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A Study of Fetal Growth Retardation in Teratological Tests: Relationship Between Body Weight and Ossification of the Skeleton in Rat Fetuses

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ABSTRACT The normal development of preterm rat fetuses (day 19.0 to day 21.0) was investigated with respect to body weight and ossification of the sacrococcygeal vertebrae, supraoccipital bone, sternebrae, and proximal phalanges in the forepaw. Normal standard growth curves were established for these indexes in rat fetuses. A method was devised for the analysis of the relationship between low body weight and retarded ossification induced by teratogenic agents. In normal fetuses, mean body weight and number of ossified sacrococcygeal vertebrae and the ossification stage of the supraoccipital bone increased approximately linearly with advancing fetal age. The number of ossified sternebrae and proximal phalanges in the forepaw increased curvilinearly with advancing fetal age. By use of the standard curves, fetal growth retardation observed in teratological experiments may be expressed as retardation in relation to the standard in hours. The characteristic pattern of growth retardation induced by an agent may be evaluated by comparison of the degree of retardation expressed in a common "hour" scale among various growth indexes. This method was applied to experimental data of fetal growth retardation induced by maternal fasting. The degree of retardation was found to differ among growth indexes; body weight and ossification of the sacrococcygeal vertebrae were the most severely retarded; ossification of the supraoccipital bone and the sternebrae was moderately retarded; ossification of the proximal phalanges in the forepaw was least retarded.

Low body weight and retarded ossification have been induced by many teratogenic agents (Ornoy et al., '69; Ornoy, '71; Brown et al., '76; Kennedy et al., '76; Dix et al., '77). However, the relationship between low body weight and retarded ossification has seldom been discussed. The present authors reported earlier that the relationship between low body weight and retarded ossification in coccygeal vertebrae observed in teratological experiments could be analyzed by measuring relative differences of body weight and ossification of the coccygeal vertebrae between the treated group and the control group with the variance within groups in normal rat fetuses ($\hat{\sigma}_0$) as a scale (Ariyuki et al., '80a).

A new method was devised for further analysis of the relationship between ossification of other bones and body weight in teratological tests. This method makes use of normal developmental patterns of various ossification indexes and body weight in preterm rat fetuses.

MATERIALS AND METHODS

For establishment of standard growth in preterm fetuses, nontreated Wistar rats (Kitayama LABES Co., Ltd., 12–14 weeks of age) were used. Each female was caged with a male overnight (from 4 PM to 9 AM). Pregnancy was considered to have started (day 0) on the next morning at 9 AM, when vaginal smears contained sperm. The pregnant animals were fed a standard diet (CLEA Japan Inc., CA-1), and allowed tap water ad libitum. Fetuses were removed by cesarean section at 12-hour inter-

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vals from day 19 (9 AM) to day 21 (9 AM) of gestation. After removal, fetuses were individually weighed and examined for gross malformations. The skeleton was stained by the method of Dawson ('26). The number of ossified sacrococcygeal vertebral bodies, sternebrae, and proximal phalanges in the forepaw was counted. Ossification in the supraoccipital bone was staged according to the criteria previously developed by us (Ariyuki et al., '80b). The number of ossified proximal phalanges was counted in the right forepaw, because there was no significant difference between the right and left sides. For each growth index at each fetal age, the mean and standard deviations were calculated. These values were then plotted and a normal growth curve was produced.

As an example of low body weight and retarded ossification in teratological tests, data of fetal growth retardation induced by maternal fasting were analyzed. The data were obtained from Wistar rat (Kitayama LABES Co., Ltd., 12–14 weeks of age) fetuses whose mothers were fasted from days 14 (12 AM) to 21 (9 AM) of gestation (detection of sperm in vaginal smears = day 0) and dissected on day 21 (9 AM) of gestation. Water was available ad libitum even during the fasting period.

RESULTS

The standard growth in preterm rat fetuses

Body weight and ossification data at each fetal age are shown in Table 1. Growth curves with respect to each growth index are shown in Figure 1. Body weight and number of ossified sacrococcygeal vertebrae and ossification stage of supraoccipital bone increased approximately linearly as age advanced. The number of ossified sternebrae and proximal phalanges in the forepaw increased curvilinearly with increased fetal age; the growth curve for sternebrae was convex; that for proximal phalanges in the forepaw was concave. Seventy-eight percent of day 19.0 fetuses already had one or more ossified sternebrae, and almost all fetuses had six ossified sternebrae on day 21.0. Proximal phalanges in the forepaw began to ossify starting on day 20.0, and about 80% of day 21.0 fetuses had four ossified proximal phalanges (the maximum number to ossify during the fetal period in rats).

