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Tetrasomy 16 in the mouse: a more severe condition than the corresponding trisomy

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SUMMARY

Although an understanding of the phenotypic similarities and differences between individuals trisomic and tetrasomic for the same chromosome or chromosome segment is of considerable theoretical interest with regard to the relationship of the phenotypic features of aneuploid states to changes in the dosage of specific genes, the data obtainable from human cases of tetrasomy do not permit firm conclusions to be drawn. However, a mouse model system based on the intercrossing of animals doubly heterozygous for two Robertsonian translocation (metacentric) chromosomes with monobrachial homology can be used to generate both tetrasomic and trisomic embryos simultaneously. With mice carrying the two metacentric chromosomes, *Rb(11.16)2H* and *Rb(16.17)32Lub*, which share chromosome 16 in common, embryos with tetrasomy 16 and trisomy 16 have been generated. The rate of non-disjunction in both males and females was 34 to 36 %, and the observed overall frequency of tetrasomics in embryos up to 10 days of age was 3·3 %, very close to the expected 3·1 %. Although trisomy 16 and tetrasomy 16 embryos could not be distinguished by size from one another or from diploid embryos at the sixth day of gestation, the tetrasomy 16 embryos were very small and delayed in development at day 10 and clearly distinguishable from the trisomics. Fusion of the anterior neuropore had not occurred, rotation of the embryo was incomplete, and somite formation was retarded. By day 12, all of the tetrasomy 16 embryos were either dead or dying. By contrast, virtually all trisomy 16 embryos were alive at day 10, although about 60 % were retarded in development. The same was also the case on day 12 for trisomy 16 embryos obtained from mothers that were naturally mated. However, superovulation of the mothers, which caused a doubling of the number of implants, resulted in a significant decrease in the frequency of trisomy 16 embryos and possibly of tetrasomy 16 embryos as well. This finding is consistent with the suggestion that the viability of aneuploid embryos, which may be at a proliferative disadvantage, is highly dependent upon intrauterine conditions. These exogenous factors notwithstanding, it is clear that tetrasomy 16 has much more deleterious effects on embryonic development than does trisomy 16 and that four doses of the genes on chromosome 16 result in developmental derangements greater than are produced by three doses of the same genes.

INTRODUCTION

While trisomy is the most commonly observed autosomal aneuploidy in man, only a few reports have been made of full or, more usually, partial autosomal tetrasomy in either mosaic or pure form (Balestrazzi *et al.* 1983; Batista, Vianna-Morgante & Richiere-Costa, 1983; Garcia-Cruz *et al.* 1982; Schinzel *et al.* 1981;

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Shapiro, Hansen & Littlefield, 1985; Wisniewski, Hassold, Heffelfinger & Higgins, 1979). Understanding the phenotypic similarities and differences between individuals trisomic and tetrasomic for the same chromosome or chromosome segment is of considerable theoretical interest with regard to attempts to relate the phenotypic features of aneuploid states to changes in the dosage of specific genes (Epstein, 1986). Unfortunately, the conclusions that can be drawn from the human tetrasomy data are quite limited because of the scarcity of cases, the difficulties in matching unrelated translocations (two translocations for a similar region do not necessarily involve exactly the same loci), and the problems associated with investigations of human prenatal development. The existence of these limitations in the study of tetrasomy in man suggests a need for an animal model system in which specific tetrasomies can be generated and analysed.

A method for generating embryos and foetuses trisomic for whole autosomes already exists (Gropp & Kolbus, 1974; White, Tjio, Van de Water & Crandall, 1974) and is based on the use of animals, usually males, doubly heterozygous for two Robertsonian translocation (metacentric) chromosomes that have monobrachial homology (i.e. share a chromosome arm in common) (Gropp, Kolbus & Giers, 1975). A high degree of non-disjunction occurs in such animals (Gropp & Winking, 1981) and results in the generation of both nullisomic and disomic gametes. The latter, upon fertilization of a normal gamete, produces trisomic progeny. Depending on the specific metacentric chromosomes used, all of the mouse autosomal trisomies can be produced, and this breeding system has been used in a variety of studies of aneuploidy in the mouse (Epstein, 1985*a*,*b*). Since the same combinations of chromosomes can be carried by both males and females, it would be predicted that a breeding scheme employing pairs of animals with identical sets of metacentric chromosomes should result in the conception of embryos tetrasomic or nullisomic for the common chromosome arm, as well as the already observed trisomics and monosomics (Fig. 1). Such a breeding scheme had previously been used to study trisomy 19 (White, Tjio, Van de Water & Crandall, 1972) and non-disjunction (Searle & Beechey, 1982) and had been suggested by Ford(1975) as a means to produce nullisomic embryos. A preliminary report of its use to generate embryos with tetrasomy 15 has recently appeared (Beechey, Kirk & Searle, 1985).

In this report, we describe the generation and phenotypic properties of mouse embryos tetrasomic for chromosome 16. These embryos, while able to implant and survive to midgestation, are much more severely affected than are siblings with trisomy 16. In addition, we describe some previously unreported aspects of the development of trisomy 16 embryos and of a possible relationship between intrauterine conditions and the survival of aneuploid embryos.

