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Charcot-Marie-Tooth Disease Type 1A: Molecular Mechanisms of Gene Dosage and Point Mutation Underlying a Common Inherited Peripheral Neuropathy

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INTRODUCTION

In the search for the molecular bases of neurological diseases, the studies on Charcot-Marie-Tooth disease (Charcot and Marie, 1886; Tooth, 1886) have been extremely informative. With an estimated frequency of 1 in 2500 (Skre, 1974), CMT is the most common inherited peripheral neuropathy. Molecular investigation of Charcot-Marie-Tooth disease type 1A (CMT1A) which is the major form of CMT, has delineated a novel mechanism responsible for autosomal dominantly inherited human disease. Furthermore, these findings have provided valuable insights into understanding the molecular mechanisms underlying other inherited peripheral neuropathies besides CMT1A.

Charcot-Marie-Tooth polyneuropathy syndrome is a clinically heterogenous disorder of the peripheral nerves which is characterized by slowly progressive weakness and atrophy of the distal muscles (Charcot and Marie, 1886; Tooth, 1886). CMT falls under the classification of hereditary motor and sensory neuropathy (HMSN I,II, and III) (Dyck et al.1992). The onset of clinical symptoms in CMT occurs at a mean age of 12.2 ± 7.3 years (Bird and Kraft, 1978). The intrinsic muscles of the foot are usually affected first, resulting in gait abnormalities and foot deformities (Lupski et al.1991a; Dyck et al.1992). Dropped foot usually occurs due to dorsiflexor weakness of the peroneal and tibial muscles, and pes cavus deformity may occur progressively with age. Atrophy of the distal legs may be a prominent feature in some patients. The loss of deep tendon reflexes occurs early in the course of the disease, and variable progressive weakness of the hand muscles may occur in the later stages. The involvement of sensory nerves is rare, although decreased pain to pinpricking in a stocking distribution is seen in some patients (Lupski et al.1991a; Dyck et al.1992; Vance, 1991). However, the severity of CMT clinical symptons may vary considerably. Differences have been noted among affected individuals within the same family (Kaku et al.1993b), and even between identical twins with CMT (Garcia et al. 1990).

Two forms of the disease can be distinguished on the basis of electrophysiologic and histologic findings. CMT type 1 (CMT1 or HMSNI) exhibits abnormally decreased motor

nerve conduction velocity (NCV below 40 meters/second) that is symmetric from nerve to nerve, and between nerve segments, with no evidence of conduction block (Kaku et al.1993a). This suggests an intrinsic defect in myelination, which is borne out by findings on peripheral nerve biopsy (Kaku et al.1993a; Lupski and Garcia, 1992). There is consistent increase in the fascicular area of the nerve, marked decrease in the number of myelinated fibers, and frequent onion bulb formations composed of concentric Schwann cell processes surrounding myelinated and demyelinated internodes (Lupski and Garcia, 1992). On the other hand, the neuronal form CMT type 2 (CMT2) is characterized electrophysiologically by normal or near-normal NCV (Lupski et al.1991a; Dyck et al.1992) and is not associated with hypertrophic demyelinating neuropathy (Lupski and Garcia, 1992). The clinically similar cases of CMT1 and CMT2 are distinguishable by the fully penetrant and essentially age-independent phenotype of uniformly reduced motor NCV, which serves as a major diagnostic criterion for CMT1 (Lupski et al.1991a; Vance, 1991; Nicholson, 1991).

Genetic heterogeneity is evident in CMT1 which demonstrates autosomal dominant, autosomal recessive, and X-linked inheritance, as well as sporadic cases (McKusick, 1992).

The most common subtype is CMT1A which shows autosomal dominant inheritance, and whose locus demonstrates linkage to DNA markers on chromosome 17p11.2-pl2 (McKusick, 1992; Vance et al.l989). The locus for autosomal dominant CMTlB shows linkage to DNA markers on chromosome lq21.2-q23 (Bird et al. 1982), while CMT1C likely represents a third autosomal dominant locus which remains to be mapped (Chance et al.1990; Chance et al.1992b). The focus of this article is on CMT1A, and the underlying molecular mechanisms that give rise to this predominant form of Charcot-Marie-Tooth disease.

CMT1A IS ASSOCIATED WITH AN INHERITED DNA DUPLICATION

We have shown that a DNA duplication in the chromosome 17p11.2-p12 region is associated with CMT1A, which provided an unprecedented example of a stable DNA rearrangement that cosegregates with a genetic disorder in an autosomal dominant manner (Lupski et al.1991b). The DNA duplication in CMT1A was independently identified in European families by Van Broeckhoven and colleagues, who reported the first case of a *de novo* duplication that arose simultaneously with the onset of disease, providing additional evidence that the duplication is a cause of CMT1A (Raeymaekers et al.1991). Additional cases of new mutation CMT1A duplication have since been reported (Wise et al.1993), including one study which showed that de *novo* CMT1A duplications accounted for nine out of ten cases of sporadic CMT1A (Hoogendijk et al.1992).

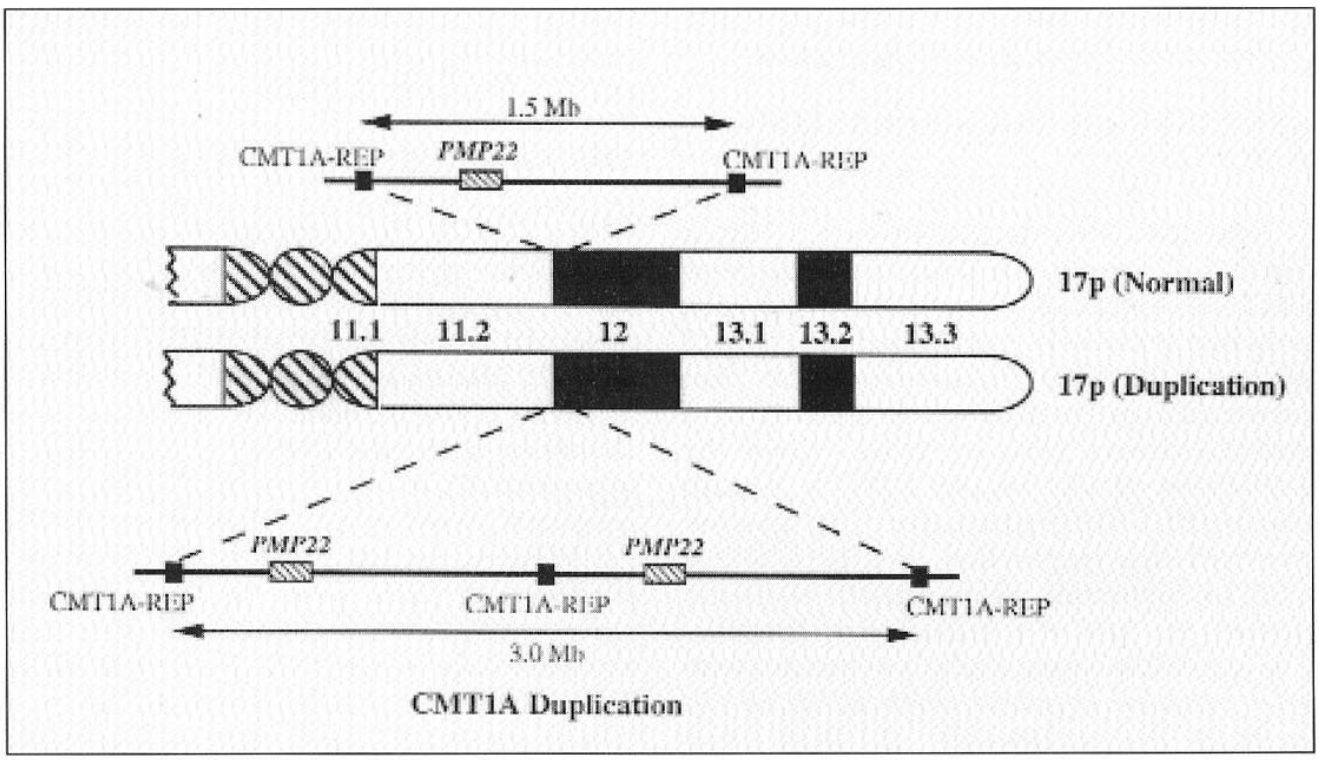
We have demonstrated through a number of molecular methods the presence of the CMT1A duplication (Lupski et al.1991b), which is not detectable by cytogenetics (Lupski et al. 1993). The analysis of short tandem repeats that map within the duplicated region provided one of the first indications for the duplication. Highly polymorphic GT_n repeat alleles at locus VAW409 (D17S122) were analyzed by the polymerase chain reaction (PCR) wherein three alleles were seen in CMT1A patients compared to the usual two alleles in normal individuals. Southern analysis using markers VAW409R1 and VAW409R3 (D17S122), VAW412R3 (D17S25), and EW401 (D17S61) that map within the duplicated region usually showed two alleles with dosage differences in CMT1A patients, which is indicative of duplication. Pulsed-field gel electrophoresis (PFGE) of patient DNA followed by Southern hybridization to probe VAW409R3 (D17S122) identified a novel 500-kb *Sac*II junction fragment that is specific to the CMT1A duplication (Lupski et al.1991b). The most graphic detection of the CMT1A duplication was provided by two-color fluorescence in situ hybridization (FISH). Probes mapping within and outside of the CMT1A duplication were hybridized to interphase nuclei prepared from lymphoblasts, and fluorescent detection revealed three dots in the case of the CMT1A duplication versus two dots in the normal case. Thus, we have molecularly identified the CMT1A duplication

by multiple independent methods (Lupski et al.1991b). We have further shown that failure to account for the duplication in linkage analysis can lead to incorrect localization of the disease locus for CMT1A (Lupski et al.1991b).

The findings on the CMT1A duplication have since been confirmed by studies on patients from different ethnic backgrounds including British (Hallam et al.1992), French (Brice et al.1992), Italian (Bellone et al.1992), U.S. ((Lupski et al.1991b, Chance et al.1992b) and Welsh (MacMillan et al.1992) populations. The CMT1A duplication has been found in approximately 70% of CMT1 patients in our recent study (Wise et al.1993). A separate study has estimated the frequency of the CMT1A duplication at about 85% of CMT1 patients (Van Broeckhoven, personal communication). A thorough molecular analysis of 63 unrelated CMT1 patients from different ethnic groups revealed the same-sized junction fragments in patients with the CMT1A duplication (Wise et al.1993). Although the possibility remains that different-sized DNA duplications may be associated with some cases of CMT1A, the majority of examined cases clearly involve CMT1A duplications of the same size as determined by PFGE (Wise et al.1993). This suggests that a structural feature of the human genome at the chromosome 17p11.2-pl2 region may provide an intrinsic mechanism for the repeated occurrence of the CMT1A duplication.

We have demonstrated by physical mapping that the CMT1A duplication of approximately 3.0-Mb consists of a tandem duplication of a 1.5-Mb region which is flanked by large homologous sequences which we referred to as CMT1A-REP (Pentao et al.1992). A schematic diagram of the CMT1A duplication region is shown in Figure 1, which depicts the structure of the normal, and the mutated chromosome 17p region containing the segmental CMT1A duplication in

FIGURE 1. The CMT1A duplication is a submicroscopic tandem duplication of a 1.5-Mb DNA region on chromosome 17p11.2-pl2 (Pentao et al. 1992; Lupski et al. 1993). The regions on chromosome 17p are depicted, with the normal chromosome shown above and the segmentally duplicated chromosome below. The cross-hatched circles represent the centromere. The normal chromosome 17p contains a 1.5-Mb region on 17p11.2-p12 flanked by homologous CMT1A-REP sequences (filled boxes)(Pentao et al.1992) of approximately 30 kb in length (our unpublished observations). The PMP22 gene (cross-hatched boxes) maps within this 1.5-Mb critical region, which is duplicated in most patients with CMT1A (lower figure). The 3.0-Mb CMT1A duplication is not detected cytogenetically, and results in two copies of PMP22 and three copies of CMT1A-REP on the duplicated chromosome (Patel et al.l992; Pentao et al.1992).



17p11.2-pl2. The CMT1A-REP sequences of approximately 30 kb (our unpublished observations) which flank the 1.5-Mb critical region appear to mediate homologous recombination. A mechanism has been proposed wherein misalignment occurring at proximal and distal CMT1A-REP sequences during meiosis, followed by unequal crossing, lead to the CMT1A duplication (Pentao et al.1992).

The same mechanism would be expected to have a reciprocal recombination product involving deletion of the 1.5-Mb region flanked by CMT1A-REP. In fact, Chance et al. have recently identified a DNA deletion of 1.5-Mb in 17p11.2-pl2 that is associated with hereditary neuropathy with liability to pressure palsies (HNPP) (Chance et al.1993), which is a clinically distinct, demyelinating peripheral neuropathy that demonstrates autosomal dominant transmission (Windebank, 1992). The HNPP deletion encompasses DNA markers that are known to be duplicated in CMT1A (Chance et al.1993), and HNPP deletion junction fragments corresponding to the sizes predicted from the physical map have been recently identified by PFGE and Southern analysis using a CMT1A-REP probe (Roa et al.1993b). Thus, the CMT1A duplication and HNPP deletion appear to represent reciprocal DNA rearrangements that are associated with distinct inherited peripheral neuropathies.

GENE DOSAGE AS A MECHANISM FOR CMT1A

How does DNA duplication of 1.5-Mb on 17p11.2-pl2 lead to the disease phenotype of CMT1A? A number of mechanisms had been proposed previously, which includes that of gene dosage. This hypothesis holds that increased copy number and expression of a dosage-sensitive gene or genes within the duplication results in the CMT1A disease phenotype. An alternative explanation proposed that interruption of a gene at the CMT1A duplication junction results in disease through the loss of a critical gene function. The identification and analysis of patients with more extensive segmental duplications on 17p enabled the evaluation of these two hypotheses. We reported an interesting clinical case of a 17p partial trisomy patient, whose complex phenotype included the characteristic CMTl feature of decreased motor NCV (Lupski et al. 1992). The retention of this electrophysiological phenotype was likely to result from increased dosage of the critical gene(s) encompassed by both the CMT1A and this larger 17p duplication. However, it is highly unlikely for the interruption of a gene at the duplication junction to cause the CMT1A phenotype, since the junctions are entirely different in the CMT1A and 17p duplications (Lupski et al.1992). These findings were confirmed in three other cases of partial 17p trisomy patients with different duplication endpoints, who exhibited decreased motor NCV and distal muscle atrophy and weakness in addition to the complex phenotype due to 17p trisomy (Chance et al.1992a; Upadhyaya et al.1993; Roa et al.1993c). The available evidence collectively supports the gene dosage hypothesis.

The finding that gene dosage is involved in generating the disease phenotype in CMT1A duplication patients leads to the question of which gene or genes within the 1.5-Mb duplication region has a direct role in CMT1A. A gene associated with CMT1A has been identified. And in further support of the gene dosage mechanism, this gene maps away from the duplication junctions and is not interrupted as a result of the CMT1A duplication (Patel et al.1992; Pentao et al.1992).

THE *PMP22* GENE IN CMT1A

The *PMP22* gene maps within the CMT1A duplication region, and encodes a peripheral nerve myelin protein with an apparent molecular weight of 22 kilodaltons (Patel et al.1992; Matsunami et al.1992; Valentijn et al.1992b; Timmerman et al.1992). The membrane-associated *PMP22* gene product is incorporated in the compact portion of peripheral nerve myelin (Snipes et al.1992). Furthermore, we have shown that *PMP22* is expressed at high levels in the peripheral nerve, which is consistent with the histopathology of CMT1A (Patel et al.1992). Thus, *PMP22* has been proposed to be a dosage-sensitive gene whose increased

FIGURE 2. Molecular mechanisms leading to CMT1A and HNPP. The genotypes involving the 1.5-Mb CMT1A duplication region and the *PMP22* gene are illustrated, alongside the phenotypes in each particular case. The status of the 1.5-Mb DNA region containing the *PMP22* gene (open box) and flanked by homologous CMT1A-REP sequences (filled boxes)(Pentao et al. 1992) are shown on the left column. The genotypes are listed in the middle column, and the corresponding phenotypes are listed in the right column. Point mutation in the *PMP22* gene is denoted by the asterisk (Valentijn et al. 1992; Roa et al. 1993a; Roa et al. 1993b). Deletion of the 1.5-Mb region associated with HNPP (Chance et al. 1993) is depicted by the dashed triangle, and reduction of the CMT1A-REP to one copy on the segmentally deleted chromosome 17p (our unpublished observations). The phenotype of nerve conduction velocity is represented by NCV, wherein an accompanying down arrow represents decreased NCV, and two down arrows represent severely reduced NCV.

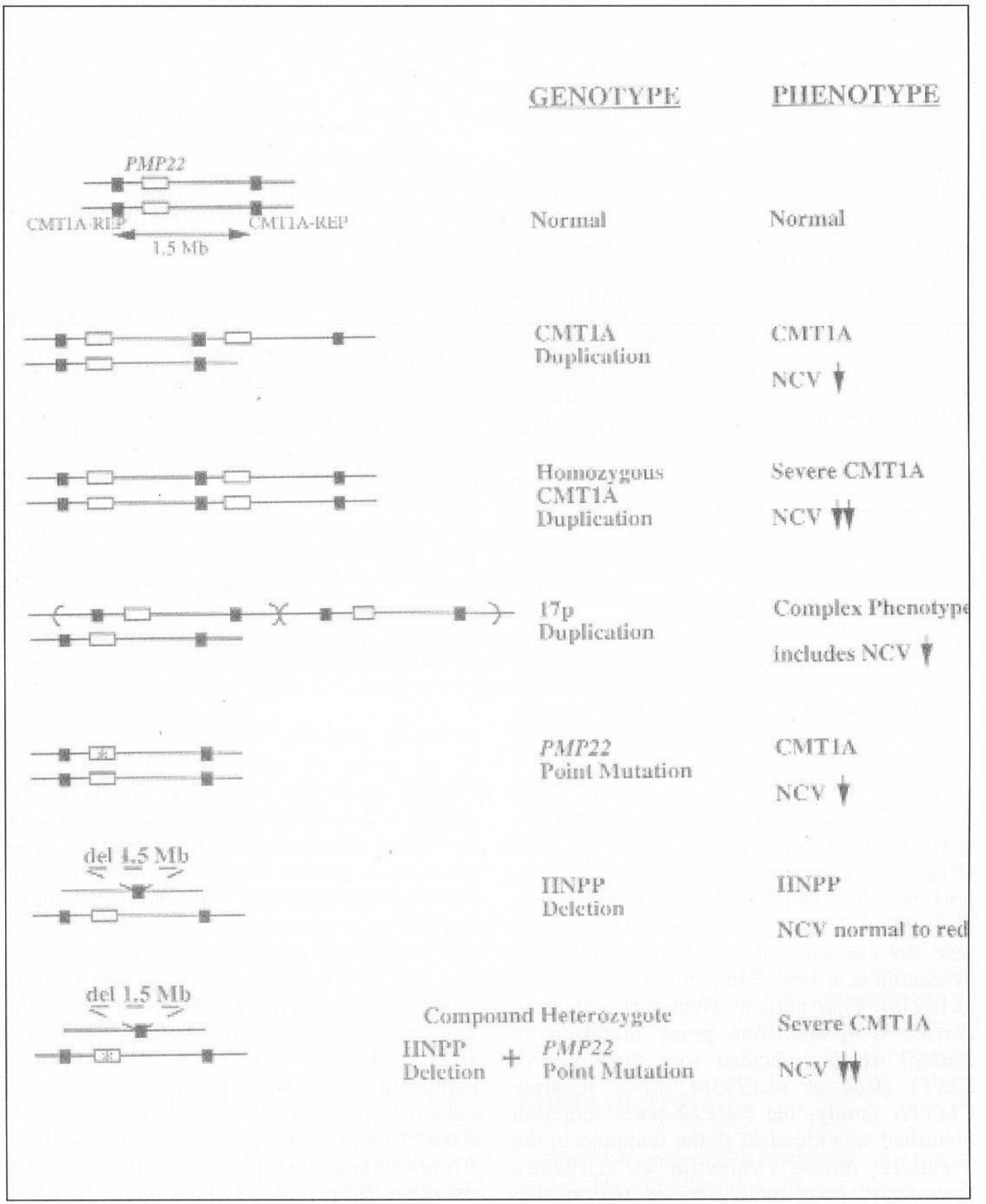


Table 1. Contrasting Findings in Charcot-Marie-Tooth disease type 1A and Hereditary Neuropathy with Liablity to Pressure Palsies		
	CMT1A	HNPP
Molecular Basis	• 1.5-Mb DNA duplication in 17p11.2-p12(>70%) • *PMP22* point mutation	• 1.5-Mb DNA deletion in 17p11.2-p12
Clinical symptoms	• slowly progressive distal muscle atrophy & weakness • pes cavus & claw hand deformity • absent deep tendon reflexes • gait disturbance	• recurrent episodes of palsy following minor trauma or nerve compression • limb weakness and sensory loss in the affected nerve
Electrophysiology	• symmetrically decreased motor nerve conduction velocity (NCV <40 meters/second) • no conduction block	• segmentally reduced motor nerve conduction velocities • conduction block in affected nerve
Histopathology	• hypertrophic demyelinating neuropathy with frequent onion bulb formation	• tomaculous neuropathy characterized by focal myelin thickening

TABLE 1. Contrasting findings in CMT1A and HNPP. These two distinct, autosomal dominantly inherited, demyelinating peripheral neuropathies are differentiated in terms of the molecular mechanism underlying each disease, and for the clinical, electrophysiologic, and histopathologic findings in CMT1A and HNPP (Lupski et al.1991a; Dyck et al.1992; Pentao et al. 1992; Windebank, 1992; Chance et al.1993).

expression presumably leads to the disease in patients with the CMT1A duplication (Patel et al.1992; Matsunami et al.1992; Valentijn et al.1992b; Timmerman et al.1992). Finally, point mutations in *PMP22* asssociated with the disease have been identified in CMT1 patients who do not carry the CMT1A duplication (Valentijn et al.1992a; Roa et al.1993a; Roa et al.1993b). One patient which we identified carried a spontaneous point mutation in *PMP22* which coincided with the onset of CMT1 (Roa et al.1993a). In a separate CMT1A family, the *PMP22* point mutation identified was identical to the mutation in the *Trembler*^{J} mouse (Valentijn et al.1992a). Previously, point mutations in the murine *PMP22* gene were identified in *Trembler* (Suter et al.1992b) and *Trembler*^{J} (Suter et al.1992a) mouse mutants, which had been considered mouse models for CMT1A. In addition, we have recently identified a third *PMP22* point mutation associated with CMT1A, which appears to be a recessive point mutation allele (Roa et al.1993b). In this family which segregates both the apparent recessive *PMP22* point mutation and the HNPP deletion, the CMT1A phenotype was expressed in only one compound heterozygote patient who carried the apparent recessive *PMP22* point mutation on one chromosome 17, in combination with the HNPP deletion on the other chromosome 17 homologue (Fig.2).

Thus, the collective genetic evidence firmly supports a direct role of the *PMP22* gene in the disease process of CMT1A.

MOLECULAR MECHANISMS UNDERLYING CMT1A

The disease phenotype in CMT1A may be elicited by two alternative molecular mechanisms: gene dosage resulting from the CMT1A duplication, or point mutation in the *PMP22* gene in patients without the CMT1A duplication. The majority (70% to 85%) of CMT1 patients carry the duplication and exhibit the disease due to increased amounts of a normal gene product, which is presumably *PMP22*. In keeping with this hypothesis, our preliminary analyses detected no mutations in the coding region of the *PMP22* gene of CMT1A duplication patients (our unpublished observations). More importantly, the gene dosage hypothesis involving *PMP22* is supported by the correlation between *PMP22* copy number and clinical phenotype. Figure 2 illustrates the genotypes and NCV phenotypes in normal individuals, and in patients heterozygous and homozygous for the CMT1A duplication. Patients who are homozygous for the CMT1A duplication were found to have a severe CMT1A phenotype (Lupski et al.1991b) which was comparable to that of Dejerine-Sottas syndrome or HMSNIII (Killian and Kloepfer, 1979; Dejerine and Sottas, 1893).

It is striking that the apparent reciprocal deletion of 1.5-Mb which encompasses the *PMP22* gene is associated with a different demyelinating peripheral neuropathy, HNPP. The genotype and phenotype for HNPP are also depicted in Figure 2. The contrasting molecular and phenotypic findings for CMT1A and HNPP are presented in Table 1 (Chance et al.1993; Windebank, 1992; Lupski et al.1991a; Dyck et al.1992). While these two diseases are clinically distinct entities, it is evident that increased gene dosage can lead to a more permanent and debilitating disease state in CMT1A, compared to the effect of the deletion in HNPP. Given the role of the *PMP22* gene product in the process of myelination which is central to the etiology of both CMT1A and HNPP, it has been suggested that underdosage of *PMP22* can also lead to a peripheral neuropathy phenotype (Chance et al.1993). Unlike the case of CMT1A however, no *PMP22* mutations have yet been identified in cases of HNPP without the deletion, which would indicate a direct role for the *PMP22* gene in the HNPP disease process. Perhaps it would be more likely for *PMP22* mutations associated with non-deletion HNPP to be nonsense mutations that would result in loss of gene function. If the HNPP phenotype associated with the 1.5-Mb HNPP deletion is caused by decreasing the gene dosage of *PMP22*, then null mutations in the *PMP22* gene would be expected to render the same effect.

As an alternative mechanism for CMT1A, point mutations in *PMP22* have been demonstrated in a subset of patients who do not carry the CMT1A duplication. The mechanism of *PMP22* point mutation is also illustrated in Figure 2. The *PMP22* point mutations that are associated with CMT1A (Valentijn et al.1992a; Roa et al.1993a; Roa et al.1993b) as well as *Trembler* (Suter et al.1992b) and *Trembler*^{J} (Suter et al.1992a) mice all cause single amino acid substitutions in the protein. These missense point mutations could conceivably alter the wild-type function of *PMP22*, thereby leading to disease. Since both the CMT1A duplication and point mutations in *PMP22* give rise to very similar CMT1A disease phenotypes, we have proposed that either mechanism can disrupt the macromolecular stoichiometry of *PMP22* on the peripheral nerve membrane which may be critical to normal nerve function. According to this hypothesis, either the overproduction of *PMP22*, or the presence of mutant *PMP22* with increased or altered function, could interfere with the normal physiology of the peripheral nerve and result in the disease phenotype of CMT1A. However, determination of the exact mechanisms leading to the disease await the elucidation of the biological function of *PMP22*.