Analysis of the relationship between low body weight and retarded ossification in a teratological test

Table 2 shows body weight and ossification data obtained from the fetuses whose mothers

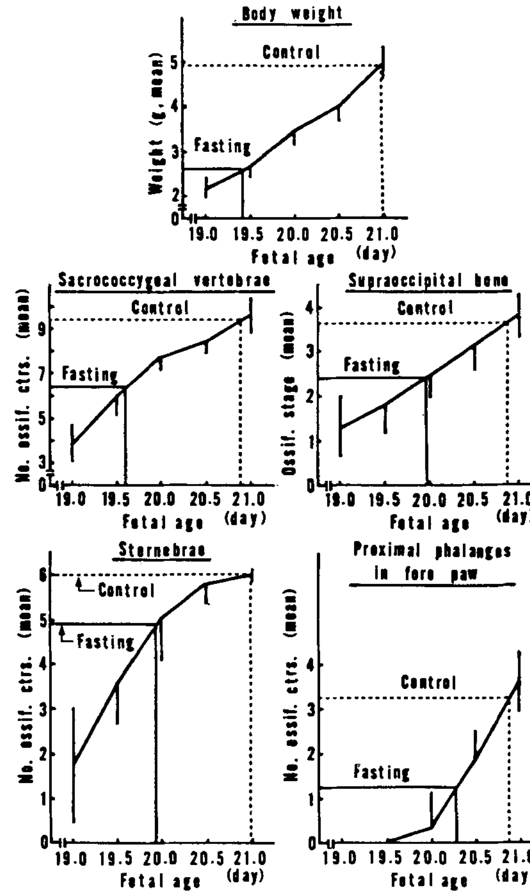

Fig. 1. Normal growth curves for body weight and ossification of the sacrococcygeal vertebrae, supraoccipital bone, sternebrae, and proximal phalanges in the forepaw in preterm rat fetuses (oblique lines) and plots of data of fasting experiment shown in Table 2. A vertical line shows the mean ± SD. Dotted and solid lines show experimental data of the control and fasting group, respectively.

were fasted. Mean body weight in the fasting group was lighter than that in the control group. Ossification in the sacrococcygeal vertebrae, supraoccipital bone, sternebrae, and proximal phalanges in the forepaw in the fasting group was also retarded as compared with that in the control group.

Figure 1 shows the way to express the degree of retardation in hours by utilizing the normal standard. Growth in the control was the same or least retarded as compared with the standard day 21.0 fetuses, within 6 hours in terms of the standard growth. Growth in the fasting group was retarded compared with that in the standard day 21.0 fetuses. Growth retardation in the fasting group, as shown in Figure 2, varied depending upon the indexes utilized. Body weight and ossification of the sacrococcygeal vertebrae were the most severely retarded, 30–36 hours in relation to the standard growth; ossification of the supraoccipital bone and the sternebrae was moderately retarded (approximately 24 hours); ossification of the proximal phalanges in the forepaw was least retarded (18 hours).

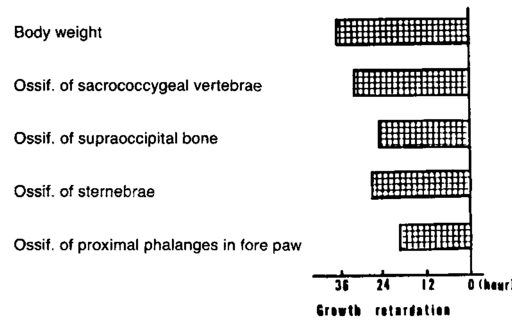

Fig. 2. Fetal growth retardation induced by maternal fasting (days 14–21 of gestation) in rats. The retardation (hatched areas) is expressed in hours of normal development. The degree of retardation in the fetuses was different among growth indexes.

DISCUSSION

Low body weight and retarded ossification in mouse and rat fetuses have been discussed individually in reports of teratological investigations, but analysis of the relationship between low body weight and retarded ossification has not been well documented. We previously discussed methods for comparative evaluation of low body weight and retarded ossification of the coccygeal vertebrae induced by teratological agents (Ariyuki et al., '80a). In the present study, we analyzed the relationship between body weight and ossification of bones other than the coccygeal vertebrae. To establish a common scale for the analysis, normal growth of preterm rat fetuses was examined. Body weight and number of ossified sacrococcygeal vertebrae showed an approximately normal distribution, whereas ossification indexes in the bones other than the sacrococcygeal vertebrae, such as ossification stages of the supraoccipital bone, deviated from a normal distribution in term fetuses. Analysis of the relationship between normally distributed measurements of growth indexes is relatively easy if one utilizes the mean and standard deviation (Ariyuki et al., '80a). However, this method cannot be applied to measurements with non-normal distribution. To overcome this difficulty, we first established normal growth curves for various ossification indexes and body weight in preterm rat fetuses. Growth retardation in a teratological experiment was then expressed on a common "hour" scale by utilizing these standard curves.

The use of means as representative values for measurements with non-normal distribution may be refutable. We examined the use of other representative values such as modes and medians, but found that the means were the most meaningful representative values in the present experiment.

Developmental velocity seems to vary depending upon the growth indexes utilized: Body weight and ossification of the sacrococcygeal vertebrae and supraoccipital bone developed steadily; ossification of the sternebrae developed more slowly near term; but ossification of the proximal phalanges in the forepaw developed rapidly near term.