MATERIALS AND METHODS

(A) *Production of aneuploid embryos*

Female mice doubly heterozygous for two metacentric Robertsonian translocation chromosomes, *Rb(11.16)2H* and *Rb(16.17)32Lub* were mated with doubly heterozygous males of the

same type to generate aneuploid embryos and diploid controls. Both homozygous strains were obtained from Dr Heinz Winking and the late Prof. Alfred Gropp, Lübeck, Germany, and were maintained in our laboratory as closed outbred colonies. The day the vaginal plug was observed was considered as day 0. At the appropriate day of gestation, the pregnant females were killed, the embryos removed and their appearance recorded. Some of them were photographed before the genotype was determined by karyotype analysis.

(B) *Karyotype analysis*

Embryos were karyotyped by modifications of the method described in Magnuson, Smith & Epstein (1982). Day-3 (preimplantation) embryos were removed from the uteri of superovulated females (4 to 5 weeks old) and incubated for 3–4 h in modified Whitten's medium (Golbus & Epstein, 1974) containing 0·08 $\mu g\,ml^{-1}$ of vinblastine (Velban, GIBCO). After incubation, the mitotically arrested embryos were individually transferred to a microdrop of a hypotonic solution (0·75 % sodium citrate) in a well of a microtitre plate and incubated at room temperature for 5 min and then on ice for up to 1 h. One embryo at a time was then transferred to a new well containing a 7:3 methanol: acetic acid fixative. After a few seconds, the embryo was transferred to a microscope slide and immediately covered with a microdrop of a 1:4:5 lactic acid (85 % syrup solution): distilled water: acetic acid solution. Once the fixed embryo was seen to break apart, it was washed with an excess of fixative and dried thoroughly. The slides were stained with 3 % Giemsa (Gurr) for 10 min. The genotype was determined by counting the number of acrocentric and metacentric chromosomes per metaphase plate (Fig. 1). Tetrasomic embryos have 42 chromosome arms distributed as 34 acrocentrics and 4 metacentrics while trisomics have 41 chromosome arms and 3 metacentrics and diploid embryos have 40 arms and 2 metacentrics.

Day-6 (early postimplantation) embryos were dissected from the uteri of hormonally induced females (8 to 12 weeks old) and incubated 4–6 h in Dulbecco's modified Eagle's medium supplemented with 10 % foetal calf serum and 0·04 $\mu g\,ml^{-1}$ of vinblastine. The karyotyping procedure was the same as that for preimplantation embryos, except that the hypotonic solution was 0·6 % sodium citrate.

Day-10 and day-12 (midgestation) embryos were dissected either from superovulated or naturally mated females (8 to 12 weeks old). Small pieces of tissue were incubated for 3–4 h in the same medium as day-6 embryos, then treated in 1 ml of 0·56 % KCl for 10 min, fixed in 1 ml of 3:1 methanol: acetic acid for 15 min, and transferred to 80 μl of 60 % acetic acid to allow the cells to break apart. After 5 min, the cell suspension was transferred to a microscope slide covered with a film of a 0·01 % Nonidet P40 solution. The slides were dried at 37 °C and stained with Giemsa for 4 min.

RESULTS

(A) *Survival of aneuploid embryos*

Forty-two superovulated *Rb2H/Rb32Lub* females were mated with *Rb2H/Rb32Lub* males and sacrificed at various stages of development. The mean litter size (number of preimplantation embryos or implantation sites) was 23·1, 14·7, 15·1, and 18·2 at, respectively, 3, 6, 10, and 12 days of gestation. Twenty-three additional naturally mated females were dissected at days 10 and 12 and had a mean of 9·8 and 10·2 implantation sites, respectively.

Among the 593 embryos successfully karyotyped, 20 were tetrasomic for chromosome 16 (3·4 %). The frequency in embryos 10 days or less in gestational age was 3·3 %. The frequency of tetrasomy per 100 *diploid* embryos ranged from 1·8 in day-12 superovulated females to 7·8 in day-10 superovulated females (Table 1).