CONCLUSIONS

CMT1A may be caused by alternative molecular mechanisms of increased gene

dosage as a result of the CMT1A duplication in the majority of cases, or by point mutation of *PMP22* in a minority of CMT1A cases where the duplication is not involved. The high frequency of CMT1A due to DNA duplication of the same size in the majority of patients appears to be a function of a structural feature of the genome at the 17p11.2-pl2 region. The 1.5-Mb DNA region subject to duplication is flanked by large, homologous CMT1A-REP repeat sequences. These could conceivably act as substrates for homologous recombination leading to DNA duplication in the case of CMT1A (Pentao et al.1992), or DNA deletion in the case of HNPP (Chance et al.1993). Although this 1.5-Mb region is likely to contain multiple genes, it is striking that duplication of such a large region results in the limited CMT1A phenotype of demyelinating peripheral neuropathy. The CMT1A phenotype can also result from point mutation of a single gene - *PMP22*. It therefore appears that *PMP22* is the dosage sensitive gene whose overexpression results in CMT1A. Moreover, the HNPP deletion involving the 1.5-Mb region containing *PMP22* also suggests that underexpression of *PMP22* may similarly lead to a demyelinating neuropathy (Chance et al.1993). The findings derived from these molecular studies on CMT1A thus appear to cross the artificial boundaries between single gene disorders that demonstrate Mendelian segregation, and malformation disorders involving chromosome abnormalities (such as segmental aneuploidies involving microduplication or microdeletion syndromes).

The results of these molecular studies have a direct impact on the diagnosis of patients with Charcot-Marie-Tooth disease. CMT1A is the predominant form of the disease, and an estimated 70% to 85% of CMT1 patients carry the duplication. Molecular analysis to detect the CMT1A duplication should therefore be considered as a primary test for patients with clinical symptoms of CMT1 regardless of family history. Molecular analysis can be performed on peripheral blood samples, and would be valuable in the differential diagnosis of patients with peripheral neuropathy, and for presymptomatic diagnosis of individuals in CMT1 families.

SUMMARY

Charcot-Marie-Tooth disease type 1A is a demyelinating, inherited peripheral neuropathy which is associated with a DNA duplication in chromosome 17p11.2-p12 in over 70% of patients with CMT1A. The CMT1A duplication is not detected cytogenetically, and constitutes a tandem duplication of a 1.5-Mb region of DNA flanked by homologous sequences designated as CMT1A-REP. Detection of the CMT1A duplication by molecular methods is a valuable diagnostic test for the majority of CMT1A cases. This duplication mutation shows stable inheritance through multiple generations, and may also arise as a new mutation in sporadic patients. The CMT1A duplication leads to the disease phenotype apparently through increased dosage of a gene(s) within the duplicated segment. A disease gene associated with CMT1A has been identified in the form of PMP22, which maps within the CMT1A duplication region, and encodes a myelin protein of the peripheral nerve. Point mutations in the PMP22 gene have been identified in CMT1A patients, including one case of a new mutation in PMP22 which coincided with the onset of the disease. Thus, two alternative molecular mechanisms are responsible for CMT1A: DNA duplication leading to increased gene dosage, and point mutation of the PMP22 gene.

RESUMEN

La enfermedad de Charcot-Marie-Tooth Tipo 1A es una neuropatía periférica desmielinizante hereditaria que se asocia, en más del 70% de los pacientes con una duplicación del DNA en el cromosoma 17p11.2-p12. La duplicación del CMT1A no se puede demostrar citogenéticamente ya que constituye una duplicación en fila de una región del DNA de

1.5-Mb bordeada por series homólogas llamadas CMT1A-REP. La demostración de la duplicación del CMT1A por métodos moleculares es un examen de laboratorio de importancia diagnóstica en la mayoría de los casos de CMT1A. Esta mutación por duplicación muestra un patrón hereditario constante y estable a través de múltiples generaciones que también se puede originar como una mutación nueva en casos esporádicos de la enfermedad. La duplicación del CMT1A produce el fenotipo de la enfermedad aparentemente a través de un aumento de dosis de un gene(s) dentro del segmento duplicado. Se ha identificado un gene asociado con el CMT1A en la forma de PMP22. Este gene se localiza dentro del segmento duplicado, que se encuentra en la mielina del nervio periférico. Se han identificado unos puntos de mutación en el gene del PMP22 en pacientes con CMT1A, incluyendo un caso de una mutación nueva en el PMP22 asociado con el comienzo de la enfermedad. Por consiguiente, dos mecanismos moleculares alternativos son responsables del CMT1A: una duplicación del DNA que conlleva a un aumento de la dosis del gene y un punto de mutación del gene del PMP22.

RÉSUMÉ

Le syndrome de Charcot-Marie-Tooth type 1A est une neuropathie périphérique héréditaire associée dans plus de 70% des cas à une duplication d'une portion du chromosome 17 située en p11.2-p12. Cette duplication, non détectable par les méthodes classiques de cytogénétique, est un agencement en tandem d'une région de 1,5 Mb. Celle-ci est limitée par 2 séquences homologues appelées CMT1A-REP. La détection de cette duplication par des méthodes moléculaires constitue un test fiable pour le diagnostic de la majorité des cas de CMT1A. Cette mutation est transmise génétiquement de manière stable et a été également observée chez quelques cas sporadiques. Le phénotype observé dans ce syndrome semble être le résultat d'un phénomène de dosage génique d'un gène(s) situé(s) dans la région dupliquée. Un gène, PMP22 codant pour une protéine de myeline du nerf périphérique, a été isolé et associé à ce syndrome. Plusieurs mutations ponctuelles dans ce gène ont été identifiées chez des patients atteints de CMT1A, parmi lesquelles une mutation de novo. En conséquence, deux mecanismes moléculaires semblent être responsables de la survenue de ce syndrome: une duplication d'une portion du chromosome 17 entraînant un phénomène de dosage génique et la mutation du gène PMP22.

ZUSAMMENFASSUNG

Die Charcot-Marie-Tooth-Erkrankung Typ 1A ist eine demyelinisierende, erbliche periphere Neuropathie, die in über 70% der CMT1A Patienten mit einer Duplikation 17p11.2-p12 einhergeht. Die Duplikation kann zytogenetisch nicht erkannt werden. Sie stellt die Duplikation einer 1.5 Mb DNA-Region dar, die von homologen Sequenzen - genannt CMT1A-REP - flankiert wird. Der Nachweis der CMT1A Duplikation mit Hilfe molekularer Methoden ist ein wertvoller diagnostischer Test für die Mehrzahl aller CMT1A Fälle. Die Duplikationsmutationen zeigen eine stabile Vererbung über mehrere Generationen . Sie können auch als Neumutationen bei sporadischen Fällen ohne Familiengeschichte auftreten. Die CMT1A Duplikation führt scheinbar über eine erhöhte Gendosis innerhalb des duplizierten Segments zu dem beobachteten Phänotyp. Ein Gen - PMP22 - wurde mit CMT1A assoziiert. Das Gen wurde innerhalb der CMT1A Duplikationsregion kartiert. Es codiert für ein Myelinprotein der peripheren Nerven. Punktmutationen im PMP22-Gen sind bei CMT1A Patienten ebenfalls gefunden worden, einschließlich eines Falles einer Neumutation in PMP22, die mit dem entsprechenden Krankheitsbeginn übereinstimmte. Es sind also zwei alternative molekulare Mechanismen für CMT1A verantwortlich: 1) DNA Duplikation verbunden mit erhöhter Gendosis und 2) Punktmutation im PMP22-Gen, das mit CMT1A assoziiert ist.

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REFERENCES

1. Bellone, E., Mandich, P. and Mancardi, G.L. Charcot-Marie-Tooth (CMT) 1a duplication in 17p11.2 in Italian families J.Med.Genet.,29: 492-493.1992.
2. Bird, T.D., Ott, J. and Gilblett, E.R. Evidence for linkage of Charcot-Marie-Tooth disease neuropathy to the Duffy locus on chromosome number 1 Am.J. Hum. Genet.,34: 388-394.1982.
3. Bird, T.D. and Kraft, G.H. Charcot-Marie-Tooth disease: Data for genetic counseling relating age to risk Clin.Genet.,14: 43-49.1978.
4. Brice, A., Ravise, N., Stevanin, G., Gugenheim, M., Bouche, P., Penet, C., Agid, Y. and French CMT Research Group, Duplication within chromosome 17p11.2 in 12 families of French ancestry with Charcot-Marie-Tooth disease type 1a J.Med.Genet.,29: 807-812.1992.
5. Chance, P.F., Bird, T.D., O'Connell, P., Lipe, H., Lalouel, J.-M. and Leppert, M. Genetic linkage heterogeneity in type 1 Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy type 1) Am.J. Hum.Genet.,47: 915-925.1990.
6. Chance, P.F., Bird, T.D., Matsunami, N., Lensch, M., Brothman, A.R. and Feldman, G.M. Trisomy 17p associated with Charcot-Marie-Tooth neuropathy type 1A phenotype: Evidence for gene dosage as a mechanism in CMT1A Neurology,42: 2295-2299.1992a.
7. Chance, P.F., Matsunami, N., Lensch, W., Smith, B. and Bird, T.D. Analysis of the DNA duplication 17p11.2 in Charcot-Marie-Tooth neuropathy type 1 pedigrees: additional evidence for a third autosomal CMT1 locus Neurology,42: 2037-2041.1992b.
8. Chance, P.F., Alderson, M.K., Leppig, K.A., Lensch, M.W., Matsunami, N., Smith, B., Swanson, P.D., Odelberg, S.J., Disteche, C.M. and Bird, T.D. DNA deletion associated with hereditary neuropathy with liability to pressure palsies Cell,72: 143-151.1993.
9. Charcot, J.-M. and Marie, P. Sur une forme particuliere d'atrophie musculaire progressive, souvent familiale, debutant par les pieds et les jambes et atteignant plus tard les mains Rev.Med.,6: 97-138.1886.
10. Dejerine, J. and Sottas, J. Sur la nevrite interstitielle, hypertrophique et progressive de l'enfance Comptes Rendus de la Societe de Biologie Paris,45: 63-96.1893.
11. Dyck, P.J., Chance, P., Lebo, R. and Carney, A.J. Hereditary motor and sensory neuropathies. In: Peripheral Neuropathy, edited by Dyck, P.J., Thomas, P.K., Griffin, J.W., Low, P.A. and Poduslo, J.F. Philadelphia: W.B. Saunders Company, 1992, p. 1094-1136.
12. Hallam, P.J., Harding, A.E., Berciano, J., Barker, D.F. and Malcolm, S. Duplication of part of chromosome 17 is commonly associated with hereditary motor and sensory neuropathy type 1 (Charcot-Marie-Tooth disease type 1) Ann.Neurol.,31: 570-572.1992.
13. Hoogendijk, J.E., Hensels, G.W., Gabreels-Festen, A.A.W.M., Gabreels, F.J.M., Janssen, E.A.M., De Jonghe, P., Martin, J.-J., Van Broeckhoven, C., Valentijn, L.J., Baas, F., De Visser, M. and Bolhuis, P.A. De-novo mutation in hereditary motor and sensory neuropathy type 1 Lancet,339: 1081-1082.1992.
14. Kaku, D.A., Parry, G.J., Malamut, R., Lupski, J.R. and Garcia, C.A. Uniform slowing of conduction velocities in Charcot-Marie-Tooth polyneuropathy type 1 Neurology, 1993a.(In Press)
15. Kaku, D.A., Parry, G.J., Malamut, R., Lupski, J.R. and Garcia, C.A. Nerve conduction studies in Charcot-Marie-Tooth polyneuropathy associated with a segmental duplication of chromosome 17 Neurology, 1993b (In Press).
16. Killian, J.M. and Kloepfer, H.W. Homozygous expression of a dominant gene for Charcot-Marie-Tooth neuropathy Ann.Neurol.,5: 515-522.1979.
17. Lupski, J.R., Garcia, C.A., Parry, G.J. and Patel, P.I. Charcot-Marie-Tooth Polyneuropathy Syndrome: Clinical Electrophysiological and Genetic Aspects. In: Current Neurology, edited by Appel, S. Chicago: Mosby-Yearbook, 1991a, pp. 1-25.
18. Lupski, J.R., Montes de Oca-Luna, R., Slaugenhaupt, S., Pentao, L., Guzzetta, V., Trask, B.J., Saucedo-Cardenas, O., Barker, D.F., Killian, J.M., Garcia, C.A., Chakravarti, A. and Patel, P.I. DNA duplication associated with Charcot-Marie-Tooth disease type 1A Cell,66: 219-232. 1991b.
19. Lupski, J.R., Wise, C.A., Kuwano, A., Pentao, L., Parke, J.T., Glaze, D.G., Ledbetter, D.H., Greenberg, F. and Patel, P.I. Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A Nature Genetics,1: 29-33.1992.
20. Lupski, J.R. and Garcia, C.A. Molecular genetics and neuropathology of Charcot-Marie-Tooth disease type 1A Brain Pathology,2(4): 337-349.1992.
21. Lupski, J.R., Pentao, L., Williams, L.I., and Patel, P.I. Stable inheritance of the CMT1A DNA duplication in two patients with CMT1 and NF Am. J. Med. Genet.,45:92-96.1993.
22. MacMillan, J.C., Upadhyaya, M. and Harper, P.S. Charcot-Marie-Tooth disease 1a (CMT1a): evidence for trisomy of the region p11.2 of chromosome 17 in South Wales families J.Med.Genet.,29: 12-13.1992.
23. Matsunami, N., Smith, B., Ballard, L., Lensch, M.W., Robertson, M., Albertsen, H., Hanemann, C.O., Muller, H.W., Bird, T.D., White, R. and Chance, P.F. Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot-Marie-Tooth 1A Nature Genetics,1: 176-179.1992.
24. McKusick, V.A. Mendelian Inheritance in Man,

Baltimore :The Johns Hopkins University Press, 1992. Ed. 10
25. Nicholson, G.A. Penetrance of the hereditary motor and sensory neuropathy Ia mutation: assessment by nerve conduction studies Neurology,41: 547-552.1991.
26. Patel, P.I., Roa, B.B., Welcher, A.A., Schoener-Scott, R., Trask, B.J., Pentao, L., Snipes, G.J., Garcia, C.A., Francke, U., Shooter, E.M., Lupski, J.R. and Suter, U. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A Nature Genetics,1: 159-165.1992.
27. Pentao, L., Wise, C.A., Chinault, A.C., Patel, P.I. and Lupski, J.R. Charcot-Marie-Tooth type 1A tandem duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit Nature Genetics,2: 292-300.1992.
28. Raeymaekers, P., Timmerman, V., Nelis, E., De Jonghe, P., Hoogendijk, J.E., Baas, F., Barker, D.F., Martin, J.J., De Visser, M., Bolhuis, P.A., Van Broeckhoven, C. and the HMSN Collaborative Research Group, Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type Ia (CMT1a) Neuromuscular Disorders,1: 93-97.1991.
29. Roa, B.B., Garcia, C.A., Suter, U., Kulpa, D.A., Wise, C.A., Mueller, J., Welcher, A.A., Snipes, G.J., Shooter, E.M., Patel, P.I. and Lupski, J.R. Charcot-Marie-Tooth disease type 1A: Association with a Spontaneous Point Mutation in the PMP22 Gene N.Engl.J.Med.,329: 96-101.1993a.
30. Roa, B.B., Garcia, C.A., Pentao, L., Killian, J.M., Trask, B.J., Suter, U., Snipes, G.J., Ortiz-Lopez, R., Shooter, E.M., Patel, P.I. and Lupski, J.R. Evidence for a recessive PMP22 point mutation in Charcot-Marie-Tooth disease type 1A Nature Genetics,1993b (In Press).
31. Roa, B.B., Garcia, C.A., Wise, C.A., et al. Gene dosage as a mechanism for a common autosomal dominant peripheral neuropathy: Charcot-Marie-Tooth disease type 1A. In: Phenotypic Mapping of Down Syndrome and OtherAneuploid Conditions, edited by Epstein, C.J. New York: Wiley-Liss Inc., 1993 (In Press).
32. Skre, H. Genetic and clinical aspects of Charcot-Marie-Tooth's disease Clin.Genet.,6: 98-118.1974.
33. Snipes, G.J., Suter, U., Welcher, A.A. and Shooter, E.M. Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13) J.Cell.Biol.,117: 225-238.1992.
34. Suter, U., Moskoco, J.J., Welcher, A.A., Snipe, G.J., Kosaras, B., Sidman, R.L., Buchberg, A.M. and Shooter, E.M. A leucine-to-proline mutation in the putative first transmembrane domain of the 22-kDa peripheral myelin protein in the trembler-J mouse Proc.Natl.Acad.Sci.USA,89: 4382-4386.1992a.
35. Suter, U., Welcher, A.A., Ozcelik, T., Snipes, G.J., Kosaras, B., Francke, U., Billings-Gagliardi, S., Sidman, R.L. and Shooter, E.M. The Trembler mouse carries a point mutation in a myelin gene Nature,356: 241-244.1992b.
36. Timmerman, V., Nelis, E., Van Hul, W., Nieuwenhuijsen, B.W., Chen, K.L., Wang, S., Cullen, B., Leach, R.J., Hanemann, C.O., De Jonghe, P., Raeymaekers, P., van Ommen, G.-J.B., Martin, J.J., Muller, H.W., Vance, J.M., Fischbeck, K.H. and Van Broeckhoven, C. The peripheral myelin protein gene PMP-22 is contained within the Charcot-Marie-Tooth disease type 1A duplication Nature Genetics,1: 171-175.1992.
37. Tooth, H.H. The Peroneal Type of Progressive Muscular Atrophy, London:H.K. Lewis, 1886.
38. Upadhyaya, M., Roberts, S.H., Farnham, J., MacMillan, J.C., Clarke, A., Heath, J.P., Hodges, I.C.G. and Harper, P.S. Charcot-Marie-Tooth disease 1A (CMT1A) associated with a maternal duplication of chromosome 17p11.2-12 Hum.Genet.,91: 392-394.1993.
39. Valentijn, L.J., Baas, F., Wolterman, R.A., Hoogendijk, J.E., van den Bosch, N.H.A., Zorn, I., Gabreels-Festen, A.A.W.M., De Visser, M. and Bolhius, P.A. Identical point mutations of PMP-22 in Trembler-J mouse and Charcot-Marie-Tooth disease type 1A Nature Genetics,2: 288-291.1992a.
40. Valentijn, L.J., Bolhuis, P.A., Zorn, I., Hoogendijk, J.E., van den Bosch, N., Hensels, G.W., Stanton, Jr.V.P., Housman, D.E., Fischbeck, K.H., Ross, D.A., Nicholson, G.A., Meershoek, E.J., Dauwerse, H.G., van Ommen, G.J.B. and Baas, F. The peripheral myelin gene PMP-22/GAS-3 is duplicated in Charcot-Marie-Tooth disease type 1A Nature Genetics,1: 166-170.1992b.
41. Vance, J.M., Nicholson, G.A., Yamaoka, L.H., Stajich, J., Stewart, C.S., Speer, M.C., Hung, W.-Y., Roses, A.D., Barker, D. and Pericak-Vance, M.A. Linkage of Charcot-Marie-Tooth neuropathy type la to chromosome 17 Exp.Neurol., 104: 186-189.1989.
42. Vance, J.M. Hereditary motor and sensory neuropathies J.Med.Genet.,28: 1-5.1991.
43. Windebank, A.J. Inherited recurrent focal neuropathies. In: Peripheral Neuropathy, edited by Dyck, P.J., Thomas, P.K., Griffin, J.W., Low, P.A. and Poduslo, J.F. Philadelphia: W.B. Saunders, 1992, p. 1137-1148.
44. Wise, C.A., Garcia, C.A., Davis, S.N., Heju, Z., Pentao, L., Patel, P.I. and Lupski, J.R. Molecular analyses of unrelated Charcot-Marie-Tooth disease patients suggest a high frequency of the CMT1A duplication Am.J.Hum.Genet.,1993 (In press).

The Inherited Epilepsies

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Introduction. The epilepsies are a heterogeneous group of disorders that are the consequence of neuronal (network) hyperexcitability (Dichter and Ayala,1987) which results in a variety seizure types. A significant proportion of seizures which occur in childhood are the result of a genetic predisposition.

As has been noted in a more detailed recent review (Dichter and Buchhalter, 1993), analysis of the inherited epilepsies is complicated by several problems. Perhaps the major issue is the criteria for inclusion as "affected" in a given family. In many past reports, only the statement that a "family history" of seizures was present. Other studies have used both clinical descriptions as well as EEG data. The use of either or both of these criteria can dramatically alter the results of linkage analysis. The diagnosis of the proband can be standardized by use of the international classification of epilepsy syndromes (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) and/or seizure types (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). Furthermore, the EEG has proven to be a useful tool to uncover family members who bear the epilepsy trait, but who may not manifest seizures. This has been demonstrated in pedigree analyses of the primary generalized epilepsies (Jeavons and Harding, 1975; Newmark and Penry, 1979). It should be noted that the expression of age dependent EEG frequencies in nonepileptic individuals appears to be under genetic control as found in twin studies (Vogel, 1970). Identification of affected family members can also be complicated by the fact the certain seizure disorders and EEG patterns have an age related onset and offset.

In the following review, those epilepsies in which evidence exists for a genetic component will be briefly summarized. Excluded from this review are those conditions in which seizures play a prominent role in the setting of metabolic or neurocutaneous syndromes (Dichter and Buchhalter, 1993).

Generalized Epilepsies

Benign Familial Neonatal Convulsions (BFNC)

BFNC is inherited in an autosomal dominant fashion (Zonana et. al., 1984) with onset in the first few weeks to months of life. The tonic or clonic seizures may appear partial or generalized. The interictal EEG is normal, whereas the ictal EEG can reveal focal or generalized epileptiform abnormalities. Remission usually occurs within several months following onset. Blood chemistries and neuroimaging are normal. Although termed "benign", a small percentage of these children develop afebrile seizures, mental retardation and learning disabilities.

Pedigree analysis has demonstrated that almost 50% of children at risk had neonatal

seizures thereby indicating that autosomal dominant transmission with high penetrance was most likely. BFNC is the first epilepsy syndrome to which a chromosomal linkage was made. Genetic linkage analysis of 3 families using the polymorphic DNA marker loci CMM6 (D20S19) and RMR6 (D20S20) indicated tight linkage to chromosome 20 (Leppert et. al., 1989; Leppert, 1990) with a combined likelihood ratio of >8,000,000 to 1 for linkage of the gene causing BFNC to the markers on the long arm of chromosome 20. Another group reported 2 families in which significant clinical heterogeneity existed in that children continued to have seizures beyond 12 months, several developed subsequent epilepsy and asymptomatic obligate carriers were found (Ryan et. al., 1991). Using the same markers as above, only one family demonstrated linkage to chromosome 20. This demonstrated that phenotypic (clinical) heterogeneity may be due to genetic heterogeneity, rather than variations of symptoms due to a single gene.

Juvenile Myoclonic Epilepsy (JME)

Genetically inherited, primary generalized epilepsy which may represent up to 30% of all epilepsies (Delgado-Escueta et. al., 1990) is composed of syndromes including; JME, childhood absence epilepsy and epilepsy with generalized tonic-clonic seizures upon awakening. Each of these forms of idiopathic generalized epilepsy appears to have a genetic component. However, factors such as a multiple seizure types, incomplete penetrance of the age dependent clinical and/or EEG manifestations complicate genetic analysis.

Of these syndromes, JME can be narrowly defined for study purposes as an epilepsy syndrome with onset between 8-20 years, normal intellect, myoclonic jerks worse in the morning and intermittent generalized tonicclonic seizures (GTCS). Absence seizures may also occur. The EEG reveals characteristic 4-6 hz, bilaterally symmetric, polyspike-wave bursts (Delgado -Escueta et. al., 1990). This syndrome has been estimated to be present in between 4% and 11% of individuals with epilepsy.

Several groups have studied the familial incidence of JME (Delgado-Escueta et. al., 1989; Weissbecker et. al., 1991; Greenberg et. al., 1988). It is clear that there is a high incidence of JME as well as several other generalized seizure types in families of individuals with JME. There is also a fairly high incidence (approximately 12% in siblings and parents) of epileptiform EEG abnormalities (Delgado-Escueta et. al., 1990) in family members who do not develop clinical seizures. Several groups have performed genetic linkage analyses with individuals with abnormal EEGs included as affected (Weissbecker et. al., 1991; Greenberg et. al., 1988), whereas earlier studies had employed only phenotypic descriptions of seizures as inclusion criteria (Panayiotopoulos and Obeid, 1989). The mode of inheritance of JME may differ within different kindreds. A dominant mode with high penetrance (approximately 90%) (Delgado-Escueta et. al., 1990), a recessive mode (Panayiotopoulos and Obeid, 1989), and a two locus model (Delgado-Escueta et. al., 1990) have been proposed. Delgado-Escueta and colleagues suggest that at the molecular level, different abnormal genes, even at the same locus, could have different modes of expression and appear more or less dominant under different circumstances (Delgado-Escueta et. al., 1990). Thus, for analysis of the genetic basis of this syndrome (e.g. which gene is involved and what the effects of the mutation are), the mode of inheritance may not be critical.

Three groups have localized the JME gene to the short arm of chromosome 6 (6p) using different forms of linkage analysis using serological (Greenberg et. al., 1990; Delgado-Escueta et. al., 1990; Weissbecker et. al., 1991; Greenberg et. al., 1988) and DNA (Durner et al, unpublished, cited in (Delgado-Escueta et. al., 1990)) markers. Combining the data from these sets of pedigrees produces a LOD score of 6.7. However, it remains possible that the clinical phenotype may have more than one genotype. No known genes for ion channels, neurotransmitters, neurotransmitter receptors, or other "neurobiologically important" molecules are currently identified with this portion of chromosome 6.

Childhood Absence Epilepsy

Childhood absence epilepsy (pyknolepsy) is a syndrome of primary generalized epilepsy which occurs predominantly in children between the ages of 4 and 8 years and is manifest by multiple absences, which may be accompanied by brief clonic movements of mouth or hands. It has been estimated that childhood absence epilepsy is present in between 13-17% of individuals with epilepsy (Obeid and Panayitopoulos, 1988; Janz, 1985). The EEGs of these individuals show characteristic 3 hz spike-wave discharges, although faster and slower frequencies can also be noted. It is generally considered these children are intellectually and neurologically normal. This form of epilepsy seems to have a female preponderance (60-75%) (Engel, 1989). The absence seizures respond to ethosuximide or valproate, although only valproate is effective against the GTCS. Some children will outgrow both seizure types by adolescence (20-30%); others stop having absences but are left with GTCS; others continue to experience both types of seizures throughout life. This syndrome needs to be distinguished from juvenile absence, a related primary generalized epilepsy occurring in a later age group, and atypical absence, which is frequently associated with neurological lesions and psychomotor retardation.

