that of the other group, which was long and smooth. It remained so until the animals approached adulthood; then both groups had smooth hair.

A large percentage of the oral dose was absorbed by the rabbits. The individual retention of Zn^{65} is shown in Fig. 2. The activity at 24 hours was considered 100 percent. The percentage of Zn^{65} retained by rabbits fed the low-zinc ration was 58 after 16 days. The rabbits fed the diet containing ZnO retained only 14 percent of the orally administered zinc after 16 days. This difference in retention indicates that the zinc in the low-zinc ration was not absorbed but that in the ZnO was available to the animal.

Our finding is in agreement with the observations that zinc-deficient animals retain a high percentage of administered Zn^{65} (*5*). The greater turnover of the Zn^{65} by the rabbits fed the ration supplemented with ZnO indicates that the animals fed the diet adequate in zinc absorb less Zn^{65} than do those fed the inadequate diet. Curves describing the retention of radioactive zinc have been established. Those describing animals fed small amounts of zinc, or zinc that is not available to the animal, have a flat slope, those describing animals fed medium rations have a medium slope, and those describing animals fed an excess of zinc have a very steep slope.

If the whole-body counting is to be used in obtaining an index of the deficiency in an element, the absorption of the element should be investigated. If the absorption is nearly normal, the shape of the retention curve will reflect, with marked accuracy, the amount of the element previously available in the diet.

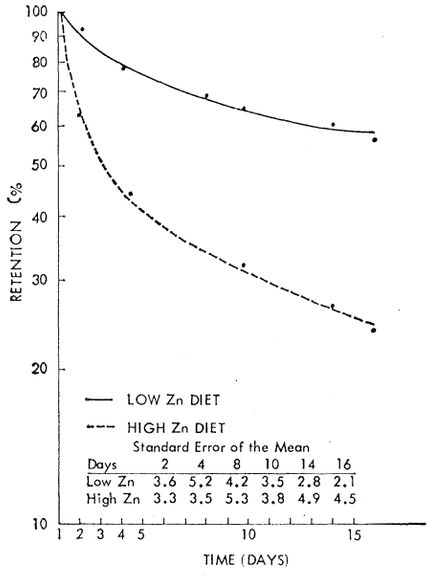

Fig. 2. Percentage of Zn^{65} retained, as a function of time.

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Autosomal Deletion Mapping in Man

Abstract. *Two families were observed in which morphologically similar deletions involving a G-group chromosome were associated in the propositi with conspicuous abnormalities in ossification of the skull. The findings suggest that specific genetic information relating to morphogenesis of the skull may be located on a G-group chromosome.*

Deletion mapping has been successfully exploited for the definition and localization of genetic elements in viruses (*1*), bacteria (*2*), and higher organisms (*3*). Study of the families reported below suggests that deletion mapping may also be useful for the chromosomal localization of autosomal genes in man (*4–6*).

The proband in the first family was a 16-year-old girl with pycnodysostosis. This condition is a rare, generalized bone disease which is usually inherited as a Mendelian recessive trait (*7*). The proband showed all of the phenotypic characteristics typical of this disorder, including short stature (138 cm), sclerotic bones with predisposition to fracture in the lower extremities (a history of 36 fractures was elicited in the present case), a double row of front teeth, hypoplasia of the ungual tufts, and hypoplasia of the mandible with flattening of the angle of the jaw. The clinical and biochemical findings have been described (*8*). A particularly striking feature of this and reported cases of pycnodysostosis is a dysplasia of the skull in which the cranial sutures did not close normally. This condition results in a large head, and not infrequently it has led to the erroneous diagnosis of hydrocephalus. In the present case, the anterior fontanel was still widely patent at the age of 16 years (Fig. 1A). The parents and three siblings were clinically normal, and no evidence of a similar bone disease has been found in any other relative. The parents were not consanguineous, their families having originated in different parts of the country. Karyotype studies of the proband revealed deletion of the short arm and satellite region of a G-group chromosome (Fig. 2A). An identical chromosome anomaly was found in the father, two siblings (Fig. 3A), the paternal grandfather, and a paternal uncle, all of whom were clinically normal. Genetic studies of this family permitted exclusion of the ABO, MNS, Rh, Kidd, Lutheran, and haptoglobin loci from the region involved in the deletion.