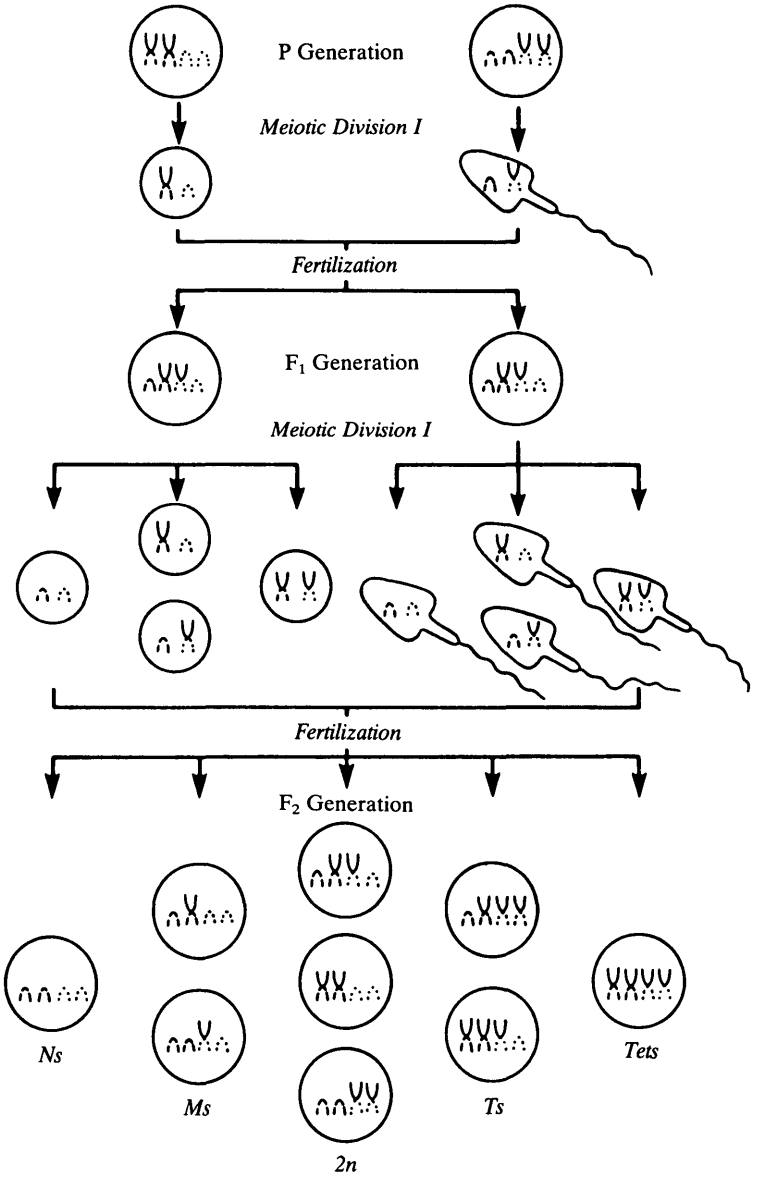

Fig. 1. Production of specific nullisomic (*Ns*), monosomic (*Ms*), diploid (*2n*), trisomic (*Ts*) and tetrasomic (*Tets*) mouse embryos. In the first cross (P generation), a female homozygous for a Robertsonian translocation chromosome is mated with a male homozygous for another Robertsonian translocation chromosome which has one chromosome arm in common with the female's metacentric. The resulting F_1 generation mice, doubly heterozygous for the two metacentrics, have a high rate of non-disjunction which will generate 25–40 % (Gropp, Kolbus & Giers, 1975) of gametes either nullisomic or disomic for the shared chromosome arm. When two F_1 mice are crossed together, four different types of aneuploids are generated (F_2 generation) together with diploid embryos. Only the three pairs of chromosomes involved in the metacentrics are shown here. Except for a very low frequency of non-disjunction involving mainly chromosomes 11 and 17 (White *et al.* 1972; Gropp & Winking, 1981), the other 34 acrocentrics segregate normally.

Table 1. *Number and frequency of aneuploid embryos in the cross* Rb(11.16)2H/Rb(16.17)32Lub×Rb(11.16)2H/Rb(16.17)32Lub

Stage	Sample size	Number of diploid embryos	Number of embryos (frequency per 100 diploid embryos) Monosomy 16	Trisomy 16	Tetrasomy 16	Other chromosome abnormalities*
From superovulated females						
Day 3	105	47	24 (51·1)	29 (61·7)	1 (2·1)	4 (8·5)
Day 6	141	97	0 (0)	36 (37·1)	5 (5·2)	3 (3·1)
Day 10	117	77	0 (0)	29 (37·7)	6 (7·8)	5 (6·5)
Day 12	66	55	0 (0)	9 (16·4)	1 (1·8)	1 (1·8)
From naturally mated females						
Day 10	30	19	0 (0)	8 (42·1)	1 (5·3)	2 (10·5)
Day 12	134	96	0 (0)	31 (32·3)	6 (6·2)	1 (1·0)

*Included are non-specific monosomic, trisomic, double trisomic, triploid and one triploid–tetrasomic embryos.

Approximately equal frequencies of monosomy 16 and trisomy 16 embryos were found at the early blastocyst (day 3) stage (Table 1). However, the mean cell number was significantly lower ($P < 0{\cdot}005$, Student's t-test) in monosomic than in diploid or in trisomic littermates (38 ± 3 [S.E.M.], 52 ± 2 and 49 ± 3 cells per embryo, respectively). The single tetrasomy 16 embryo karyotyped at this stage had 51 cells. At day 6, no monosomic embryos were found and the ratio

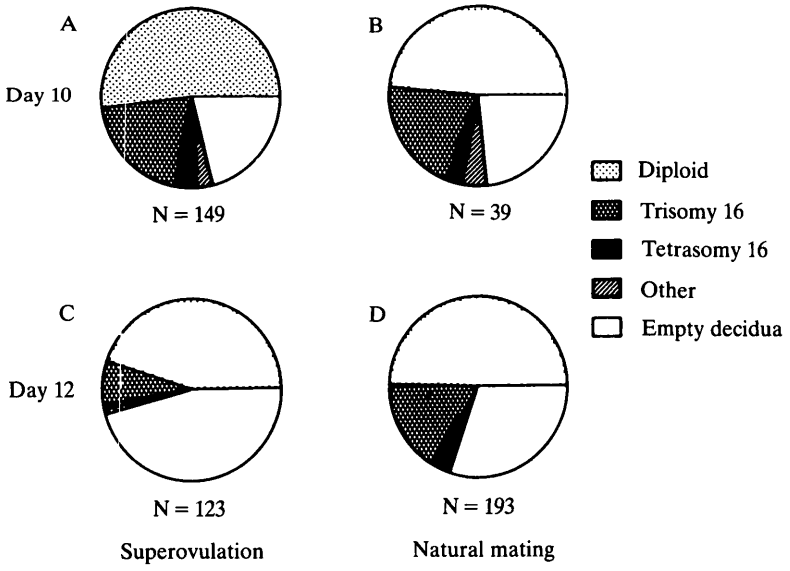

Fig. 2. Frequency of aneuploid and diploid embryos from superovulated (A,C) and naturally mated (B,D) *Rb2H/Rb32Lub* females mated with males of the same genotype, after 10 days (A,B) and 12 days (C,D) of gestation. N indicates the number of implants analysed.