Childhood absence epilepsy has a clear genetic basis with a proposed autosomal dominant mode of inheritance (Metrakos and Metrakos, 1961; Serratosa et. al., 1990). Complicating the genetic analysis is the observation that among family members of children with this form of epilepsy, generalized spike wave bursts are common, even in the absence of clinical seizures. In fact, one study of children with generalized epilepsy demonstrated that among those mothers who clearly had abnormal EEGs, only 26% had experienced seizures (Doose and Baier, 1989). The spike wave bursts occur more frequently in young relatives (Doose and Baier, 1987; Doose and Baier, 1989) and such EEG patterns may disappear in later life.

Concordance rates for monozygotic twins with absence epilepsy have been recorded at 75% if only absence seizures are considered, and as high as 84% if 3 hz spike wave EEGs are also included in the analysis (Lennox, 1951) suggesting a high degree of penetrance for this particular gene(s). In a series of 12 extended pedigrees of children initially diagnosed as having pure childhood absence epilepsy (Delgado-Escueta et. al., 1990), not using the EEG to count affected individuals, 20% of siblings had epilepsy. If the proband is added to each family, (30%) of "at risk" individuals in generations in which one individual had childhood absence epilepsy, developed epilepsy. Similarly, 35% of children born into families where one parent had generalized epilepsy developed generalized epilepsy. Studies are currently underway to obtain EEGs on family members in order to perform linkage analyses. The chromosomal localization childhood absence has not been identified.

Early Childhood Myoclonic Epilepsy (ECME)

Early childhood myoclonic epilepsy (ECME) (Delgado-Escueta et. al., 1990) or epilepsy with myoclonic astatic seizures (Engel, 1989) is another form of primary generalized epilepsy consisting of atonic drop attacks with or without myoclonic jerks, absences or generalized tonic-clonic seizures. This is a much less common form of epilepsy. Individuals with ECME have EEGs which show 2-3 hz spike-wave or polyspike-wave complexes and/or 4-6 hz polyspike-wave complexes (Delgado-Escueta et. al., 1990). These individuals are intellectually and neurologically normal and do not have demonstrable CNS lesions. The seizures are usually, but not always, responsive to valproate.

Preliminary data from family studies indicate 20% of family members had some type epilepsy (Delgado-Escueta et. al., 1990). Of interest only about 80% of those with seizures had primary generalized epilepsy indicating that the inherited predisposition was not expressed with a single phenotype. These data are based only on seizures, and EEG data are currently being collected. A more complete description of the inheritance pattern of this form of epilepsy will await

determination of how many "at risk" individuals without seizures demonstrate abnormal EEGs. Similarly, linkage analyses will depend on clear definitions of the complete syndrome.

Other forms of myoclonic epilepsy

The myoclonic seizure type occurs as a component of other epilepsy syndromes. Different classification schemes have been proposed for these syndromes (Jeavons, 1977) which are heterogeneous with regard to age of onset, other seizure types, intellectual impairment and extent of organ involvement outside the nervous system. Those syndromes in which information is available indicating genetic transmission will be discussed.

Severe Myoclonic Epilepsy in Infancy (SME)

Severe myoclonic epilepsy in infancy is an uncommon syndrome characterized by a combination of mixed myoclonic or clonic seizures, generalized tonic-clonic seizures and complex partial seizures. These seizures are often precipitated by fevers, with onset usually during the first two years of life. These seizures may partially respond to valproate but are generally intractable. Individuals with this form of epilepsy often develop significant psychomotor retardation. Whether this occurs as a result of the intractable seizures or due to some other neurodegenerative disorder is not known.

A high incidence (25-50%) of a positive family history for seizures (both with and without fever) has been reported in this syndrome (Dravet et. al., 1985; Dalla Barnardina et. al., 1982). In addition, Fujiwara et al (Fujiwara et. al., 1990) reported a pair of monozygotic twins who both showed very similar onsets and patterns of seizures. Nothing more is known about the mode of inheritance or chromosomal localization.

Progressive Myoclonus Epilepsy (PME)

There are a group of syndromes (at least three of which are clearly delineated) in which a general neurological deterioration is associated with both sporadic non-epileptic myoclonus and epileptic seizures, which can be myoclonic, clonic or generalized tonic-clonic. These epilepsies have in common progressive neurologic deterioration (including cerebellar ataxia and dementia) and a proposed autosomal recessive mode of inheritance.

The *Lafora* type of PME is a rapidly progressive syndrome which often begins in the second decade with generalized tonic-clonic or myoclonic seizures followed by a relatively rapid deterioration in intellectual function, psychosis, and muscle atrophy. These individuals have PAS positive inclusion bodies (containing acid mucopolysaccharides) throughout the grey matter of the CNS and in skeletal and heart muscle and in liver. Within the brain, the cerebellum, olives, red nucleus, substantia nigra, thalamus, and cortex are most involved.

The *Unverricht-Lundborg* type of PME has been reported among Scandinavian individuals with a younger age of onset and less neurological and cognitive impairment than noted with Lafora type (Norio and Koskiniemi, 1979). No consistent diagnostic pathological features (i.e. Lafora bodies) are present. In a study of over 100 affected Finnish patients, 26% of siblings were affected (Norio and Koskiniemi, 1979). A linkage analysis has recently been reported. Using a variety of markers, Lehesjoki et al (Lehesjoki et. al., 1991) have localized the gene for the Unverricht-Lundborg syndrome on chromosome 21 (band q22.3). This is the only "pure" PME syndrome for which a chromosomal localization has been made.

The *Baltic* type of PME is similar to Unverricht -Lundborg, but with a more varied seizure component, including photosensitive and, occasionally violent, myoclonus, absence and generalized tonic -clonic seizures, and a more pronounced cerebellar component, has been described as Baltic myoclonus epilepsy (Eldridge et. al., 1983; Koskiniemi, 1986). No Lafora bodies are present. This syndrome is important to recognize, as phenytoin, which is commonly employed as an antiepileptic drug in these patients, may produce an acceleration in the neurological deterioration.

Localization-Related (Partial) Epilepsies

Benign Epilepsy of Childhood with Rolandic Spikes (BECRS):

BECRS may be the most common identifiable type of childhood epilepsy. It was found in 25% of epileptic children in a large prospective population study (Blom and Heijbel, 1989). It is characterized by normal developmental, neurological and neuroradiologic examinations. The interictal EEG reveals a characteristic uni- or bi-hemispheric, shifting monomorphic epileptiform discharge, usually with an amplitude maximum over the inferior Rolandic cortex with a normal background. Nocturnal seizures predominate and are characterized by hemifacial or lingual sensory phenomena, drooling and a motor-based dysphasia and occasionally, hemi-clonic or tonic-clonic seizures. Seizures abate by 20 years of age in greater than 95% of cases. A "family history" of epilepsy is cited in most series with a range from 9-68% (Degen and Degen, 1990; Heijbel et. al., 1975; Lerman and Kivity, 1975; Aicardi, 1986). Age related expression (penetrance) and recession is clearly indicated by the onset and resolution of the seizures and the EEG features at approximately four years and 20 years, respectively. The clinical and EEG heterogeneity is illustrated by a recent study (Degen and Degen, 1990) in which a positive family history for epilepsy was present in 40%. Of note, among the five siblings with seizures, one had BECRS, three were febrile, and two afebrile generalized tonic-clonic (Degen and Degen, 1990). The waking and sleep EEG of the siblings was epileptiform in 51%, 6% of which were focal and 32% had generalized spike-wave discharges. This study illustrates that a family history of seizures does not necessarily indicate that the same types of seizures are present in all affected family members. Furthermore, the EEG may serve as a marker for epileptogenicity in both symptomatic and unaffected family members, although all individuals may not have a similar"epileptiform" abnormality.

An autosomal dominant inheritance with age dependent, variable penetrance has been considered the most likely mode of transmission. A recent study used a "priori" methods to analyze a cohort selected on the basis of characteristic clinical and EEG features and having a sibling between 3-13 years old (Heijbel et. al., 1975). A family history of seizures in childhood in 68% of cases with seizures in 11% and 15% of the parents and siblings, respectively. 34% of the siblings had characteristic centrotemporal spikes. Assumptions of dominant inheritance provided a high correlation between the expected and observed cases.

Childhood Epilepsy with Occipital Paroxysms (CEOP):

CEOP as described in the 1989 ILAE Revised Proposal for Classification of Epilepsies and Epileptic Syndromes (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) is a localization-related, idiopathic syndrome characterized by ictal symptomatology involving visual phenomena, hemi-clonic and occasionally, generalized tonic-clinic seizures often followed by a headache occurring in an otherwise normal child. The EEG reveals a normal background and bi- or uni-lateral occipital spike-wave pattern which attenuates with eye opening. The reports upon which this description was based also indicated an excellent outcome in the majority of cases (Gastaut, 1982; Gastaut, 1950; Gibbs and Gibbs, 1952). However, when one of the most influential early series (Gastaut, 1982) was updated (Gastaut and Zifkin, 1987), patients were included with developmental retardation and hemiparesis as well as complex partial seizures. A family history of epilepsy was present in 37% of patients in the latter series with no additional characterization of the mode of transmission.

The importance of clearly defined inclusion criteria is illustrated by a report which described children with occipital lobe foci. One group corresponded to the ILAE definition of CEOP, but the other included children with brain lesions (Terasaki et. al., 1987). In the former, a family history of "convulsive disorders" was noted in 44% of first and second degree relatives. In the latter

group, this history was present in only 1 case. A low incidence of a positive family history (10%) in children with abnormal development or neuroimaging studies (Aicardi and Newton, 1987) and otherwise similar clinical and EEG features has been noted elsewhere.

Finally, another "benign" occipital lobe epilepsy syndrome exists with normal cognition, development and epileptiform EEG transients abated by visual fixation and absence of structural abnormalities that different ictal features (Panayiotopoulos, 1989). In these children, the seizures are nocturnal and are characterized by vomiting and tonic deviation of the eyes. Complete seizure relief occurred by 12 years. No significant family history was reported for either group. Thus, it is likely that multiple childhood occipital lobe epilepsy syndromes exist which have a variety of outcomes. These differ in the ictal manifestations and likely genetic transmission.

Acquired partial (focal) epilepsy

Acquired partial epilepsy (simple and complex) can be defined as seizures which occur in an otherwise normal individual after a specific provocation (e.g. head trauma, a stroke, a brain tumor). The seizures are focal (partial) seizures with or without secondary generalization. A less restrictive definition could include patients with a suspected, although not demonstrated, lesion which may ultimately be demonstrated pathologically by biopsy or autopsy. It has been suggested that a genetic predisposition may exist in some individuals who develop acquired partial epilepsy (Ottman, 1989; Treiman, 1989), specifically with regard to brain tumors (Lund, 1952), brain trauma (Evans, 1962), and children with infantile hemiplegia (Rimoin and Metrakos, 1963). A family history of "temporal lobe epilepsy" has been reported to range from 11-30% (Holowach et. al., 1958; Currie et. al., 1971; Falconer, 1971; Jensen, 1975; Mattson et. al., 1985). However, in a recent critical review of this literature (Ottman, 1989), it is noted that the prevalence of seizures in relatives of individuals with partial epilepsy is probably less than 5%, although this may be slightly greater than that found in appropriate control groups.

Several inherited syndromes exist in which seizures are a major presenting feature of the disorder and have a genetic basis.

Febrile Convulsions (FC)

FC are defined as "seizures accompanied by fever, occurring between the ages of 1 month and 7 years, not symptomatic of recognized acute neurological illness" (Nelson and Ellenberg, 1976). FC are called "simple" if the seizures are brief generalized tonic-clonic seizures or "complex" if prolonged (>30 minutes) or focal or recur within 24 hours. Children with simple FC may have a slightly greater likelihood of developing epilepsy than the general population, whereas a complex FC significantly increases that probability depending upon the number of complex features and length of follow-up of the study population (Annegers et. al., 1987; Nelson and Ellenberg, 1976).

The syndrome of febrile convulsions represents the most common form of seizures seen in childhood with males and females equally affected. A positive family history of epilepsy vs. FCs is reported in 7-25% of children with FC (Tsuboi, 1989; Nelson and Ellenberg, 1976). Twin studies have shown concordance rates for monozygotic twins, ranging from 31-70% (Schiottz-Christensen, 1972; Lennox-Buchthal, 1971; Lennox-Buchthal, 1973; Tsuboi and Okada, 1985). In dizygotic twins, the concordance rate ranges from 14-18% in all of the above studies. These studies indicate that FC do have a genetic component and that either a partial penetrance for the FC trait or a polygenic pattern of inheritance exists. Given the number of biochemical aspects of neuronal function that are temperature dependent, it is likely, that seizures in the presence of fever could have multiple mechanisms influenced by several genes.

Myoclonus Epilepsy and Ragged Red Fibers (MERRF)

MERRF is a progressive neurological disorder characterized by myoclonus,

epilepsy, dementia, ataxia, and spasticity. The "ragged red fibers" are the manifestation of the abnormal mitochondria detected in muscle biopsies. A maternal mode of transmission suggested that it might be an X-linked disease (Wienker et. al., 1979). However, analysis of larger kindreds indicated that a mitochondrial DNA mutation was most likely (Rosing et. al., 1985). The specific gene defect associated with this syndrome is a single A to G transition mutation at nucleotide pair 8344 in human mitochondrial DNA (mtDNA) coding for the tRNA(Lys) gene (Shih et. al., 1991; Shoffner et. al., 1990). The mutation causes enzymatic defects in mitochondrial oxidative phosphorylation. The relationship of this defect to the expression of an epileptic phenotype is not yet known, but many possibilities exist, as so much of the physiology of the brain is dependent on maintenance of ion gradients, both across cell membranes and between intracellular organelles and the cytoplasm.

Pyridoxine-dependent Seizures

Pyridoxine-dependent seizures are the only human epilepsy which is known to be due to a specific biochemical abnormality (Engel, 1989). As opposed to pyridoxine deficiency, "dependency" indicates a greater than normal brain requirement for pyridoxine by specific enzymes (Dakshinamurti et. al., 1990). It has been proposed that the glutamic acid decarboxylase, for which B6 is a co-factor, is deficient, thereby decreasing the synthesis of the inhibitory neurotransmitter GABA (Scriver and Whelan, 1969; Scriver and Hutchison, 1963). Diagnosis is made by noting a rapid improvement in an epileptiform EEG and consequent seizures in response to intravenous administration of pyridoxine. The seizures usually present in the neonatal period although significant heterogeneity exists for the age of presentation (Goutieres and Aicardi, 1985) and dose required for seizure relief (Clarke and Saunders, 1979). As children of both sexes within a generation could be affected in the absence of parental disease an autosomal dominant mode of inheritance has been suggested (Bejsovec et. al., 1967). The pedigrees that have been studied are small and ascertainment of affected siblings has been by either documentation of pyridoxine-dependent seizures or a history of an infant who developed refractory seizures and died (Bankier et. al., 1983).

Conclusion

The understanding of the genetic factors underlying human epilepsy is one of the most exciting areas of current epilepsy research. Advances are being made in the identification of chromosomes likely to be associated with individual epilepsy syndromes. Further studies will undoubtedly lead to localization at the gene level. The next challenge will be to understand how particular genetic defects lead to the heterogeneous group of neuronal hyperexcitability states which manifest as the epilepsies. Perhaps, principles and mechanisms gleaned from study of the genetic animal models of the epilepsies will provide the necessary insights. In the future, new treatment strategies may be directed at the genetic mechanisms responsible for expression of seizure phenotype, or the timing of onset and remission.

SUMMARY

Our knowledge regarding the genetic bases of the human epilepsies is in a state of rapid flux. For some forms of epilepsy, epidemiologists are still trying to determine whether there is any familial (hereditary) predisposition. Other forms of epilepsy are known to run in families, but the mode of inheritance and degree of penetrance of the trait are still in doubt. Three forms of epilepsy have been tentatively localized to specific chromosomes, and several others are being analyzed with linkage analyses. In no case, has the gene responsible for any human epilepsy been isolated or characterized and we are not yet close to understanding how any specific genetically controlled biochemical or physiological parameter is responsible for the development of an epilepsy syndrome or of any specific epileptogenic increase in brain excitability.

However, an extensive animal literature indicates that the mechanisms will be complex as a common phenotype can be the expression of multiple genes, and a single gene can be associated with several phenotypes (Buchhalter, 1993).

SUMARIO

Nuestro conocimiento concerniente a las bases genéticas de las epilepsias en el ser humano está en un estado de constante fluctuación. Para algunas formas de epilepsia, los epidemiólogos están todavía tratando de determinar si hay una predisposición familiar (hereditaria). Se conocen otras formas de epilepsia que ocurren en las familias, pero el modo de herencia y el grado de penetración de sus características son todavía dudosos. Tres formas de epilepsia han sido tentativamente localizadas a específicos cromosomas, y varias otras están siendo estudiadas con análisis de uniones. En ningún caso el gen responsable por cualquier epilepsia humana ha sido aislado o caracterizado y nosotros no estamos todavía cercanos a la comprensión de cómo cualquier específico genéticamente controlado bioquímico o parámetro fisiológico es responsable del desarrollo de un síndrome epiléptico o de cualquier aumento específicamente epileptogénico en la excitabilidad cerebral.

Sin embargo, una extensa literatura animal indica que los mecanismos serán complejos ya que un común fenotipo puede ser la expresión de múltiples genes, y un solo gen puede estar asociado con varios fenotipos (Buchhalter, 1993).

RÉSUMÉ

En ce qui concerne les bases génétiques de l'épilepsie chez l'homme, nos connaissances sont en constant état de fluctuation.

Pour quelques unes des formes, les épidemiologues en sont encore à déterminer s'il y a ou non une prédisposition familiale (héréditaire). On connait d'autres formes qui aparaissent dans certaines familles; mais le mécanisme de l'hérédité et le degré de pénétration sont encore douteux. Trois formes d'épilepsie ont été raprochées de certains cromosomes, et certaines autres ont été étudiées à travers l'analyse des unions. En aucun cas un gène déterminé a put être responsabilisé d'une épilepsie déterminée; et nous ne sommes pas près de comprendre comment n'importe quel agent génétiquement controlé, biologique ou paramètre physiologique, peut-être responsable du déroulement d'un syndrome épileptique ou de n'importe quelle augmentation, spécifiquement épileptogénique, de l'excitation cérébrale. Malgrës tout, une ample littérature (sur l'animal) indique que le mécanisme en est complexe puisque un fenotype peut être l'expression de gènes divers (ou multiples), et qu'un seul gène peut être associé à plusieurs feno types.(Buchhalter, 1993).

ZUSAMMENFASSUNG

Unsere Kenntnisse ueber die genetichen Grundlagen der Epilepsie (E) der Menschen sind einem staendigen Wandel unterworfen. Fuer einige Formen der E. versuchen die Epidemiologen festzustellen, ob eine familiaere erbliche Praedisposition vorliegt.

Man kennt andere Formen der E., die familiaer auftreten, bei denen jedoch die Art der Vererbung und der Grad der Penetration ihres Charakters noch weithin unbekannt sind.

Drei Formen der E. hat man versuchsweise in spezifischen Chromosomen lokalisiert, mittels der Unions-Analyse; und verschiedene andere Formen wurden mittels der Unions-Analyse studiert. In keinem Fall hat man das verantwortliche Gen irgend einer menslichen Form isoliert, und wir sind noch weit weg davon zu wissen, auf welche Weise irgend ein spezifisch genetisch kontrollierter, biochemischer oder physiologischer Parameter verantwortlich fuer die Entwicklung eines epileptichen Syndroms oder fuer irgend eine spezifische epileptogene Vermehrung der zerebralen Erregbarkeit sein kann.

Immerhin eine ausgedehnte Tierliteratur zeigt an, das die Mechanismen kompliezieert sind, denn ein gewoehnlicher Phaenotypus kann Ausdruck einer Vielheit von Genen sein, und ein einziges Gen kan Ursache Phaenotypen sein (Buchhalter, 1993).

REFERENCES

1. Aicardi, J. Epilepsy in Children, New York:Raven Press, 1986.
2. Aicardi, J. and Newton, R. Clinical findings in children with occipital spike wave complexes suppressed by eye opening. In: Migraine & Epilepsy, edited by Andermann, F. and Lugaresi, E. Stoneham: Butterworth, 1987, p. 111 - 124 .
3. Annegers, J.F.,Hauser, W.A. and Shirts, S.B. Factors prognostic of unprovoked seizures. New England Journal of Medicine,316:493-498, 1987.
4. Bankier, A.,Turner, M. and Hopkins, I.J. Pyridoxine dependent seizures-a wider clinical spectrum. Arch. Dis. Child.,58:415-418, 1983.
5. Bejsovec, M.I.R.,Kulenda, Z. and Ponca, E. Familial intrauterine convulsions in pyridoxine dependency. Arch Dis Child,42:201-207, 1967.
6. Blom, S. and Heijbel, J. Benign epilepsy of childhood with centro-temporal spikes. In: Genetics of the Epilepsies, edited by Mannagetta, G.B., Anderson, E.V., Doose, H. and Janz, D. Berlin: Springer-Verlag, 1989, p. 67-72.
7. Buchhalter, J.R. Animal models of inherited epilepsy. Epilepsia,34 (S3):S31-S41, 1993.
8. Clarke, T.A. and Saunders, B.S. Pyridoxine-dependent seizures requiring high doses of pyridoxine for control. Am J Dis Child, 133:963-965, 1979.
9. Commission on Classification and Terminology of the International League Against Epilepsy, Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia,22:489-501, 1981.
10. Commission on Classification and Terminology of the International League Against Epilepsy, Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia,30:389-399, 1989.
11. Currie, S.,Healthfield, K.,Henson, R. and Scott, D. Clinical course and prognosis of temporal lobe epilepsy. A survey of 666 patients. Brain,94:173-190, 1971.
12. Dakshinamurti, K.,Paulose, C.S.,Viswanathan, M.,Siow, Y.L. and Sharma, S.K. Neurobiology of Pyridoxine. Ann.NY Acad Sci.,585:128-144, 1990.
13. Dalla Barnardina, B.,Capovilla, G. and Gattoni, M. Epilepsie myoclonique grave de la premiere annee. Rev. Electroenceph. Neurophys.,12:21-25, 1982.
14. Degen, R. and Degen, H.E. Some genetic aspects of rolandic epilepsy: waking and sleep EEGs in siblings. Epilepsia,31:795-801, 1990.
15. Delgado-Escueta, A.V.,Greenberg, D.A.,Treiman, L.,et al. Mapping the gene for juvenile myoclonic epilepsy. Epilepsia,30 Suppl 4:S8-18, 1989.
16. Delgado-Escueta, A.V.,Greenberg, D.,Weissbecker, K.,et al. Gene mapping in the idiopathic generalized epilepsies: juvenile myoclonic epilepsy, childhood absence epilepsy, epilepsy with grand mal seizures, and early childhood myoclonic epilepsy. Epilepsia,31 Suppl 3:S19-S29, 1990.
17. Dichter, M. and Ayala, G.F. Cellular mechanisms of epilepsy: A status report. Science,237:157-164, 1987.
18. Dichter, M.A. and Buchhalter, J.R. The genetic epilepsies. In: The molecular and genetic basis of neurological disease, edited by Rosenberg, R.N., Prusiner, S.B., DiMauro, S., Barchi, R.L. and Kunkel, L.M. Boston: Butterworth-Heinemann, 1993, p. 925-948.
19. Doose, H. and Baier, W.K. Epilepsy with primarily generalized myoclonicastatic seizures: a genetically determined disease. Eur.J.Pediatr.,146:550554, 1987.
20. Doose, H. and Baier, W.K. Generalized Spikes and Waves. In: Genetics of the Epilepsies, edited by Mannagetta, G.B., Anderson, E.V., Doose, H. and Janz, D. Berlin: Springer-Verlag, 1989, p. 95-103.
21. Dravet, C., Bureau, M. and Roger, J. Severe myoclonic epilepsy in infants. In: Epileptic Syndromes in Infancy, Childhood and Adolescence, edited by Roger, J., Dravet, C., Bureau, M., Dreifuss, F. and Wolf, P. London: John Libbey, 1985, p. 58-67.
22. Eldridge, R.,Iivanainen, M.,Stern, R.,Koerber, T. and Wilder, B.J. "Baltic" myoclonus epilepsy: hereditary disorder of childhood made worse by phenytoin. Lancet,2:838-842, 1983.
23. Engel, J.Jr. Seizures and Epilepsy, Philadelphia:F. A. Davis Company, 1989.
24. Evans, J. Post-traumatic epilepsy. Neurology, 12:665-674, 1962.
25. Falconer, M. Genetic and related aetiological factors in temporal lobe epilepsy. A review. Epilepsia, 12:13-31, 1971.
26. Fujiwara, T.,Nakamura, H.,Watanabe, M.,Yagi, K. and Seino, M. Clinicoelectrographic concordance between monozygotic twins with severe myoclonic epilepsy in infancy. Epilepsia,31:281-286, 1990.
27. Gastaut, H. Evidence electrographique d'un mecanisme sous-cortical dans certaines epilepsies partielles-la signification clinique des "secteurs areothalamiques". Rev Neurol,83:396-401, 1950.
28. Gastaut, H. A new type of epilepsy: Benign partial epilepsy of childhood with occipital spike-waves. Clin. Electroenceph.,13:13-22, 1982.
29. Gastaut, H. and Zifkin, B.G. Benign epilepsy of childhood with occipital spike and wave complexes. In: Migraine and Epilepsy, edited by Anderman, F. and Lugaresi, E. Boston: Butterworths, 1987, p. 47-82.
30. Gibbs, F. and Gibbs, E. Atlas of Electroence phalography, Cambridge:Addison-Wesley Press, 1952. pp. 222-224.
31. Goutieres, F. and Aicardi, J. Atypical presentations of pyridoxinedependent seizures: A treatable cause of intractable epilepsy in infants. Ann. Neurol., 17:117-120, 1985.
32. Greenberg, D.A.,Delgado-Escueta, A.V.,Widelitz, H.,et al. Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. Am.J.Med.Genet.,31:185-192, 1988.
33. Greenberg, D.A.,Durner, M.,Delgado-Escueta, A.V. and Janz, D. Is juvenile myoclonic epilepsy an autosomal recessive disease? [letter; comment]. Ann. Neurol.,28: 110-111, 1990.
34. Heijbel, J.,Blom, S. and Rasmuson, M. Benign epilepsy of childhood with centrotemporal foci: a genetic study. Epilepsia,16:285-293, 1975.
35. Holowach, J.,Thursten, D. and O'Leary, J. Jacksonian seizures in infancy and childhood. J. Pediat., 52:670-686, 1958.
36. Janz, D. Epilepsy with impulsive petit mal (juvenile myoclonic epilepsy). Acta Neurol Scand,72:449-459, 1985.
37. Jeavons, P. Nosological problems of myoclonic epilepsies in childhood and adolescence. Dev. Med. Child Neurol.,19:3-8, 1977.
38. Jeavons, P.M. and Harding, G.F.A. Photosensitive Epilepsy, London: Heinemann, 1975.
39. Jensen, I. Genetic factors in temporal lobe epilepsy. Acta. Neurol. Scand.,52:381-394, 1975.
40. Koskiniemi, M. Baltic myoclonus. Adv. Neurol., 43:57-64, 1986.
41. Lehesjoki, A.E.,Koskiniemi, M.,Sistonen, P.,et al. Localization of a gene for progressive myoclonus epilepsy to chromosome 21q22. Proc.Natl.Acad.Sci.U.S.A.,88:3696-3699, 1991.

