TRISOMY IN THE NEWBORN

EXPERIMENTS ON MICE HETEROZYGOUS FOR THE T_6BnR TRANSLOCATION

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The T_6BnR translocation in the heterozygous state does not cause any significant increase in embryonic mortality but gives rise to trisomy in 10% of fetuses at the 16th-18th day of development. All 19 embryos with trisomy were considerably retarded in development and 9 of them had developed mental anomalies: 6 had malformations of the brain of the exencephaly and hydrocephalus types, 1 had a cleft hard palate, 2 had extensive hemorrhages and edema of the subcutaneous cellular tissue. Underdevelopment of the vascular network and villi of the yolk sac was found in all the trisomics. It is postulated that mice with the T_6(9, 14)BnR translocation can be used as a convenient biological model for studying inherited anomalies of the central nervous system and, in particular, exencephaly.

Because of the high frequency with which the chromosomes fail to separate in meiosis, mice heterozygous for the Robertson translocations (centric fusion of the autosomes) are a convenient model with which to study the effect of numerical chromosomal aberrations on embryonic development.

Trisomy in mice with centric fusion T_1IEM (8th-17th pairs) and T_1ALD (6th-15th pairs) leads to characteristic changes in morphogenesis and is manifested as dominant lethals in the period of active organogenesis, the 8th-12th day of pregnancy [1, 2]. Meanwhile the additional 19th pair of autosomes in mice heterozygous for the Robertson translocation T_{163}H (9th-19th pairs) and T_1Wh (5th-19th pairs) in some cases is compatible with completion of the intrauterine period. Such trisomics, however, are retarded in development and they often have anomalies of the facial skull (cleft palate) and they die soon after birth.

This paper describes another trisomy in mice compatible with completion of the antenatal period of development. This trisomy is found in the progeny of mice heterozygous for the T_6BnR translocation – one of 7 translocations of the Robertson type present in the karyotype of the tobacco mouse Mus poschiavinus.* These mice are genetically similar to the ordinary laboratory mouse Mus musculus, they can mate together, but they have only 26 chromosomes in their diploid set, including 7 pairs of metacentrics [7]. The fundamental number (NF) – the number of chromosome arms – in Mus musculus and Mus poschiavinus is the same namely 40. It has been shown that metacentric chromosomes in the karyotype of tobacco mice are the result of centric fusion of different pairs of autosomes in the normal karyotype of the house mouse Mus musculus. In particular, chromosomes involved in the T_6BnR translocation correspond to the 9th and 14th pairs [6, 9].

EXPERIMENTAL METHOD

CBA mice, with a normal karyotype, and mice heterozygous for the Robertson translocation T_6BnR were used. The latter were obtained by crossing F_1 hybrids (Mus musculus × Mus poschiavinus), heterozygous for 7 Robertson translocations with CBA females. As a result of a series of successive matings, mice

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TABLE 1. Fertility of CBA Mice with Normal Karyotype and of Mice (♂) Heterozygous for the T_6BnR Translocation (results for the 18th day of pregnancy)

Line of mice		Females		No. of corpora lutea	No. of implantation sites	Number of dying embryos						Number of living embryos							
						total		before implantation		after implantation		total	normal		delayed development		malformed	
♂	♀	inseminated	pregnant				%	abs.	%	abs.	%		abs.	%	abs.	%	abs.	%
T_6BnR/CBA	CBA	26	22	198	160	58	29,2	28	14,1	30	15,1	130	118	90,7	8	6,1	4	3,2
CBA	CBA	20	18	163	142	41	24,9	21	12,8	20	12,2	122	120	98,3	2	1,6	—	

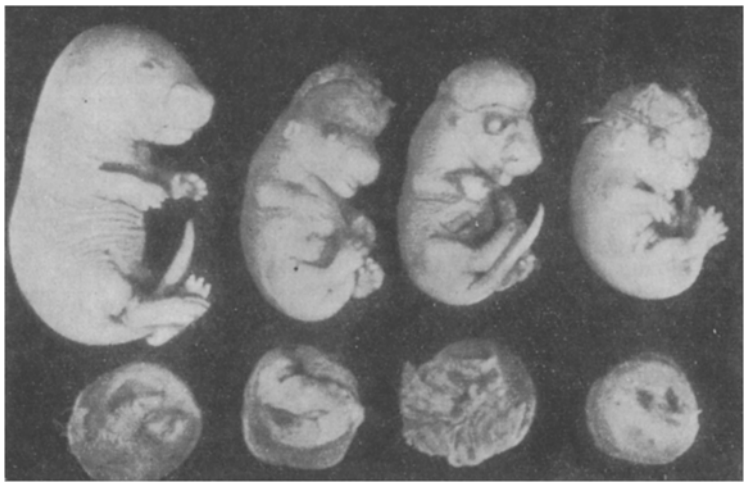

Fig. 1. Control embryo on 18th day of development with diploid karyotype (NF = 40) and trisomic embryos (NF = 41) with similar brain anomalies: exencephaly (♂T_6/9,14/BnR/+ × ♀ CBA).

heterozygous for the translocation T_6BnR were obtained, using the method of bone marrow biopsy for selection [4].

