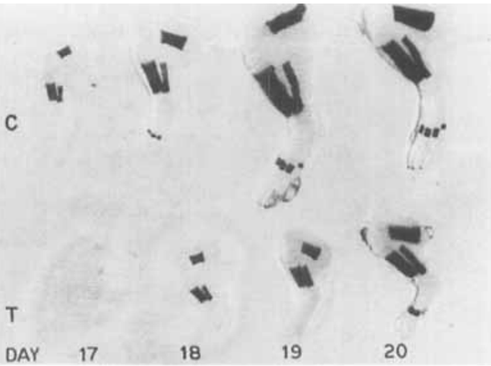

Figure 2. Hindlimb skeletons of 17- to 20-day-old rat fetuses. Note the marked reduction in the length of the long bones and the delayed appearance of the ossification centres for the metatarsals in the treated groups.

Bones of the lower limb (Table 2, Figure 2). In the control group, the ilium, femur, tibia and fibula were ossified in 100 per cent of cases on the 17th day and the ischium and pubis on the 19th day. The ossification was initially inhibited in the treated group and was complete in a comparable percentage of cases after a delay of 3 days in the case of the ilium and 2 days in the case of the other bones. The ischium and pubis in the treated group did not show ossification in all cases even on the 20th day. In the drug-treated group, the metatarsals were ossified in only 67.7 per cent of cases by the 20th day whereas in the controls all were ossified on the 19th day. There were a greater number of metatarsals with retarded ossification in the treated group. Ossification centres for the phalanges were not seen in any of the controls or in the treated group.

Table 2. Effect of maternal i.p. injection of CPZ (100 mg/kg on the 14th day of gestation) on the fetal skeleton—Bones of the lower limb

	Control—Percentage ossified					Treated—Percentage ossified				
Day	16	17	18	19	20	16	17	18	19	20
Ilium	—	100.0	—	—	—	—	9.4	78.8	91.2	100.0
Ischium	—	—	—	100.0	—	—	—	—	67.6	96.8
Pubis	—	—	—	100.0	—	—	—	—	35.3	96.8
Femur	—	100.0	—	—	—	—	15.6	57.6	100.0	—
Tibia	—	100.0	—	—	—	—	15.6	54.4	100.0	—
Fibula	—	100.0	—	—	—	—	15.6	54.4	100.0	—
Tarsals	—	—	—	—	—	—	—	—	—	—
Metatarsals	—	—	2.3	100.0	—	—	—	—	35.3	67.7
Phalanges	—	—	—	—	—	—	—	—	—	—