42. Lennox, W. The heredity of epilepsy as told by relatives and twins. JAMA,146:529-536, 1951.
43. Lennox-Buchthal, M. Febrile and nocturnal convulsions in monozygotic twins. Epilepsia,12:147-156, 1971.
44. Lennox-Buchthal, M.A. Febrile convulsions. A reappraisal. EEG Clin Neurophys,32:Suppl: 1-38, 1973 .
45. Leppert, M.,Anderson, V.E.,Quattlebaum, T.,et al. Benign familial neonatal convulsions liked to genetic markers on chromosome 20. Nature,337:647648, 1989.
46. Leppert, M. Gene mapping and other tools for discovery. Epilepsia,31 Suppl 3:S11-S18, 1990.
47. Lerman, P. and Kivity, S. Benign Focal Epilepsy of Childhood: A follow-up study of 100 recovered patients. Arch. Neurol.,32:261-264, 1975.
48. Lund, M. Epilepsy in association with intracranial tumor. Acta. Psychiat Neurol. Scand . , 81:3-149 , 1952.
49. Mattson, R.,Cramer, J.,Collins, J.,et al. Comparison of carbamazepine, phenobarbital, phenytoin and primidone in partial and secondarily generalized tonic-clonic seizures . NEJM,31:145-151, 1985 .
50. Metrakos, K. and Metrakos, J. Genetics of convulsive disorders. II. Genetic and electroencephalographic studies in centrencephalic epilepsy. Neurology, 11:464-483, 1961.
51. Nelson, K. and Ellenberg, J. Predictors of epilepsy in children who have experienced febrile seizures. NEJM,295:1029-1033, 1976.
52. Newmark, M.E. and Penry, J.K. Photosensitivity and Epilepsy: A Review, New York:Raven Press, 1979.
53. Norio, R. and Koskiniemi, M. Progressive myoclonus epilepsy: genetic and nosological aspects with special reference to 107 Finnish patients. Clin.Genet., 15:382-398, 1979.
54. Obeid, T. and Panayitopoulos, C. Juvenile myoclonic epilepsy: a study in Saudi Arabia. Epilepsia, 29:280-282, 1988.
55. Ottman, R. Genetics of the partial epilepsies: a review. Epilepsia,30: 107111, 1989.
56. Panayiotopoulos, C.P. Benign childhood epilepsy with occipital paroxysms: A 15 year prospective study. Ann. Neurol.,26:1:51-56, 1989.
57. Panayiotopoulos, C.P. and Obeid, T. Juvenile myoclonic epilepsy: an autosomal recessive disease. Ann.Neurol.,25:440-443, 1989.
58. Rimoin, d. and Metrakos, J. The genetics of convulsive disorders in the families of hemiplegics. In: Proceedings of the II International Congress on Human Genetics, Unknown, 1963, p. 1655-1658.
59. Rosing, H.S.,Hopkins, L.C.,Wallace, D.C.,Epstein, C.M. and Weidenheim, K. Maternally inherited mitochondrial myopathy and myoclonic epilepsy. Ann.Neurol.,17:228-237, 1985.
60. Ryan, S.G.,Wiznitzer, M.,Hollman, C.,Torres, M.C. Szekeresova, M. and Schneider, S. Benign familial neonatal convulsions: Evidence for clinical and genetic heterogeneity. Ann Neurol,29:469-473, 1991.
61. Schiottz-Christensen, E. Genetic factors in febrile convulsions. An investigation of 64 same- sexed twin pairs. Acta. Neurol. Scand.,48:538546, 1972.
62. Scriver, C.R. and Hutchison, J.H. The vitamin B6 deficiency syndrome in human infancy: Biochemical and clinical observations. Pediatrics,240-250, 1963 .
63. Scriver, C.R. and Whelan, D.T. Glutamic acid decarboxylase (GAD) in mammalian tissue outside the central nervous system, and its possible relevance to hereditary vitamin B6 dependency with seizures. Ann NY Acad Sci.,166:83-96, 1969.
64. Serratosa, J.,Weissbecker, K. and Delgado-Escueta, A. childhood absence epilepsy: an autosomal recessive disorder? Epilepsia,31:651 (Abst.), 1990.
65. Shih, K.D.,Yen, T.C.,Pang, C.Y. and Wei, Y.H. Mitochondrial DNA mutation in a Chinese family with myoclonic epilepsy and ragged-red fiber disease. Biochem. Biophys. Res.Commun. , 174:1109-1116, 1991.
66. Shoffner, J.M.,Lott, M.T.,Lezza, A.M.,Seibel, P.,Ballinger, S.W. and Wallace, D.C. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell,61:931-937, 1990.
67. Terasaki, T., Yamatogi, Y. and Ohtahara, S. Electroclinical delineation of occipital lobe epilepsy in childhood. In: Migraine & Epilepsy, edited by Andermann, F. and Lugaresi, E. Stoneham: Butterworths, 1987, p. 125-138.
68. Treiman, D.M. Genetics of the Partial Epilepsies. In: Genetics of the Epilepsies, edited by Mannagetta, G.B., Anderson, E.V., Doose, H. and Janz, D. Berlin: Springer-Verlag, 1989, p. 73-82.
69. Tsuboi, T. and Okada, S. The genetics of epilepsy. In: Genetic Aspects of Human Behavior, edited by Sakai, T. and Tsuboi, T. Tokyo: Igaku-Shoin,1985
70. Tsuboi, T. Genetic analysis of febrile convulsions: twin & family studies. In: Genetics of the Epilepsies, edited by Mannagetta, G.B. Berlin: SpringerVerlag, 1989, p. 25-33.
71. Vogel, F. The genetic basis of the normal human electroencephalogram(EEG).Humangenetik,10:91-11 4, 1970.
72. Weissbecker, K.A.,Durner, M.,Janz, D.,Scaramelli, A.,Sparkes, R.S. and Spence, M.A. Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. Am.J.Med.Genet., 38:32-36, 1991.
73. Wienker, T.F.,von Reutern, G.M. and Ropers, H.H. Progressive myoclonus epilepsy. A variant with probable X-linked inheritance. Hum.Genet.,49:8389, 1979.
74. Zonana, J.,Silvey, K. and Strimling, B. Familial neonatal and infantile seizures: an autosomal-dominantdisorder.Am.J.Med.Genet.,18:45 5-459, 1984.

Neurological Diseases Due to Mitochondrial DNA Mutations: Concepts and Problems in Pathogenesis

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1 .INTRODUCTION

"Mitochondrial molecular genetics", a new chapter in human pathology that deals with mutations in mitochondrial DNA (mtDNA), began only seven years ago with the discovery of the first mtDNA mutations related to human diseases (1,2). It is perhaps not surprising, therefore, that there are so many enigmatic questions regarding pathogenesis. Although this review will identify some of these questions, it seems appropriate to begin by summarizing the central concepts of mitochondrial genetics and their clinical relevance (3).

(i) **MtDNA**. Mitochondria are unique mamalian organelles in that they contain distinct genetic material. The DNA is a 16,569 base-pair circular structure tightly packed with no introns within the genes encoding 13 polypeptides, two ribosomal RNAs, and punctuated by 22 transfer RNAs (Figure 1).

(ii) **Polyplasmy**. In contrast to nuclear DNA (nDNA) genes, which are each represented by two alleles, multiple mitochondria are present in most cells and, in turn, each mitochondrion contains multiple genomes, consequently each cell contains hundreds or thousands of copies of mtDNA. At cell division, mitochondria and mtDNAs segregate randomly among daughter cells.

(iii) **Maternal inheritance**. At fertilization, virtually all mtDNA derives from the oocyte (Figure 2). Therefore, the mode of transmission of mtDNA (and of mtDNA point mutations) differs from mendelian inheritance. A mother carrying a mtDNA point mutation will pass it on to all her children, males as well as females, but only her daughters will transmit it to their children. Maternal transmission may not be easy to detect at the clinical level because of heteroplasmy and the threshold effect, which explain how different individuals in the matrilinear lineage of the same family may be severely affected, oligosymptomatic, or apparently healthy.

(iv) **Heteroplasmy**. In normal tissues, all mtDNA molecules are identical (homoplasmy). A mutation may be present in all mtDNA genomes (homoplasmic mutant). Altematively, the mutation might exist in some of the genomes, so that the cells harbor two populations of mtDNA, wild-type mtDNA and mutant mtDNA: this situation is called heteroplasmy. Generally, neutral polymorphisms of mtDNA are homoplasmic, whereas most, but not all, deleterious mutations are heteroplasmic.

(v) **Threshold effect**. The clinical (phenotypic) expression of a pathogenic mtDNA mutation is determined largely by the relative proportion of wild-type and mutant genomes in different tissues. In other words, for a mtDNA mutation to impair energy metabolism to an extent sufficient to cause dysfunction of that particular organ or tissue, a minimum critical number of mutant mtDNA is

From left to right: Dr. Víctor Soriano, Mrs. Clara Soriano and Dr. Salvatore DiMauro who is holding in his hand a bronze plaque given by the "American Neurological Association" after delivering "The Soriano Lecture" in San Francisco.

necessary; a concept aptly termed the "threshold effect" (4). Thus, when assessing a clinical syndrome due to mtDNA mutation, it is important to keep in mind that the vulnerability of different tissues to a given degree of heteroplasmy depends on the oxidative metabolism requirements.

(vi) **Mitotic segregation**. At cell division, the proportion of mutant mtDNAs in daughter cells may shift, and the phenotype may change accordingly. Furthermore, the proportion of mutant mtDNAs might change in different tissues. This phenomenon are exemplified by some children with Pearson's bone marrow/pancreas syndrome, who survive this usually fatal hemopoietic disorder only to develop, later in life, symptoms and signs of the Kearns-Sayre syndrome (KSS), a mitochondrial encephalomyopathy (5,6). The molecular basis of both conditions is generally single deletion in mtDNA which arises during oogenesis or embryogenesis. It is likely that the number of deletions decreases in successive generations of rapidly replicating blood cells when selective survival of normal cells occurs. By contrast, the number of deletions increases with time in postmitotic tissues such as brain and muscle.

2. DOCUMENTING THE PATHOGENICITY OF mtDNA POINT MUTATIONS

Since 1988, when the first point mutation in mtDNA was discovered in several pedigrees with Leber's hereditary optic neuropathy (LHON) (2), more than thirty distinct mutations have been associated with human diseases (7), and the number keeps growing. Because the rate of spontaneous mutation of human mtDNA is quite high and its capacity for repair is limited, numerous neutral polymorphisms have become fixated in the human mitochondrial genome (8,9), a phenomenon that has been exploited to trace the origin of the human species (10) and of human populations (11). The high abundance

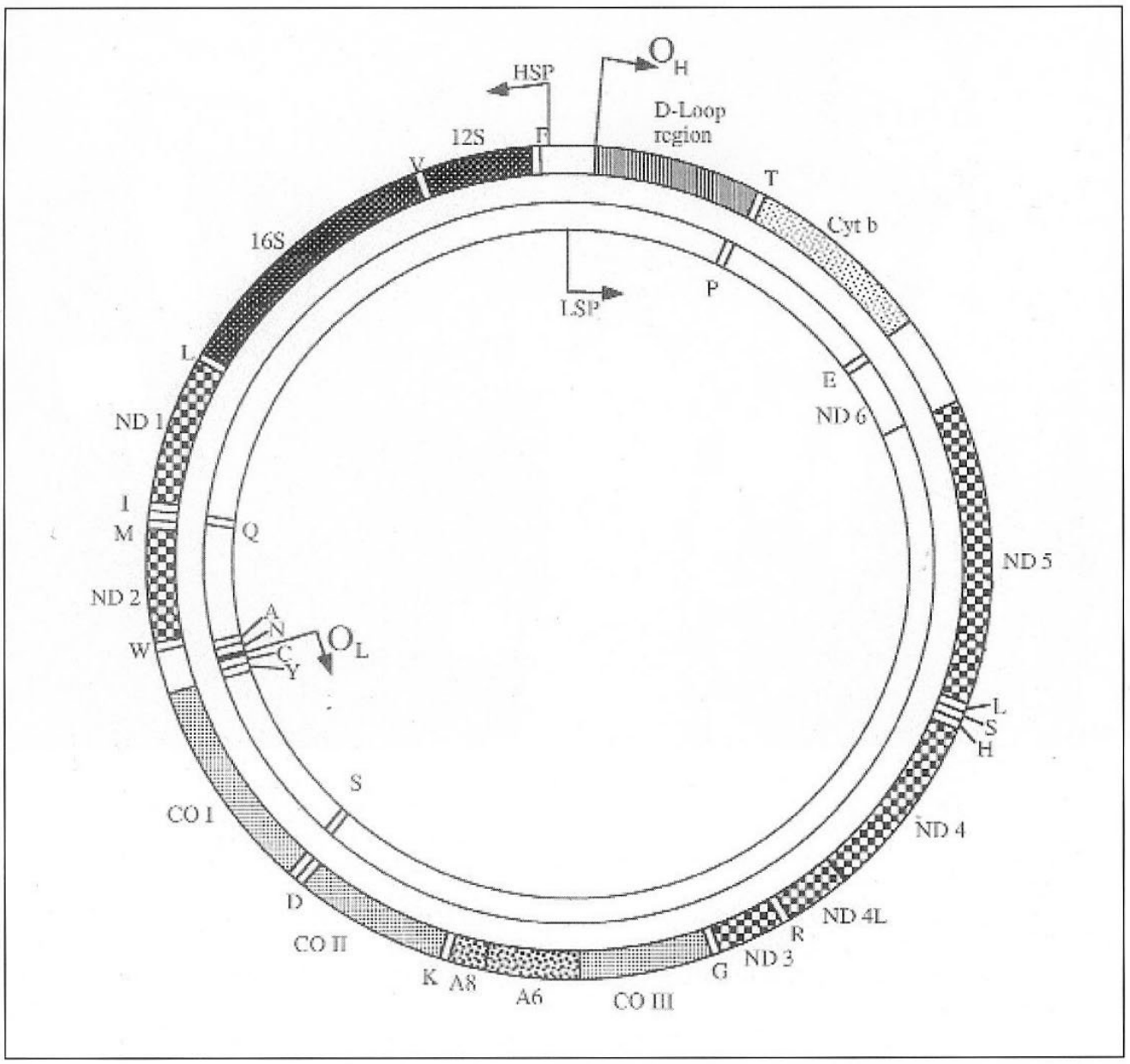


Figure 1. Schematic representation of mitochondrial DNA (MtDNA)
Map of the human mitochondrial DNA. Genes are represented by differently shaded areas. Individual patterns identify genes encoding the rRNAs, the tRNAs, or the subunits of the same respiratory complex. The inner circle represents the light strand, and the outer circle the heavy strand. Abbreviations: OH, OL, origin of replication of the heavy (H) or light (L) strand (arrows indicate the direction of duplication or transcription); Cyt b, cytochrome b; ND1-ND6, subunits of NADH dehydrcgenase complex (complex I); COI-COIII, subunits of cytochrome c oxidase (complex IV). Single capital letters indicate tRNA genes and identify the corresponding aminoacids by conventional single-letter code.

of benign polymorphism, however, imposes on the clinical scientist the burden of proving the pathogenicity of any newly discovered mtDNA alteration in a patient with suspected mitochondrial encephalomyopathy.

There are three "canonical" criteria that support a pathogenic role for a newly identified mutation:

(i) the base change in question has never been encountered in normal individuals. To confirm this point, control individuals, preferably from multiple ethnic backgrounds, should be screened and should not harbor the putative mutation.

(ii) the base change involves a region of the mitochondrial genome that has been conserved during evolution and is, therefore, presumably functionally important.

(iii) the base change is heteroplasmic. This is not an absolute requirement, because some

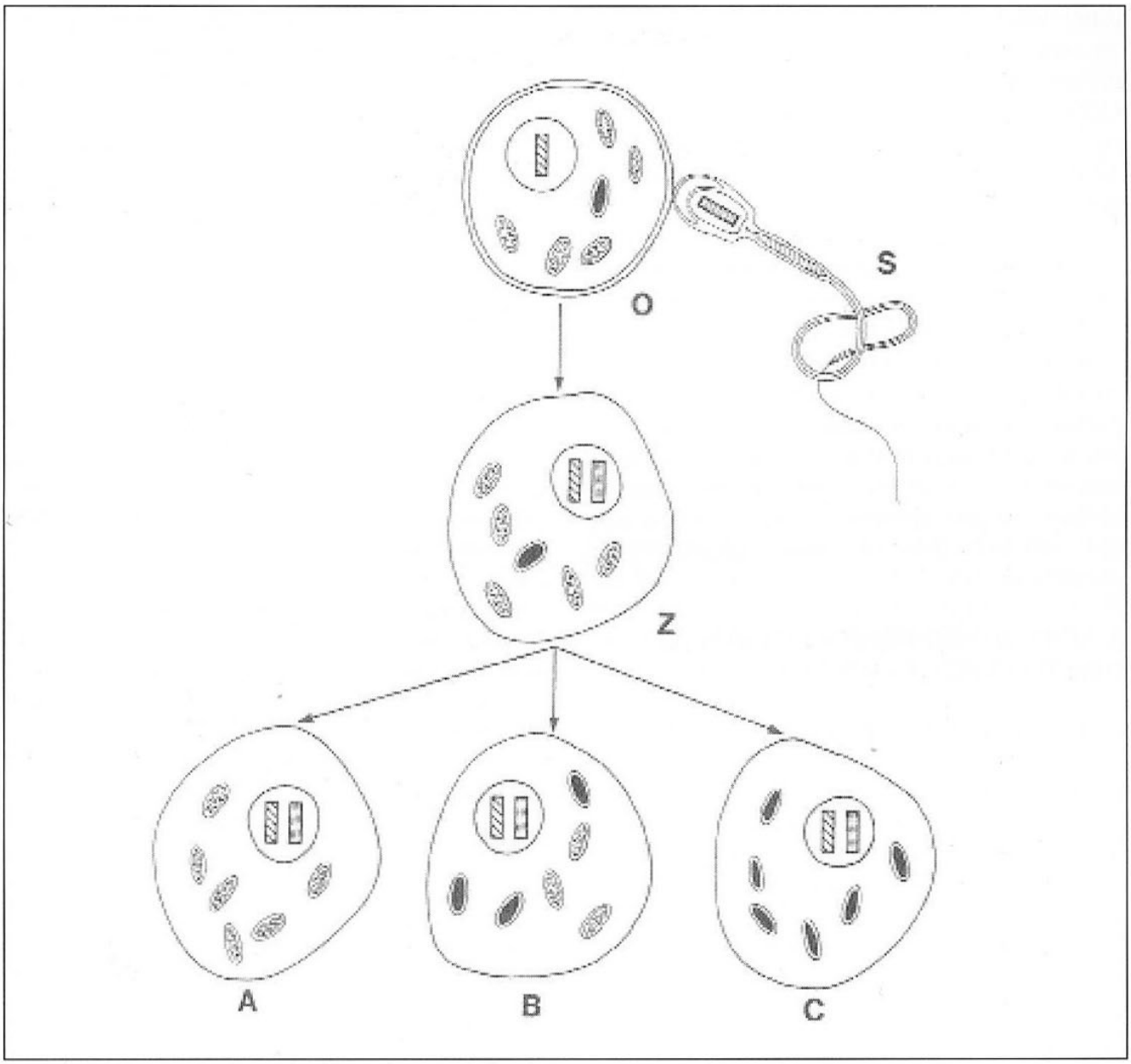


Figure 2. Di Mauro, Hirano.
Cartoon illustrating the maternal inheritance of mitochondrial genomes and the random distribution of mutant and wild-type genomes in daughter cells of the zygote. For simplicity, the relative sizes of the oocyte and the sperm have not been respected, and it has been assumed that individual mitochondria contain either a single mitochondrial genome or uniform populations of mutant (filled mitochondria) or wild-type (open mitochondria) genomes. Abbreviations: 0, oocyte; S, sperm; Z, zygote; A,B,C, daughter cells representing stem-cells of different tissues.

pathogenic mutations (including the very first described by Wallace et al in LHON (2)) have been homoplasmic. It is likely, however, that homoplasmy for most deleterious mutations would be lethal. Conversely, heteroplasmy has never been shown for neutral mutations.

Additional evidence for pathogenicity is provided by demonstration of the mutation in maternal relatives of the proband, especially if there is a good correlation between abundance of the mutation and severity of symptoms and signs.

(iv) To these criteria, we would like to add, whenever possible, a fourth criterion of pathogenicity based on single-fiber PCR. If the muscle biopsy from the patient shows mitochondrial proliferation with ragged-red fibers (RRF) (Figure 3a), these patently dysfunctional fibers (which usually also fail to stain or stain weakly for cytochrome c oxidase [COX][Figure 3b]) can be mechanically removed from thick cross-sections, transferred to a test-tube and processed for polymerase chain reaction (PCR) amplification and

detection of the mutation. Using this technique, we have been able to document higher abundance of mutations in RRF or COX-negative fibers than in "normal" fibers not only in patients with point mutations (12-14) but also in patients with large-scale deletions (15).

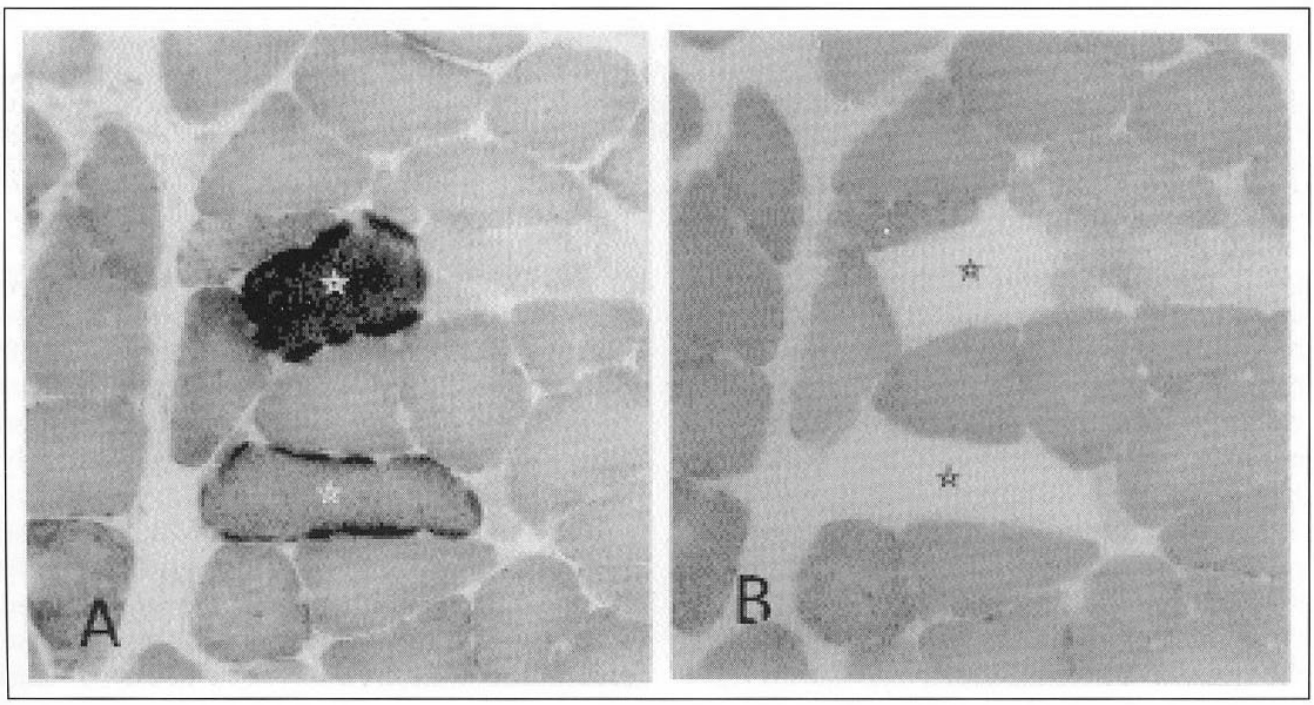
(v) A fifth criterion can be applied when a structural gene encoding a polypeptide is thought to contain a mtDNA mutation; correlation with a biochemical defect in the mutated enzyme strongly supports the pathogenicity of the mutation. Unfortunately, we frequently fail to detect the biochemical abnormality with our conventional assays, perhaps in part because in vitro systems may not reflect the in vivo physiological conditions.

