Identification of Triploid Genome by Fluorescence Microscopy

Abstract. *Fluorescence markers on chromosome numbers 3, 13, and 14 gave cytological evidence of the paternal origin of the extra haploid set in a premature triploid infant with XXY sex chromosome complement. The mother had discontinued the use of oral contraceptives 13 months prior to conception. It is not possible to tell whether the mechanism involved was fertilization by dispermy or by a diploid sperm.*

Triploidy is relatively common in plants but occurs spontaneously only occasionally in animals, the classical examples being drosophila and salamanders. It can be induced by delayed fertilization (*1*). Instances of triploid abortuses have been observed in humans (*2*); one cause has been traced to conception occurring during the 7-month period after termination of oral contraceptives (*3*).

Although triploidy may be seen frequently in abortions, only a few fetuses are known to have reached, or to have almost reached, term (*2*). No such infant has lived more than a few hours. A triploid infant who lived only 4 hours offered the opportunity to obtain, for the first time, cytological evidence for the parental origin of the extra genome.

The male infant, delivered after 6½ months gestation, had multiple congenital malformations including cleft lip and palate, hydrocephalus, and poorly developed external genitalia. Autopsy revealed right dilatation of the heart with patent auricular septum and patent ductus arteriosus; the corpus callosum was absent. The paternal and maternal ages were 27 and 24 years, respectively. The mother had been taking oral contraceptives for 1 year, but had discontinued them because of harmful side effects. She became pregnant 13 months later.

Because some of the infant's features were suggestive of chromosomal trisomy, skin samples were obtained postmortem, and were cultured for chromosomal evaluation. Air-dried slides were prepared for analysis. The infant was found to be triploid with an XXY sex chromosome complement; there was no indication of mosaicism.

In order to trace the origin of the extra haploid set, we analyzed the chromosomes in the lymphocytes of both parents. No marker chromosomes could be identified with orcein staining. Preparations from all three subjects were stained with fluorochromes to reveal the banding patterns (*4*). Fluorescent variants in the human karyotype that we are now able to identify are bands located near the centromeres of chromosomes 3, 4, 13, and 22, as well as the satellites of Nos. 13, 14, 21, and 22.

The relevant chromosomes of the family studied are shown in Fig. 1. The father is heterozygous for the fluorescent variant on chromosome No. 3, one being brightly fluorescent and the other, negative. This band is also present on both maternal No. 3 chromosomes but they differ from each other in size and intensity, and both are smaller and weaker than the paternal positive band. Among the paternal group D chromosomes, the satellites of both No. 13 chromosomes are brightly fluorescent. In addition, each can be identified since one No. 13 has a brightly fluorescent band near the centromere, while the other has a weaker band in this area. Both No. 14 chromosomes have fluorescent satellites of equal intensity, but they are smaller and weaker than those of No. 13. The equivalent maternal chromosomes have no fluorescent markers. No distinctive variants could be identified on chromosome Nos. 4, 15, 21, or 22, in either parent.

In the triploid infant, the origins of chromosome Nos. 3, 13, and 14 can be identified. They are comprised of one maternal and two paternal chromosomes, an indication of fertilization of a single ovum by two sperms or a diploid sperm (diandry).