The probands in the second family were brothers (aged 5 and 3 years), who were referred for study because of the occurrence in both of craniostenosis. There was a history of absence of the "soft spot" in both children at birth. We confirmed by radiography the existence of premature closure of the sagittal suture, and the films also revealed the "beaten metal" appearance characteristically found in this condition (Fig. 1B). Except for moderate asymmetry of the skull, the children appeared to be physically and mentally normal. Chemical analysis of their blood, including calcium, phosphorus, alkaline phosphatase, acid phosphatase, total protein, and plasma amino acid determinations, gave no evidence of a generalized metabolic disorder. The mother had marked dolichocephaly, confirmed radiographically, which may well have resulted from early closure of the cranial sutures. There was no history of an abnormally shaped skull in her seven siblings, her parents, or any other family member. The father was clinically normal, and there was no history of bone disease or skull deformity in his family. There was no evidence of consanguinity.

Karyotype studies in both probands revealed G-group short-arm deletions which were morphologically indistinguishable from the one observed in family 1 (Fig. 2B). An identical chromosome anomaly was detected in the clinically normal father. Preliminary radioautographic studies suggest that the abnormal chromosome is an early replicator.

Nearly 30 years ago Haldane emphasized the importance of considering autosomal hemizygosity as a possible alternative form of recessive inheritance in man (*9*). He clearly predicted that, among diseases thought to be transmitted as Mendelian recessive traits, cases would be discovered in which full expression of the trait was the consequence of a single recessive gene in combination with deletion of the corresponding region in the homologous chromosome. This type of inheritance could and should be suspected, Haldane argued, in those cases of rare recessive traits in which there was no history of parental consanguinity. In the first family, full expression of a single recessive gene for pycnodysostosis located in the region of autosomal hemizygosity caused by the deletion is a plausible interpretation for the clinical findings in the proband. Alternatively, it might be assumed that the proband is an aneuploid segregant of a cryptic translocation carrier and that the findings in the proband result solely from aneuploidy.

The failure to observe miscarriage, abortion, or pycnodysostosis in any of the other ten offspring of the apparent deletion carriers in this family renders a cryptic translocation less likely. Nevertheless, the actual extent of the genetic material that has been lost or altered cannot be stated with certainty, since some or all of the missing short arm could have been inserted into another portion of the genome. The co-occurrence of the chromosome anomaly and a rare recessive trait could, of course, be a coincidence. A history of parental consanguinity would have favored this interpretation, but none was found. The presence of a deleted chromosome in many clinically normal members of this family raises the question of how frequently such variations may occur in the general population. Variation of the size of the short arm and of the satellite region of G-group chromosomes has been reported to constitute a chromosomal polymorphism (*10*). Apparent complete deletion of the short arm, however, was not among the variations encountered by Court-Brown, Jacobs, and Brunton in their study of 438 randomly chosen men and women (*11*), or by Summitt in his 50 normal controls (*12*). Consequently, the frequency of this anomaly among G-group chromosomes may be estimated as less than approximately .0005 or, at the 95 percent confidence level, as less than .002, when a Poisson distribution is assumed.

Other examples of morphologically similar G-group short-arm deletions have been reported (*13–16*), which have been shown to be familial in some instances (*15*, *16*). We are aware of no cases, however, that were not ascertained through an abnormal proband. Nevertheless, the question of what proportion of "recessive alleles" in man are actually minute deletions remains unsettled. This problem is of theoretical interest since deletion hemizygosity would appear to be one mechanism, showing a negative correlation with inbreeding, whereby recessive phenotypes may be fully expressed and acted upon by selection.