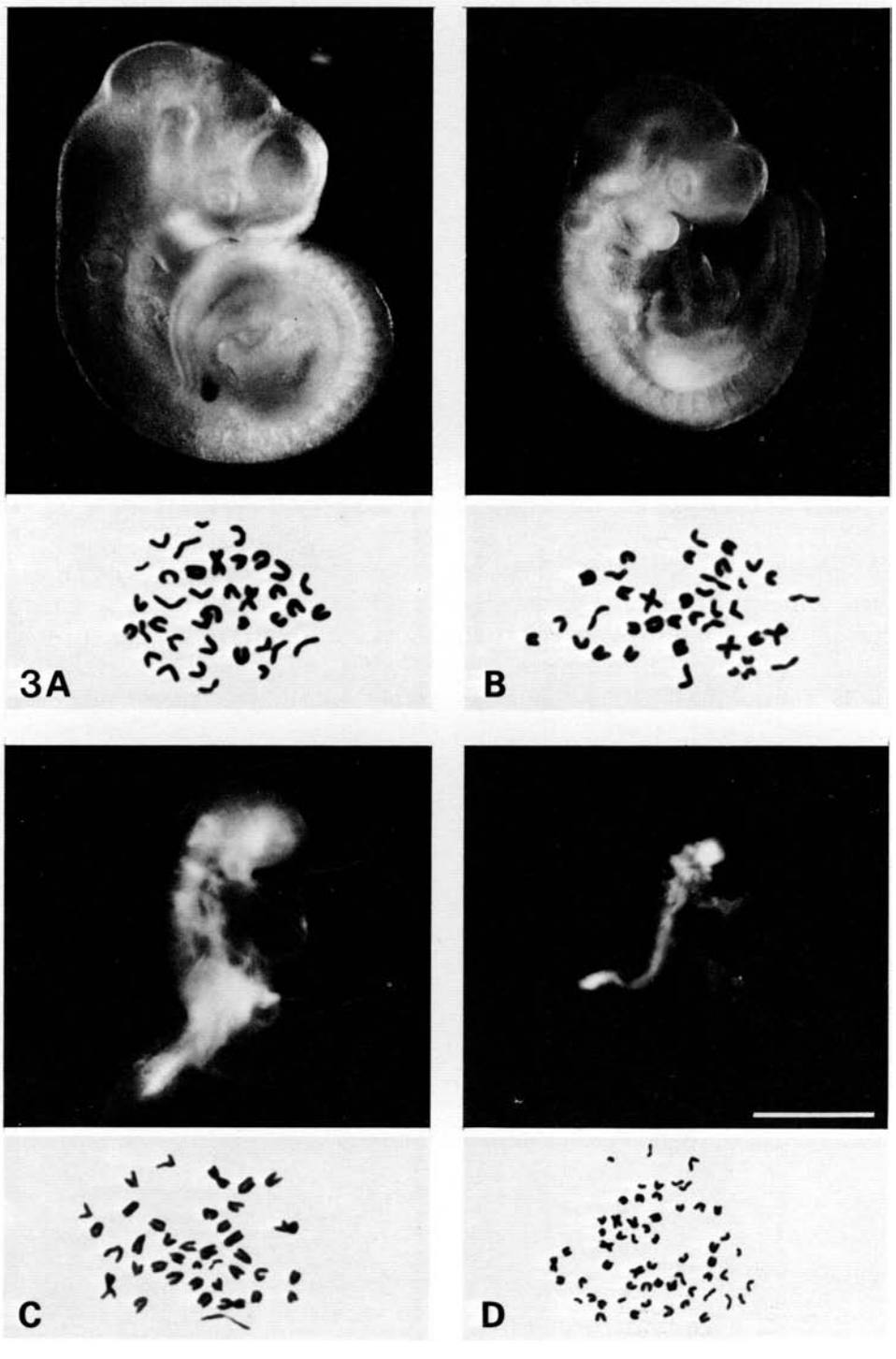
3A
B
C
D

Table 2. *General appearance of the trisomic 16 (Ts) and tetrasomic 16 (Tets) embryos and their diploid littermates (2n) at midgestation*

Appearance of the embryos	Superovulated females						Naturally mated females		
	Day 10			Day 12			Day 12		
	2*n*	Ts	Tets	2*n*	Ts	Tets	2n	Ts	Tets
Normal to slightly small	73	12		48	4		94	16	
Retarded, alive	4	17	6	4	1		2		1
Dying				3	4			14	4
Membranes only						1		1	1

of trisomics to disomics was lower, although not significantly, than before implantation.