3. WHY DO DIFFERENT CLINICAL PHENOTYPES EXIST?

The clinical classification of disorders due to mtDNA mutations has been marked by heated debates between "lumpers" and "splitters" even before it was recognized that these syndromes (most notably, Kearns-Sayre syndrome [KSS]; myoclonus epilepsy with RRF [MERRF]; and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS]) were, in fact, due to distinct mtDNA mutations (16-18). The "splitters" tried to define distinct and separate clinical disorders, while, by contrast, the "lumpers" argued that because of the many overlapping features of patients with respiratory chain defects, discrete syndromes were unidentifiable. Considering that all mtDNA mutations presumably impair oxidative/phosphorylation and energy production, the question of why should we see different clinical phenotypes seems legitimate, even to die-hard "splitters" like the authors of this review. There are several possible explanations:

(i) **Type of gene affected**. We and others have observed that massive mitochondrial proliferation, histologically evident as RRF, occurs almost invariably in patients with mtDNA alterations causing impairment of mitochondrial protein synthesis, such as mtDNA deletions encompassing tRNA or rRNA genes, point mutations in tRNA genes, or mtDNA depletion. By contrast, RRF are

Figure 3. Di Mauro, Hirano.
Histochemical stains for succinate dehydrogenase (SDH, panel A) and cytochrome c oxidase (COX, panel B) activities in serial sections from a patient with mitochondrial encephalomyopathy. Two ragged-red fibers are identified by excessive SDH stain (white stars). The same fibers in the serial section show lack of COX activity (black stars). x150 (Courtesy of Dr. Eduardo Bonilla).



almost never seen in patients with point mutations in polypeptide-encoding (structural) genes, such as those observed in LHON or in NARP (neuropathy, ataxia, and retinitis pigmentosa) which generally affect, respectively, complex I and ATPase subunits. Because all pathogenic mtDNA mutations ought to affect oxidative phosphorylation, the signal for mitochondrial proliferation may not be the impairment of oxidative phosphorylation per se, but rather a response to defective mitochondrial protein synthesis. The nature of this signal, however, remains elusive.

In addition, one would expect that mutations in structural genes cause decreased activity of the specific enzyme affected by the mutation, whereas mutations in genes controlling mitochondrial protein synthesis ought to result in decreased activities of all complexes of the respiratory chain except complex II (which is encoded entirely by nuclear genes). Both situations have been verified. Repeatedly, we have found abnormal activities for complexes I, III, and IV in patients with mtDNA deletions (19), point mutations affecting tRNA genes (14,20,21), and mtDNA depletion (22,23). On the other hand, isolated defects of complex I activity have been documented in platelets (24), leukocytes (24a), and muscle (25) from patients with LHON and point mutations in the genes for ND6, ND1, and ND4, respectively. Also, a defect in ATP synthesis has been documented in mitochondria from patients with the NARP mutation in the ATPase 6 gene of mtDNA (26,27).

(ii) **Abundance of the mutation**. The influence of the abundance of a heteroplasmic mutation on the clinical phenotype is exemplified by the T-to-G transversion at nt8993 in the ATPase 6 gene. When present in relatively low proportion, this mutation is associated with NARP, a mitochondrial encephalomyopathy typically manifesting in young adult life (28). However, when the mutation is very abundant, it causes maternally-inherited Leigh syndrome (MILS) (29,30).

(iii) **Distribution of the mutation in different tissues**. Large-scale mtDNA single deletions (that is, each patient harbors only one type of deletion) are associated, among others, with two syndromes that share ocular myopathy as a common feature, but differ strikingly in severity. The first is Kearns-Sayre syndrome, a devastating multisystem disorder characterized by the triad of: (1) onset before age 20; (2) progressive external ophthalmoplegia (PEO); and (3) pigmentary retinopathy, plus at least one of the following: heart block, cerebellar syndrome, or cerebrospinal fluid protein above 100mg/dl. The second syndrome is sporadic PEO with RRF, a clinically benign condition characterized by ophthalmoplegia, ptosis, and proximal limb weakness.

While both type and abundance of mtDNA deletions in skeletal muscle overlap in the two conditions (19), the deletions are present in all tissues in KSS, as documented in several postmortem studies (19,31-33), whereas indirect evidence suggests that in PEO deletions must be absent or present in low abundance in tissues other than muscle. The evidence is indirect because no postmortem study has been conducted in this benign condition and mtDNA has been studied only in readily accessible tissues, such as blood, and cultured fibroblasts.

(iv) **Distribution of the mutation within a single tissue**. It is conceivable that very high concentrations of a mutation in specific areas of a given tissue might also "specify" the clinical phenotype. This concept would apply especially to heterogeneous tissues, such as the brain, although direct evidence is still lacking. For example, it would be interesting to see whether brain nuclei that are particularly affected in MERRF, such as the dentate and olivary nuclei (34), harbor higher levels of the mutation than surrounding brain areas. It would be equally interesting to see if, in patients with MELAS, cortical areas that are commonly affected by "strokes", such as the occipital cortex, have abundant mutations. Unfortunately, these studies are made difficult by the fact that targeted areas obtained postmortem are likely to show secondary changes (neuronal loss, gliosis) and may, therefore, not reflect the original levels of a given mutation.

Indirect evidence for the validity of the above concept has come from studies of muscle. We had noticed that a substantial number of patients with PEO and RRF harbored the "MELAS mutation" at nt-3243 in the tRNALeu(UUR) gene (35). We also noticed that, whereas most RRF in patients with MELAS are COX-positive (a distinguishing feature of MELAS from other mitochondrial encephalomyopathies), many RRF in patients with PEO were COX-negative. In an attempt to understand the basis for such striking clinical heterogeneity, we have compared the distribution of the nt-3243 mutation in individual muscle fibers from patients with typical MELAS and from patients with PEO (36). We found a direct correlation between the concentration of the nt-3243 mutation and impairment of COX activity at the single fiber level: the levels of mutant mtDNAs were relatively low (56±21%) in non-RRF, high (95±3%) in COX-positive RRF, and the mutation was virtually homoplasmic in COX-negative RRF (36). The presence of these quasihomoplasmic RRF seems to distinguish patients with PEO from patients with MELAS and may explain the predominantly muscular phenotype. It is tempting to speculate that, conversely, patients with MELAS may have almost homoplasmic concentrations of the mutation in localized areas of the brain and that these areas may be prone to decompensate when faced with sudden increases in energy demand. This, however, remains to be documented.

4. SAME GENOTYPE - DIFFERENT PHENOTYPES

We have already mentioned three apparently puzzling situations where the same mutation is associated with different phenotypes, and we have proposed possible explanations. These mutations are: (i) **single deletions of mtDNA**, which can cause Pearson's bone marrow/pancreas syndrome, KSS, or sporadic PEO with RRF; (ii) the **A-to-G mutation at nt-3243**, which can cause MELAS, maternally-inherited PEO with RRF, or diabetes mellitus and deafness (DAD); and (iii) the **T-to-G mutation at nt-8993**, which can cause NARP or MILS. It has been proposed that the different clinical expressions of a single mtDNA mutation may be due to differences in the nuclear genome or to the coexistance of other mtDNA polymorphisms, which would, in isolation, be clinically silent. While the second "scenario" has been hypothesized in patients with LHON (37), we think that the situations listed above can be adequately explained by the peculiar features of mtDNA genetics, including heteroplasmy, mitotic segregation, and the threshold effect.

5. DIFFERENT GENOTYPES - SAME PHENOTYPE

Another apparent puzzle is represented by different mutations giving rise to very similar clinical syndromes. The most impressive example is probably Leigh's syndrome (LS), a devastating encephalopathy of infancy or early childhood characterized by symmetrical necrotizing lesions in the basal ganglia, pons, medulla, and spinal cord. The three major etiologies of LS represent three different modes of inheritance: (i) COX deficiency, which is partial and generalized (38), is transmitted as an autosomal recessive trait (39); (ii) pyruvate dehydrogenase (PDH) deficiency, which is transmitted most commonly as an X-linked recessive trait (40); and ATP synthase deficiency due to the T-to-G mutation at nt-8993 of mtDNA and is transmitted matemally (MILS) (29,30,41). Although the main clinical and laboratory features associated with the three mutations are very similar, some interesting clinical differences emerge from the comparison of large series of patients. Onset tends to be earlier and seizures are more common in patients with PDH and MILS than in patients with COX deficiency, and pigmentary retinopathy is seen only in patients with MILS (30).

Five mutations at different sites in the tRNALeu(UUR) gene have been associated with MELAS (42-46). While this may not appear too surprising because the same gene is involved in all cases, the MELAS phenotype has also been associated with two mutations in structural genes. The pathogenicity of the A-to-G transition at nt-11084 in the gene encoding subunit 4 of complex I (ND4) (47), has been questioned because the mutation has been found in normal Asian individuals (48).

The second structural gene mutation is a T-to-C transition at nt-9557 in the gene encoding COX-III (49).

Two mutations at different sites in the tRNALys gene have been associated with MERRF, a more common one at nt-8344 (50) and a rare one at nt-8356 (51).

At least 15 different mutations have been associated with LHON, all in structural genes encoding subunits of complex I, III, or IV (37). However, while some of these mutations are sufficient to cause LHON per se (primary mutations), others (secondary mutations) behave as neutral polymorphisms in isolation but seem to act synergistically to increase the probability that primary mutations cause blindness (37).

A variation on the theme of "different mutations - same phenotype" is represented by different mutations not only in the same gene, but at the very same site of the same gene, such as the T-to-C substitution identified at nt-8993 in some patients with MILS instead of the more common T-to-G mutation (52,53). Although in this situation a common phenotype would appear hardly surprising, we have preliminary evidence suggesting that, in fact, the T-to-C mutation results in a less severe clinical syndrome than the T-to-G mutation (Santorelli et al, in preparation). This might be explained by the different amino acid substitutions in the ATPase 6 polypeptide. While the leucine at position 156 is converted to arginine by the T-to-G mutation, and it is converted to proline by the T-to-C mutation. Tatuch et al. suggested that the positively charged arginine is likely to alter proton translocation through the F_1-F_0 proton channel of ATP synthase, thus hindering ATP synthesis (29). The much less drastic change in electrical charge induced by the proline substitution may explain the milder phenotype associated with the T-to-C mutation, but this remains to be documented at the biochemical level.

6. PATHOGENESIS OF STROKES IN MELAS

A major problem in mitochondrial encephalomyopathies regards the pathogenesis of the "stroke-like episodes" that are the clinical hallmark of the MELAS syndrome. Controversy exists between two hypotheses, one postulating that the strokes are vascular in origin, the other postulating that they are "metabolic strokes".

The vascular hypothesis is based essentially on morphological evidence showing marked accumulation of mitochondria in endothelial and smooth muscle cells of arterioles both in muscle (54-56) and in brain (57,58). In brain, these lesions affected mostly pial arterioles and small arteries up to 250 μm in diameter and were less frequent in intracerebral vessels (57). The same alterations were seen in the choroid plexus (58).

There are three versions of the vascular hypothesis. (i) "Indirect" involvement of the brain due to cardiomyopathy leading to chronic anoxia or embolization. This mechanism is unlikely because cardiomyopathy is not a consistent feature of MELAS, thromboembolization has not been documented in postmortem studies, and the stroke-like lesions do not respect large arterial perfusion territories. (ii) Occlusion of small vessels due to the proliferation of mitochondria in endothelial cells (59). However, mitochondrial proliferation severe enough to obliterate the lumen has not been observed in a careful morphological study of muscle biopsies from several patients (54). (iii) The most attractive hypothesis is that a primary "mitochondrial angiopathy" affecting small arteries of the brain may lead to impairment of autoregulation in the cerebral circulation (54,57). The observation that, at least in muscle biopsies, the mitochondrial angiopathy is seen much more often in MELAS than in other encephalomyopathies (55) could explain why strokes are a clinical hallmark of MELAS. In addition, the involvement of small arteries and capillaries could explain why strokes in MELAS usually do not conform to the distribution of major cerebral arteries (60).

The "metabolic stroke" hypothesis envisions groups of neurons in the cortex harboring very high percentages of mutant mtDNAs, just below the threshold needed to cause irreversible cell dysfunction. A sudden

increase in energy demand imposed on these cells, such as that accompanying a seizure, could cause the cells to decompensate. There are several lines of evidence supporting a non-vascular pathogenesis, including normal angiograms and cerebral blood flow studies revealing generalized cerebral hyperperfusion (56,60-62). These studies suggest that focal brain damage occurs in areas with preserved circulation. The high lactate concentration documented by nuclear magnetic resonance proton spectroscopy in vulnerable areas of the brain in patients with MELAS further supports a metabolic rather than vascular pathogenesis. One puzzling aspect of the "metabolic stroke" hypothesis is the relative specificity of strokes for the MELAS mutation at nt3243. Why shouldn't other mutations impairing brain metabolism, such as the "MERRF" mutation at nt-8344 or mtDNA deletions, cause strokes? While strokes do, in fact, occur in rare patients with deletions (63) and the "MERRF" mutation (64), their prevalence in association with the MELAS mutation can only be explained by postulating a particularly high frequency of this mutation in cortical neurons, and especially in areas that are prone to show "metabolic infarcts", such as the occipital cortex. Such localized concentration of the MELAS mutation in certain neurons could be the counterpart of the extremely high concentration of the same mutation found in individual muscle fibers in patients with myopathy rather than stroke-like episodes (36). However, this remains to be documented and, in any case, it begs the question of which factors are responsible for the differential localized abundance of distinct mutations in different areas of the brain.

CONCLUSIONS

In contrast to the rapid growth in our understanding of the molecular basis of mtDNA-related diseases, we have only a rudimentary grasp of the pathogenic mechanisms responsible for the protean clinical expression of these genetic defects. Functional studies are hindered by the absence of animal models, and by the shifts in the percentages of mutant mitochondrial genomes observed in subsequent generations of cells in primary fibroblast and muscle cultures (65). An extremely useful tool to study the functional consequences of mtDNA mutations is represented by the rho° cells, human cell lines completely devoid of mtDNA after prolonged exposure to ethidium bromide (66). By fusing these cells with enucleated cell (cytoplasts) from patients, the rho° cells can be repopulated with mitochondria containing mutant genomes to form cybrids. Several biochemical features, including oxidative phosphorylation and mitochondrial protein synthesis, can be studied in cybrid cell lines containing varying proportions of mutant and wild-type mtDNAs. These experimental approaches together with increasingly sophisticated and noninvasive neuroradiological techniques provide promising new approaches to a better understanding of pathogenetic mechanisms.

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SUMMARY

Although the first mutations of mitochondrial DNA (mtDNA) were reported in 1988, progress in the new field of "mitochondrial genetics" has been spectacular and numerous syndromes ("mitochondrial encephalomyopathies") have been associated with mtDNA rearrangements (deletions or duplications) or with point mutations in tRNA genes or in structural genes. The principles of mitochondrial genetics are briefly reviewed.

In contrast to our rapid progress in elucidating the molecular basis of these disorders, understanding of the pathogenetic mechanisms remains incomplete. We discuss several pathogenic puzzles, including: (a) why are there so many distinct clinical phenotypes?; (b) why does the same mutation cause different phenotypes?; (c) why do different mutations cause the same phenotype?; (d) what is the pathogenesis of strokes in MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes)?

RESUMEN

En 1988 las primeras mutaciones en el DNA mitocondrial fueron reportadas, desde entonces los progresos en el campo de la "genética mitochondrial" han sido asombrosos resultando en numerosos sindromes ("Encefalomiopatias mitochondriales") asociados con reordenamientos del DNA mitocondrial (deleciones y duplicaciones) o con diferentes puntos de mutacion en los tRNA genes o en genes estructurales. Las bases de la genética mitocondrial serán brevemente discutidas. En contraste con el rápido avance en la detección de las bases moleculares en estos transtornos, el progreso logrado en lo referente a los mecanismos patogénicos subyacentes es aún limitado, y numerosas preguntas esperan por respuestas. Discutiremos entre ellas: (a) ¿Porqué existen tantos y tan diferentes fenotipos clínicos? (b) ¿Cuál es el mecanismo por el cual una misma mutación genera diferentes fenotipos clínicos o su inversa? (c) ¿Porqué diferentes mutaciones causan un mismo fenotipo clínico? y finalmente (d) ¿Cuál es la patogénesis de los fenómenos vasculares en MELAS (encefalomiopatía mitocondrial con acidosis láctica y episodios tipo stroke)

RÉSUMÉ

Bien que les premières mutations de l'ADN mitochondrial (ADNmt) fûrent décrites en 1988, les progrès réalisés dans le nouveau champ d'investigations que représente la génétique de l'ADNmt ont été spectaculaires et de nombreux syndromes ("cytopathies mitochondriales") ont été associés à des remaniements de l'ADNmt (délétions ou duplications) ou à des mutations ponctuelles localisées dans des gènes codant pour des ARNt ou des protéines. Les principes généraux de la génétique de l'ADNmt sont rapidement exposés.

Contrairement à nos progrès rapides ayant permis l'établissement des bases moléculaires de ces pathologies, la compréhension des mécanismes pathogéniques reste cependant incomplète. Les questions en suspens pour lesquelles nous proposons une discussion sont les suivantes: (a) pourquoi il y a t'il tant de phénotypes cliniques distincts? (b) pourquoi la même mutation induit-elle des phénotypes differents? (c) pourquoi différents mutations entrainent-elles le même phénotype? (d) quelle est la pathogénie des attaques cerebrales dans le syndrome appelé MELAS ("Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes")?

ZUSAMMENFASSUNG

In 1988, wurden die ersten mitochondrialen DNA Mutationen beschrieben, seither ist der Fortschritt in diesem neuen Gebiet der "mitochondrialen Genetik" beeindruckend und eine Vielzahl von Syndromen (sogenannte "mitochondriale Encephalomyopathien")konnten mit mitochondrialen "DNA-rearrangements" (Deletionen oder Duplikationen), oder mit Punktmutationen in den tRNA-Genen oder in Strukturgenen assoziiert werden. Die Grundlagen der mitochondrialen Genetik werden kurz wiederhohlt. Im Gegensatz zu unserem raschen Fortschritt in der Aufklärung der molekularen Basis mitochondrialer Erkrankungen, bleibt unser Verständnis für den pathogenetischen Mechanismus inkomplett. Es werden einige pathogenetisch schwierige Fragen diskutiert, unter anderem: (a) Warum gibt es so viele verschiedene klinische Phänotypen?; (b) warum kann ein und dieselbe Mutation verschiedene Phänotypen hervorrufen?; warum können verschiedene Mutationen den gleichen Phänotyp hervorrufen?; was ist der pathogenetische Hintergrund der Schlaganfälle in MELAS (Mitochondriale Encephalomyopathie mit Laktat Acidose und Schlaganfällen).

REFERENCES

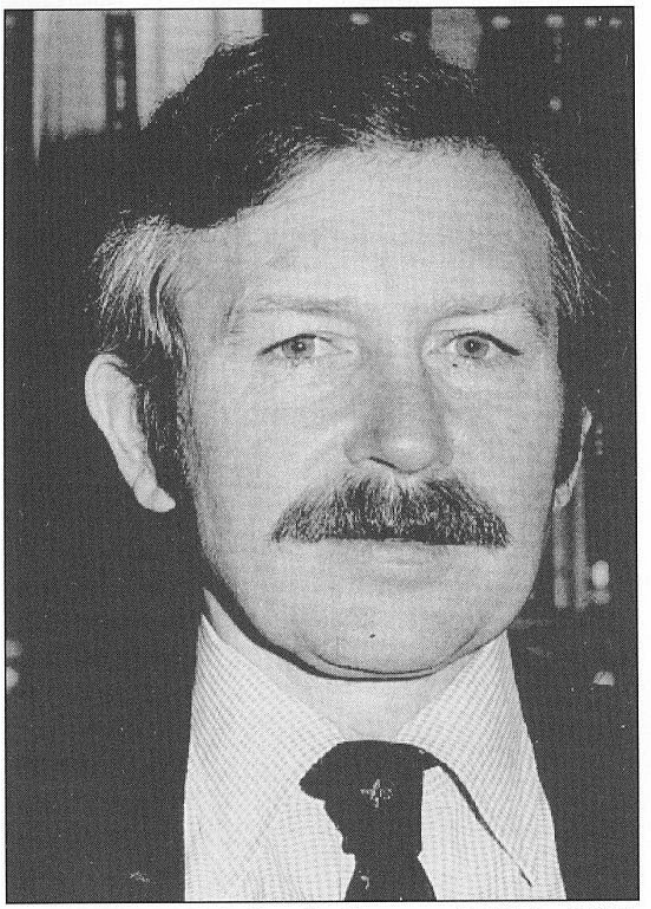
1. Holt, I. J., Harding, A. E., and Morgan Hughes, J. A. (1988) Nature 331, 717-719
2. Wallace, D. C., Singh, G., Lott, M. T., Hodge, J. A., Schurr, T. G., Lezza, A.M. S., Elsas, L. J., and Nikoskelainen, E. K. (1988) Science 242, 1427-1430
3. DiMauro, S. and Moraes, C. T. (1993) Arch. Neurol. 50, 1197-1208
4. Wallace, D. C., Zheng, X., Lott, M. T., Shoffner, J. M., Hodge, J. A., Kelley, R.I., Epstein, C. M., and Hopkins, L. C. (1988) Cell 55, 601-610
5. Larsson, N. G., Holme, B., and Kristiansson, B. (1990) Pediatr-Res. 28, 131-136
6. McShane, M. A., Hammans, S. R., Sweeney, M., Holt, I. J., Beattie, T. J., Brett, E. M., and Harding, A. E. (1991) Am. J Hum. Genet. 48, 39-42
7. Schon, E. A., Hirano, M., and DiMauro, S. (1994) J. Bioenerg. Biomembr. 26,291-299
8. Wallace, D. C. (1989) Cytogenet. Cell Genet. 51, 612-621
9. Lertrit, P., Kapsa, R. M. I., Jean-Francois, M. J. B., Thyagarajan, D., Noer, A.S., Marzuki, S., and Byrne, E. (1994) Hum. Mol. Genet. 3, 1973-1981
10. Stoneking, M. (1994) J. Bioenerg. Biomembr. 26, 251-259.
11. Torroni, A., Wallace, D.C. (1994) J. Bioenerg. Biomembr. 26, 261-271.
12. Moraes, C. T., Ricci, E., Bonilla, E., DiMauro, S., and Schon, E. A. (1992) Am.J. Hum. Genet. 50, 934-949
13. Moraes, C. T., Ciacci, F., Bonilla, E., Ionasescu, V., Schon, E. A., and DiMauro, S. (1993) Nature Genet. 4, 284-288
14. Moraes, C. T., Ciacci, F., Bonilla, E., Jansen, C., Hirano, M., Rao, N., Lovelace, R. E., Rowland, L. P., Schon, E. A., and DiMauro, S. (1993) J. Clin. Invest. 92, 2906-2915
15. Sciacco, M., Bonilla, E., Schon, E. A., DiMauro, S., and Moraes, C. T. (1994) Hum. Mol. Genet. 3, 13-19
16. Berenberg, R. A., Pellock, J. M., DiMauro, S., and Rowland, L. P. (1977) Ann. Neurol. 1, 37-43
17. DiMauro, S., Bonilla, E., Zeviani, M., Nakagawa, M., and DeVivo, D. C. (1985) Ann-Neurol. 17, 521-538
18. Petty, R. K., Harding, A. E., and Morgan Hughes, J. A. (1986) Brain 109, 915-938
19. Moraes, C. T., DiMauro, S., Zeviani, M., Lombes, A., Shanske, S., Miranda, A.F., Nakase, H., Bonilla, E., Werneck, L. C., Servidei, S., Nonaka, I., Koga, Y., Spiro, A. J., Brownnel, A. K. W., Schmidt, B., Schotland, D. L., Zupanc, M., DeVivo, D. C., Schon, E. A., and Rowland, L. P. (1989) N. Engl. J Med. 320, 1293-1299
20. Ciafaloni, E., Ricci, E., Shanske, S., Moraes, C. T., Silvestri, G., Hirano, M., Simonetti, S., Angelini, C., Donati, A., Garcia, C., Martinuzzi, A., Mosewich, R., Servidei, S., Zammarchi, E., Bonilla, E., DeVivo, D. C., Rowland, L. P., Schon, E. A., and DiMauro, S. (1992) Ann. Neurol. 31, 391-398
21. Silvestri, G., Ciafaloni, E., Santorelli, F., Shanske, S., Servidei, S., Graf, W. D., Sumi, M., and DiMauro, S. (1993) Neurology 43, 1200-1206
22. Moraes, C. T., Shanske, S., Tritschler, H-J., Aprille, J. R., Andreetta, F., Bonilla, E., Schon, E. A., and DiMauro, S. (1991) Am. J. Hum. Genet. 48, 492-501
23. Tritschler, H. J., Andreetta, F., Moraes, C. T., Bonilla, E., Arnaudo, E., Danon, M. J., Glass, S., Zelaya, B. M., Vamos, E., Telerman-Toppet, N., Shanske, S., Kadenbach, B., DiMauro, S., and Schon, E. A. (1992) Neurology 42, 209-217
24. Parker, W. D., Oley, C. A., and Parks, J. K. (1989) New Engl. J. Med. 320, 1331-1333

24a. Howell, N., Bindoff, L. A., McCullough, D. A., Kubacka, I., Poulton, J., Mackey, D., Taylor, L., Turnbull, D. M. (1991) Am. J. Hum. Genet. 49, 939-950.

