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Development of rib-vertebrae: a new mutation in the house mouse with accessory caudal duplications*

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Summary. The new recessive mutation rib-vertebrae (rv) causes fusions of lower ribs and malformations of vertebrae, which results from disturbed somite arrangement. In addition, duplications of the caudal neural tube and sometimes unilateral suppression of kidney formation can be observed. The new mutation is compared with the six already known mutations in mice with "Wirbel-Rippen-Syndrome" and with a similar syndrome in man. From the various effects of the rv-gene observed, it is suggested that the gene causes abnormal inner and outer surface formation, producing manifold secondary effects.

Key words: Malformation – Skeleton – Neural tube – Mouse

Introduction

The developmental analysis of mutants in inbred mice offers the possibility to study the pathogenesis of malformations from the very beginning. This paper describes a new gene producing the "Wirbel-Rippen-Syndrom" (Theiler 1968), in addition to duplications of the caudal neural tube.

The mutation is an experiment produced by nature which would be impossible to perform by technical means. It gives insight into the interdependence of embryonic organs during development, as for instance notochord, somites, hind gut and neural tube. It is interesting to note that in the mutation "rib-vertebrae" (rv) described here, somites may develop sometimes without notochord. A similar situation has been observed in the mutation "truncate" (Theiler 1959). It is concluded that the notochord seems not to be necessary for the development of somites. However, it is responsible for their bilateral arrangement: in the mutation "truncate", the somites fuse across the midline whenever the separating notochord is lacking. In the tail bud of some "rv"-embryos lacking a notochord, the somites are irregularly arranged. The "rv"-gene also shows that the floor plate of the spinal cord is induced by the notochord.

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Materials and methods

This mutation was found in inbred strain C57BL/J at the Jackson Laboratory and by repeated backcrosses was transferred onto strain C57BL/6J. Five skeletons of newborns were cleared and stained in Alizarin red and examined with a dissecting scope. Skeletal preparations from 19 homozygous and 19 normal littermates 4 to 5 weeks of age were examined for skeletal abnormalities. Forty five homozygous and 45 normal littermate controls 4 to 5 weeks of age were examined for cystic or missing kidneys. Three 13-day-fetuses were examined with a dissecting scope and 46 embryos from 11 to 13 days of gestation were fixed in Vandegrifts (modified Bouin) solution, serially sectioned and stained with hematoxylin and eosin.

Results

1. Inheritance

The results of genetic crosses made to determine the inheritance of rib-vertebrae (*rv*) are summarized in Table 2. The data are consistent with the inheritance of *rv* as a recessive autosomal mutation with good penetrance. Negative linkage results obtained through the courtesy of Eva M. Eicher and Jan Southard are summarized in Table 3. Test for allelism with vestigial-tail (*vt*) and fleck (*Fk*) were negative.

Table 1. Malformations in clearance preparations of 4 newborn

Specimen no.	Cervical vertebrae	Ribs	Lumbar vert.
1	Irregular neural arches Fusion Atlas-Axis unilateral	13 ribs. Left cervical rib. Right lumbar rib. fusion 6/7, 9/10, 11/12	Irregular
2	Left arch of atlas bifurcated	12 ribs	Irregular
3	Left arch of atlas enlarged	12 ribs left; 14 ribs right. Fusion 11/12 left; fusion 13/14 right	Irregular
4	Fusion arches 3/4 right. Supraoccipitale doubled	13 ribs	Irregular

Table 2. Crosses made to determine the inheritance of *rv*

Mating	Offspring				Total	*rv*
	Normal ♀	Normal ♂	*rv* ♀	*rv* ♂		(%)
rv/*rv* × *rv*/+	29	25	30	15	99	45.45
+/+ × *rv*/*rv*	151	129	0	0	280	0
+/*rv* × +/*rv*	262	282	55	49	648	16.5
rv/*rv* × *rv*/*rv*	0	0	32	41	73	100

Table 3. Negative linkage results

Chromosome	Gene name	Gene symbol
1	Delayed-splotch	*fx*
	Leaden	*ln*
	Delayed-splotch	Sp^{d}
2	Ragged	*Ra*
	Lethargic	*lh*
3	Varitint	*Va*
	Carbonic anhydrase-1	*car*
4	Depilated	*dep*
5	Hammer toe	*Hm*
	Viable spotting	W^{v}
6	Sightless	*Sig*
	White	mi^{ru}
7	Pink eye	*p*
	Taupe	*tp*
	Chinchilla	c^{ch}
	Shaker-1	*sh-1*
	Frezzey	*fr*
8	Sombre	E^{so}
9	Rough coat	*rc*
	Scant hair	*sch*
10	Steel	*Sl*
11	Waved-2	*wa-2*
	Shaker-2	*sh-2*
12	Hyperthyroid	*hyt*
13	Furless	*fs*
	Extra toes	*xt*
14	Piebald	*s*
15	Underwhite	*us*
	Hairy ears	*Eh*
18	Twirler	*Tw*
	Shaker with syndactylism	*sy*