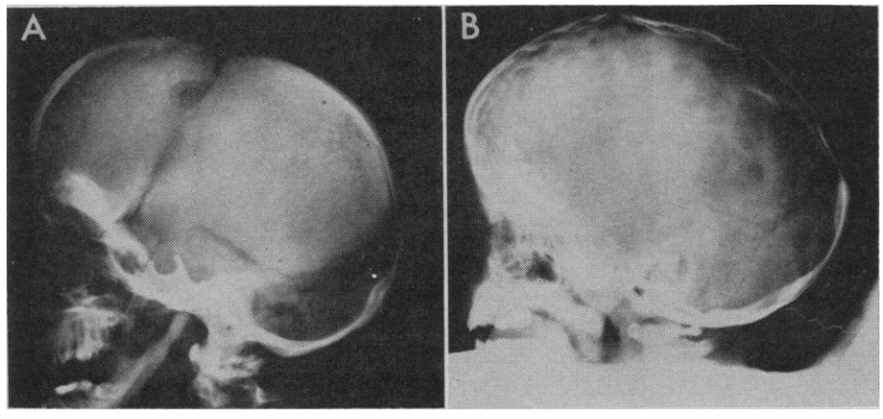

Fig. 1. Lateral x-rays of skulls of the proband in family 1 (A) and of one of the probands in family 2 (B), showing open sutures in the former and absence of the sagittal suture in the latter; in (B) the skull has a "beaten metal" appearance.

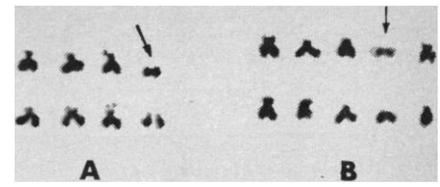

Fig. 2. Abstracts of two sets of G-group chromosomes from the proband in family 1 (A) and one of the probands in family 2 (B). Arrows indicate deletion chromosome.

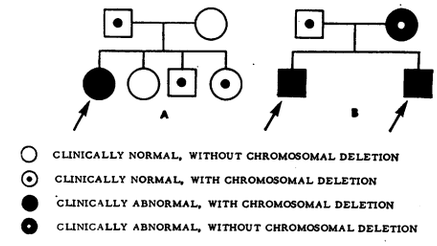

Fig. 3. Pedigrees showing transmission of deletion chromosomes in family 1 (A) and family 2 (B).

The mode of inheritance of craniostenosis is not as well established as that of pycnodysostosis. Familial cases have been described, however, suggesting a genetic etiology. Several reported kindreds show male-to-male transmission with affected members in two or even three successive generations, an indication of autosomal dominant inheritance. In other pedigrees, recessive inheritance is suggested by the occurrence of multiple affected siblings with normal parents (*17*). In family 2, the increased severity of the trait in the children could be explained by modification of the expression of a dominant gene in the presence of a deletion. It is striking that the only two families in which we have observed G-group short-arm deletions, among hundreds of families studied, were both ascertained through probands who showed conspicuous abnormalities in the ossification of the skull. Unless these associations are entirely fortuitous, it appears that specific genetic information relating to the morphogenesis of the skull is carried on the short arm of one (or both) of the G-group chromosomes, tentatively identified as G-22 in one case.

If closure of the cranial sutures is defined as the phenotype under consideration, analysis of these two families would suggest that dominance relationships (Fig. 4) may exist. Pycnodysostosis, the normal phenotype, and cranio-

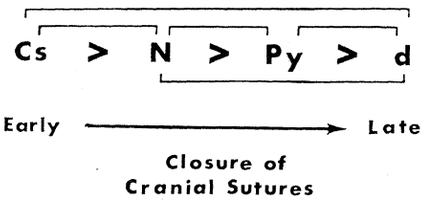

Fig. 4. Postulated dominance relationships of the genes for craniostenosis (*Cs*) and pycnodysostosis (*Py*), to their normal allelomorphs (*N*) and the deletion (*d*), with respect to closure of the cranial sutures. Brackets indicate genotypes that have been observed.