Two series of experiments were carried out. In series I the fertility of the males heterozygous for the T_6BnR translocation was studied. For this purpose they were mated with CBA females and these were autopsied on the 18th day of pregnancy. The number of corpora lutea in the ovaries and the number of implantation sites in the uterus were counted. The number of living and dead embryos was noted. Living embryos were separated from the membranes, weighed on torsion scales, their cranio-caudal length measured, and examined under the MBS-1 binocular loupe for anomalies. In series II a cytogenetic analysis was made of 75 fetuses, including 59 normal and 16 retarded in development and deformed, from the progeny of CBA females mated with T_6BnR/+ males. All embryos with trisomy were fixed with Bouin's fluid and investigated microanatomically [3]. Six trisomics were studied by examination of serial histological sections stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The results of the experiments of series I (Table 1) show that the embryonic mortality in CBA females mated with T_6BnR males was 29.2%; moreover about equal numbers of embryos died before and after implantation (about 15%). Spontaneous embryonic mortality in the control group was a little lower (24.9%), but the difference is not statistically significant.

The cranio-caudal length of 120 embryos on the 18th day of development in CBA mice was 18 ± 2 mm and their weight about 800 mg. No anomalies were found in any of this series and only two were visibly

TABLE 2. Cytological Analysis of Normal Embryos, Embryos with Delayed Development, and Malformed Embryos from CBA Females Mated with T_6BnR/+ Males (results for 18th day of pregnancy)

Embryos	Karyotype	
	diploid NF = 40	heteroploid (trisomy) NF = 41)
Normal		
Length 14 mm		
Weight 600 mg	57	1*
With delayed development but no visible anomalies		
Length 15 mm		
Weight 600 mg	2	6
With delayed development and with anomalies		9
With exencephaly		5
With hydrocephalus		1
With cleft palate		1
With edema and hemorrhages		2
Total	59	16

*Fused placentas.

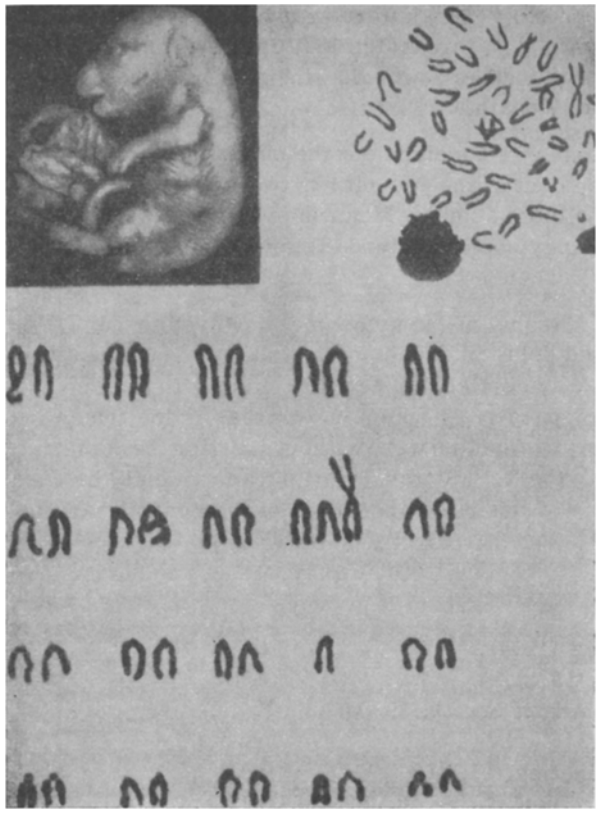

Fig. 2. Embryo at 18th day of development (♂T_6/9,14/BnR/+ × ♀ CBA). Trisomy 2n = 40, NF = 41. General delay in development, hemorrhages into subcutaneous cellular tissue.

retarded in development. By contrast, in 10% of the embryos (12 of 130) in the experimental series the cranio-caudal length was less than 15 mm and their weight did not exceed 500 mg; 8 of the embryos were externally completely normal, but the other 4 showed similar anomalies of the brain (exencephaly; Fig. 1).

Cytogenetic analysis showed that the karyotype of these embryos always contained 40 chromosomes, including 1 submetacentric chromosome, whereas the number of chromosome arms was 41, i.e., all these embryos were trisomics. The frequency of appearance of embryos with trisomy in the progeny of CBA females mated with T_6BnR/+ males was thus almost 10%.