Table 4. Forty-five animals checked for abnormal kidneys at weaning (4 weeks)

Missing		Bilateral
L	R	
8	8	0

2. Appearance of the adult mutant

Affected adults have reduced body length and distal tail kinks of various degrees of severity. Some animals classified as normal by tail kink criteria were found to be homozygous when their skeletons were cleared and stained. The appendicular skeleton appeared normal while affected animals of both sexes breed, females often are poor breeders.

3. Skeletal malformations of newborns

Fusions of ribs are usually located in the lower half of the thoracic basket (Table 1) and are often asymmetric. Often only the heads of two successive ribs are united or, more rarely, the peripheral parts of the ribs are fused (Fig. 1 A). Generally, the neural arches of the same region are irregularly formed and often fused (Fig. 1 B). Usually the neural arches and occasionally the transverse processes in the cervical region are asymmetrically fused and a cervical rib may be present. The vertebral bodies, especially in the lower thoracic and the lumbar region, are irregularly arranged (Fig. 1 A). However, the tail vertebrae are seldom affected.

4. Embryos of 13 days

Macroscopic examination of five embryos reveals an unusual shape of the tail in three specimens (Fig. 2). The tail tip is enlarged and seemed to be budding off with several branches (Fig. 3). The somites appear to be unevenly distributed at the tail tip, but in the upper, undisturbed tail they are regularly arranged and shaped. The remnants of the neural tube, visible in the terminal part, do not show any duplications in these specimens.

Histological examination of the *tails* shows that the "buds" arising at the tail tip are filled with mesenchyme

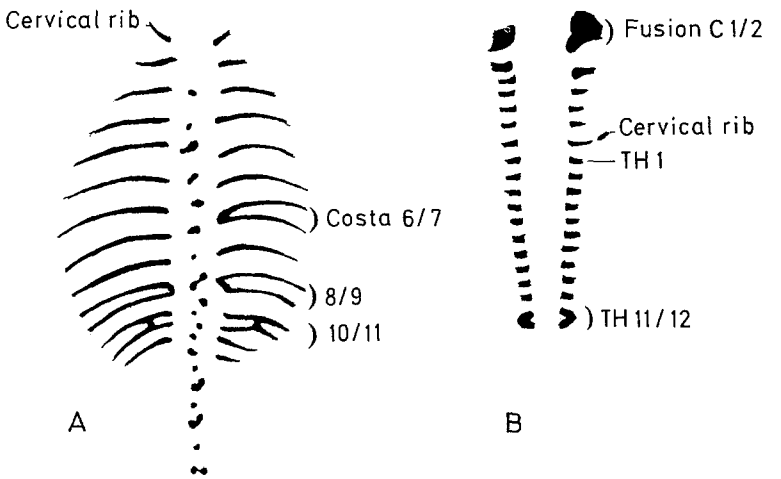


Fig. 1 A, B. Typical skeletal malformations in rv/rv. **A** Ventral view, newborn, clearance preparations. Irregular vertebral bodies and rib fusions. **B** Dorsal view. Irregular neural arches

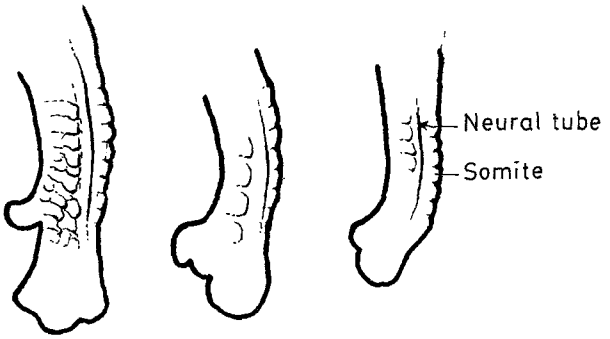


Fig. 2. Various types of sprouting tails of 13 days embryos. Some irregularities of somite arrangement are visible in left specimen

only. Neither notochord nor neural tube penetrate into the new branches. Near the tail tip the neural tube is already degenerating in some specimens. The notochord, which ends shortly before the terminal part of the tail, shows a massive pycnosis. While the upper part of the tail does not show any irregularity at the base of the tail, two partially fused somites were observed in one specimen.