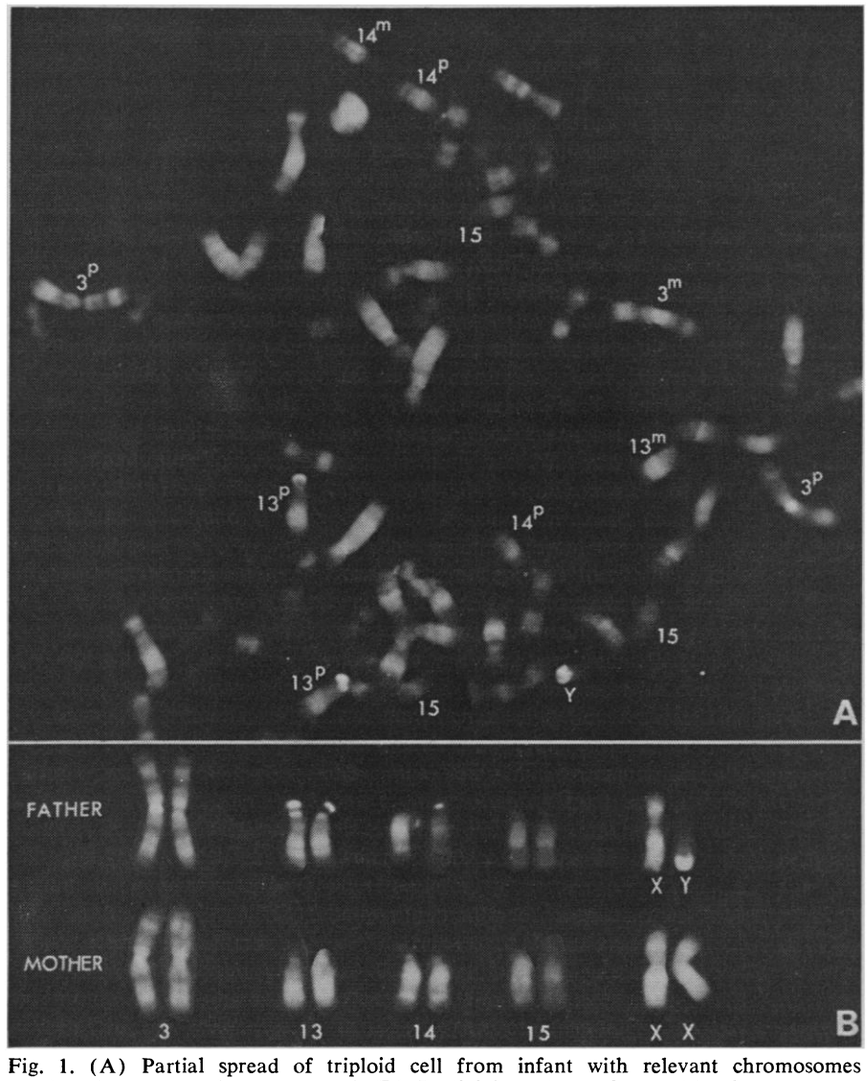

Fig. 1. (A) Partial spread of triploid cell from infant with relevant chromosomes numbered; *p*, paternal; *m*, maternal. (B) Partial karyotype of parents' cells.

Edwards *et al.* (*5*) have suggested that digyny (fertilization of a diploid ovum) is probably the more common mechanism involved in man and they present blood group data as evidence. Penrose and Delhanty (*6*) reported a triploid fetus to be derived from one sperm and an unreduced ovum; however, they present no real evidence for this interpretation.

Statistical analyses of sex chromosome distribution (*7*) support diandry because the reported ratio of 17 XXX : 30 XXY approximates the expected ratio 1 : 2. The very low frequency of XYY triploids (4 cases) suggests that strong selective forces may be at work if dispermy, rather than diploid sperm, is the more common mechanism. There is proof that polyspermy does occur in the rat (*1*). An investigation of the DNA content of sperm obtained from men attending a subfertility clinic (*8*) revealed a significant proportion of diploid sperm and indicated that these sperm could be a cause of triploid embryos.

In the case reported here, delayed fertilization may have allowed penetration of the ovum by more than one sperm. However, a period of 13 months without oral contraceptives appears to be sufficient time for recovery of normal function. In the three triplet sets, Nos. 3, 13, and XXY, where the two paternal homologs are different both are present in the triploid cells. This adds to the possibility that the paternal contribution was a diploid gamete formed by both genomes. When markers on more chromosomes are identified, it should be possible to determine which of the two mechanisms is more commonly involved.

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A Calcium Pump in Vascular Smooth Muscle

Abstract. *A microsomal cell fraction derived from the intimal-medial layer of rabbit aorta takes up calcium in the presence of magnesium and adenosine triphosphate. The rate of uptake of calcium is slower than that observed in skeletal muscle microsomes. Uptake of calcium by mitochondria from the aorta is even more limited and, unlike microsomal uptake, is inhibited by azide.*

The mode of regulation of vascular muscle tone is of medical importance. At the cellular level the agent that ultimately controls the mechanical activity of smooth muscle fibers is the calcium ion (*1*). Isolation of subcellular units that are involved in the transport of calcium into and out of the cytoplasm is useful for elucidating mechanisms by which vascular tone is regulated in health and disease. Two energy-dependent calcium sequestration systems have now been isolated from the smooth muscle of the rabbit aorta.