Altogether 19 trisomics, including 16 embryos on the 18th day of development, 2 on the 16th day of development, and 1 newborn mouse, were identified and subjected to morphological analysis. The most constant distinguishing feature of the embryos with trisomy was generalized delay in development (Table 2). For instance, the cranio-caudal length of 15 of the 16 trisomics on the 18th day of development was about 15 mm while their weight was below 600 mg (Fig. 2). The only exception was a trisomic sharing a common placenta with another embryo with normal karyotype. Both embryos were phenotypically indistinguishable from the controls and their identification was possible only by cytogenetic analysis. All the other trisomics were almost one-third smaller in length and weight than normal; considerable retardation in development

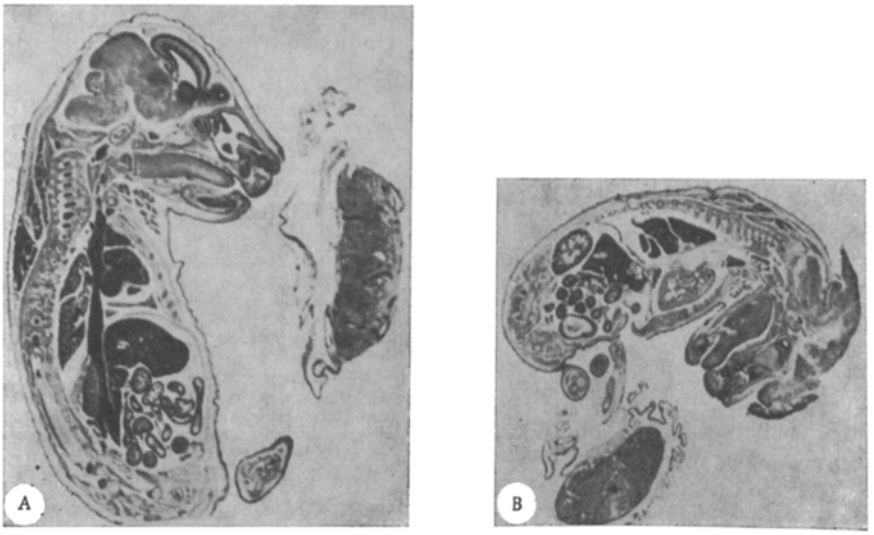

Fig. 3. Sagittal histological sections through embryos: A) control embryo on 18th day of development, with diploid karyotype (2n = 40, NF = 40); B) trisomic embryo with excencephaly (2n = 40, NF = 41).

could be noted as early as on the 15th day of pregnancy. It is important to note, however, that the weight of the placentas of the trisomics differed only a little from the control (135 mg and 147 mg, respectively). Consequently, this trisomy does not affect growth of the chorio-allantoic placenta, but it does lead to marked delay of embryonic development. This trisomy also gives rise to frequent and severe developmental anomalies. For instance, 9 of the 16 trisomics had developmental anomalies on the 18th day; 6 had brain anomalies (exencephaly, hydrocephalus), 1 had a defect of the hard palate (cleft palate), and 2 had extensive hemorrhages and edema of the subcutaneous cellular tissue. Finally, it is interesting to note that the only newborn mouse with trisomy, although alive, died soon after birth. Microanatomical examination of this mouse revealed hydrocephalus and cleft palate. The uninflated lungs revealed that the newborn trisomic was unable to breathe, and this was the direct cause of its early death.

During the investigation of 6 trisomics on the 16th-18th day of development the only abnormality discovered in the histological sections was marked underdevelopment of the vascular network and villi of the yolk sac, indicating a disturbance of the function of the omphaloid placenta. Meanwhile no other disturbance of morphogenesis specific for this particular karyotype pathology could be found either in the embryo itself or in the placenta (Fig. 3).

Just as with trisomy of the 19th pair of autosomes [8], trisomy of the autosomes involved in the T_6BnR translocation (the 9th and 14th pairs) thus leads to generalized delay in development and to death soon after birth. Meanwhile there are definite differences between these trisomies. In newborn mice with trisomy of the 19th pair a defect of the hard palate (cleft palate) is comparatively common, whereas in the trisomics in the experiments described in this paper deformities of the nervous system were without question the dominant feature and exencephaly was particularly frequent. Mice with the T_6BnR translocation can evidently be used as a convenient biological model for studying the pathogenesis of inherited anomalies of the central nervous system and, in particular, of exencephaly.

LITERATURE CITED

1. V. S. Baranov and A. P. Dyban, Ontogenez, 2, No. 2, 164 (1971).
2. V. S. Baranov and A. P. Dyban, Arkh. Anat., No. 8, 67 (1972).
3. A. P. Dyban, V. S. Baranov, and I. M. Akimova, Arkh. Anat., No. 10, 89 (1970).
4. L. D. Udalova, Arkh. Anat., No. 9, 87 (1971).
5. B. M. Cattanach, C. E. Williams, and H. Bailey Cytogenetics, 11, 412 (1972).
6. "Committee on Standarized Genetic Nomenclature for Mice," J. Hered., 63, 69 (1972).
7. A. Gropp, U. Tettenborn, and E. von Lehmann, Cytogenetics, 9, 9 (1970).
8. B. J. White, J. H. Tjio, L. van de Water, et al., Cytogenetics, 11, 363 (1972).
9. L. Zech, E. P. Evans, E. Ford, et al., Exp. Cell Res., 70, 263 (1972).