On the tenth day of development, the proportion of trisomy 16 embryos from either superovulated or naturally mated females was the same as at day 6. Two days later, no change was found in the naturally mated females, but superovulated one carried significantly fewer ($P < 0{\cdot}05$, chi-squared test) trisomy 16 embryos (Table 1). The same difference was observed when the numbers of diploid and chromosomally abnormal embryos were related to the total number of implantation sites (Fig. 2).

(B) *Severity of the trisomic and tetrasomic states*

Trisomic and tetrasomic embryos could not be distinguished by size from normal littermates at the 6th day of gestation. However, the tetrasomic embryos were very delayed in development at day 10 (Fig. 3, Table 2). Although a beating heart and onset of somite and allantois formation was noted, fusion of the anterior neuropore had not occurred (Fig. 4B) and the rotation of the embryo was incomplete (Fig. 3C, Fig. 4A,B). The most advanced day-10 tetrasomic embryo had only 17 somites (Fig. 4D), compared with $29{\cdot}8 \pm 1{\cdot}4$ (S.D.) and $26{\cdot}8 \pm 1{\cdot}9$ somites in diploid and trisomic siblings, respectively. At day 12, the tetrasomic embryos were either dying (Table 2, Fig. 5D,E,F) or were so resorbed that only extraembryonic membranes were found. At this stage, only one out of seven tetrasomics still had a beating heart.

An extremely deformed and retarded day-10 embryo (Fig. 3D) had 53 acrocentric and 4 metacentric chromosomes, giving a total of 61 chromosome arms. We assume that this triploid–tetrasomic embryo resulted from the combination of diploid and haploid–disomic gametes. Alternatively, it could have been generated by a dispermic fertilization, with one of the three gametes being disomic.

Fig. 3. Light micrographs of day-10 embryos from superovulated (A,B,C) and naturally mated (D) females and their respective karyotypes. (A) Normal diploid embryo with 36 acrocentric and 2 metacentric chromosomes; (B) trisomy 16 with 35 acrocentric and 3 metacentric chromosomes; (C) tetrasomy 16 with 34 acrocentric and 4 metacentric chromosomes; and (D) triploid–tetrasomy 16 with 53 acrocentric and 4 metacentric chromosomes. The slight growth retardation of the trisomic embryo and the severe retardation of the tetrasomic and triploid–tetrasomic embryos are apparent. Bar equals 1 mm.

At day 10 about 60% of the trisomics were smaller than the diploid controls (Table 2, Fig. 3). Two days later, the discrepancy between the normal-appearing trisomics (Fig. 5B) and the retarded ones (Fig. 5C) was even greater, the latter being in an advanced stage of resorption (Table 2). Surprisingly, no intermediate form of abnormal phenotype was observed, suggesting the existence of two distinct populations of trisomics. The existence of these populations was not correlated with the sizes of the litters or the ages of the females.

DISCUSSION

As expected, tetrasomic embryos were actually generated by an intercross between mice doubly heterozygous for two metacentric chromosomes that share one arm in common (Fig. 1). Nullisomic embryos, also predicted by the breeding

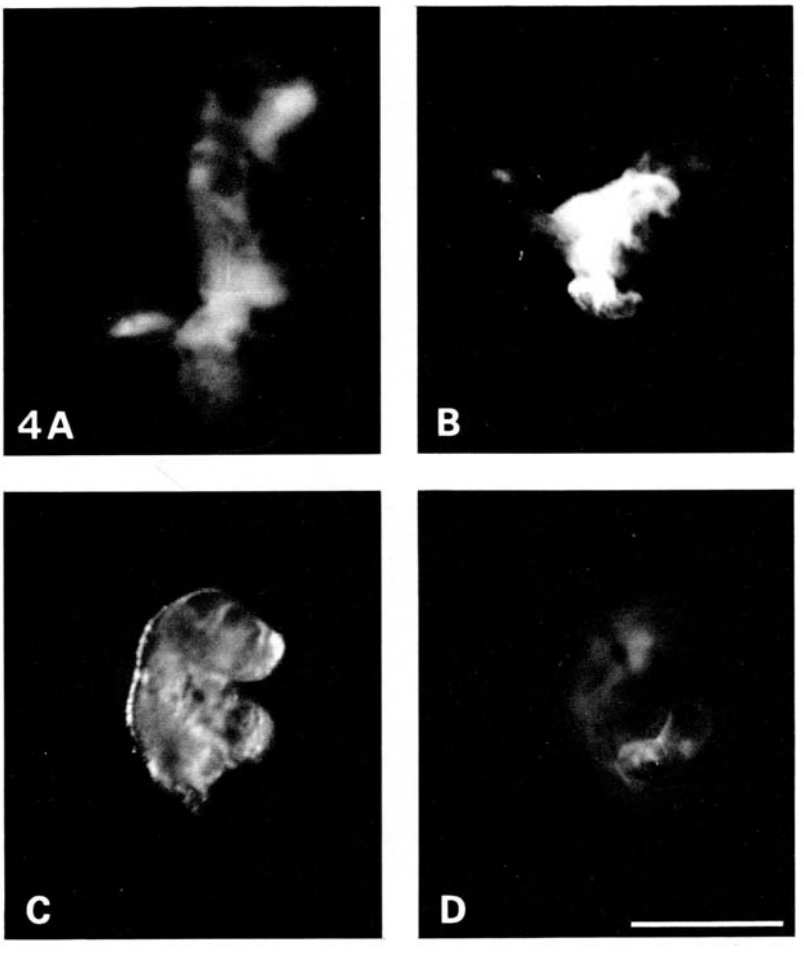

Fig. 4. Tetrasomy 16 embryos at the 10th day of gestation, from hormonally induced (A,C,D) and naturally mated (B) *Rb2H/RB32Lub* females. The embryo in (D) was the most advanced day-10 tetrasomic observed. Bar equals 1 mm.