By utilizing the standard shown in Figure 1, growth retardation in rat fetuses obtained from teratological tests can be expressed in hours of normal development. It is thus possible to compare the degree of retardation among several growth indexes by a common scale in hours.

This method was applied to experimental data of fetal growth retardation induced by maternal fasting, and the degree of retardation was found to differ among indexes. Aliverti et al. ('79) described examples of inconsistency of retardation in fetal body weight with that in ossification, and stressed that the stage of fetal skeletal development could provide a reliable index. Our results support their view. The fact that there are considerable differences even among indexes of skeletal development indicates the importance of observation on several skeletal regions.

The normal growth curve may be affected by various genetic and environmental factors. It should be noted that our developmental data cannot be used as "normal" in other laboratories. The data reported here were obtained from one experiment in one group of rats. It is expected that different patterns of growth retardation may be induced by different agents. Comparative studies on growth retardation induced by different fasting periods and other agents are in progress in our laboratory.

TABLE 1. Body weight and ossification in normal rat fetuses

Fetal age (day)	Number of mothers	Number of fetuses	Litter size Mean ± SD	Body weight (g) Mean ± SD	Number of ossified sacro-coccygeal vertebrae Mean ± SD	Supraoccipital: % Fetuses with any stage[1] of ossification					
						0	1	2	3	4	5
19.0	20	252	12.6 ± 3.7	2.180 ± 0.223	3.65 ± 0.87	10	54	35	1	0	0
19.5	20	283	14.2 ± 2.4	2.685 ± 0.270	5.81 ± 0.91	1	32	57	10	0	0
20.0	20	266	13.3 ± 3.2	3.455 ± 0.331	7.50 ± 0.56	0	1	52	47	0	0
20.5	20	291	14.6 ± 2.7	4.008 ± 0.340	8.18 ± 0.51	0	0	11	66	23	0
21.0	20	273	13.7 ± 3.3	4.973 ± 0.380	9.48 ± 0.81	0	0	0	27	69	4

[1]According to the criteria of Ariyuki et al. ('80b).

TABLE 2. Low body weight and retarded ossification in 21-day-old rat fetuses induced by maternal fasting

Group	Number of mothers	Number of fetuses	Litter size Mean ± SD	Body weight (g) Mean ± SD	Number of ossified sacro-coccygeal vertebrae Mean ± SD	Supraoccipital: % Fetuses with any stage[1] of ossification					
						0	1	2	3	4	5
Control[2]	10	144	14.4 ± 1.6	4.19 ± 0.30	9.3 ± 0.7	0	0	0	36	59	5
Fasting[3]	10	144	14.4 ± 1.9	2.63 ± 0.43	6.3 ± 1.0	1	5	49	42	3	0

[1]According to the criteria of Ariyuki et al. ('80b).
[2]Nontreated control.
[3]Fasting from day 14 to 21 of gestation.

Fortunately, no malformations were observed in the bones analyzed in the fasting experiment. If the bones are malformed, the number or stage of ossified bones may not be used as an index of state of ossification.

Hannah and Moore ('71) observed that retardation of ossification of the fetal axial skeleton was induced by maternal fasting on days 9 and 10 of gestation in rats. Maternal dietary restriction also induced low fetal body weight and retardation of ossification in rats (Berg, '65). However, these two reports did not deal with the relationship between the low body weight and the retarded ossification. The application of the present method to these types of experiments may yield useful information for understanding the nature of growth retardation.

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TABLE 1. (continued)

bone	Sternebrae								Proximal phalanges in forepaw					
Mean stage[1] of ossif. ctrs. with SD	% Fetuses with any number of ossification 0	1	2	3	4	5	6	Mean number of ossif. ctrs. with SD	% Fetuses with any number of ossification 0	1	2	3	4	Mean number of ossif. ctrs. with SD
1.28 ± 0.66	22	24	21	26	7	0	0	1.71 ± 1.26	100	0	0	0	0	0
1.76 ± 0.64	1	1	3	38	47	9	1	3.57 ± 0.88	100	0	0	0	0	0
2.45 ± 0.53	0	0	0	6	23	31	40	5.04 ± 0.96	75	10	15	0	0	0.39 ± 0.73
3.12 ± 0.57	0	0	0	0	2	14	84	5.81 ± 0.47	7	2	88	1	2	1.91 ± 0.61
3.77 ± 0.52	0	0	0	0	0	3	97	5.97 ± 0.16	0	0	12	9	79	3.65 ± 0.72

TABLE 2. (continued)

bone	Sternebrae								Proximal phalanges in forepaw					
Mean stage[1] of ossif. ctrs. with SD	% Fetuses with any number of ossification 0	1	2	3	4	5	6	Mean number of ossif. ctrs. with SD	% Fetuses with any number of ossification 0	1	2	3	4	Mean number of ossif. ctrs.with SD
3.68 ± 0.58	0	0	0	0	0	1	99	6.00 ± 0.08	0	0	31	15	54	3.22 ± 0.90
2.41 ± 0.67	0	0	1	6	23	43	27	4.90 ± 0.90	35	10	55	0	0	1.27 ± 0.94

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