25. Larsson, N. G., Anderson, O., Holme, E., Oldfors, A., and Wahlstrom, J. (1991) Ann. Neurol. 30, 701-708
26. Tatuch, Y. and Robinson, B. H. (1993) Biochem. Biophys. Res. Comm. 192, 124-128
27. Trounce, I., Neill, S., and Wallace, D. C. (1994) Proc. Nat. Acad. Sci. (USA) 91, 8334-8338
28. Holt, I. J., Harding, A. E., Petty, R. K., and Morgan Hughes, J. A. (1990) Am. J Hum. Genet. 46, 428-433
29. Tatuch, Y., Christodoulou, J., Feigenbaum, A., Clarke, J. T. R., Wherret, J., Smith, C., Rudd, N., Petrova-Benedict, R., and Robinson, B. H. (1992) Am. J. Hum. Genet. 50, 852-858
30. Santorelli, F. M., Shanske, S., Macaya, A., DeVivo, D. C., and DiMauro, S. (1993) Ann. Neurol. 34, 827-834
31. Zeviani, M., Gellera, C., Pannacci, M., Uziel, G., Prelle, A., Servidei, S., and DiDonato, S. (1990) Ann. Neurol. 28, 94-97
32. Shanske, S., Moraes, C. T., Lombes, A., Miranda, A. F., Bonilla, E., Lewis, P., Whelan, M. A., Ellsworth, C. A., and DiMauro, S. (1990) Neurology 40, 24-28
33. Ponzetto, C., Bresolin, N., Bordoni, A., Moggio, M., Meola, G., Bet, L., Prelle, A., and Scarlato, G. (1990) J. Neurol. Sci. 96, 207-210
34. Sparaco, M., Bonilla, E., DiMauro, S., and Powers, J. M. (1993) J. Neuropath. Exp. Neurol. 52, 1-10
35. Moraes, C. T., Ciacci, F., Silvestri, G., Shanske, S., Sciacco, M., Hirano, M., Schon, E. A., Bonilla, E., and DiMauro, S. (1993) Neuromusc. Disord. 3, 43-50
36. Petruzzella, V., Moraes, C. T., Sano, M. C., Bonilla, E., DiMauro, S., and Schon, E. A. (1994) Hum. Mol. Genet. 3, 449-454
37. Wallace, D. C. and Lott, M. T. (1993) in Mitochondrial DNA in Human Pathology (DiMauro, S. and Wallace, D. C., eds) pp. 63-83, Raven Press, New York
38. DiMauro, S., Servidei, S., Zeviani, M., DiRocco, M., DeVivo, D. C., DiDonato, S., Uziel, G., Berry, K., Hoganson, G., Johnsen, S. D., and et al, (1987) Ann-Neurol. 22, 498-506
39. Miranda, A. F., Ishii, S., DiMauro, S., and Shay, J. W. (1989) Neurology 39, 697-702
40. Fujii, T., Van Coster, R. N., Old, S. E., Medori, R., Winter, S., Gubits, R. M., Mattwes, P. M., Brown, R. M., Brown, G. K., Dahl, H. H. M., and De Vivo, D. C. (1994) Ann. Neurol. 36, 83-89
41. Ciafaloni, E., Santorelli, F., Shanske, S., Deonna, T., Roulet, E., Janzer, C. Pescia, G., and DiMauro, S. (1993) J. Pediat. in press, (Abstract)
42. Goto, Y., Nonaka, I., and Horai, S. (1990) Nature 348, 651-653
43. Goto, Y. I., Nonaka, I., and Horai, S. (1991) Biochim. Biophys. Acta 1097, 238-240
44. Morten, K. J., Cooper, J. M., Brown, G. K., Lake, B., Pike, D., and Poulton, J. (1993) Hum. Mol. Genet. 2, 2081-2087
45. Sweeney, M. G., Bundey, S., Brockington, M., Poulton, J., Weiner, J. B., and Harding, A. E. (1993) Quart. J. Med. 709, 713
46. Goto, Y., Tsugane, K., Tanabe, Y., Nonaka, I., and Horai, S. (1994) Biochem. Biophys. Res. Comm. 202, 1624-1630
47. Lertrit, P., Noer, A. S., Jean-Francois, B., Kapsa, R., Dennett, X., Thyagarajan, D., Lethlean, K., Byrne, E., and Marzuki, S. (1992) Am. J. Hum. Genet. 51, 457-468

48. Sakuta, R., Goto, Y. I., Nonaka, I., and Horai, S. (1993) Am. J. Hum. Genet. 53, 964-965
49. Manfredi, G., Schon, E. A., Moraes, C. T., Bonilla, E., Berry, G. T., and DiMauro, S. (1995) Neuromusc. Disord. (in press)
50. Shoffner, J. M., Lott, M. T., Lezza, A. M. S., Seibel, P., Ballinger, S. W., and Wallace, D. C. (1990) Cell 61, 931-937
51. Silvestri, G., Moraes, C. T., Shanske, S., Oh, S. J., and DiMauro, S. (1992) Am. J. Hum. Genet. 51, 1213-1217
52. De Vries, D. D., van Engelen, B. G. M., Gabreels, F. J. M., Ruitenbeek, W., and van Oost, B. A. (1993) Ann. Neurol. 34, 410-412
53. Santorelli, F. M., Shanske, S., Jain, K. D., Tick, D., Schon, E. A., and DiMauro, S. (1994) Neurology 44, 972-974
54. Sakuta, R. and Nonaka, I. (1989) Ann. Neurol. 25, 594-601
55. Hasegawa, H., Matsuoka, T., Goto, I., and Nonaka, I. (1991) Ann. Neurol. 29, 601-605
56. Fujii, T., Okuno, T., Ito, M., Mutoh, K., Horiguchi, Y., Tashiro, H., and Mikawa, H. (1991) J. Neurol. Sci. 103, 37-41
57. Ohama, E., Ohara, S., Ikuta, F., Tanaka, K., Nishizawa, M., and Miyatake, T. (1987) ActaNeuropath. 74, 226-233
58. Ohama, E. and Ikuta, F. (1987) ActaNeuropath. 75, 1-7
59. Kishi, M., Yamamura, Y., Kurihara, T., Fukuhara, N., Tsuruta, S., Matsukara S., Hayashi, T., Nakagawa, M., and Kuriyama, M. (1988) J-Neurol-Sci. 86, 3140
60. Hirano, M., Ricci, E., Koenigsberger, M. R., Defendini, R., Pavlakis, S. G., DeVivo, D. C., DiMauro, S., and Rowland, L. P. (1992) Neuromusc. Disord. 2, 125-135
61. Seyama, K., Suzuki, K., Mizuno, Y., Yoshida, M., Tanaka, M., and Ozawa, T. (1989) Acta Neurol. Scand. 80, 561-568
62. Ooiwa, Y., Uematsu, Y., Terada, T., Nakai, K., Itakura, T., Komai, N., and Moriwaki, H. (1993) Stroke 24, 304-309
63. Zupanc, M. L., Moraes, C. T., Shanske, S., Langman, C. B., Ciafaloni, E., and DiMauro, S. (1991) Ann. Neurol. 29, 680-683
64. Hammans, S.R., Sweeney, M.G., Brockington, M., Lennox, G.G., Lawton N.F., Kennedy, C.R., Morgan-Hughes, J.A., and Harding, A.E. (1993)

BENTO P.M SCHULTE, M.D. PH.D.



Professor Schulte died on april 11th, 1991, at the age of 63. He held the chair of Neurology at the Catholic University of Nijmegen, the Netherlands, and was an editor to the International Journal of Neurology from 1979 onward.

The son of a well-knownsurgeon-gynaecologist he was born and got his education in the city of Maastricht. He began his medical studies in Louvain, Belgium, then moved on to Utrecht where he studied from 1947 to 1954, leaving only for three periods of clinical clerkship, at Munnich, Vienna and Paris. In 1954 he qualified in Medicine and began his training to become a neurologist in the St.Ursula Clinic at Wassenaar, The Hague. Here he worked under Hoelen (psychiatry), Tans (neurology), De Vet (neurosurgery) and Magnus (clinical neurophysiology).

After he got his certification as a neurologist, in 1959, he defended his Ph. D.-thesis, "Hermanni Boerhaave Praelectiones de morbis nervorum 1730-1735", under the aegis of Professor Carp.

From 1960 to 1980 he headed the Department, first of Psychiatry, later of Neurology, of the St.Elizabeth Hospital in Tilburg. With his dynamic leadership he reorganised and invigorated health care in such a way that the neurological department became an affiliated teaching centre. He particularly furthered close cooperation with the neurosurgeons.

Having been inspired by William Fields, together with a team of experts, he organised the "Tilburg Epidemiological Study of Stroke", with the help of the regional family physicians. This epidemiological interest brought him into contact with Bruce Schoenberg who made him co-editor of "Neuroepidemiology". Together they organised an International Workshop on Clinical Neuro-epidemiology at Nijmegen University in 1982.

From 1980 to 1991 Schulte held the chair of Clinical Neurology in the Institute of Neurology of the Catholic University of Nijmegen, the Netherlands. He reorganised and consolidated the Institute that had been founded in 1953 by his predecessor Professor Dr.J.J.G. Prick. The managerial commitments of this task required much of his energy, especially so, when in the mid-eighties, the university was faced with restricted financial resources. Yet he steered his Institute safely through these difficult times.

He fulfilled the lion's share of the Institute's teaching duties and he was a respected and gifted pedagogue who meticulously prepared his lectures, insisting also on the presence of a patient at every session with undergraduate students. During clinical rounds he paid due attention to the attitude maintained by the interns and

residents towards the patients, emphasising the importance of the patient's subjective experience of his own situation. In matters of doctor-patient relationship he advised a maximal involvement on the part of the doctor, while maintaining his distance. As a physician and as a neurologist he felt strongly his responsibility as to the maintenance of high ethical professional standards. He presided over the University Hospital's Ethical Committee and was a member of the Regional Court of Justice for the Medical Profession.

His main scientific achievements were in the field of cerebro-vascular diseases as witnessed by a vast number of publications, beginning with the series issuing from the Tilburg epidemiological study, continuing, among other writings, with a chapter in the ***Handbook of Neurology*** *and ending with his contribution to the World Congress of Neurology in New Delhi (1989) where he was invited to open the Congress with his paper on "Clinical Epidemiology of Cerebro-Vascular Disease".*

The history of medicine was of continual interest to Schulte. Together with L.J. Endtz he wrote "A short history of neurology in the Netherlands" the booklet offered to the participants of the 11th World Congress of Neurology in Amsterdam in 1977. During his Nijmegen years Schulte fertilized the work of a great number of students writing on medical-historical subjects and on medicine in relation to philosophy. Under his direction a thesis was prepared on "Brown-Séquard" by Koehler.

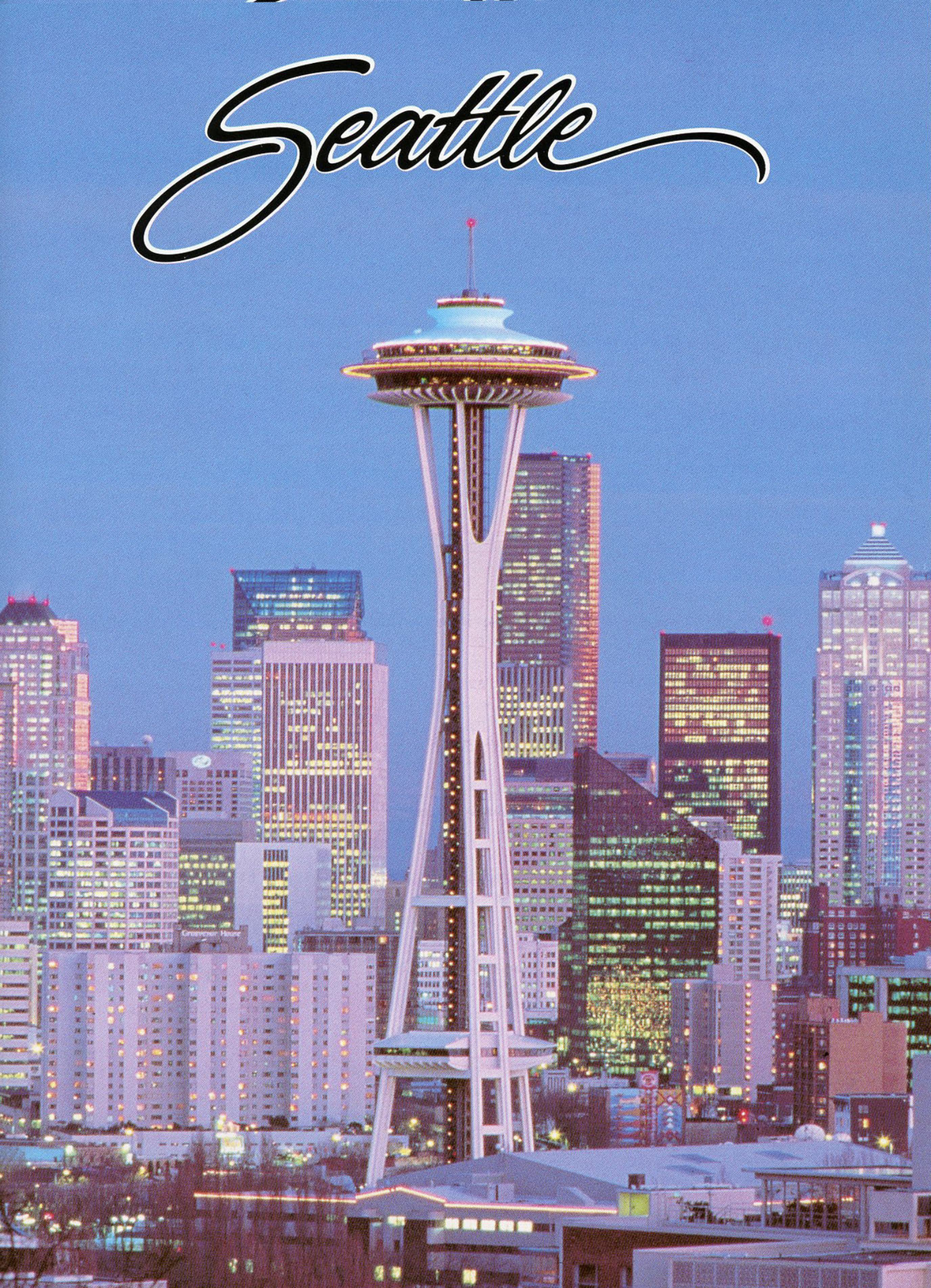
Schulte was essentially a modest man of great integrity, and with a scrupulous concern for fairness. He was kind and courteous, ready to give his time to anyone who approached him to discuss a problem. On the eve of his retirement the symptoms and signs of a neurological disease became apparent and were increasingly a hindrance to his daily functioning.

In 1962 Bento Schulte married Teresa Ravensloot who enabled him to combine a happy family life with busy professional commitments. Their home and hospita]ity were always available to the many friends and guests from abroad.

With Professor Doctor Bento Schulte the international neurological community has lost a distinguished member and an outstanding neurologist who contributed to many aspects of the field.

We extend our sympathy to his wife.

A.K.


Seattle

Seattle

An inspired writer said that Seattle was built like Rome on seven hills.

Its cradle was settled amid the most magnificent scenery of natural beauty. The onlooker is surrounded by a true symphony of colours and sounds in which participate the emerald green of the forests, rumorous cascades and the imposing snowcapped mountains.

Flanked by the Cascade Range to the east and the Olimpic Mountains to the west, Seattle is located on a slender isthmus of land, having westwards Puget Sound, a mighty salt water inlet from the Pacific and on the Eastern margin the freshwater Lake Washington.

No matter how big the city has grown in more than a century, how impressive its many skyscrapers project their massive structures on the Seattle's skyline, still, the snow crowned summit of Mount Rainer, emerging majestically like a giant, remains the indisputable sovereign domineering the city.

THE EXPLORERS

The Fog. In an epoch when the world had many uncharted areas and several nations were greedily sending audacious explorers to extend their dominions, twice the fog has played an important role favoring young United States' interests.

The first one was when Sir Francis Drake on board "The Golden Hind" anchored in 1579 at the North of San Francisco Bay. His stay there was a short one, just enough to await good weather that allowed him to cross most of the so called Pacific Ocean.

Veiled by a fog Drake missed the entrance to San Francisco Bay. Thus the fog preserved San Francisco for the United States.

The second time was in 1792 when Captain George Vancouver, a well seasoned 35 years old English explorer commanding the appropriately named "Discovery", surveyed the coastline from San Francisco to present day British Columbia sailing along the Island that now bears his name.

It would be impossible to miss the entrance to the Columbia River unless he had found its sight completely obliterated by the most dense fog.

This was a fortunate event for the United States, because the same year it was discovered by Robert Gray, an American sea captain born in Tiverton on May 10, 1755, who during the American Revolution served in the Continental Navy.

In the employ of Boston investors Gray became a pioneer in the China fur trade that enriched residents of New England.

He was the first seaman to carry the flag of the new United States around the world returning to Boston in 1790.

In 1791 Gray reassumed the sea trade commanding the 212 ton "Columbia".

On May 12, 1792, he discovered the mouth of the river that flows between Oregon and Washington and named it "Columbia River" for his ship. Others had seen the entrance of the Columbia River but nobody had explored it. Happily, Gray directed his ship course up the river searching for a Village.

According to his description "The beach was lined with natives, who ran along the shore following the ship".

This fact was of utmost importance when the question was raised as to whom pertained the explored territories: if to his Majesty King George or to the United States.

But even if the United States won the territories of the Oregon country it is out of question that Vancouver left an imperishable mark on the American map.

This young and intrepid British explorer honored his crown and highly placed officers of the Admiralty giving their names to any important geographical manifestation his eyes laid on. Thus, while charting the new territories Vancouver reached the Sound which he christened with the name of his junior officer Peter Puget, and other names like Rainier, Vashon, Bambridge were added and remained in these territories.

Some of them like the name of Mount Rainier have originated bitter discussions, since the Indians were adamant concerning the change of name of the mountain which from immemorial times they have known as "mountain that is god" and was called "Takhoma". The settlers decided that the Indian name should be respected.

But this decision originated lasting feudal quarrels between Tacoma and Seattle, that reached an end long after the Federal Board of geographical names declared Rainier as the official name.

It is narrated that when Vancouver reached the Sound he caused a great impact on the natives of that region, some were startled, others dazzled at the sight of the oncoming "Discovery", all sails navigating on the clear waters of the Sound.

This was for them a heavenly vision; they may have thought that the gods had fulfilled their promise to visit the earth..

Once recovered from the surprise and full of curiosity they readily surrounded the ship in their canoes.

Vancouver kindly took some of them aboard. Within the group of natives who entered the ship was a leader of the Suquamish tribe and his son Sealth, a child who never forgot this event.

Years later Sealth would recall that the English captain had to remove his jacket and shirt to convince them that he was all white and not a red man with a painted face.

This was the first encounter that the Indians of that region had with the white man.

Vancouver left the Sound and greatly impressed by its beauty wrote: "The serenity of the climate, the innumerable pleasing landscapes and the abundant fertility that unassisted nature puts forth, require only to be enriched by the industry of man... to render the most lovely country that can be imagined".

These words were prophetic indeed, Seattle grew in force and splendor to match the grandeur and beauty of its surroundings. In time it became the largest metropolis the Pacific Northwest location has. Its privileged geographical surroundings favoured its growth as a trade and transportation hub.

Its surroundings many railroad lines, highways and ferries criss-cross the city's map with routes connecting many Seattle locations and beyond its borders.

It is worthwhile to mention the magnificent Lake Washington floating Bridge built in 1940. The bridge, which spans the Lake on floating pontoons is considered an engineering feat.

Seattle´s panoramic view is greatly enhanced by its spectacular Elliot Bay.

The Pioneers
We detachment steady throwing
Down the edges through the passes up the mountains steep
Conquering, holding, daring, venturing, as we go, the unknown ways,
Pioneers! O pioneers!
Walt Whitman

Seattle was born from a seed of faith that germinated in the soul of Arthur Denny a prospective pioneer, twenty nine years old, with an Irish ancestry.

He was already married with Mary Ann Boren, and was possessed by the idea of leaving Illinois for the Pacific Coast.

The United States Government, like the one of other nations, after having considerably extended their dominions, was confronted with the problem to populate it with their own citizens not considering the Indians as possessors of their earth. Thus, they have established "The Donation Claim Act", which entitled those pioneers who had first cast their eyes on a land to own it.

But nothing is really free in this world, and the price to be paid for an unexplored land's possession was really high.

It demands courage to fare the unknown dangers, and all the hardships of building something from nothing.

On April 10, 1851, Denny's family left Illinois to venture the risks of a long journey crossing the Great Plains.

A group of four wagons carrying five families integrated by twelve adults and a dozen of children, travelled several months traversing towns and rivers. It is presumed that they were heading for Oregon, when one Saturday in July, they had their first encounter with hostile Indians who fired upon them several times. Unwanted, Denny swiftly traversed the enemies' ground and the dangerous situation was overcome. Unfortunately others were not so lucky and several days later, a wagon was attacked and its people were shot dead.

Finally Denny's caravan reached Portland.

To Denny's dismal Portland was overcrowded with people. He had dreamed with an empty land to forge upon it his family future.

He was entertaining these depressive thoughts when a man named Brock began to talk to the pioneers' group about an enchanting place called Puget Sound which was like paradise on earth. His words filled their spirits with enthusiasm. It was like destiny were pointing out the place were Seattle ought to be founded. The curious fact was that Brock had never set a foot in that area.

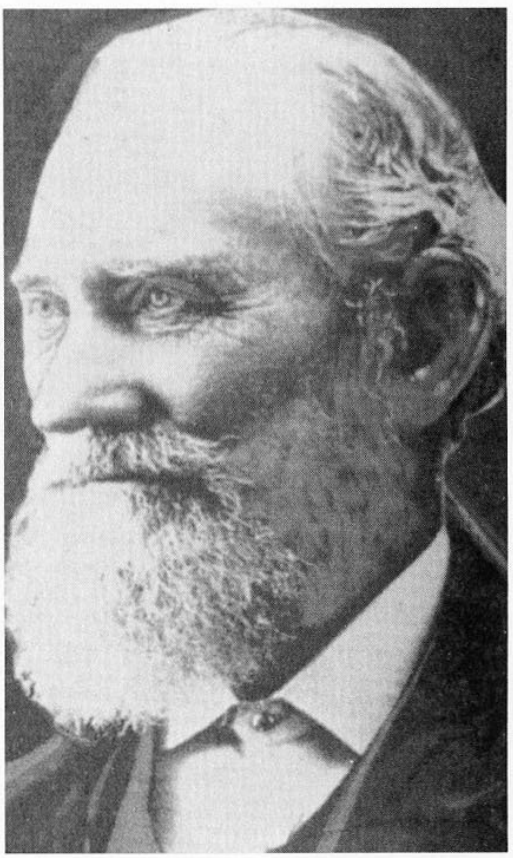
Arthur Denny was eager to know Puget Sound but his plans were hampered when his wife, one of his children and himself were stricken with malarial fever.

Impatient by these impediments, and wishing to learn about Puget Sound as soon as possible Arthur ordered his younger brother, the nineteen years old David to explore this area along with Terry and Low.

Carrying on his orders they reached what would be known as Elliot Bay and decided to settle in Alki Point. Here the story of Seattle began and in this historic landmark years later grateful Seattleiters erected a simple granite shaft on which the names of the twenty four founders were engraved.

In this place, on the eastern shore of Puget Sound they found what they wanted. A benign climate, an incomparable harbor, vast tracts of timber and salmon filled waters.

Wishing to report Arthur the good news, Low returned to Portland; while Terry and David Denny remained on the claim.



Arthur Denny led the city's early settlers.

Arthur Denny was very much excited hearing Low narration, and even if he was not fully recovered from his illness, he fervently desired to be settled in his own land.

Happily in those days Captain Folger was filling out his schooner "Exact" for a voyage to Queen Charlot Islands where gold had been found.

Captain Folger kindly agreed to touch lower Puget Sound on his way north.

On the 5th of November of 1851 the "Exact" left Portland carrying among its passangers the pioneers party.

This was integrated by Arthur Denny and family, John N. Low and family, William N. Bell and family, and Charles C. Terry.

The 13th of November they landed in Alki Point.

The arrival could not had been more disheartening. It was pouring rain.The ladies sunbonnets,so out of place, were soon pasted to their cheeks. All of them broke down, and cried desperately while they rowed for shore.They found David Denny in a very poor condition; feverish, lying down in a roofless cabin, without food, since voracious skunks had eaten all his provisions. Arthur Denny still pale and shaking from malaria bravely faced the situation. Rain or not rain, they had to finish David's cabin,to take refuge from the most pertinacious rain and begin to build a larger one so that in the two log cabins give shelter to twelve adults and a dozen children.

They proudly called their small community "New York".Others less optimistic added "Alki" which means "by and by" in Indian jargon.

On Alki windy point Denny's party built their homes under the scrutiny of a hundred diminutive Duwamanish and Suquamish people.

Sometimes consumed by curiosity they intruded into the cabins handling the few but intriguing objects. Generally, they leave quietly the place.The neat pioneer women tried to hide their concern at their unwelcomed visitors, wishing to maintain good relationship with their feared neighbors.

Captain Dan Howard commanding the brig "Leonora" was sailing near Alki and seeing white men on the beach when ashore. Soon they were doing business. Captain Howard was interesed in a cargo of piles for San Francisco at that time booming with new millionaires anxious to build luxurious residences.

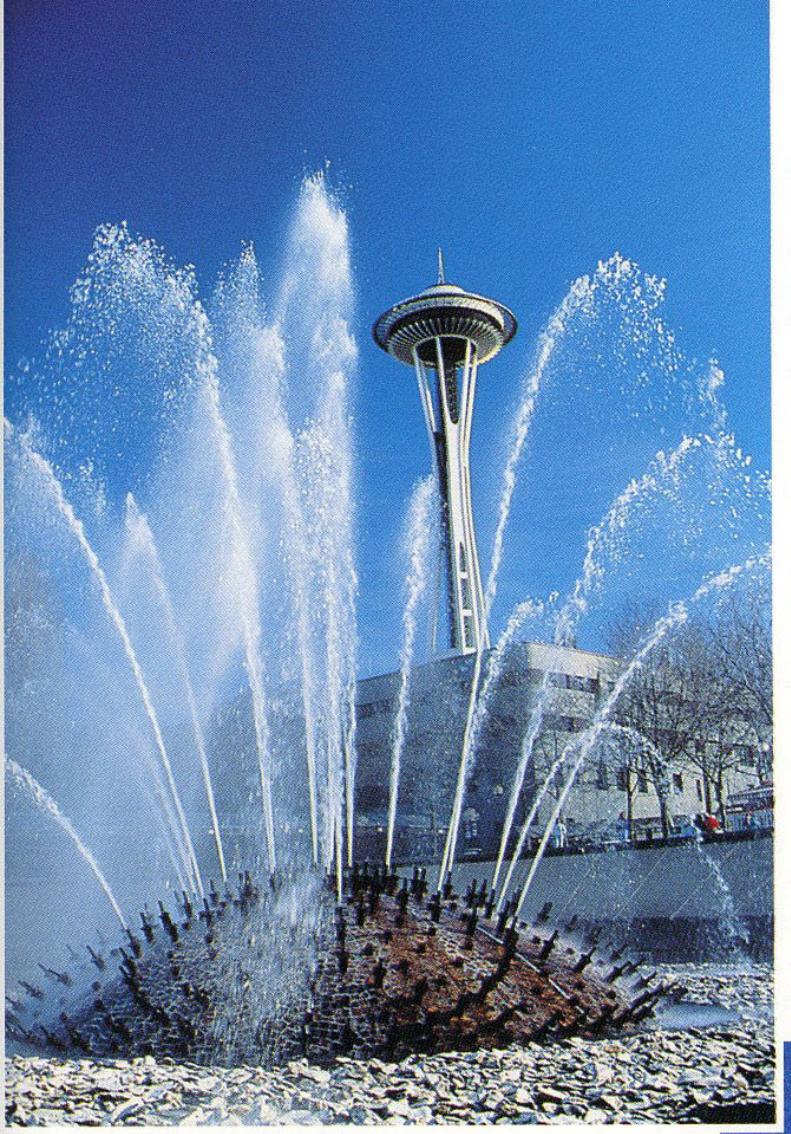
The pioneers began a work enthusiastically cutting, chopping, sawing, trimming and rolling the logs tide waters.