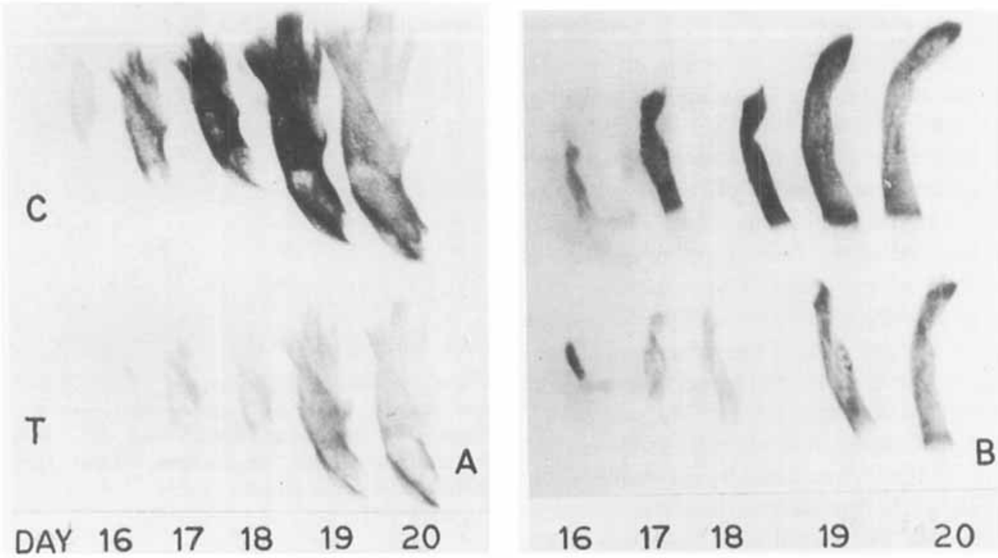

Figure 3. Mandibles (A) and clavicles (B) of 16- to 20-day-old rat fetuses. Note the scanty and delayed ossification in the treated group in contrast to the control group.

Axial skeleton (Tables 3 and 4, Figure 3). Of the skull bones, the mandible was the first to ossify both in the control and the treated groups and on the 16th day ossification was seen in 100 per cent and 63 per cent of cases, respectively. In the treated group, 100 per cent of the mandibles were not ossified until the 18th day. The premaxilla, maxilla, frontal, squamosal, palatine, basioccipital and exoccipital bones were ossified in 100 per cent of cases in the control group on the 17th day whereas in the treated group a comparable percentage of bones was not ossified until the 19th day, i.e., there was a delay of 2 days.

Table 3. Effect of maternal i.p. injection of CPZ (100 mg/kg on the 14th day of gestation) on the fetal skeleton—Skull bones

	Controls—Percentage ossified					Treated—Percentage ossified				
Day	16	17	18	19	20	16	17	18	19	20
Mandible	100.0	—	—	—	—	63.0	64.5	100.0	—	—
Maxilla	42.9	100.0	—	—	—	—	9.4	90.9	100.0	—
Premaxilla	—	100.0	—	—	—	—	—	75.6	100.0	—
Nasal	—	85.3	100.0	—	—	—	15.6	57.6	100.0	—
Frontal	21.4	100.0	—	—	—	—	13.5	78.8	100.0	—
Parietal	—	91.2	100.0	—	—	—	—	21.2	94.0	100.0
Squamosal	—	100.0	—	—	—	—	—	15.2	100.0	—
Palatine	—	100.0	—	—	—	—	—	78.2	100.0	—
Basioccipital	—	100.0	—	—	—	—	6.3	75.6	100.0	—
Exoccipital	—	100.0	—	—	—	—	9.4	87.9	100.0	—
Supraoccipital	—	—	—	100.0	—	—	—	—	59.2	74.2
Basisphenoid	—	91.2	100.0	—	—	—	—	75.6	100.0	—
Hyoid	—	—	—	96.8	100.0	—	—	—	56.0 (Rudi.)	87.1 54.8 (Rudi.)

Table 4. Effect of maternal i.p. injection of CPZ (100 mg/kg on the 14th day of gestation) on the fetal skeleton—Vertebrae, ribs and sternebrae

	Controls—Percentage ossified					Treated—Percentage ossified				
Day	16	17	18	19	20	16	17	18	19	20
1. Vertebrae										
Arches[a]	21.4	100.0	—	—	—	—	78.1	100.0	—	—
	(C_1–C_3)	52.9	39.5	41.9	45.0	—	50.0	21.2	29.4	38.7
		(C_1–L_3)	(C_1–L_6)	(C_1–S_5)	(C_1–CO_1)		(C_1)	(C_1–C_3)	(C_1–S_2)	(C_1–S_5)
Bodies[b]	—	—	—	100.0	—	—	—	—	100.0	—
				41.9	72.5				11.8	22.3
				(T_1–S_5)	(T_1–CO_3)				(T_1–S_3)	(T_1–CO_3)
									(T_5–S_2)	
2. Ribs[c]	92.9	100.0	—	—	—	17.1	100.0	—	—	—
	21.4	55.9	100.0	—	—	17.1	18.8	57.6	100.0	—
	(10)	(13)	(13)			(3–6)	(9)	(12)	(13)	
3. Sternum[d]	—	—	—	96.8	100.0	—	—	—	29.4	100.0
				38.7	97.5	—	—	—	17.6	6.3
				(5)	(6)				(2)	(6)

a & b—parenthesis indicates the ranges of ossified vertebral arches and bodies respectively.
c—parenthesis indicates the number of ossified ribs.
d—parenthesis indicates the maximum number of ossified sternebrae.

