The long, flexible neck which makes it possible for a lophophore to bend away from chimneys in cheilostomes is essentially absent in modern stenolaemates, in which the lophophore seldom protrudes far from the skeletal aperture (*8*). This suggests that if chimneys analogous to those in *Membranipora* exist in stenolaemates, their location would be reflected in some modification of the skeleton.

We propose that monticules in fossil stenolaemate Bryozoa are skeletal reflections of areas in which functional lophophores were absent or modified so as not to resist the excurrent flow of water. This hypothesis would explain a number of observations. (i) The consistency of distances between monticules would be expected if the number of cilia per unit area of colony surface is approximately equal among species possessing monticules. (ii) A rhombic arrangement of monticules would be expected, since water movement would be most facilitated by having lophophores located no farther than some relatively constant distance from a chimney. (iii) The sizes of monticules would be expected to be roughly consistent if assumption (i) is correct. Too big a chimney would take up too much space that could otherwise be occupied by a feeding lophophore; too small a chimney would reduce feeding efficiency. (iv) The heterogeneity of structures comprising monticules among taxa of fossil stenolaemates can be explained because monticules could be comprised of a variety of structures, depending on the evolutionary backgrounds of the taxa. For chimneys to be functional the only requirement is that no zoids effectively resist the excurrent water stream. (v) Monticules would be expected to develop only in the exozone where functional polypides were present. (vi) One would expect monticules to develop some distance from a growing edge, where lophophores were not yet functional and water could escape. Delicate colonies would therefore lack monticules.

Many Recent and fossil cheilostomes with robust colonies possess regularly distributed mammillate areas which resemble monticules in gross appearance. Perhaps such structures represent skeletal reflections of chimneys. One genus with many such protuberances, *Hippoporidra*, was studied by Cook (*10*), who reported that in some species the areas contain specialized zoids (called "cortical zoids") with reduced apertures. In *H. senegambiensis*, cortical zoids possess only half the usual number of tentacles and lack cilia altogether. This suggests that protuberances are areas in which excurrent water flow is not resisted by functional lophophores. In view of later research (*11*), it seems likely that such zoids are males which release sperm into the water to be carried to other colonies for fertilization. If the protuberances represent chimneys, male zoids would be in a suitable location for sperm dispersal.

The hypothesis proposed here does not necessarily exclude previous theories. In some species, for example, monticules may have been brooding or budding regions and still represent chimneys.

The hypothesis suggests similar explanations for some Bryozoa with unusual colony forms. Colonies of *Scalaripora, Glyptopora, Evactinopora, Radiofascigera, Centronia* (*12*), and others have regularly arranged regions which apparently lacked functional autozoids in life. These regions may represent avenues for excurrent water, and it may be that providing for efficient water circulation has had an important effect on colony form in Bryozoa.

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5. Photographs of 96 longitudinal thin sections of about 80 species of Paleozoic stenolaemate Bryozoa exhibiting recognizable monticules were examined at random. Eleven specimens (11.4 percent) exhibited budding in monticular regions and were counted as supporting the theory of Anstey and Delmet (*4*) even if budding was common elsewhere in the section.
6. The distance between monticules (2 to 3 mm) was more nearly constant among Ordovician Trepostomata than among any of 20 other variates measured in 24 species [F. McKinney, *Bull. Am. Paleontol.* **60**, 218 (1971)]. We measured numerous specimens from 20 species of cystoporates and cryptostomes chosen at random from the reference collections at the Smithsonian Institution, Washington, D.C. Monticule diameter is difficult to measure because monticules ordinarily blend into the colony surface without a definite border. Within the limits of precision of measurement (± 0.2 mm), monticule diameter in any one species is constant; the diameter varies from 1 to 2 mm among taxa in Cystoporata and Cryptostomata.
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Computer-Assisted Analysis of Chromosomal Abnormalities: Detection of a Deletion in Aniridia/Wilms' Tumor Syndrome