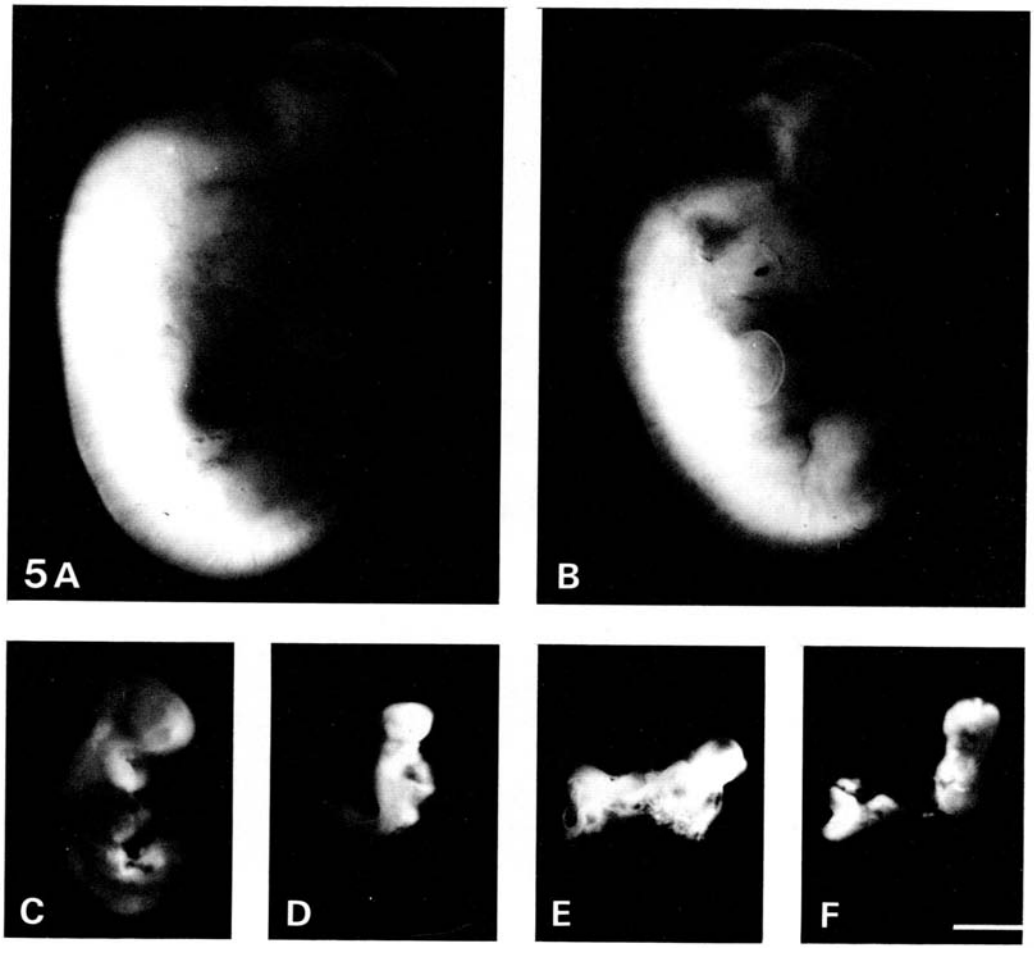

Fig. 5. Diploid (A), trisomy (B,C) and tetrasomy 16 (D,E,F) mouse embryos from naturally mated females at 12 days of gestation. All tetrasomic embryos show severe developmental retardation and are apparently moribund. Bar equals 1 mm.

scheme to be generated in equal numbers, were not detected at the early blastocyst stage. Preliminary results from the karyotyping of earlier stages suggest that nullisomics might undergo one, perhaps, at a maximum, two divisions, before they die (S. Debrot, A. Zweig, C. J. Epstein, unpublished observations). In previous experiments (Magnuson *et al.* 1985) we found that the *Rb2H/Rb32Lub* males, when mated with ICR females, generate 17 % monosomics and 17 % trisomics when analysed after 2 days of development, giving a male non-disjunction rate of 34 %. Using these data, we calculated that a 36 % rate of non-disjunction in the doubly heterozygous females best fits with our day-3 results. We therefore conclude that superovulated *Rb2H/Rb32Lub* females have a rate of non-disjunction similar to that of doubly heterozygous males and that when intercrossed *Rb2H/Rb32Lub* heterozygotes will generate about 3 % tetrasomic embryos (or 6 tetrasomics per 100 diploids). The frequency of tetrasomics found at day 10,

7·8 per 100 diploids (Table 1), is consistent with this expectation and indicates that all tetrasomics probably implant and survive to midgestation.