In the nasal and basisphenoid bones the ossification was delayed by 1 day in the treated group while in the parietal bones it was delayed by 2 days. The hyoid bone in the treated group showed ossification in 87.1 per cent of cases of which 54.8 per cent were rudimentary as compared with 100 per cent with prominent ossification in the controls, when examined on the 20th day. Ribs were ossified in 92.9 per cent of the control cases on the 16th day as compared with 17.1 per cent in treated rats while on the 17th day ossification of the ribs was seen in 100 per cent in both groups. However, by then 13 ribs could be observed in 55.9 per cent of the controls as compared with only 9 ribs in 18.8 per cent of the treated cases. By the 19th day the ossification of the ribs was comparable in the treated group, i.e., 1 day later than the control group. The sternebrae were the worst affected. On the 20th day only 6.5 per cent of cases in the drug group had six sternebrae as against 97.5 per cent in the control group.

All fetuses showed ossification centres for some vertebral arches on the 17th day in the control and on the 18th day in the treated groups, the ossification proceeding in a cephalocaudal direction. However, the number of vertebrae with ossification centres for the arches was always less in the drug-treated group when compared with the corresponding controls. Though 100 per cent of cases in both the control and treated groups showed ossification centres for the vertebral bodies on the 19th day, the number of bodies ossified was never comparable.

DISCUSSION

Skeletal ossification in the rat begins with the mandible on the 16th to 17th day of gestation (sperm positive = day 0) (Strong 1926, Walker & Wirtschafter 1957, Wright et al. 1958, Witschi 1962, Brock & Kreybig 1964). Fritz & Hess (1970) suggested that in teratological studies ossification of the fetal skeleton could be considered as an indication of maturity which could be studied by designing a chronological investigation and recording the ossified and nonossified parts of the skeleton and distinguishing the incomplete ossification from the pathological or the abnormal.

The present chronological study of the effect of a single dose (100 mg/kg) of chlorpromazine administered on the 14th day of gestation has demonstrated that rat fetal skeletogenesis can be remarkably retarded by this drug (Figures 4 and 5). The appearance of ossification centres for the long bones of the upper limb was delayed by 1 to 3 days in most of the cases. Even in the few cases in which the centres appeared a bit earlier, the degree of ossification was inhibited. By the 20th day of gestation, the 5th metacarpal had not appeared in the drug-treated group and in only 70.6 per cent of cases were the metacarpals 2, 3, 4 ossified, as against 100 per cent in the control group. The phalangeal ossification was totally inhibited in the treated group. Femur, tibia and fibula were ossified in 100 per cent of control fetuses by the 17th day as compared with the 19th day in the drug-treated group, i.e., a delay of 2 days. In the case of the ilium the delay was even greater. The metatarsals 2, 3, 4, 5 were ossified by the 20th day in 98 per cent of the control fetuses as compared with only 32 per cent of the treated fetuses, the suppression of ossification being highly significant ($P < 0.001$). In the skulls of the drug-treated fetuses, besides there being a significant delay in the appearance of the ossification centres for the various bones, the rate of ossification was also found to be slower as evidenced from the wide sutures persisting until late in the period of gestation, a sign of incomplete ossification (Murphy 1962, McColl et al. 1963, 1965). In most of the treated cases (54.8 per cent) the hyoid bone was represented only by a rudimentary centre of ossification. Both in the control and treated groups, the ossification of vertebral arches progressed cephalocaudally, but in the drug group, the ossification was always restricted to a lesser number of vertebrae. Unlike the