In sixteen working days they cut 13.458 feet of timber. Stimulated by the gold rush the building boom in San Francisco raised lumber prices to $ 200 per board feet.

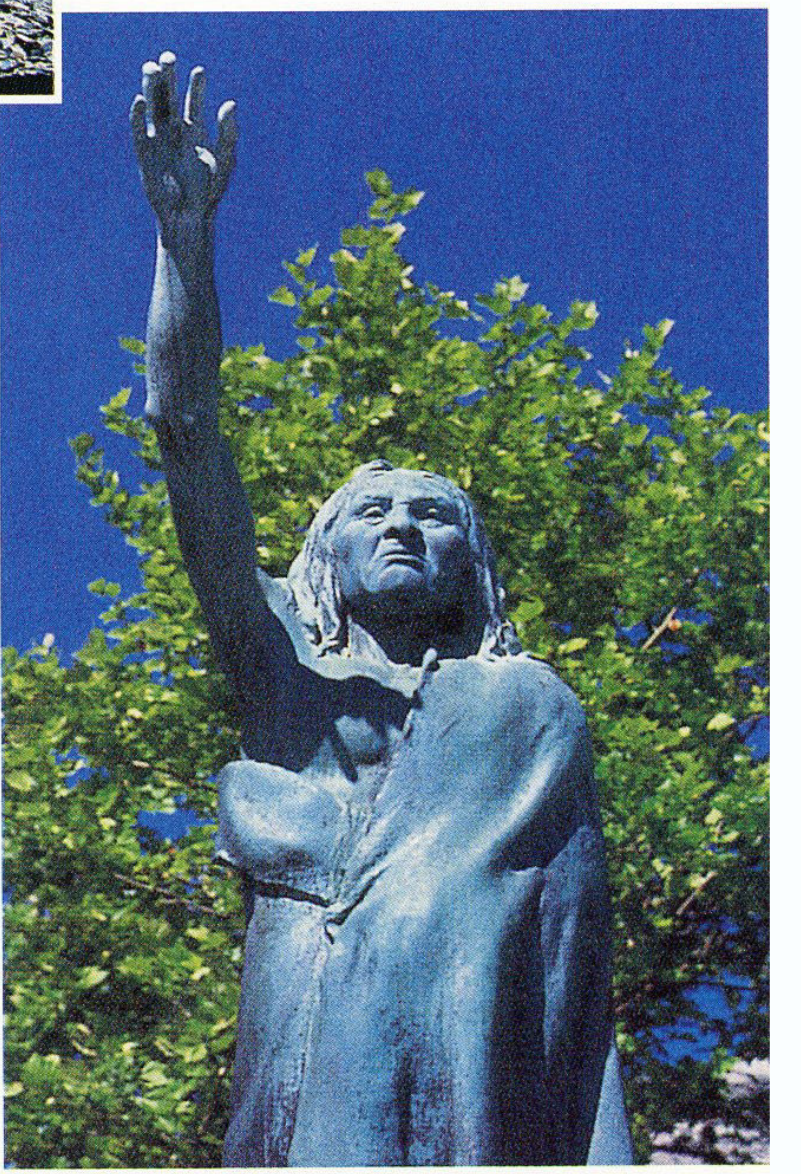
Thus, began one of the most important industries in Seattle: timber. Because the beach at Alki Point proved to be unsuitable for loading timber, the colonists decided to search for another place on the coast, deep enough to allow the ships to approach the shore. Borrowing the women clothline and with horseshoes attached for a weight, they set out in a canoe and sounded the coastline looking for a better location. They were overwhelmed when their rig showed deep waters close to the shore on what is now Elliot Bay. This was not only an ideal place for their purposes but they were surprised by its beauty. The harbour framed the Olimpic Mountains so perfectly, that they were elated at its sight.

On February 15, 1852, most of the pioneers moved to the east of Elliot Bay, where the city's business section now stands. The colonists staked claims along the shore and back deep into the forest. This was how Seattle was born; emerging from a selvatic, mountainous and watery environment, that would grow embracing Alki Point and spread towards a huge body of waters to be called Lake Washington.

Scarcely a year had passed after their move across the bay when a canoe with two strangers approached the settlement. One of them was SEALTH, the Chief of the Duwamanish and the



In the background a panoramic view of Seattle with the Space Needle taken from the foaming waters of the "INTERNATIONAL FOUNTAIN".



Statue of Chief Sealth, near Seattle Center, appears to offer benediction to his namesake city.

Suquamish tribes, the other was a white man, Dr. Davis S. Maynard, a general practitioner with spectacles and a parsimonious chin whisker.

His contenance greatly impressed the Denny's who hurried to conquer him for the settlement with a gift of 640 acres of land, including 300 feet of prime waterfront.

It was not treating the patients that "Doc" Maynard earned his reputation but with other enterprises such as, starting a general store, and the first hospital.

He envisioned one of the most successful of Seattle's economical resources being the first to try packing and shipping salmon.

Maynard was a vivacious man liked by everybody, gaining friends among the Indians and settlers.

He was fond of drinking and after imbibing his generosity was without limits reducing the prices of his merchandise until he ended giving them away.

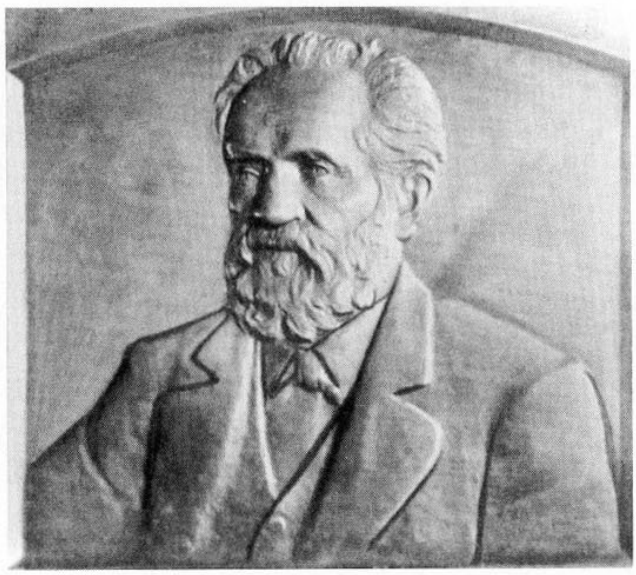
It was Maynard's idea to give the name of Chief SEALTH to the new settlement,changing it for Seattle. But he had to convince his Indian friend who feared that this use of his name would offend his spirit after death.

Without Maynard's intervention Seattle may still have been named Duwamps as this was its original name.

Excepting the time Maynard was drunk, which was very often, he and Denny got along very well.

Arthur Denny and his associate founders approved any iniciative, especially if the individual was willing to work and had an idea that meant a new commercial enterprise. Thus, they readily welcomed Maynard's idea to pack salmon aided by his Indian friends whom he hired as fishermen.

With this purpose in mind, Maynard examined the terrain and declared that the best location was a southern point of Arthur Denny's place, declaring that he only wanted a temporary site where he could salt down fish. Later on he accepted Arthur's suggestion of making of this a permanent location. In spite of that the founders backed his fish packing plan allowing him a series of rearrangements to facilite his endeavor; Maynard's first venture at industrialism ended disastrously. Something went wrong; either there was not enough salt in the packing or the barrels were defective, the fish got spoiled and Maynard lost his investment.



Henry Yesler established its first major business, a sawmill.

Maynard's life was a succession of business failures.

He lost money in many attemped enterprises such as a blacksmith shop, a salmon packing concern and a general store until his death, which occurred in 1873.

But it was Maynard who promoted Seattle's most important acquisition luring Henry L. Yesler to establish his steam sawmill in Seattle with a donation of prime Harborfront proprietary..

Yesler, unlike Maynard, was a well seasoned industrialist who had full knowledge of his business. He had his sawmill stored in Portland and liking the place he promptly brought it to Seattle.

Newcomers were steadily populating other parts of the Sound 's shoreline. But many of them were attracted to the settlement on Elliot Bay maybe because they were impressed by the white plume of Yesler steam engine. That feather against the sky seemed a promise of a coming city.

The mill was the first of its kind in the sound and raised Seattle's prestige as a lumber exporter, mainly to San Francisco where many of its early residences were built with Yesler's lumber.

The advent of Yesler steam sawmill introduced some changes in the communal life.

Stimulated by its noise and clatter, the founders began the building of frame houses, as they wished to vacate their log cabins as soon as possible.

The new house looked more like home and facilitated the commerce. Soon the mill cookhouse and mess was the last log structure in use.

Nevertheless, the mill cookhouse remained for many years the center of the town communal activities. The building was the place of election for every kind of entertainment, and the pioneers enjoyed very much the visits of wandering charlatans posing as "professors" dressed in tailcoats and stiff collars narrating to them their adventurous voyages in exotic regions, or about esoteric subjects such as phrenology.

Nearly ten years had slipped by since the pioneers founded Seattle when the University of Washington was established in 1861.

This was a dearing enterprise and called for an imaginative, audacious and energetic man, ready to spare no efforts to sow the seeds of Seattle's intelectual life.

Asa Mercer stepped into the brief story of the youngest of the actions as a kind of hero of astonishing stature.

He was the younger brother of Judge Thomas Mercer, a highly respected pesonality who arrived at Seattle in 1852.

Asa came from college fresh and full of spirits, with the aim to build a house of learning. To fulfil his dream he ought to work from the bottom up. Waist deep in the wet marsh, Asa fiercely chopped at the woods of great fir and cedar stumps to clear the ground for the building. Once this was attained and the University with his help erected, he crossed its lintel, acclaimed as its first President and only teacher.

Then Asa ought to face the problem to populate the University with students. Without thinking twice he toured the Puget country in his canoe to entice the prospective pupils exhalting the profits of academic learning. This was reinforced with promises of jobs of cutting firewood at $ 1.50 a cord, which ended in persuading some of them to enter the University.

Not only Asa was pouring his energies in scholarly efforts to increase the mental level of the Seattleites, but he became aware of another shortcoming of Seattle's emerging society. His keen eyes discovered that the settlement was mostly populated by men and the possibility of getting a bride was difficult.

This had also been noticed by Charles Prosch, who highly concerned, published in his periodical the "Puget Sound Herald" the following announcement which appeared in February 24, 1860:

Attention bachelors: "Believing that our only chance of the realization of the benefits and early attainments of matrimonial alliances depends upon the arrival in our midst of a number of the fair sex from the Atlantic States, and that, to bring about such an arrival, a united effort and action are called for on our part, we respectfully request a full attendance of all eligible and sincerely desirous bachelors in this community to assemble on Tuesday evening next in Delin and Shorey's building to devise ways and means to secure this much needed and desirable inmigration to our shores".

But in spite of his efforts the plan of Prosch did not prosper; among other reasons, the main impediment was the lack of money.

To bring a bride from the Atlantic accross the Rockies, would mean a lot of money beyond the means of any bachelor of the Puget Coast.

When Asa learned of Prosch's project he became sincerely interested in it, maybe because he ranked among the bachelor who dreamed of a suitable bride to be his life's companion.

Thus, the young President of the Territorial University approached the newspaper editor to join efforts and exchange ideas about the best way to bring to reality that which seemed to be an unsurmountable task, to provide any desirous bachelor with a dignified and decent wife.

The situation demanded celerity since the proportion of males to females were nine to one. Many of the men became involved with Indians girls, some temporarily, others permanently, marrying them.

A great majority become customers of a rascal who profited the occasion installing a house of entertainment, later on known as the "Mad House" where the bachelors enjoyed music, dancing, drinking, and eventually the favors of an Indian girl, selected among the ones recruited by the owner of the place.

Asa Mercer was a man of action, once an idea had germinated in his mind he would employ all his energy, resourcefulness and sagacity to bring it to completion.

He thought that the best plan to procure a bride for every bachelor was that he should go to the east and come back with several hundreds of young ladies to satisfy the need of the settlement.

But it was easier said than done. As it always happens, the need of money was one of the many obstacles he would find in carrying out his project.

Mercer tried to get economical resources from Governor Pickering and the members of his legislature, but he only got good words of appreciation for his service to the country, but no money.

He had better a response from some members of the settlement who placed in his hands enough money to go to Boston.

The results of this first expedition were not the ones he expected.

The hundreds of ladies he hoped to bring back to Seattle were reduced to eleven girls ranging from fifteen to twenty five.

They arrived at San Francisco wan from seasickness, and were frightened by the harshness of the new environment.

They continued their voyage and after stopping at Port Gamble sailed for Seattle.

On May 16, 1864 on board of the sloop "Kidder" the first group of the Mercer Girls, as they would be forever called, arrived at the shores of the Elliot Bay.

They couldn't have had a better reception. It was already midnight and everybody was at the port. A wave of excitement travelled through the onlookers. The girls were arriving.

The bachelors with their hair slicked down like sea otters were hanging around hoping that their countenances, in their best garbs would make a good impression in some of the girls.

It was a joyous welcome. The three pianos of the settlement did not stop the music the whole night. There was singing and dancing until the rising sun iluminated with its yellowish rays the scene.

Everbody was exhausted and happy.

The girls were placed under the care of some ladies of the town who offered their homes and protection until they eventually found a job or a husband.

The fact that there were only eleven prospective wives instead of the several hundred expected did not diminish their enthusiasm and admiration they had for Asa Mercer.

But these were not Asa's feelings who felt frustated by the meager results of his first expedition. So, he began to plan a second one.

This time the trip was financed by the bachelors who stimulated by the arrival of the eleven girls were willing to deposit in his hands a certain amount of money which would assure everyone of them a suitable mate.

Asa's confidence in this new adventure was in part sustained by the fact that Abraham Lincoln whom he remembered as a dear friend in his childhood, actually was the President of the United States.

But once more destiny put Mercer's endurance to test. On arriving at New York on his way to Washington he had the impression that something ominous was floating in the environment; everybody looked sad and silent; he had the opressive sensation that a tragedy had happened.

His feelings were confirmed when he learned that Abraham Lincoln was assassinated the night before at Ford's Theater.

This was a great blow for Asa Mercer. His dearest dreams were shattered. Nevertheless he tenaciously clinged to his purposes spending some weeks in Washington seeking help for his enterprise from President Johnson down, to all the authorities of his government, with no avail.

Until he met General Grant who was more receptive to his words, promising help.

This came in the form of an order to the United States Army addressed to the Quartermaster General Meigs to provide Mercer a vessel completely fitted and manned ready to carry its cargo of brides to the Puget Sound.

Asa was exhilarated, but his buoyancy cooled down when General Meigs shouted his indignation at General Grant's orders considering them illegal.

Already tried by life's ups and downs, Asa employed all the arguments and methods possible to induce Meigs to change his mind.

Finally the Quartermaster yielded to Mercer's siege to the point of allowing him to have his vessel upon payment of eighty thousands dollars to the general government.

Lady Luck seemed to be smiling at Mercer when a man named Ben Holladay approached him proposing him an agreement; he would buy his vessel and would take the five hundred girls. Mercer said he was willing to make the trip, as far as San Francisco, at the going rate.

Things couldn't be better. Yet, Mercer would still have to face what according to his own words would be the worst of the obstacles encountered in fulfilling his mission.

A few days before the date settled for the departure, in the New York Herald appeared a long vituperative article defamating Mercer and stating that all the men in Puget Sound were corrupted and dissolute and the girls ran the risk of being turned into houses of ill fame, advising them to stay at home.

Before Asa Mercer could react to this infamy and essay a defense, the article was reprinted everywhere. Its effects were devastating. Two thirds of the girls desisted of the voyage, sending Mercer letters enclosing copies of the article and refusing further considerations about the matter.

On the other hand, Ben Holladay, who expected to have five hundred passengers in his newly acquired vessel instead of the two hundred which remained after the girl's mass dessertion, dryly informed Mercer that the going rate for a smaller group would be proportionally increased. This placed Mercer in many distressing situations which he managed to solve with his limitless resourcefulness.

The trip was a hazardous one. It was a long journey via the Strait of Magallanes, Rio de Janeiro, and the Galapagos.

The girls were suffering from seasickness, longing for home, and bordering desperation.

After arriving at San Francisco and paying Holladay his debt, he had two hundred girls and no money to send them to Seattle.

He anxiously sent a telegram to Governor Pickering asking financial aid to pay the girls' passage to Elliot Bay.

He received for an answer words of congratulation for his service to the country but no money.

Thus, to complete his mission Mercer did not hesitate to sacrifice four farm wagons he bought in New York with the aim to obtain some profits selling them in Puget Sound. But his financial investment ended in passages covering the trip of the prospective two hundred brides to destiny.

The "Mercer Girls" have embellished Seattle's history with a flavor of poetry and romance that has come down to our days giving arguments to novelists and screen writers who have charmed audiences around the world with the story of the "Mercer Girls" narrated in the novel "Cargo of Brides" and a television series entitled "Here come the Brides".

The "Mercer Girls" forever linked to the roots of a growing nation helping to build its future must be remembered with respect and gratitude. They were very courageous, of utmost importance to Seattle's progress in a very crucial time.

Married to Anne Stephen from Baltimore, one of the "brides" he recruited and leaving behind an adventurous life and his dreams as President of the Territorial University, Asa Mercer and his wife settled down at the foothills of the Rocky Mountains to become a rancher.

Mercer had left Seattle; but his achievements remained there forever.

The "Mercer Girls" quietly merged with the population acting as yeast in the growing society. Time has shed a curtain of silence about them, and Seattleites had lost track of their lives. It was difficult to spot their descendents. Happily, to certify the veracity of their existance Mrs. Betty (Engle) Engstrom of Greenbank, Washington identifield herself as the grandaughter of Flora Pearson Engle, who was only fifteen years old, when she arrived at Seattle, accompanied by her mother and a younger brother on Mercer's second trip. Her father and an older sister came on the first expedition.

Mr. Pearson, was the lighthouse keeper at Fort Casey, and when Flora arrived she became the lighthouse assistant. The family, like others, brought along a grand piano. Flora used to teach music the children of the community, and to entertain the social reunions playing the piano.

Engstrom treasured among her possessions Flora's beautiful grand piano, where keys became concave at the center for being used for such a long time.

She was specially fond of Flora's lovely yellow taffeta formal dress complete with hoop skirts.

The true character of the "Mercer Girls" came alive with these mementos.

These were trying beginnings.

The Territorial University of Washington that Asa Mercer erected from the bottom upon a ten acre site on a hilly wilderness, has suffered an astonishing transformation through the years. What they pompously called "University" was little more than a backwoods school subjected to the ups and downs that menaced its very existence. During these early years it had to close several times for lack of funds.

The first Faculty consisted of just one Professor whose task was to take care of a curriculum which included Latin, Greek, English, History, Algebra and Physiology.

And this situation remained the same for many years.

Within three decades the University had outgrown its downtown site and moved to a large forested area on the shores of Lake Washington on Unions Bay with fine views of the Cascade Range and Mt. Rainer.

Farsighted, the regents saw to it that the University retained little of its downtown acreage, which is now the Metropolitan Track, and it is an important source of income to the University.

With the arrival of the statehood in 1889 the University was firmly established as an institution of higher education, yet, there still prevailed a significant shortage of Professors of different subjects.

This is easily demonstrated by the following anecdote: When President Charles W. Elliot of Harvard visited the campus in 1892 he inquired about academic progress in the Northwest. Meeting Professor Orson B. Johnson he asked what chair the professor occupied. "I don't know what chair you would call it, "was the reply. "I teach zoology, botany, physiology, physics, astronomy and....."

"You don't occupy a chair" interrupted President Elliot. "You occupy a settee".

Growth soon called for a larger campus and in 1895 classes opened in Denny's Hall, the first building on the present Seattle campus.

The University's future was decisively linked to the Alaska Yukon Exhibit that was being programmed by a few Seattle leaders at a luncheon.

The group was integrated by enthusiastic members of the Chamber of Commerce decided to push the project to its completion. This was conceived to celebrate the tenth anniversary of the discovery of gold in the Klondikes, located in Canada, not in Alaska, but it was the precursor of other strikes in the Far North.

By this time, the University of Washington embraced three modest buildings so hidden in the wilderness that the newcomers ought to search for them among the tall firs and cedars.

Professor Edmond Meany was an early historian of great prestige and maybe the most popular faculty member in the annals of the University of Washington.

With admirable intuition Meany envisioned the profits the University would derive from this event if the so much desired Exhibit would take place in the spacious University's campus.

Thus, he readily decided to propose campus to the Exhibit's organizers.

Not everybody was convinced that Meany's offer was a good one.

Many believed that the campus was too far away to be easily reached by foreign people wishing to attend the fair.

Meany tried to conquer them arguing that the trip in the trolley car in their journey to the Alaska Yukon Exhibit, would mean a sightseeing of real interest for the visitors.

As a university member, Professor Meany expected that after the Exhibit was finished the University of Washington would inherit some of the buildings erected for the fair. More than that, the selvatic campus would look much better after being trimmed for the occasion, beautifying the landscape.

The state legislature after a short debate agreed to Meany's wishes.

Maybe because they had endured an almost permanent demand for buildings from the University of Washington and this seemed to be a wonderful occasion to favour it. Once this was established others adhered; everyone wished that Seattle would have a University to be proud of it.

The organizers shed a contagious enthusiasm that was easily transmitted from city to city until in compromise almost all the North Pacific adhered and cooperated in this endeavor.

The plan of the fair was carefully studied. The Olmsted Brothers, already famous for their beautiful design of most of Lake Washington Boulevard were asked to take care of the plan of the whole fair.

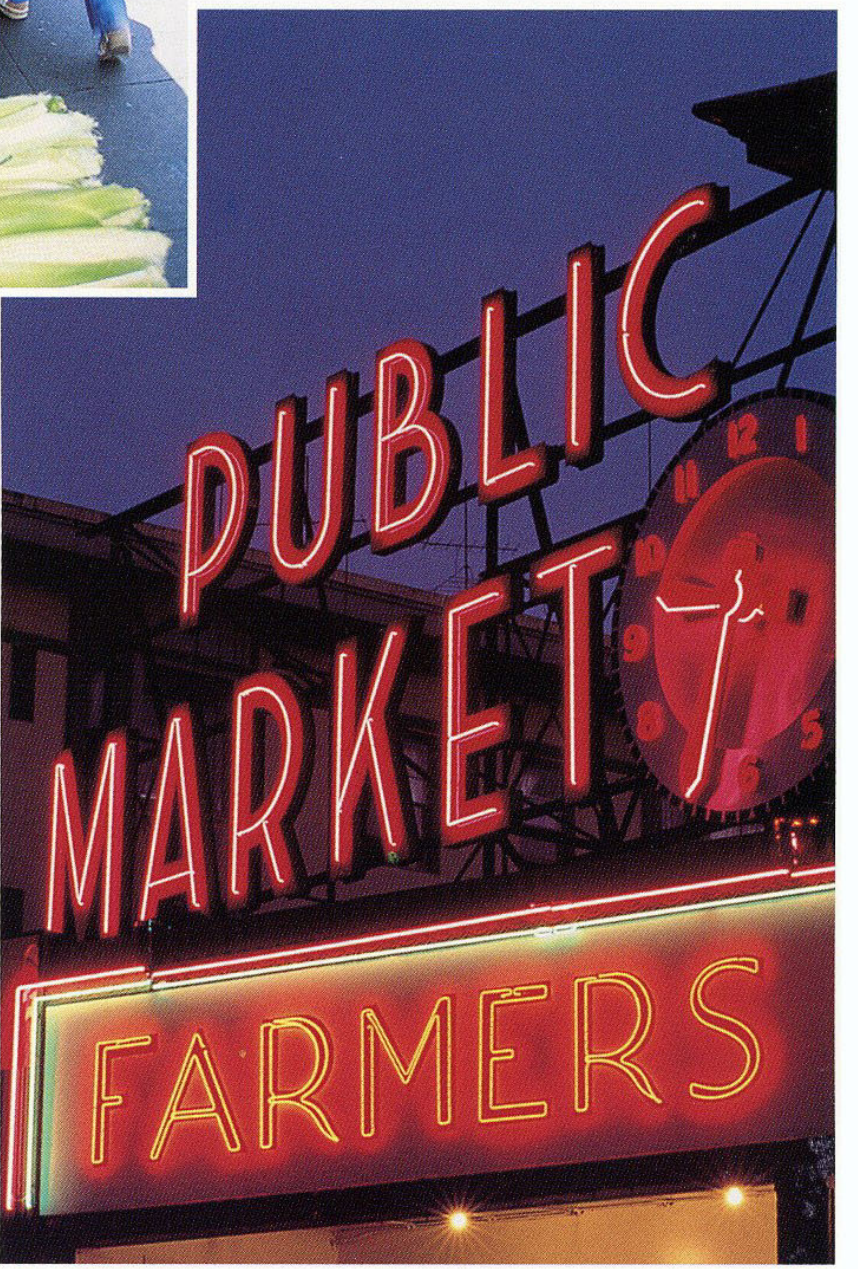
They assigned this task to James Frederick Dawson, who took full advantage of the charming environment with forests crowned by mountains and bathed by cristaline waters.

The Seattleites wished to have a wonderful setting for their fair; and they were not deceived.

As a center piece of the fair, Dawson built a long pool which descended gently by means of short waterfalls. This was under light at night and its sight was so fascinating, that the visitors remained for hours in front of it under the spell of its beauty.

The fair was a great success. Seattle looked at its best. A feeling of joyous festivity permeated the environment. The streets downtown were arched with banners. The buildings were ornated with a multitude of flags of national colors. An expectant crowd was greatly impressed by a colorful parade mostly military honored by the presence of the Duke Connaught's Own Rifles.

A fresh produce stand at the PIKE PLACE MARKET.



PIKE PLACE MARKET.

The climax of the excitement occurred when on June lst, at ten o'clock in the morning in Washington D.C. President William Howard Taft, pressing an Alaskan gold nugget, by Telegraph signaled the opening of the Alaska Yukon Pacific Exposition.

All at once the bells of the Pacific Standart Tim began to ring whistles on the waterfront loudly screamed in different tunes a throng of 80.000 jubilant people traversed the gates of the Expo on that memorable day.

Newspaper writers were sent to Seattle to cover the fair; they were surprised at the achievements of a frontier community, the youngest of the American Nations.