Abstract. *A chromosome translocation, t(8p+;11q−), in a patient with aniridia and Wilms' tumor, appeared balanced by standard techniques, including trypsin banding. Computer analysis of optical microscope scanning profiles of chromosome pairs 8 and 11 revealed an interstitial deletion of the short arm of 8. Computer analysis coupled to the new banding techniques provides greater resolution for the detection of subtle chromosomal variations not recognized by banding methods alone.*

Newly developed chromosomal banding techniques not only permit ready identification of all human metaphase chromosomes, but also assist in the detection of complex chromosomal rearrangements (*1–4*). However, variation in the state of chromosome contraction (or elongation) within and among metaphase preparations may obscure subtle translocations or deletions when they are analyzed by visual scanning alone. Neurath and colleagues (*5*) have developed a high-resolution microscope scanning system coupled

to a computer which is programmed to analyze and compare size, area, and banding patterns of human metaphase chromosomes. An initial study of banded chromosomes from normal individuals, including monozygotic twins, indicates that these measurements appear to have a genetic basis and may serve as useful genetic markers (*5*). This report describes a child with severe sporadic aniridia and Wilms' tumor who was discovered to have a chromosomal translocation, t(8p+;11q−), which appeared balanced by routine techniques. Further study included computer analysis of trypsin banded preparations and permitted detection of a small deletion of the short arm of chromosome 8. This study illustrates the value of computer analysis of human metaphase chromosomes as a means of detecting subtle chromosomal anomalies unrecognized by methods currently in common use.

T.M. (MGH 167-19-15) was born with bilateral aniridia and was found to have bilateral glaucoma, megacorneas, and anterior polar cataracts shortly thereafter. No other malformations were apparent except for partial malrotation of the right kidney, as shown by an intravenous pyelogram. The child was reexamined at 2- to 3-month intervals. Abdominal x-ray at 14 months was considered normal, but during an admission at 19 months the abdomen appeared prominent and a right-sided mass was palpated. A unilateral Wilms' tumor was removed without complications, and chemotherapy was instituted. The child is now 34 months of age, and, although motor development has been normal, speech has been severely delayed. Family history and examination of parents and siblings revealed no similarly affected individuals.

Initial standard metaphase spreads prepared prior to chemotherapy revealed a translocation, which appeared to be balanced, of a portion of the long arm of chromosome 11 to the short arm of chromosome 8. Subsequent study of peripheral leukocyte and skin fibroblast cultures with a modification of the trypsin banding technique of Seabright (*6*) further suggested that a portion of the short arm of chromosome 8 remained unaccounted for, but the exact segment could not be determined by visual inspection (Fig. 1).

Ten high-quality trypsin banded metaphase preparations of chromosomes 8 and 11 were scanned optically by the PIQANT system (*5*) which is connected to a general-purpose computer

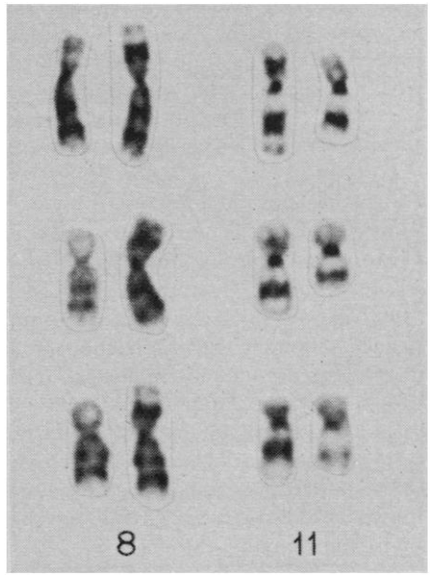

Fig. 1. Trypsin-Giemsa treated chromosome pairs 8 and 11.

programmed to measure and compare chromosome pairs (*5, 7*). The averaged computer-optical scanning profiles of the normal and abnormal trypsin banded chromosomes 8 and 11 are represented in Fig. 2. Total length of the chromosomes studied was normalized to 200 units with each unit equal to approximately 0.14 μm in a typical metaphase (*7*). Relative lengths of chromosomes 8 and 11 are summarized