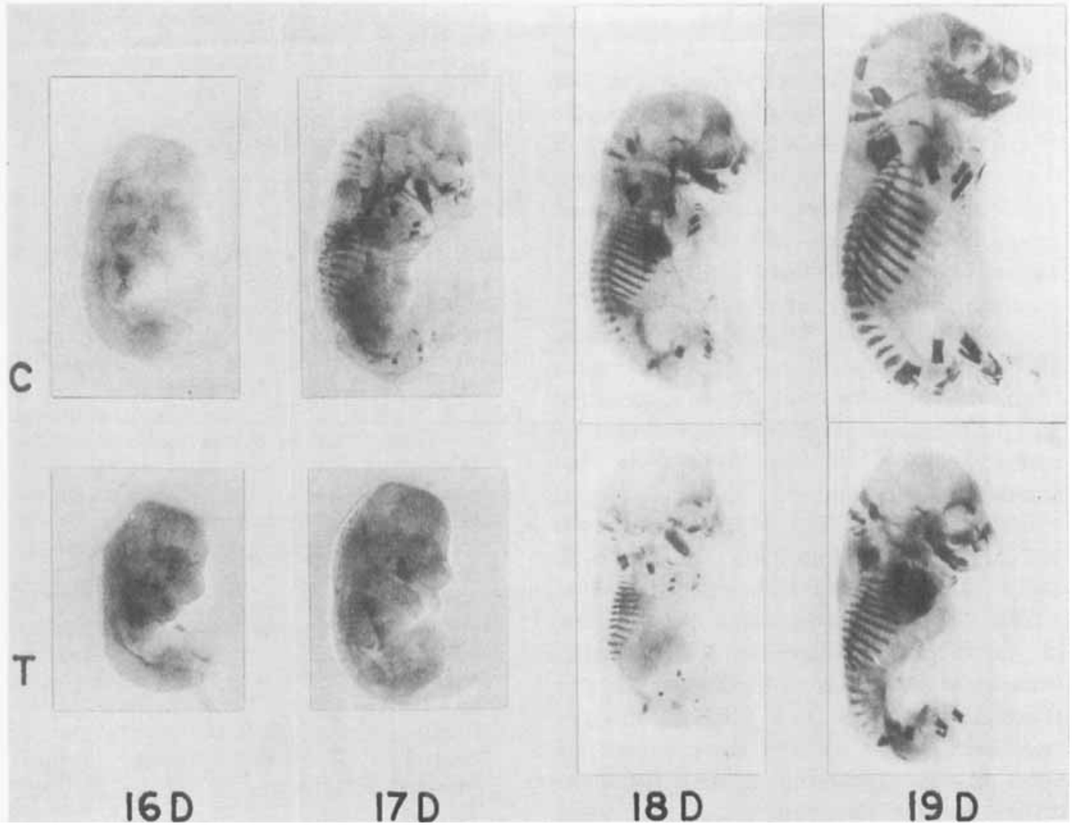

Figure 4. Skeletons of 16- to 19-day-old rat fetuses showing a general inhibition of skeletogenesis, i.e., late appearance of the various ossification centres and reduced size of the bones in the treated cases.

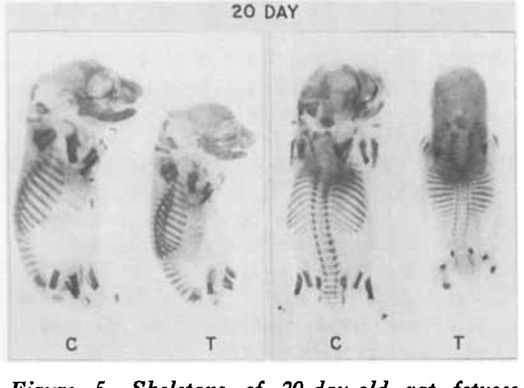

Figure 5. Skeletons of 20-day-old rat fetuses showing markedly stunted size and delayed ossification in the treated cases, especially in the skull, vertebrae and digital bones.