Vertebral column. Irregularities of the cervical and thoraco-lumbar regions appear in all homozygous specimens examined. In the cervical part, fusions of the vertebral bodies are encountered (Fig. 4). Fusions of the anterior arch of the Atlas with the Axis are common. The transverse processes may be irregularly spaced; e.g., in embryo no. 3 the distance between the 2nd and 3rd transverse processes is unilaterally widened and a small accessory ganglion is added to the ganglion C3.

In the lower thoracic and in the lumbar region, some vertebral bodies are reduced, others enlarged and the segments of the notochord are irregularly spaced (Fig. 6). The articular processes are often fused. In embryo no. 1, articular processes of vertebrae th 8–11 form one single longitudinal bar of cartilage, and the heads of ribs 11–12 and 8–10 are fused too. Similar malformations of the skeleton are encountered in all embryos of this age. However, two out of five embryos do not show any branching of the tail tip.

5. *Embryos of 12 days*

Eleven embryos of this age group were sectioned. All exhibited similar malformations of the developing skeleton. In frontal sections, the condensations of the primitive intervertebral discs often appear distorted (Fig. 7), and the developing vertebral bodies take an irregular outer shape. This anomaly is preferentially localized in the cervical and thoracolumbar regions. The main part of the tail develops normally, but the tail tip of most embryos exhibits a peculiar outer contour, having irregular and various sized projections (Fig. 10). These projections are filled with mesenchyme only. The adjacing end of the notochord is sometimes enlarged, as is the end of the hind gut. Both seem to be sprouting, but they never enter the projections of the tail.

The terminal neural tube is normal in some embryos. In contrast, in 3 out of 11 embryos of this age group the neural tube is doubled or sprouting and in 2 embryos it is in a chaotic state (Fig. 8). Starting at the lumbar level, the anomaly is first visible by a ventrolaterally displaced group of matrix cells, beneath the anlage of the motor column. The outer shape of the neural tube is initially not altered. More caudally, a second displaced group of matrix cells appears, surrounding a small accessory lumen, which does not communicate with the central canal of the neural tube. In adjacent sections, the neural tube is bulging out at the anomalous region, and a small secondary tube arises, which sprouts in a disorganized manner and gives rise to many accessory neural tubes (Fig. 8). The larger tubes in the lumbo-sacral region often are accompanied by irregular masses of spinal ganglia.

The notochord is generally not doubled, and can easily be located by the site of the thin floor plate of the neural tube which it has induced (Fig. 8). The chaotically sprouting neural tissue fills more and more of the tail towards its tip. The tail, in these cases, is much shortened and may be irregularly bifurcated, the branches sometimes being filled with neural tissue. In less severe cases, the less sprouting slender neural tubes are accompanied by irregularly shaped, occasionally fused somites.

6. *Embryos of $11^1/_2$ days*

Sixteen embryos of this age group were serially sectioned, and in 3 of them the posterior neural tube was disorganized, like the one described in the day 12 group. In one embryo, the neural tube appeared to be normal, as far as it had formed in the tail while the hind gut was slightly enlarged, forming several small bulges, and the tip of the adjoining notochord was club-shaped and fusing with the hind gut blastema. Exactly at the level of the starting anomalies of gut and notochord, the outer shape of the tail started to bulge irregularly. The terminal somites, in the prominent bulge, were not accompanied by notochordal process.

In the sacral region, where gut, notochord and neural tube were perfectly normal, some irregularly fused somites could be observed. In spite of a perfectly straight notochord and neural tube in the terminal part of the tail, near the tip, fused somites may sometimes be observed (Fig. 11).