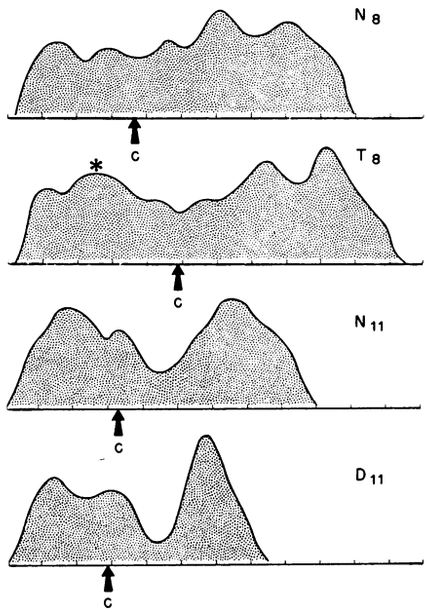

Fig. 2. Computer profiles of optical scans of normal (*N*), translocated (*T*), and deleted (*D*) chromosomes 8 and 11. Note the additional "hump" in the short arm of T8 (represented by asterisk), which is the interstitial deletion of the long arm of D_{11}. Location of centromere is denoted by arrow *C*.

in (*8*). Inherent variability of the state of chromosome contraction and banding is normalized by the computer, so that the values for length of chromosome pairs and banding peaks are equalized from cell to cell. Thus, the deleted 11 is 11.96 units shorter than the normal 11, and the abnormal 8 only 4.02 units longer than the normal 8. The sum of the abnormal chromosomes 8 and 11 is 7.94 units less than the combined lengths of the normals, indicating a loss of 14.6 percent of chromosome 8. No significant differences in the measurements of the parental chromosome 8 homologs were found to account for this discrepancy. Inspection of the optical profiles and comparison of bands by superimposition supports as a possible explanation of this complex translocation and deletion a four-break interstitial deletion and translocation event (see Fig. 3). Breaks in 11q apparently occurred at bands 11q21 and 11q24. The distal portion of 11q reattached to the more proximal 11q21 break point, resulting in the abnormal 11, designated del 11 (11pter→11q21::11q24→11qter) (*3*). Breaks in 8p apparently occurred at two points, one break between bands 8p12 and 8p21 and one in band 8p22. Intercalation of the inverted 11 (:11q21 →11q24:) segment occurred in 8p, resulting in 8(8pter→8p22::11q24→ 11q21::8p12 or 8p21→8qter). Segment 8(:8p12→8p22:) appears to be deleted. Therefore this karyotype shows an unbalanced interstitial translocation with monosomy for a portion of 8p.

All 250 cells studied in leukocyte and skin fibroblast cultures contained the unbalanced karyotype. Both parents and five siblings had balanced karyotypes.

We know of no previous report of monosomy for a portion of the short arm of 8. Niebuhr (*9*) reported a familial translocation, t(6p+;8q−), resulting in multiple spontaneous abortions, presumably because of the lethality of monosomy for a large portion of the long arm of 8 in the unbalanced fetus. In Niebuhr's case, approximately 90 percent of the long arm was missing in the unbalanced state. In our case approximately one-third of the short arm of 8 is missing, which accounts at most for a loss of about 15 percent of the entire chromosome.

Epidemiological studies have established that the association of aniridia and Wilms' tumor occurs much more frequently than expected by chance alone (*10*). Thus Miller *et al.* (*10*)

