Figs. 1 – 6 – forelimbs. (1) – normal forelimb of an SHR/SHR, +/+ control foetus. This type of limb can be seen in all +/+ and +/*Lx* control foetuses regardless of the genetic background. (2) – forelimb of an SHR/BN, +/+ foetus following RA treatment, syndactyly of digits 2 and 3, shortened zeugopodium, ossification of ulna is lacking. (3) – oligodactylous forelimb of an SHR/BN, +/+ foetus after RA treatment with shortening of zeugopodium bones. A similar picture can be seen in all +/+ and +/*Lx* foetuses following RA treatment. Tuberositas deltoidea is missing in treated foetuses regardless of the foetus genotype. (4) – forelimb of an SHR/BXH2, *Lx/Lx* control foetus. Five triphalangeal digits and metacarpals due to the *Lx/Lx* genotype and BN modifying genes. A similar picture can be seen in about 71% of SHR/BXH2, 17% of SHR/BN and 92% of BN/BN, *Lx/Lx* control foetuses, but in none of the SHR/SHR, *Lx/Lx* control foetuses. (5) – forelimb of an SHR/BXH2, *Lx/Lx* foetus following RA treatment. Five triphalangeal digits, 3rd metacarpal missing, ulna and radius shortened. (6) – oligodactylous forelimb of an SHR/BN, *Lx/Lx* foetus following RA treatment with four triphalangeal digits and underdeveloped and shortened zeugopodium bones without any sign of ossification. This type of digital defect is most frequent in BN/BN, *Lx/Lx*, and only in few cases in SHR/BXH2, *Lx/Lx* foetuses.
Figs. 7 – 16 – hind limbs. (7) – normal hind limb of an SHR/SHR, +/+ control foetus. This type of limb can be seen in all +/+ foetuses regardless of the genetic background and in +/*Lx* foetuses with SHR/SHR, SHR/BN and SHR/BXH2 genetic backgrounds. (8) – oligodactylous hind limb of a BXH2/BXH2, *Lx/Lx* control foetus with four triphalangeal toes and malformed leg bones, which are of the same width. (9) – polydactylous hind limb of a BN/BN, *Lx/Lx* control foetus with six triphalangeal toes and afflicted zeugopodium. (10) – polydactylous hind limb of an SHR/BXH2, +/*Lx* foetus following RA treatment with six triphalangeal toes and six ossified metatarsals. (11) – oligodactylous hind limb of an SHR/BN, +/*Lx* foetus following RA treatment with four triphalangeal toes. Zeugopodium is not afflicted after RA treatment in any group of +/*Lx* foetuses. (12) – polydactylous hind limb of an SHR/SHR, *Lx/Lx* control foetus with six triphalangeal toes, six metatarsals and unafflicted zeugopodium bones. (13) – polydactylous hind limb of an SHR/BXH2, *Lx/Lx* control foetus with five triphalangeal toes, five metatarsals and moderately afflicted zeugopodium (thickened fibula and narrowed tibia). (14) – oligodactylous hind limb of an SHR/BXH2, *Lx/Lx* foetus following RA treatment with three toes and severe affliction of zeugopodium, where only one thick shortened bone instead of tibia and fibula is present. (15) – oligodactylous hind limb of a BN/BN, *Lx/Lx* foetus following RA treatment with two toes and severe affliction of zeugopodium bones, from which one bone is missing. (16) – extreme reduction of hind limb in a BN/BN, *Lx/Lx* foetus following RA treatment. Only cartilaginous remnants of the whole limb with one toe can be seen, ossification is only in the pelvis.

Lx genotype of foetuses: +/Lx

Both pairs of limbs were affected by the RA influence on heterozygous progeny. The fore- and hind limb autopodium affliction is summarized in Table 3.

The frequency of central OD or SD in forelimbs in groups with SHR/SHR, SHR/BN and SHR/BXH2 genetic backgrounds was approximately 26, 93 and 29% of limbs, respectively. In foetuses with the SHR/BN genetic background the frequency of these defects was significantly higher as compared with the two remaining groups.

Hind limbs of RA-treated +/*Lx* foetuses were afflicted on the one hand with preaxial PD (triphalangy of hallux or 6 toes, Fig. 10), on the other hand with preaxial OD (4 toes, hallux missing or rudimentary, without metatarsus, Fig. 11). The triphalangy of hallux represented the prevailing defect of hind foot in all experimental groups of +/*Lx* foetuses.

The lowest frequency of toe defects was found in foetuses with the SHR/SHR genetic background (only 2 cases of triphalangeal hallux, approximately 1% of limbs affected). In the SHR/BN group the frequency was significantly higher (nearly 39%) and in the SHR/BXH2, *Lx/Lx* group significantly highest (62% of limbs afflicted).

Hind limb zeugopodium was unaffected in all RA-treated +/*Lx* foetuses.

Lx genotype of foetuses: Lx/Lx

Both autopodium and zeugopodium in both pairs of limbs were affected by RA administration to females carrying homozygous *Lx/Lx* progeny. Table 4 shows the effect of RA on forelimb autopodium.

In addition to central OD or SD like in +/+ and +/*Lx* groups, preaxial forelimb affliction appeared in *Lx/Lx* foetuses. Forelimbs with 4 triphalangeal digits occurred (Fig. 6), resulting from preaxial OD or from central OD and pollex triphalangy (Fig. 5). On the other hand, limbs with preaxial PD (5 triphalangeal digits or 6 digits) appeared.