earlier reports (Horvath & Druga 1975, Horvath et al. 1976), the full-term fetuses of the drug-treated group showed no defects but only a very low percentage (22 per cent) showed the first thoracic to the third coccygeal vertebral bodies as against 72.5 per cent in the control group. There were no supernumerary ribs either in the control or in the treated fetuses though a significantly large number of ribs were rudimentary in the latter group. In only 6.5 per cent of the drug-treated fetuses could all six sternebrae be observed, as compared with 98 per cent in the controls, though there was no abnormal ossification in any group. If missing vertebral bodies and sternebrae are considered malformations (Kimmel & Wilson 1973, Raju et al. 1976), the rudimentary ribs and smaller long bones must be considered a retardation of growth (Kimmel & Wilson 1973). Though not well established to be teratogenic, tranquilizers are suspect because of their categorical association with thalidomide—a known teratogen (Wilson 1973). Recent experimental works (Horvath & Druga 1975, Horvath et al. 1976, Singh & Padmanabhan 1978c) and prospective studies in humans (Rumeau-Rouquette et al. 1977) support the fact that chlorpromazine is teratogenic. Its marked growth retarding effect is one common observation in all experimental studies (Ordy et al. 1963, Walker & Patterson 1973, Horvath & Druga 1975, Horvath et al. 1975, 1976, Singh & Padmanabhan 1978b, 1978c). The present chronological observation of rat fetal skeletogenesis adds further evidence and provides an explanation for this effect. In a species (rats) with 21 days of gestation, a delay of 1–3 days, caused by CPZ, in the appearance of the ossification centres and the slow progress of ossification also explains the reduced birth weight and retarded postnatal growth in rats reported by Singh & Padmanabhan (1978b, 1978c).

REFERENCES

Brock, N. & Krebig, T. von (1964) Teratogenese als pharmakologisch—toxikologisches problem. I. Allgemeine Grundlagen: Nomale Embryonalentuicklung von Ratte und Meerschweinchen. *Arzneimittel-Forsch.* **14,** 655–664.

Chambon, Y. (1955) Action de la chlorpromazine sur l'evolution et l'avenir de la gestation chez la rate. *Ann. Endocr. (Paris)* **16,** 912–922.

Chambon, Y. (1956) Effect abortif de la chlorpromazine chez l'animal. *Bull. Féd. Soc. Gynéc. Obstét. franç.* **8,** 447.

Courvoisier, S. & Ducrot, R. (1954) Recherche des effets de la chlorpromazine (4560) sur la sphère génitale et la croissance. *C.R. Soc. Biol. (Paris)* **148,** 462–466.

Eyal, Z. & Eyal-Giladi, H. (1963) Growth retarding and growth promoting effect of chlorpromazine on developing Ambystoma mexicanum (axolotl) embryos. *Exp. Cell Res.* **29,** 394–399.

Eyal-Giladi, H. & Eyal, Z. (1962) The effect of chlorpromazine on the embryonic development of the axolotl (Ambystoma mexicanum). *J. Embryol. exp. Morph.* **10,** 357–372.

Eyal-Giladi, H., Eyal, Z. & Eshel, S. (1964) A microscopic study of chlorpromazine (Largactil) treated axolotl larvae. *J. Embryol. exp. Morph.* **12,** 447–456.

Fratta, I., Zak, S. B., Greengard, P. & Sig, E. B. (1964) Fetal death from nicotinamide-deficient diet and its prevention by chlorpromazine and imipramine. *Science* **145,** 1429–1430.

Fritz, H. & Hess, R. (1970) Ossification of the rat and mouse skeleton in the perinatal period. *Teratology* **3,** 331–338.