studied 440 cases of Wilms' tumor and found aniridia in 1 : 73, in comparison to the occurrence of aniridia in the general population of about 1 : 50,000. Aniridia alone is known to be transmitted as an autosomal dominant trait (*11*). A similar genetic causation is suspected for isolated Wilms' tumor from reports of twin concordance and sib aggregation (*10*). Characteristically the aniridia associated with Wilms' tumor is almost always sporadic and very severe, often accompanied by glaucoma and cataracts (*12*). Fraumeni and Glass (*12*) estimated that about 33 percent of patients with severe sporadic aniridia developed Wilms' tumor within the first year of life. In a detailed statistical analysis of cases of Wilms' tumor, Knudson and Strong (*13*) concluded that the association of aniridia and Wilms' tumor fits a mutational model essentially identical to isolated inherited Wilms' tumor. According to their analysis, "the mutation causing aniridia could be an unusual aniridic allele which also results in Wilms' tumor or . . . the mutation could be a chromosomal abnormality involving more than one genetic locus—one locus relating to aniridia and the other to Wilms' tumor." The question posed by our case is, does the defined chromosomal mutation, partial deletion of 8p, represent a unique biological event supporting the latter hypothesis? One can only speculate that certain critical genetic loci are located within or near this particular segment of 8p, namely, segment 8(:8p12→8p22). Further chromosome banding studies in patients with Wilms' tumor with and without aniridia will be essential before linkage can be suggested. Unfortunately, at the present time lack of linkage assignments to chromosome 8 prevents further testing of this possible association. Thus it is possible that the occurrence of the chromosome abnormality in this one patient may be completely coincidental and have no relationship to the abnormal phenotype.

Although chromosome banding has greatly extended our ability to detect chromosome anomalies unrecognized by older methods, this case of aniridia and Wilms' tumor stresses the importance of the need for further precision in our analysis. The combined approach of banding and computer-assisted scanning adds yet a new dimension in human chromosome analysis. Application of these analytic tools to the large group of patients with malformations and no abnormality on standard analysis is expected to reveal new defects. Furthermore, the sensitivity of this system to detect variation in chromosome banding profiles now permits for the first time the means to search for familial banding polymorphisms which may prove useful in genetic counseling and prenatal diagnosis (*5*).

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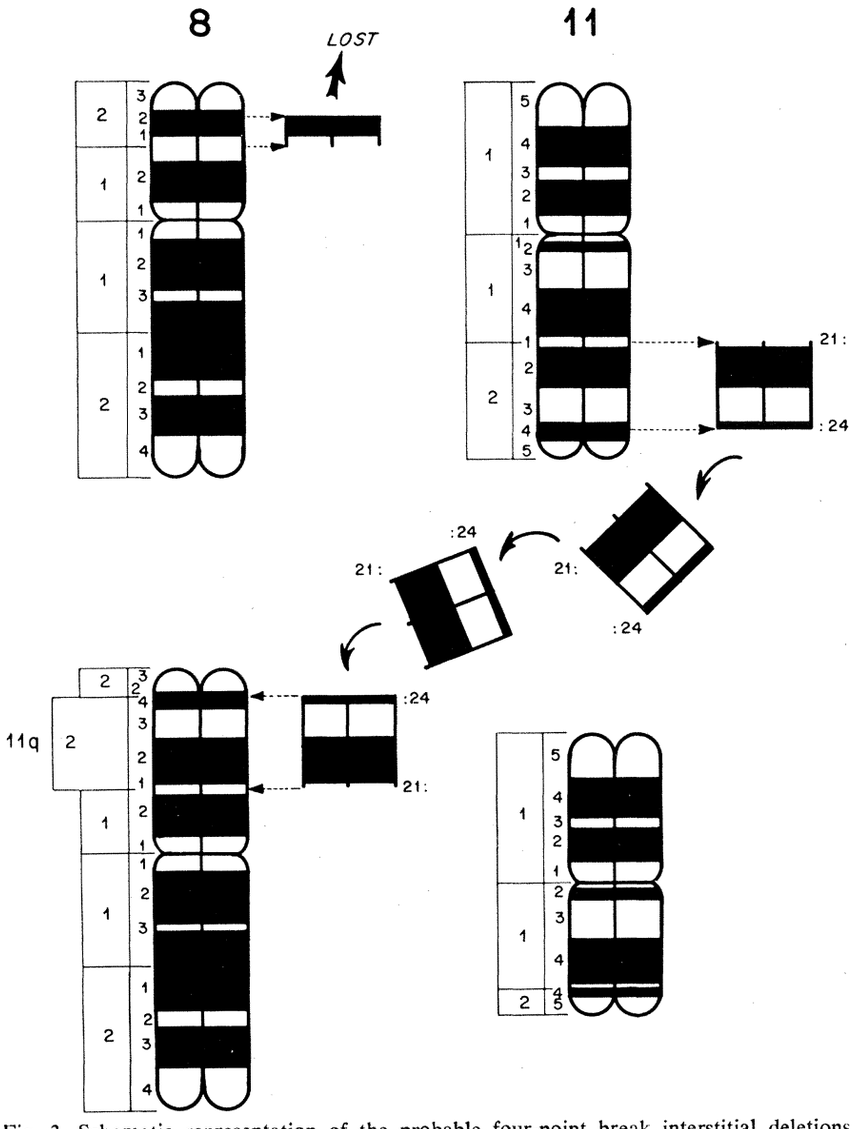