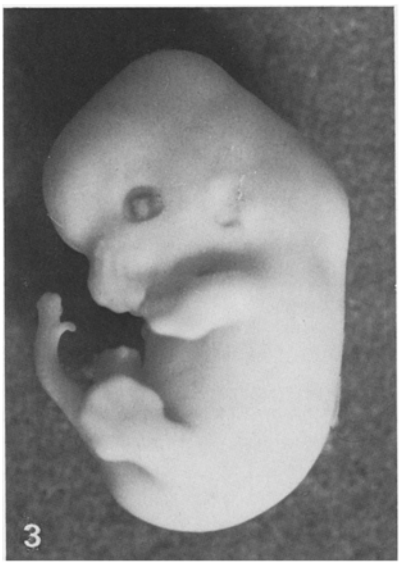
7. *Embryos of 11 days*

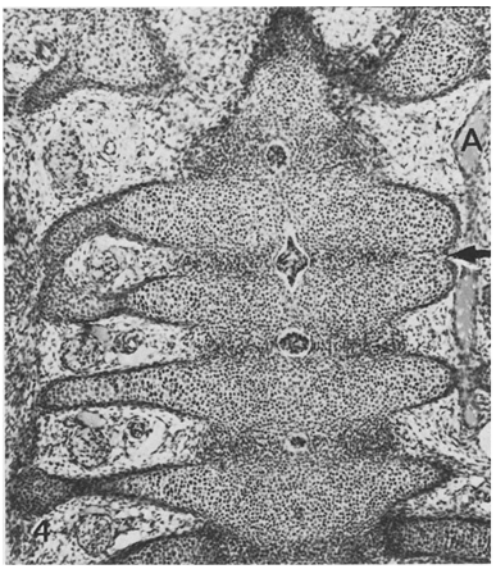
The tail tips of 6 out of 12 embryos of this age group already showed the characteristic "budding" of the tips. The neural tubes in these specimens were normal, but the terminal hind guts were sprouting. Only one specimen showed the enlarged terminal notochord.

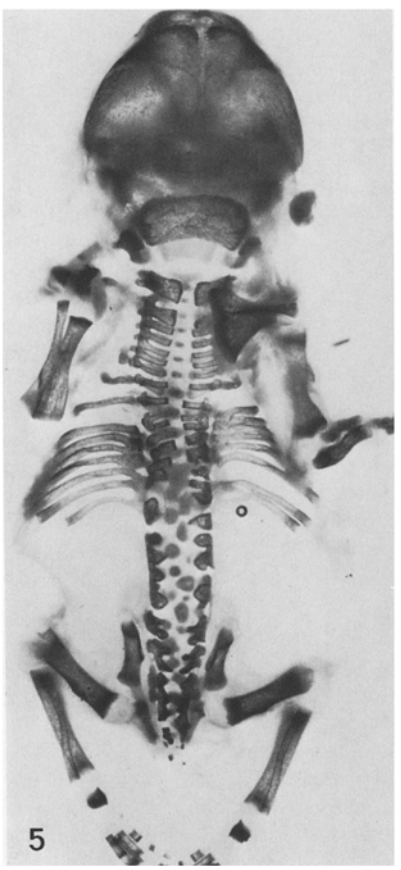
At this stage the anlage of the vertebral column already shows irregular condensations of mesenchyme. In one embryo, an abnormal fusion of the anlage of the transverse processes of vertebrae C3–C4 was seen (Fig. 9), and the intercalated intersegmental vessel was nearly completely reduced.

Discussion

The recessive gene *rv* affects the axial skeleton, the caudal neural tube and sometimes the development of the kidneys. It somewhat resembles the dominant mutation Fused (Theiler and Gluecksohn-Waelsch 1956). However, fused animals are easily recognized by their kinky tails, a symptom which is frequently lacking in *rv*-homozygotes. Many *rv*/*rv* animals show only rib fusions or deformation of the lower thoracic vertebrae. These malformations are caused