In all *Lx/Lx* groups except for BN/BN *Lx/Lx* foetuses, RA treatment increased the frequency of PD compared to the controls. On the contrary, in the BN/BN, *Lx/Lx* group the frequency of PD limbs was decreased following RA treatment from 92.4% to 27.6%, while the frequency of OD limbs was the significantly highest from all *Lx/Lx* groups (69%). The significantly highest frequency of PD limbs (91.7%) and the lowest frequency of OD limbs (3.8%) after RA treatment was found in SHR/BXH2, *Lx/Lx* foetuses.

In hind feet the number of toes was reduced in all *Lx/Lx* foetuses following RA treatment (Table 5). The reduction started preaxially (in limbs with 4 triphalangeal toes the hallux was missing). In limbs with 4 and 3 toes, a rudimentary hallux was present in some cases.

In SHR/SHR, *Lx/Lx* foetuses, which have 6–7 toes without teratogenic influence (Fig. 12), the number of

Table 1. Embryotoxic RA influence in 11 groups of progeny differing in the genotype

Genotype of foetuses		Dose mg/kg	L.F.[a]	Res.[b]	% of res.	Mean body weight	Number of litters
Lx locus	background						
+/+	SHR/SHR	0	50	3	4.8	4.0 ± 0.3	5
		100	87	7	7.4	3.6 ± 0.4[c]	9
	SHR/BN	0	42	1	2.3	4.7 ± 0.3	4
		100	83	0	0.0	3.9 ± 0.5[c]	9
	BN/BN	0	16	3	15.8	3.9 ± 0.4	4
		100	2	27	93.1[c]	3.5 ± 0.6	7
+/*Lx*	SHR/SHR	0	56	2	3.4	4.1 ± 0.4	6
		100	94	3	3.1	3.6 ± 0.5[c]	10
	SHR/BN	0	67	3	4.3	4.7 ± 0.4	7
		100	93	3	3.1	3.9 ± 0.6[c]	10
	SHR/BXH2	0	60	2	3.2	4.3 ± 0.4	6
		100	98	8	7.6	3.8 ± 0.7[c]	11
Lx/*Lx*	SHR/SHR	0	52	2	3.7	4.1 ± 0.4	6
		100	58	5	7.9	3.2 ± 0.5[c]	7
	SHR/BN	0	42	3	6.7	4.7 ± 0.4	4
		100	61	5	7.6	3.9 ± 0.7[c]	7
	SHR/BXH2	0	70	3	4.1	4.4 ± 0.6	7
		100	66	12	15.4[d]	3.4 ± 0.7[c]	8
	BXH2/BXH 2	0	48	2	4.0	4.2 ± 0.4	7
		100	3	40	93.0[c]	3.7 ± 0.2[d]	6
	BN/BN	0	33	5	13.2	4.8 ± 0.2	8
		100	23	37	61.7[c]	3.2 ± 0.4[c]	12

[a]L.F. – living foetuses
[b]Res. – resorptions and dead foetuses
[c,d] the experimental groups differ from controls at significant levels: c – $P < 0.001$, d – $P < 0.05$

Table 2. RA influence on fore- and hind limbs in +/+ progeny

Genetic background	Dose mg/kg	Forelimbs ND[a]	OD[b]	%OD	Hind limbs ND[a]	OD[b]	%OD	Number of limbs	foetuses	litters
SHR/SHR	0	100	0	0.0	100	0	0.0	100	50	5
	100	154	20	11.5[c]	174	0	0.0	174	87	9
SHR/BN	0	84	0	0.0	84	0	0.0	84	42	4
	100	13	153	92.2[d]	166	0	0.0	166	83	9

[a]ND – number of normodactylous limbs
[b]OD – number of limbs with central oligodactyly or syndactyly
[c,d] the values c and d differ at a significant level, $P < 0.001$

toes was reduced to 5, 4 or 3. The prevailing number of toes was 4 (64 limbs, 55 %). In SHR/BN, *Lx*/*Lx* foetuses, the original number of 6–7 toes was decreased to 5, 4, 3 or 2 triphalangeal toes. Limbs with 3 toes prevailed (84 limbs, 69 %). In SHR/BXH2, *Lx*/*Lx* foetuses, where limbs with 5 triphalangeal toes are prevailing in controls (Fig. 13), the reduction to 4, 3, 2 or 1 triphalangeal toe was found following RA treatment (Fig. 14). A limb with 3 toes was the most common type (103 cases, 78%). Limbs with 2 and 1 toe appeared with equal frequency (nearly 10 % each of them). In BN/BN, *Lx*/*Lx* foetuses, which have 6 or 7 toes without treatment (Fig. 9), the number of toes was reduced to 3, 2 or 1 (Figs. 15, 16). Limbs with 2 and 1 toe prevailed, appearing with the same frequency (approximately 47% each of them).

In comparison with controls, hind limb zeugopodium was bilaterally affected in all experimental *Lx*/*Lx* foetuses. After staining it was ascertained that in each group the affliction involved different types of zeugopodium reduction, namely, narrowing of tibia, shortening and malformation of tibia and fibula, joining of both bones, or one bone entirely missing. The ossification was often diminished or missing, the bones being represented only by cartilage.