Fig. 3. Schematic representation of the probable four-point break interstitial deletions and insertion between chromosomes 8 and 11. Based on Paris Conference Standardization of Chromosome Nomenclature (*3*).

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7. Chromosomes are optically scanned from 35-mm negatives of metaphase spreads and are converted into a magnified digital image representing length and density variation per unit area. Digital images are then analyzed by the computer. Variation of homologs in each metaphase spread is computed and the variations are compared to one another and to the grand mean variation derived from a large sample population. The PIQUANT computer system is arbitrarily divided into 200 units for statistical analysis. Each unit by convention equal 0.14 μm and therefore represents the first-order resolving power of the system. Additional resolution is determined

by the number of chromosomes compared. For instance, small variation between 2 chromosomes or 2 pairs of chromosomes normalized to 200 units would be more easily detected than variation among 23 pairs normalized to 200 units. Sensitivity to variation therefore increases as the total amount of the genome to be analyzed decreases. Details of the statistical methods are given in previous reports (*5*). For chromosome 1, Selles, Marimuthu, and Neurath (*5*) determined that a variation of 2.3 percent from the grand mean would be significant with $P < .05$. The deleted segment in our case in comparison represents approximately a four- to sixfold greater variation than that ultimately detectable.

8. Chromosome lengths are given in units defined as 0.14 μm per unit: normal 8(N_8), 54.14 units; translocated 8(T_8), 58.16 units; normal 11(N_{11}), 49.83 units; deleted 11(D_{11}), 37.87 units. $(N_8 + N_{11}) - (T_8 + D_{11}) = 7.94$ units difference. Measurements represent 20 scans for each chromosome pair (40 separate chromosomes).

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Toxic Polyneuropathy Produced by Methyl N-Butyl Ketone

Abstract. *A polyneuropathy affecting a large number of workers was recently observed at a plant producing plastic-coated and color-printed fabrics. Epidemiological data suggested strongly that methyl N-butyl ketone (MBK) was responsible for the outbreak. This hypothesis is now supported by the development of a peripheral neuropathy in chickens, rats, and cats exposed to MBK at atmospheric concentrations of 200 to 600 parts per million, 24 hours per day, 7 days per week. Although the animals were exposed continuously and the affected workers were exposed intermittently, the averages of the total number of hours of exposure for development of the peripheral neuropathy in the animals and workers were remarkably close.*