The findings with the tetrasomic embryos make it clear that four copies of chromosome 16 have a greater adverse effect on embryonic development than do three copies. Van der Hoeven & de Boer (1984) found a similar situation when the translocation *T(1;13)70H* was used to generate tetrasomy for the small regions of chromosome 1 and 13 involved in the 1^{13} translocation chromosome. This tetrasomy (actually a double partial tetrasomy) is a prenatal lethal, while mice that are trisomic for the same regions survive and are fertile. Similarly, Beechey, Kirk & Searle (1985) found that tetrasomy 15 results in death by day 9–9·5, whereas trisomy 15 embryos at the same stage were still alive although they were about a third smaller than normal in size and had exencephaly. Therefore, for the two whole chromosome tetrasomies and one double partial tetrasomy studied to date, the tetrasomic state results in developmental abnormalities more severe than do the corresponding trisomies. On the other hand, Beechey & Speed (1981) described a male tetrasomic for the extra small 5^{12} translocation chromosome of *T(5;12)31H*. This animal, although viable, was infertile, smaller than normal, and had a shortened head and small testes. A slightly retarded foetus with a twisted spine and the same genotype was also found at 12·5 days of gestation (Beechey, Kirk & Searle, 1980). These abnormalities are not much more severe than were found with the equivalent trisomy which resulted in male sterility and mild growth retardation. Overall, the situation in the mouse is reminiscent of what has been reported in man, with the effects of aneuploidy ranging from little or no specific phenotypic differences between tetrasomics and trisomics to the existence of clearly distinguishable syndromes with the tetrasomics being more severely affected (Epstein, 1986).

In addition to its usefulness for producing tetrasomic (and, potentially, nullisomic) embryos, the breeding system described here, with both parents bearing doubly heterozygous chromosomes, may also be of value in the study of trisomy (White *et al.* 1972). Trisomic embryos will be generated in a higher proportion than in the traditional mating of a doubly heterozygous male with a normal 'all acrocentric' female. For example, with the *Rb2H/Rb32Lub* heterozygotes used in this study, 47 rather than 27 trisomics per 100 diploids can be expected when two double heterozygotes are intercrossed rather than just one being mated to a wild-type animal.

Our data do not reveal any differences in the incidence of aneuploidy between superovulated and naturally mated females in embryos analysed at 10 days of gestation (Fig. 2A,B). However, a significant loss of aneuploid embryos is observed two days later in superovulated females (Fig. 2C). We interpret this result as indicating that a selection process may be operating within the uterus. In a crowded situation (20·5 implants/female after superovulation *versus* 10·2 after natural mating), a greater competition for either nutrients or space could occur at midgestation which could place trisomic and tetrasomic embryos at a proliferative disadvantage. This conclusion would be consistent with the suggestion of Boué,

Philippe, Giroud & Boué (1976) and Gropp (1981) that the time of death of aneuploid foetuses could be determined in part by the ability of the aneuploid placenta to serve the metabolic needs of the embryo or foetus. In a marginal situation, with an already hypoplastic placenta (Gearhart, Oster-Granite & Hatzidimitriou, 1985), trisomy 16 embryos could be quite vulnerable to the local effects of uterine crowding.

The separation of the trisomy 16 embryos into two populations, some being grossly normal or only mildly abnormal while the others are severely delayed, could suggest a critical developmental threshold about or shortly before the 10th day of development. Some trisomics may be so delayed in development at this stage that they do not recover and are resorbed, while the others are able to cross this threshold and continue through a programme of fairly normal development up to and beyond day 12. Trisomy 16 foetuses have been reported to survive until or even shortly beyond birth (Miyabara, Gropp & Winking, 1982). It is of interest to note, in the context of a barrier to embryonic development, that the 9th day of gestation is often considered in the mouse to be a critical one, since it appears to be the stage most sensitive to various exogenous insults (Rugh, 1968). Furthermore, it also represents the time at which the most lethal of the mouse trisomies result in embryonic death (Gropp, 1978).

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REFERENCES

Balestrazzi, P., Croci, G., Frassi, C., Franchi, F. & Giovannelli, G. (1983). Tetrasomy 9p confirmed by GALT. *J. med. Genet.* **20**, 396–399.

Batista, D. A. S., Vianna-Morgante, A. M. & Richieri-Costa, A. (1983). Tetrasomy 18p: tentative delineation of a syndrome. *J. med. Genet.* **20**, 144–147.

Beechey, C. V., Kirk, M. & Searle, A. G. (1980). A reciprocal translocation induced in an oocyte and affecting fertility in male mice. *Cytogenet. Cell Genet.* **27**, 129–146.

Beechey, C. V., Kirk, M. & Searle, A. G. (1985). Personal communication: trisomy and tetrasomy for chromosome 15. *Mouse News Letter* **72**, 103.

Beechey, C. V. & Speed, R. (1981). Personal communication: double tertiary trisomy in T(5;12)31H. *Mouse News Letter* **64**, 56.

Boué, J., Philippe, E., Giroud, A. & Boué, A. (1976). Phenotypic expression of lethal chromosomal anomalies in human abortuses. *Teratology* **14**, 3–20.

Epstein, C. J. (1985*a*). Mouse monosomies and trisomies as experimental systems for studying mammalian aneuploidy. *Trends in Genetics* **1**, 129–134.