stenosis are all expressed when there is a deletion. Pycnodysostosis is recessive to the normal phenotype, however, while craniostenosis appears to be dominant to it. It is not known whether the genes for pycnodysostosis and craniostenosis are alleles, members of a pseudoallelic series, or representatives of independent loci on the short arm of the same or different G-group chromosomes. Contemporary hypotheses concerning regulatory mutations (*18*), polarity (*19*), subunit interactions (*20*), allosteric effects (*21*), and genetic duplication (*22*) provide a wealth of possible mechanisms by which closely linked mutations might have antithetical effects. In the absence of more detailed knowledge about the primary actions of the genes in question, our crude attempt at deletion mapping does not permit us to distinguish between these possibilities.

It should be emphasized that craniostenosis is in all probability a polygenic trait. Craniostenosis has, for example, been observed in other cytogenetically distinct aneuploid states (*23*). However, our observations suggest that at least one gene locus which influences this trait is present on the short arm of a G-group chromosome, and they provide additional support to existing evidence that specific genetic information affecting bone formation is carried on a G-group chromosome (*14, 15, 24*).

Autosomal deletions in man may be the cause of more or less characteristic clinical syndromes, as in the case of the B, G, and E partial monosomes. Of perhaps greater genetic significance, however, are those deletions which are in themselves compatible with a normal phenotype, but which may permit or alter the expression of genes carried on the intact homolog. In informative families, deletions of this type may permit the chromosomal localization of rare autosomal genes, as is suggested by our cases. In addition, deletions provide an opportunity to map common genetic markers. Thus, any gene transmitted by a deletion carrier along with the deletion chromosome cannot be located in the region of the deletion; similarly, any locus at which a deletion carrier is heterozygous for a co-dominant allele cannot be so located; finally, in situations where the protein product of a gene can be studied, dosage effects in deletion heterozygotes may reasonably be anticipated. By these criteria, genetic studies in the present families have permitted the exclusion of the ABO, MNS, Rh, Kidd, Lutheran, haptoglobin, hemoglobin, and lactate dehydrogenase loci from the regions involved by the deletions. It is apparent that the indications for karyotype studies should be broadened to include instances of rare recessive traits in which there is no evidence of parental consanguinity. As noted by Haldane, autosomal hemizygosity should be considered a possible alternative to homoallelism in cases of this type.

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5 December 1966

Tetrahymena: Effect of Freezing and Subsequent Thawing on Breeding Performance

Abstract. Tetrahymena pyriformis, *syngen 1, frozen in 10 percent dimethylsulfoxide, stored for 2 months, and then thawed could conjugate normally. More significantly, they were viable, in normal numbers, through two sexual reorganizations. The strains apparently did not sustain genetic damage during the treatment. The techniques offer considerable promise for the maintenance of breeding stocks in ciliated protozoans.*

Stocks of *Tetrahymena pyriformis*, grown in various ways, gradually lose their ability to produce viable progeny at conjugation, perhaps because of accumulated micronuclear defects (*1*). Also associated with defective micronuclei is the phenomenon of genomic exclusion (*2*), in which the entire genome of one parent is lost at conjugation. Strains of *Tetrahymena* that have been maintained in laboratories by vegetative growth and many found in natural habitats are amicronucleate and incapable of sexual reorganization (*3*). Breeding stocks must be carefully selected and revitalized constantly by the time-consuming process of crossing and then testing the progeny of a new generation at least once a year. This procedure limits the number of stocks that can be maintained and restricts genetic studies on these forms.

The possibility of storing breeding stocks of *Tetrahymena* in the frozen state is intriguing since this process might eliminate the need for continued inbreeding. Viability has been maintained in four sterile strains and one genetically active strain of these ciliated protozoans frozen in 10 percent dimethylsulfoxide and subsequently stored at temperatures below −170°C (*4*). We have tested whether breeding performance of two strains of syngen 1