In June 1973, one worker from a plant producing plastic-coated and color-printed fabrics was examined at Ohio State University Hospital and found to have a polyneuropathy. Extensive screening of 1161 employees from that plant confirmed that there were 79 persons with clinical evidence of polyneuropathy and 182 persons with abnormal electrodiagnostic examinations (*1*). In all cases the onset of the disease was insidious. In severely affected individuals, symmetrical distal muscle weakness and loss of deep tendon reflexes were present in upper and lower extremities. Sensory impairment consisted of loss of sensitivity to superficial pain, temperature change, and light touch and vibration stimuli in toes, feet, and fingers. Electromyographic (EMG) findings consisted regularly of prolonged insertional activity, numerous positive waves, and fibrillations. Moderate slowing of nerve conduction velocities (NCV) were frequently observed. Workers with clinical peripheral neuropathy were found to have been regularly exposed to methyl *N*-butyl ketone (MBK) used as a dye solvent and cleaning agent. This solvent was introduced into the plant in August 1972, and reached full use by December 1972. No cases of polyneuropathy appeared prior to December 1972. Attack rates were highest in the work area with greatest usage of this solvent and were also correlated with increasing hours of exposure. These data suggested that MBK was related to the development of this polyneuropathy. On this basis laboratory studies were designed to simulate the environmental conditions of the affected workers by exposing animals to an atmosphere containing MBK at a concentration of 200 to 600 ppm, 24 hours per day, for 7 days per week. The results of these studies have demonstrated that several species of animals exposed to MBK under these conditions consistently developed a peripheral neuropathy as judged by clinical, physiological, and morphological criteria.

Animals used in our study included five domestic chickens, four Sprague-Dawley rats, and four domestic cats. They were held in environmental chambers with a volume close to 1000 liters. Delivery of room air at approximately 200 liter/min through the chamber maintained a normal environment with respect to O_2 (21 percent), CO_2 (less than 0.2 percent), relative humidity (less than 60 percent), and temperature (within 1° and 2°C of the normal 21° to 25°C). Solvent vapor was added to the chamber by diverting a metered portion of the airflow through the headspace of a container of MBK before it entered the chamber. Concentrations of MBK monitored by gas chromatography were initially at 200 ppm for chickens and 600 ppm for rats and cats. Food and water intake and body weights were measured regularly, and the MBK concentration was adjusted to 100 and 400 ppm, respectively, to minimize complications from inanition and weight loss. Pair-fed controls were killed when the exposed animals were killed.

To date we have observed the development of a peripheral neuropathy in all rats, cats, and chickens tested. The earliest development of overt clinical signs occurred at 4 to 5 weeks in chickens that showed an inability to stand on their legs. The cats were next to develop clinical weakness, as manifested at 5 to 8 weeks by dragging of their hind limbs and later by forelimb

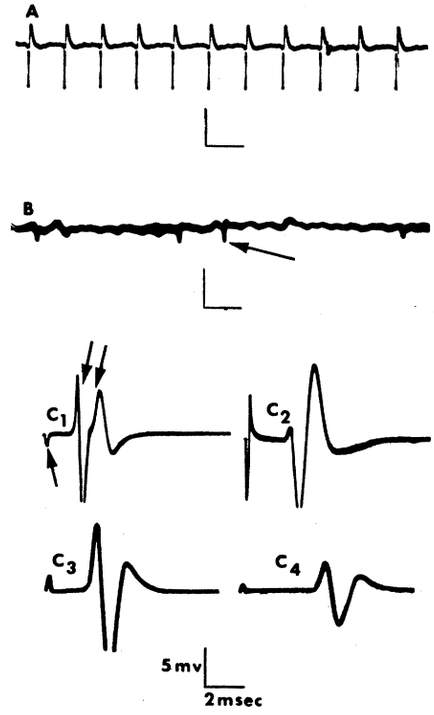

Fig 1. (A) Train of positive waves in anterior tibialis after 5 weeks of MBK exposure. Vertical scale, 200 μv; horizontal scale, 10 msec. (B) Fibrillation potentials (arrow) in gastrocnemius after 9 weeks of MBK exposure. Vertical scale, 200 μv; horizontal scale, 5 msec. (C_1) Control recording showing stimulus artifact (arrow) and recorded footpad muscle action potential (arrows) from stimulation of ulnar nerve (wrist to footpad); (C_2) control recording from elbow joint to footpad; (C_3) prolonged latency between stimulus and recorded muscle action potential from ulnar nerve stimulation (wrist to footpad); and (C_4) prolonged latency of ulnar nerve conduction from elbow joint to footpad.