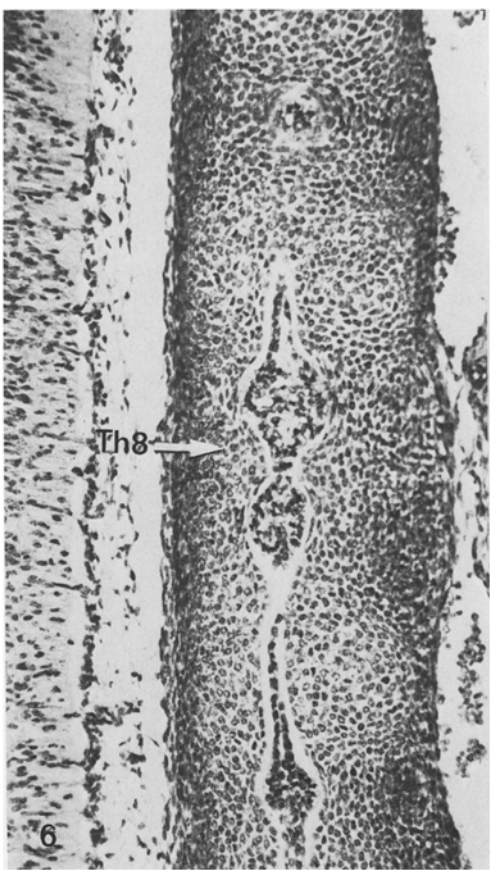


Fig. 3. rv/rv, 13 days (9 mm). Budding tail

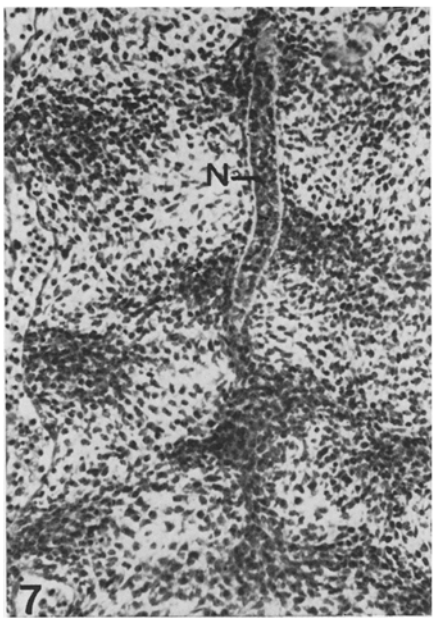
Fig. 4. rv/rv, 13 days. Cervical fusion C3/4. Fused bodies (*arrow*, right) and fused transverse processes (left). *A*, arteria vertebralis. × 50

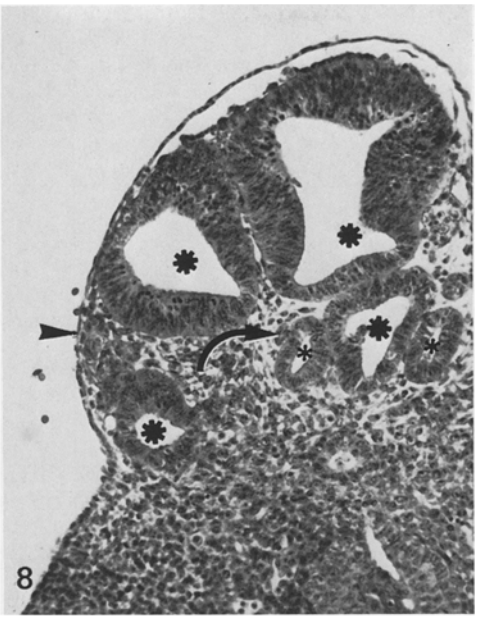
Fig. 5. rv/rv, newborn, clearance preparation, dorsal view. Skeleton severely malformed

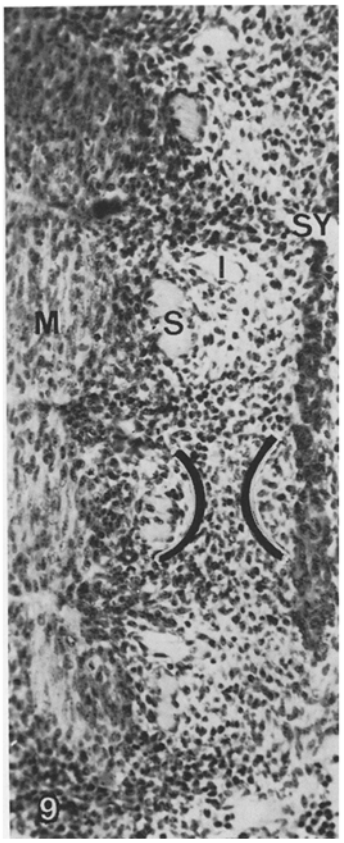
Fig. 6. rv/rv, 13 days. Sagittal section of thoracic vertebrae 7–9. Body of Th 8 reduced (*arrow*). × 120

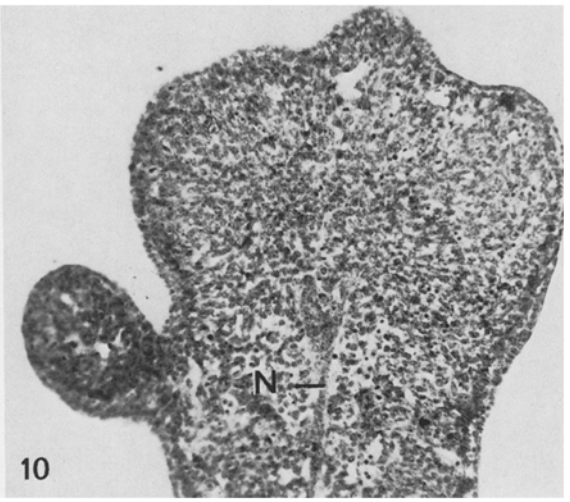
by early somite deformities. In Fig. 11, the fusion of two successive somites can be seen. This fusion probably results in the vertebrae deformities observed in later stages of development. A combination of rib fusions and vertebral malformations which results from disturbed somite development at the thoracic level has been called "Wirbel-Rippen-Syndrom" (Theiler 1968).