Epstein, C. J. (1985*b*). The mouse trisomies: experimental systems for the study of aneuploidy. In *Issues and Reviews in Teratology*, vol. 3 (ed. H. Kalter), pp. 171–217. New York: Plenum.

Epstein, C. J. (1986). *The Consequences of Chromosome Imbalance: Principles, Mechanisms, and Models*. New York: Cambridge University Press (in press).

Ford, C. E. (1975). The time in development at which gross genome unbalance is expressed. In *The Early Development of Mammals* (ed. M. Balls & A. E. Wild), pp. 285–304. Cambridge: Cambridge University Press.

GARCIA-CRUZ, D., VACA, G., IBARRA, B., SÁNCHEZ-CORONA, J., OCAMPO-CAMPOS, R., PEREGRINA, S., MOLLER, M., RIVERA, H., RIVAS, F., GONZÁLEZ-ANGULO, A. & CANTÚ, J. M. (1982). Tetrasomy 9p: clinical aspects and enzymatic gene dosage expression. *Ann. Génét.* **25**, 237–242.

GEARHART, J. D., OSTER-GRANITE, M. L. & HATZIDIMITRIOU, G. (1985). Placental development in the trisomy 16 mouse. *Pediatr. Res.* **19**, 325A.

GOLBUS, M. S. & EPSTEIN, C. J. (1974). Effect of 5-bromodeoxyuridine on preimplantation mouse embryo development. *Differentiation* **2**, 143–149.

GROPP, A. (1978). Relevance of phases of development for expression of abnormality. Perspectives drawn from experimentally induced chromosome aberrations. In *Abnormal Fetal Growth: Biological Bases and Consequences* (ed. F. Naftolin), pp. 85–100. Berlin: Dahlem Konferenzen.

GROPP, A. (1981). Clinical and experimental pathology of fetal wastage. In *Human Reproduction. Proceedings of III World Congress* (ed. K. Semm & L. Mettler), pp. 208–216. Amsterdam: Excerpta Medica.

GROPP, A. & KOLBUS, U. (1974). Exencephaly in the syndrome of trisomy No. 12 of the foetal mouse. *Nature, Lond.* **249**, 145–147.

GROPP, A., KOLBUS, U. & GIERS, D. (1975). Systematic approach to the study of trisomy in the mouse. II. *Cytogenet. Cell Genet.* **14**, 42–62.

GROPP, A. & WINKING, H. (1981). Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. *Symp. zool. Soc. Lond.* **47**, 141–181.

MAGNUSON, T., DEBROT, S., DIMPFL, J., ZAMORA, T. & EPSTEIN, C. J. (1985). The early lethality of autosomal monosomy in the mouse. *J. exp. Zool.* (in press).

MAGNUSON, T., SMITH, S. & EPSTEIN, C. J. (1982). The development of monosomy 19 mouse embryos. *J. Embryol. exp Morph.* **69**, 223–236.

MIYABARA, S., GROPP, A. & WINKING, H. (1982). Trisomy 16 in the mouse fetus associated with generalized edema and cardiovascular and urinary tract anomalies. *Teratology* **25**, 369–380.

RUGH, R. (1968). *The Mouse, its Reproduction and Development.* Minneapolis: Burgess Publishing Co.

SCHINZEL, A., SCHMID, W., FRACCARO, M., TIEPOLO, L., ZUFFARDI, O., OPITZ, J. M., LINDSTEN, J., ZETTERQVIST, P., ENELL, H., BACCICHETTI, C., TENCONI, R. & PAGON, R. A. (1981). The "cat eye syndrome": dicentric small marker chromosome probably derived from a No. 22 (tetrasomy 22pter→q11) associated with a characteristic phenotype. Report of 11 patients and delineation of the clinical picture. *Hum. Genet.* **57**, 148–158.

SEARLE, A. G. & BEECHEY, C. V. (1982). The use of Robertsonian translocations in the mouse for studies on non-disjunction. *Cytogenet. Cell Genet.* **33**, 81–87.

SHAPIRO, S. D., HANSEN, K. L. & LITTLEFIELD, C. A. (1985). Non-mosaic partial tetrasomy and partial trisomy 9. *Am. J. med. Genet.* **20**, 271–276.

VAN DER HOEVEN, F. A. & DE BOER, P. (1984). Personal communication: Double trisomy for the T(1;13)70H derived small marker chromosome 1^{13} is a prenatal lethal. *Mouse News Letter* **70**, 104.

WHITE, B. J., TJIO, J. H., VAN DE WATER, L. C. & CRANDALL, C. (1972). Trisomy for the smallest autosome of the mouse and identification of the T1Wh translocation chromosome. *Cytogenetics* **11**, 363–378.

WHITE, B. J., TJIO, J. H., VAN DE WATER, L. C. & CRANDALL, C. (1974). Trisomy 19 in the laboratory mouse. I. Frequency in different crosses at specific developmental stages and relationship of trisomy to cleft palate. *Cytogenet. Cell Genet.* **13**, 217–231.

WISNIEWSKI, L., HASSOLD, T., HEFFELFINGER, J. & HIGGINS, J. V. (1979). Cytogenetic and clinical studies in five cases of inv dup(15). *Hum. Genet.* **50**, 259–270.

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