Horvath, C. & Druga, A. (1975) Action of the phenothiazine derivative methophenazine on prenatal development in rats. *Teratology* **11,** 325–330.

Horvath, C., Szonyi, L. & Mold, K. (1976) Preventive effect of riboflavin and ATP on the teratogenic effects of the phenothiazine derivative T-82. *Teratology* **14,** 167–170.

Hurley, L. S. (1965) Demonstration "A"—Alizarin staining of bone. *Supplement to Teratology Workshop Manual Second Workshop in Teratology* pp. 121–122, Berkely, California.

Kimmel, A. C. & Wilson, J. G. (1973) Skeletal deviations in rats: Malformations or variations? *Teratology* **8,** 309–316.

McColl, J. D., Globus, M. & Robinson, S. (1963) Drug induced skeletal malformations in the rat. *Experientia (Basel)* **19,** 183–184.

McColl, J. D., Globus, M. & Robinson, S. (1965) Effect of some therapeutic agents on the developing rat fetus. *Toxicol. appl. Pharmacol.* **7,** 409–417.

Murphy, M. L. (1962) Teratogenic effects in rats of growth inhibiting chemicals, including studies on thalidomide. *Clin. Proc. Child. Hosp. (Wash.)* **18,** 307–322.

Ordy, J. M., Latanick, A., Johnson, R. & Massopust, L. C. (1963) Chlorpromazine effects on pregnancy and offspring in mice. *Proc. Soc. exp. Biol. Med.* **113,** 833–836.

Raju, P. B., Singh, S. & Sanyal, A. K. (1975) Ossification patterns of sternum in rat fetuses after maternal administration of cyclophosphamide. *Cong. Anom.* **15,** 99–106.

Rumeau-Rouquette, C., Goujard, J. & Huel, G. (1977) Possible teratogenic effect of phenothiazines in human beings. *Teratology* **15,** 57–64.

Singh, S. & Padmanabhan, R. (1978a) Growth retardation in rat fetuses induced by chlorpromazine hydrochloride (CPZ). *Anat. Anz.* (In press).

Singh, S. & Padmanabhan, R. (1978b) Prolongation of gestation and retarded postnatal growth and mortality induced by chlorpromazine in rats. *Indian J. Exp. Biol.* **16,** 542–545.

Singh, S. & Padmanabhan, R. (1978c) Teratogenic effects of chlorpromazine hydrochloride in rat fetuses. *Indian J. Med. Res.* **68,** 300–309.

Staples, R. E. & Schnell, V. L. (1964) Refinements in rapid clearing technic in the KOH Alizarin red S method for fetal bone. *Stain Technol.* **39,** 61–63.

Strong, R. M. (1926) The order, time and rate of ossification of the albino rat *(Mus norvagicus albinus)* skeleton. *Amer. J. Anat.* **36,** 313–355.

Walker, D. G. & Wirtschafter, Z. T. (1957) *The genesis of the rat skeleton. A laboratory atlas,* Charles C Thomas, Springfield, Ill.

Walker, B. E. (1969) Relation of embryonic movements to formation of cleft palate. *Teratology* **2,** 272 (abstract).

Walker, B. E. & Patterson, A. (1974) Induction of cleft palate in mice by tranquilizers and barbiturates. *Teratology* **10,** 159–164.

Wilson, J. G. (1973) *Environment and birth defects.* Academic Press, New York.

Witschi, E. (1962) *Growth, including reproduction and morphological development.* Ed. Altman, P. L. & Dittmer, D. S., Federation of American Societies for Experimental Biology, pp. 304–314, Washington, D.C.

Wright, H. V., Asling, C. W., Dougherty, H. L., Nelson, M. M. & Evans, H. M. (1958) Prenatal development of the skeleton in Long-Evans rat. *Anat. Rec.* **130,** 659–672.

Correspondence to: Dr. Shamer Singh, B.Sc., M.B.B.S., M.S., F.A.M.S., Professor & Head of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India.