In human, a similar combination was observed by Gassner (1982) and described as "kosto vertebrale Dysplasie". He examined eight subjects with this malformation, in four interrelated families. The pedigree of kindred over eight generations was consistent with autosomal recessive inheritance. All showed analogous generalized malformations of vertebrae and ribs. At birth, the children were already characterized by short bodies and "missing neck". No decrease in life expectancy or any other defects have been observed. In our opinion, it is likely that the human syndrome is also caused by somite malformations. However, it cannot be postulated that the genes are homologous: up to now, there are six different genes described which cause in mice costovertebral dysplasia: Crooked tail (Theiler 1956), fused (Theiler 1956), rib fusions (Theiler and Stevens 1960), pudgy (Grüneberg 1961), rachiterata (Theiler et al. 1974), and malformed vertebrae (Theiler et al.









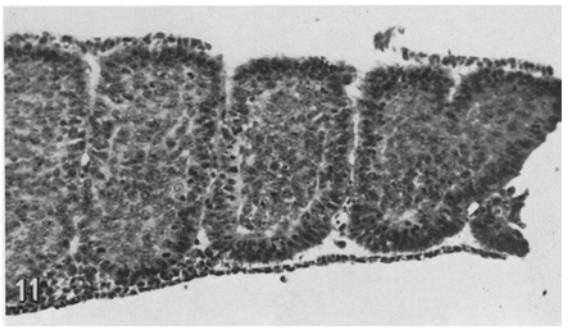


Fig. 7. rv/rv, 12 days. Frontal section of irregular cervical blastema. *N*, Notochord. ×120

Fig. 8. rv/rv, 12 days. Cross section of sacral region. The notochord (*arrow*) induces the thin floor plates in the two neural tubes above. *Arrowhead* spinal ganglion. *Asterisks* accessory neural tubes. ×120

Fig. 9. rv/rv, 11 days, frontal section. *Outlined:* fusion of mesenchymous transverse processes C3/4. *M* myotom; *S* spinal nerve; *I* intersegmental vessel; *Sy* sympathetic trunc. ×120

Fig. 10. rv/rv, 12 days. Sprouting tail tip. *N* notochord. ×120

Fig. 11. rv/rv, $11^1/_2$ days. Tail somites, sagittal section. On the right, fusion of 2 somites. ×120

1975). All these genes and probably the human gene, too, have an effect on somite formation, acting very early in development.

The rv-neural tube sometimes produces secondary lumina in the posterior portion, and secondary accessory neural tubes arise. As many as six accessory neural tubes have been observed in rv homozygotes (Fig. 8). The accessory neural tubes are not induced by accessory notochords. Except for some slight deviations, the notochord is of normal shape.

The gene fused also produces accessory neural tubes, without duplications of the notochord. However, the fused – neural tubes always remain in the sagittal plane and never form a group of several tubes as observed in rv. In the latter, the caudal neural tube sometimes sprouts in a chaotic manner. The tail tips sometimes (Fig. 10) sprout and the newly formed branches are filled with only mesenchyme. In rare cases the hind gut may also be doubled. The accessory gut does not penetrate the accessory mesenchyme of the sprouting tail. It appears that the irregularity of the tail gut is not the cause of the sprouting tip.

Unilateral suppression of kidney formation in some

fused animals (Theiler and Gluecksohn-Waelsch 1956) is a result of the failure of the ureteric bud to grow and reach the metanephric blastema. It is likely that in rv/rv animals abnormal sprouting of the ureteric bud disturbs the development of the kidneys, causing cystic or sometimes lacking kidneys.

From the various effects of the *rv* gene observed, it is probable that the gene *rv* causes abnormal inner and outer surface formation (somites, neural tube, tail tip, ureteric bud, tail gut), producing manifold secondary effects One may speculate that the surface is influenced by a variation of the adhesiveness of the cells forming the surface.

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