We prepared subcellular fractions of rabbit aorta by homogenizing approximately 1.5 g of the freshly excised vascular tissue in ten volumes of a cold (0 to 2°C) isotonic sucrose medium by means of a Potter homogenizer fitted with a Teflon pestle. We prepared the microsome fraction by centrifuging the homogenate at 1500*g* for 10 minutes, the supernatant at 27,000*g* for 10 minutes, and the new supernatant at 105,000*g* for 60 minutes. The final pellet was resuspended in 2 ml of isotonic sucrose for immediate use. Only fresh microsomal preparations were used. We prepared mitochondria by centrifuging the homogenate for 10 minutes at 1500*g* and the supernatant for 10 minutes at 9500*g*. This pellet was also resuspended in 2 ml of isotonic sucrose for immediate study.

Figure 1 shows an electron micrograph of the microsomal pellet. The fraction consists of vesicular structures enclosed, for the most part, by smooth membrane but with an occasional vesicle enclosed by rough endoplasmic reticulum. No intact mitochondria were encountered, and the preparation is free of succinate dehydrogenase, which served as a biochemical indicator of the presence of mitochondrial membranes.

Calcium uptake was studied at 37°C in a 3-ml incubation mixture containing tris(hydroxymethyl)aminomethane (tris-HCl, *p*H 7.4), 30 μmole; KCl, 300 μmole; the magnesium salt of adenosine triphosphate (Mg-ATP), 9 μmole;

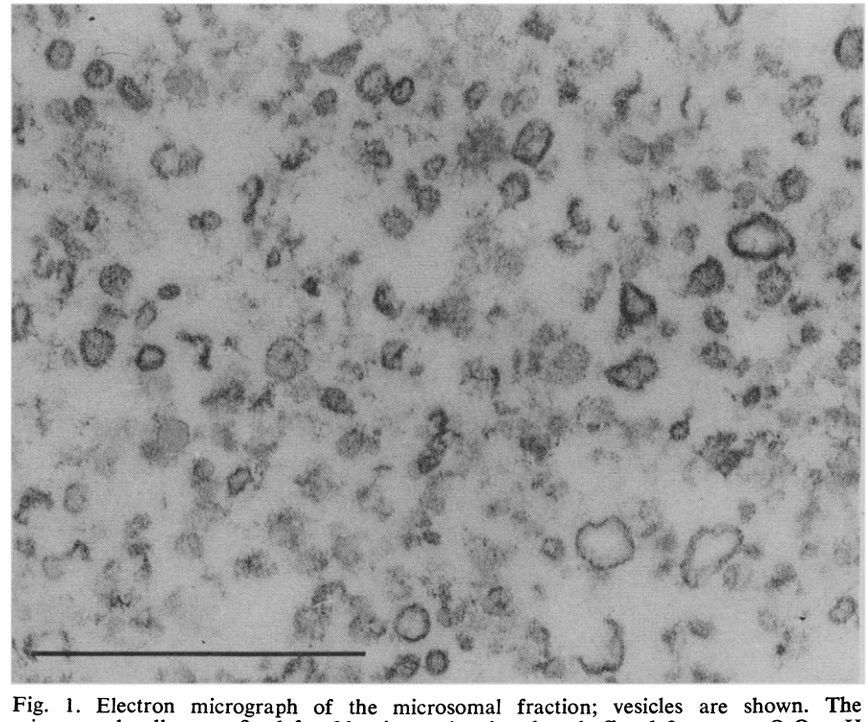

Fig. 1. Electron micrograph of the microsomal fraction; vesicles are shown. The microsomal pellet was fixed for 90 minutes in phosphate-buffered 2 percent O_8O_4, *p*H 7.5. The pellet was then dehydrated in ethanol and propylene oxide and embedded in araldite. The sections were stained in uranyl acetate and lead citrate. The scale marker is 0.5 μm.