Many of the visiting reporters praised the fair as the most beautiful exposition ever held anywhere in the world.

Thoughtfully designer Dawson included in the fairgrounds the statue of Secretary William H. Seward, the cabinet minister of Lincoln's administration.

It was the most fitted homage to the person who instrumented the purchase of Alaska from Russia.

When the Alaska Yukon Pacific Exposition was over Seattleites felt that they had accomplished a big task with a great mystery and they were very happy about it.

During the hundred and thirty eight days that lasted the fair a total of 3.740.551 visitors passed its gates which amounted in terms of money to $ 1.096.475 a fabulous sum for that time.

But more valuable than money was what the exposition did to the spiritual growth of Seattle's society, enriched with an experience that filled their lives with confidence and pride.

The University of Washington inherited from the Alaska Yukon Pacific Exposition the most unusual and beautiful campus in the nation, and no fewer than twenty buildings for the use of the University.

Remote seems to be the times when Asa Mercer toured in his canoe the Puget Sound trying to recruit students for his recently born University.

Nowadays, about 35.000 undergraduate and graduate students apply every year for the different courses dictated at the University.

They can enjoy a library of 5.355.140 volumes, 56.335 seriales and 6.070.069 microforms, 1.500 microcomputers located in dormitories, classrooms and computer centers.

Since World War II the University of Washington has increased its well earned reputation for its outstanding research and graduate programs. It has been recognized in its leadership in the fields of drama, dentistry, medicine, nursing, health services, astronomy, geography, computer science, molecular biotechnology, oceanography, sociology and social work.

The undergraduates are greatly benefited by learning from professors who are at the forefront in generating new knowledge. The University of Washington's graduate programs are some of the most highly qualified in the United States.

In its beautifully lanscaped main campus of 703 acres, located at the North Central area of Seattle stand 220 buildings of varied styles; mostly Gothic. Among them as a mute testimony of a distant past are four Grecian columns, which pertain to the first University erected by Asa Mercer.

Soon after approving the Organic Act, by which the Territory of Washington was officially separated from Oregon, President Franklin Pierce appointed Major Isaac Stevens, an Engineer, as Governor of the New Territories.

He arrived at Elliot Bay on December 1854, and one of his first duties was to make a treaty with the Indians in order to establish legal rights to the settlers' claims.

It was a momentous event when thousands of Indians pertaining to the local tribes were assembled to hear what the white man had to say.

Governor Stevens informed them that the great White Father was willing to buy their lands offering sums ranging from a hundred thousand dollars to twice that amount for some tribes' lands.

They would live in reservations where they would enjoy the Government's protection, who would provide schools for their children, mills, and some other concessions and favours for those who signed the treaties.

As soon as Stevens finished his speech, from the gathering emerged a tall Indian whose height towered the small one of his kindred. His white long hair was dangling, and his strong feature denounced the firmness of his character. Regally wrapped in unkempt blanket, he had the countenance and dignity of a statesman.

Sealth was endowed with a powerful voice, and in a calm and persuasive way he delivered a memorable and poignant address in which he poured all his regret at the present condition of his people, once numerous and powerful, now vanishing and residing into the past.

With a tinge of fine irony, Sealth expressed his gratitude to the White Chief in Washington who offered them fatherly protection buying the lands they really didn'd need.

He ended bitterly affirming that in spite of all their promised brotherly love, they would always remain two different races, with two different Gods. He didn't believe their God would love and care for his red children, who seemed to have been forsaken by their own God: The Great Spirit.

He expressed his grief and desperation affirming: "We seem to be orphans who can look nowhere for help".

This is just a brief introduction to Sealth's famous address.

CHIEF SEALTH SPEECH

Addressed to Governor Stevens and the settlers of Seattle in the presence of the assembled Indian tribes, on the occasion of the treaty signing, December, 1854.

Yonder sky that has wept tears of compassion upon our fathers for centuries untold, and which to us looks eternal, may change. Today it is fair, tomorrow it may be overcast with clouds.

My words are like the stars that never set. What Sealth says the Great Chief at Washington can rely upon with as much certainty as our paleface brothers can rely upon the return of the seasons.

The son of the White Chief says his father sends us greetings of friendship and good will. This is kind of him, for we know he has little need of our friendship in return because his people are many. They are like the grass that covers the vast prairies, while my people are few; they resemble the scattering trees of a storm-swept plain.

The Great -and I presume- good White Chief, sends us word that he wants to buy our lands but is willing to allow us to reserve enough to live on comfortably. This indeed appears generous, for the Red Man no longer has rights that he need respect, and the offer may be wise, also, for we are no longer in need of a great country.

There was a time when our people covered the whole land as the waves of a wind-ruffled sea covers its shell-paved floor, but that time has long since passed away with the greatness of tribes now almost forgotten. I will not dwell on, nor mourn over our untimely decay, nor reproach my paleface brothers with hastening it, for we, too, may have been somewhat to blame.

Youth is impulsive. When our young men grow angry at some real or imagined wrong, and disfigure their faces with black paint, their hearts also are desfigured and turn black, and then they are often cruel and relentless and know no bounds, and our old men are unable to restrain them.

Thus it has ever been. Thus it was when the white man first began to push our forefathers westward. But let us hope that the hostilities between the Red Man and his palefaced brother may never return. We would have everything to lose and nothing to gain.

It is true that revenge by young braves is considered gain, even at the cost of their own lives, but old men who stay at home in times of war, and mothers who have sons to lose, know better.

Our good father at Washington -for I presume he is now our father as well as yours, since King George has moved his boundaries farther north- our great and good father, I say, sends us word that if we do as he desires he will protect us.

His brave warriors will be to us a bristling wall of strenght, and his great ships of war will fill our harbors so that our ancient enemies far to the northward -the Sinsians, Hydas and Tsimpsians- will no longer frighten our women and old men. Then will he be our father and we his children.

But can that ever be? Your God is not our God! Your God loves your people and hates mine! He folds His strong arms lovingly around the white man and leads him as a father leads his infant son -but He has forsaken His red children, if they are really His. Our God, the Great Spirit, seems, also, to have forsaken us. Your God makes your people wax strong every day- soon they will fill all the land.

My people are ebbing away like a fast receding tide that will never flow again. The white man's God cannot love His red children or He would protect them. We seem to be orphans who can look nowhere for help.

How, then, can we become brothers? How can your God become our God and renew our prosperity and awaken in us dreams of returning greatness? Your God seems to us to be partial. He come to the white man. We never saw him, never heard His voice. He gave the white man laws, but had no word for His red children whose teeming millions once filled this vast continent as the stars fill the firmament.

No. We are two distinct races, and must ever remain so, with separate origins and separate destinies. There is little in common between us.

To us the ashes of our ancestors are sacred and their final resting place is hallowed ground, while you wander far from the graves of your ancestors and, seemingly, without regret. Your religion was written on tablets of stone by the iron finger of an angry God, lest you might forget it. The Red Man could never comprehend nor remember it. Our religion is the traditions of our ancestors -the dreams of our old men, given to them in the solemn hours of the night by the Great Spirit, and the visions of our Sachems, and is written in the hearts of our people.

Your dead cease to love you and the land of their nativity as soon as they pass the portals of the tomb - they wander far away beyond the stars, are soon forgotten and never return.

Our dead never forget this beautiful world that gave them being. They still love its winding rivers, its great mountains and its sequestered valleys and they ever yearn in tenderest affection over the lonely-hearted living, and often return to visit, guide and comfort them.

Day and Night cannot dwell together. The Red Man has ever fled the approach of the white man, as the changing mist on the mountain side flees before the blazing sun.

However, your proposition seems a just one, and I think that my people will accept it and will retire to the reservation you offer them. Then we will dwell apart in peace, for the words of the Great White Chief seem to be the voice of Nature speaking to my people out of the thick darkness, that is fast gathering around them like a dense fog floating inward from a midnight sea.

It matters little where we pass the remnant of our days. They are not many. The Indian's night promises to be dark. No bright star hovers above his horizon. Sad-voiced winds moan in the distance. Some grim Fate of our race is on the Red man's trail, and wherever he goes he will still hear the sure approaching footsteps of his fell destroyer and prepare to stolidly meet his doom, as does the wounded doe that hears the approaching footsteps of the hunter.

A few more moons, a few more winters - and not one of all the mighty hosts that once filled this broad land and that now roam in fragmentary bands through these vast solitudes or lived in happy homes, protected by the Great Spirit, will remain to weep over the graves of a people once as powerful and as hopeful as your own!

But why should I repine? Why should I murmur at the fate of my people? Tribes are made up of individuals and are no better than they. Men come and go like the waves of the sea. A tear, a tamanamus, a dirge and they are gone from our longing eyes forever. It is the order of Nature. Even the white man, whose God walked and talked with him as friend to friend, is not exempt from the common destiny. We may be brothers, after all. We will see.

We will ponder your proposition, and when we decide we will tell you. But should we accept it, I here and now make this the first condition - that we will not be denied the privilege, without molestation, of visiting at will the graves of our ancestors, friends and children.

Every part of this country is sacred to my people. Every hillside, every valley, every plain and grove has been hallowed by some fond memory or some sad experience of my tribe. Even the rocks, which seem to lie dumb as they swelter in the sun along the silent sea shore in solemn grandeur thrill with memories of past events connected with the lives of my people.

The very dust under your feet respons more lovingly to our footsteps than to yours, because it is the ashes of our ancestors, and our bare feet are conscious of the sympathetic touch, for the soil is rich with the life of our kindred.

The noble braves, fond mothers, glad, happy-hearted maidens, and even the little children, who lived and rejoiced here for a brief season, and whose very names are now forgotten, still love these somber solitudes and their deep fastnesses, which, at eventide, grow shadowy with the presence of dusky spirits.

And when the last Red Man shall have perished from the earth and his memory among the white men shall have become a myth, these shores will swarm with the invisible dead of my tribe; and when your children's children shall think themselves alone in the fields, the store, the shop. upon the highway, or in the silence of the pathless woods, they will not be alone. In all the earth there is no place dedicated to solitude.

At night, when the streets of your cities and villages will be silent and you think them deserted, they will throng with the returning hosts that once filled and still love this beautiful land.

The white man will never be alone. Let him be just and deal kindly with my people, for the dead are not powerless.

Dead - did I say?
There is no death.
Only a change of worlds!

Fully convinced of the futility of their resistance Sealth and other chieftains signed the treaty.

Not all the Indians agreed with this solution, thus, the treaty sealed an uneasy peace.

The Indians were troubled watching the endless streams of white people invading their territories.

Gradually, it began to germinate in the young Indians' mind the idea that Stevens had stolen their lands.

The natives of Elliot Bay were friendly and kind hearted and no harm could be feared from them. But, there were tribes more belligerant surrounding Seattle and, through the dense forest of the east. Waves of hostility were easily transmitted to the really warlike red man still further East accross the cascades.

The Indians felt themselves cheated by a treaty that deprived them of their birthplace lands to confine them in reservations far from their familiar places where they used to fish and hunt and the whereabouts where they carried out their daily life.

Fires of rebellions began to be ignited all around Seattle and several episodes of violence evidenced the Indians' growing animosity.

There were ominous rumors that the most exhalted tribes planned a general attack to wipe out the white men from their lands. This was known by the settlers who fearing their menaces hurried to build a blockhouse as a fortress to find refuge in case of danger.

The inevitable clash between the red men and the white occurred on January 26, 1856. The Indians hidden in the forest surrounded Seattle, began to fire their muskets. The feared attack found the Seattleites fully asleep. There were scenes of panic.

All of a sudden, the cabins' doors were wide opened. The people half dressed desperately ran towards the brickhouse for safety.

The "Decatur", a naval war vessel, under the command of Captain Gansevoort, whose mission was to protect the settlement, began to counterattack the Indian firing. The battle lasted all day long.

The exploding shells literally covered the ground.

The brickhouse was stuffed with frightened people, who could hardly breath, hearing the duel of firing between the "Decatur" and the Indian muskets. During the short periods of calm, most of the women and children were transferred to the "Decatur".

By the evening the Indian firing and yelling were decreasing and convinced that they were reaching nowhere, they left by ten o'clock at night, not without enjoying first at all the food they found on their way and damaging the empty cabins.

Thus ended Seattle's one day battle.

In 1870 thrilling news arrived at Seattle: the Northern Pacific had planned to put forth its railroads through the Puget Sound. The Seattleites jubilantly thought that it was no doubt that Seattle would be a place of election for the railroad terminal.

But they were not alone, every populated spot in the Sound had the same aspirations.

There were a lot of ups and downs, while they were awaiting the final decision. They regarded Olympia as the most feared rival, but several unexpected circumstances like having a magnate from Portland, Oregon, called Ainsworth, who had a close relationship with the Northern Pacific, proposed to them Commencement Bay, which means Tacoma for the projected terminal, which was accepted.

The announcement of this resolution caused a great commotion in the settlement, and many of the Seattleites were for several days stupefied. Then came the reaction from those who never admitted defeat. They felt an inner urgency to reestablish all the expectancies they had about Seattle linked with a railroad that would bring more progress and shine to their beloved city.

With renewed energies a group of corageous Seattleites met to discuss the way to make a railroad of their own, which would traverse the Cascades connecting Seattle with the East.

This caused an outburst of indignation in the North Pacific tycoons and they became fierce enemies of Seattle's project and caused all kinds of impediments to it.

But the people of Seattle would not let anybody discourage them. They were accustomed to endure all kinds of hardships to fulfill their plans.

Selucious Garfield, elected as territorial delegate to the Congress, was their spokesman, and remarked that a route through Snoqualmie pass to the fertile Walla Walla territories would bring a lot of benefits, with its connections besides offering a route that would mean a cheaper way of transportation than the one the North Pacific offered.

Then the Seattle and Walla Walla Railroad Transportation Company was established. The people from Walla Walla demonstrated great enthusiasm and friendliness, and that was all.

Unhappily all the economical problems concerning such a vast project remained within Seattle's own responsability.

The Seattleites resolved to solve it the way they used to do it: Working. Everybody felt part of this endeavor, donating one day's work each week. No man was exempted from this task.

This corageous attitude was of great benefit for Seattle. The newspapers praised the spirit of a population determined to construct with their own effort a railroad that was one of their most cherished dreams.

The spread of this news raised Seattle's prestige and many were the young men that, charmed by the vibrating energy of this population, chose Seattle as a place worth to live in and to share its inhabitants' enterprise.

This was just the beginnings of Seattle's railroad story. The rivalry and enmity with the North Pacific lasted fifteen years after Seattle has its own railroad; as a result, the latter, suffered all kinds of impediments in carrying out its operations.

As the years went by, many providential men like Burke appeared in Seattle's railroad evolution, helping to raise the money so badly needed.

Running towards the future, the railroad adventure had moments of elations and frustations, but they were tracing on the map the routes of a new America united by the rails' shining parallel lines.

The progress evidenced by Seattle's railroad, forming new companies and giving new impulse to its endeavor, did not pass unnoticed to the Northern Pacific magnates who could not but recognize that their efforts to push Seattle into the Elliot Bay were null, and that Seattle was in the railroad business to stay and intented to become a terminal of great importance.

Then, the incredible happened, putting an end to nearly seventeen years of warfare. The North Pacific made a purchase and took control of the Seattle Lake Shore and Eastern from stockholders in the East.

This proved to be of great benefit for Seattle, whose first impression was of alarm. The wheels of progress crisscrossed the country bringing great prosperity to a growing population of about 40.000 Seattleites in the beginning of the century.

Years later further progress was introduced in Seattle's railroad adventure when James Hill a stocky and huge Canadian wearing a black patch over one eyeless socket approached Judge Thomas Burke.

Jim was heading the Great Northern railroad and was a kind of genious in solving problems, and finding economical resources derived from the railroad goods transportation. He was anxious to meet Judge Thomas Burke and to make him some proposals. The association of Burke and Hill proved to be a fructiferous one.

Bent over some maps and drawings, one-eye Jim, unrolled over the table; Burke attentively followed its description of a tunnel under Seattle running north and south, Burke's eyes were ignited with enthusiasm.

The railroad story followed the usual way of fulfilling its aims, sometimes unifying efforts, others submitted to the clashing of the wills, to end in a glorius reality.

By 1885, the Northern Pacific and the Great Northern were chugging side by side through a vast tunnel.

Later on other railroad lines were sharing the tunnel that was first just a design on Seattle's map.

Weaved into the loom of the railroad history are the names of railroad magnates like Hill, Harriman, Villar and others who helped Brook's early efforts to place Seattle among the most important cities served by major railroad lines coming from and going to distant places.

By 1889 Seattle's population had climbed to 33.500, but the city had grown haphazardly. It was originally constructed over a sea of mud, most of the houses at the front of Elliot Bay sat on pilings.

Timber was at hand and plenty, thus, ninety per cent of the buildings were made of wood, including many sin houses that populated the area. Some sidewalks and streets were paved with the same material.

To this we may add a very queer sewer system, ingeniously built with hollowed out scraps logs which by gravity brought water from the springs that fluently ran down the high forest behind

Vintage Australian Trolleys clang along.

Sailing on Elliot Bay with the Seattle skyline in background.

the town. This curious sewer system had its pipes erratically wandering on stilts, which at first sight startled the newcomers.

This arrangement has its nuisances, toilets became overflowed at high tides. Sometimes its force produced an unpleasant experience on the person who happen to be seated on it. To solve this problem some toilets were raised, and the people needed to climb three or four steps to feel safe-guarded from a sudden unwanted touch of stinking waters.

In spite of those uncomfortable conditions the city grew and expanded. A great business sections faced Elliot Bay, and the Seattleites were proud of having an Opera House and beautiful Hotels.

Thus, commerce, art and sin flourished with and coexisted such an awkward sewer system; then something happened that resulted to be a blessing in disguise.

It was a very warm summer, with no rain for many days, fires sporadically started in several points of the forest.

On June 6, 1889 early in the afternoon, in the basement of a wooden house facing Elliot Bay was the assistant of James McCough's paint and cabinet shop busily working. All of a sudden a glue pot boiled over the stove and the flames reached the floor covered with shavings. The assistant frightened tried to end the fire pouring water over it, which was the worse thing he could have done.

The flames began to take alarming proportions and with incredible speed the flames began its devastating performance.

The fire so unconspicuously started became a giant of uncontrollable force. Its voracity found plenty of food in the houses' wooden structures. And this was the beginning of the end for the pioneer's old Seattle.

All the settlement's men were desperately fighting trying to subdue the fire; but it was an uneven fight.

The fire department's equipment was irremediably ineficient, and the water lacked pressure. Thus it was resolved to fight the fire by the oldest system, forming buckets brigades. As the wall of fire relentlessly advanced, many people hurried to save as many goods as they could.

Then a steamer pump appeared in the Bay, trying to save the situation by pumping water from the bay to dominate the fierce fire. But even this failed in its intents, because the tide was hopelessly low.

Soon all downtown business district was in flames. The Opera House along with important firms, selling different kinds of merchandise were engulfed by the implacable fire; the same fate suffered the Continental Hotel and the Arlington which was the Seattleites' pride. Among the red flames and black clouds of smoke the Commercial Mills vanished.

There were moments of alarming intensity when the fire reached the Seattle Hardware Building and beside it nearly twenty tons of cartridges were stored. The valiant firefighters ran as far as they could to escape the fusilade and remain at a prudent distance, until the ammunition was exhausted. It was a hellish nightmare, the flames and the detonations were dramatically grandiose; the soil was covered with shells.

Next the warehouses and docks were reached by the fire, one by one the warehouses with thundering noise, collapsed.

Thanks to the indefatigable buckets brigades' efforts the Boston Block, one of Seattle's finest structure was saved. The fire which so implacable destroyed downtown business section, happily spaced the residential one.

By eight o'clock in the evening, the fire that had started at 2:30 in the afternoon wiped from the face of the earth, significant testimonies of the life and doings of the earliest Seattleites. Theater, Banks, Hotels, Court Houses, Shops, all was gone with the fire.

William Boeing an aviation pionner

William Boeing was a twenty two handsome young fellow facing his last year at Yale when he decided to quit the University and to explore newer horizons for his life.

His father was from a German stock, his mother was a Viennesse who carefully raised her only son who was just eight years old when his father died.

He was a restless young man anxious to exploit his energies in building his own future, even though he belonged to a wealthy family.

His mother has remarried and he disliked his stepfather. He was lured by the idea to go to west, and more specifically to Seattle which seemed to be a place full of opportunities.

Thus, Seattle saw the arrival of a tall, bespectacled young man endowed with a sensitive face which came as many others to try fortune.

Future aviation tycoon William E. Boeing and pilot Eddie Hubbard deliver the mail on Lake Union.

It didn't escape to Bill's perspicacity that the main business in Seattle was to equip searching gold expeditions to Alaska.

Since that memorable Saturday, July 17, 1897 when the Portland steamer came from the Yukon traversing the Street of San Juan de Fuca with its cargo of gold, which exaggeratedly was considered "tons of gold"; Seattle had entered into an era of prosperity influenced by the boom of the "Golden Rush".

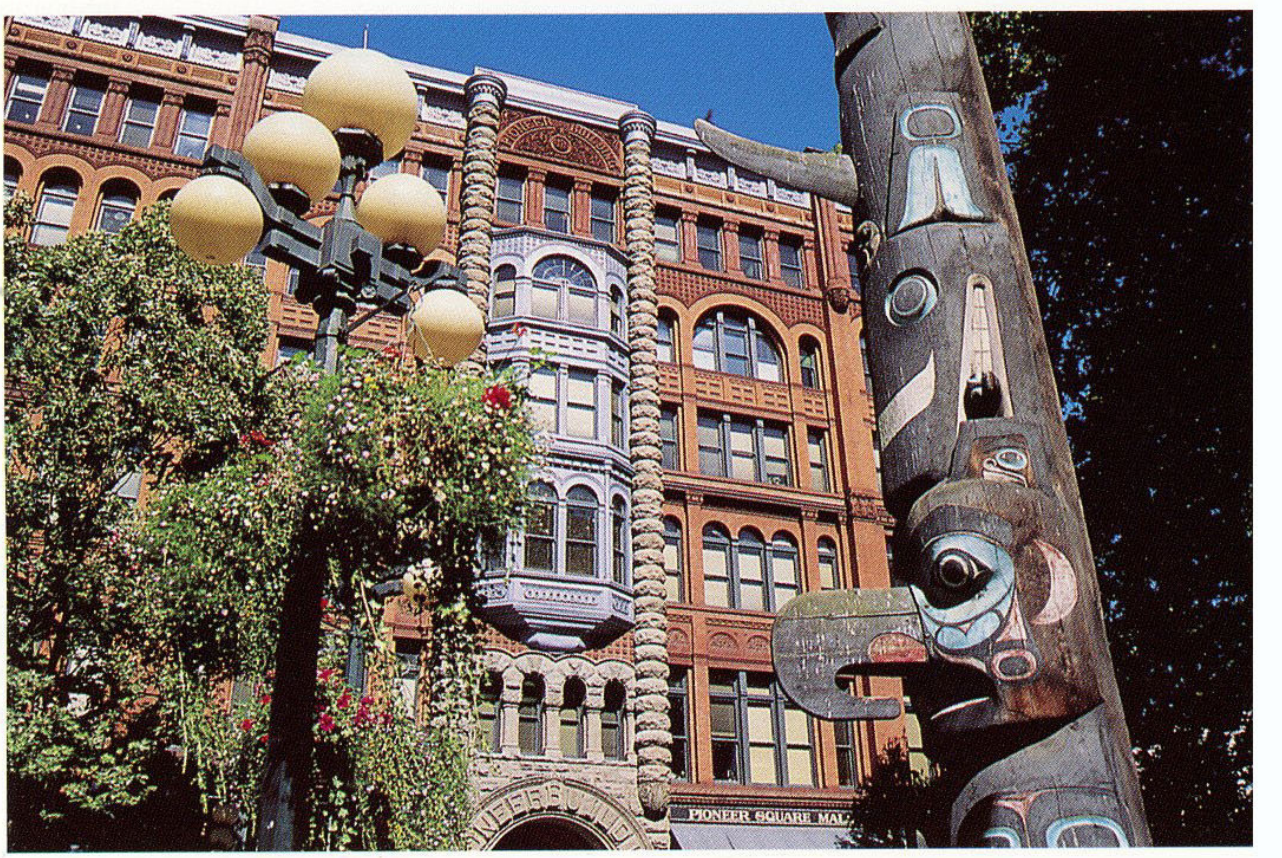
The town was invaded by Alaska-bound prospectors. This gave Seattle a great economical uplift, since they had to be fed, clothed, entertained and outfitted for their enterprise.

An intense marine traffic began carrying and bringing lots of adventurous gold seekers to and from the north. Ever since, Seattle has been benefited by the commercial advantage of being a gateway to Alaska and the Orient.

Boeing prepared himself to become one of the many providers of goods for the multitude of miners swarming into the area. Thus, he began buying a shipyard in the Dunamish River, and timber, and became fully committed to his task.

But destiny had greater plans for him, and this was revealed when a flier brought a Curtis, type of hydroplane to Seattle. Since the Wright brothers invented the airplane, many young men had his fantasy ignited by this feat; and Bill was one of them.

Legacy of "The Boeing Company", the Museum of Flight chronicles the history -and future- of aviation



Seattle's historic Pionner Square.

He didn't miss any of his exhibitions; needless to say, he asked the pilot to let him accompany in one of his rides.

At first the aviator terminantly refused to accede to his wishes, but after suffering Boeing's persecution, he reluctantly accepted to have him as his only passenger.

Then, the day arrived when Boeing was going to fly for the first time, the weather was fine, and the sun shone over a beautiful panorama.

Without thinking twice Bill climbed into the precarious flying machine whose wings were covered with thin muslin. Witnessing the scene was his friend Conrad Westerveldt who was assistant naval constructor at the Navy Yard in Bremerton and actually was assigned to the great Moran shipyard in Seattle.

When the hydroplane rose into the air, Bill was at the same time elated and frightened, but immensely happy of having this unusual experience whose impressions would decide the future of his life.

Westerveldt was also very much impressed by the man's feat in rising like a bird into the air. Afterwards both men commented about the idea of building a better plane of their own.

The seed of this wish germinated into action and became the origin of the well-known Boeing Company, the first to build planes in Seattle, which became a national pride and a source of economical power.

On July 15, 1916 the Boeing Company was founded and Bill Boeing and Conrad Westerveldt made real their dreams of building a plane of their own.

By that time airplanes were built of wood and there was plenty of that in Seattle's surroundings.

Westerveldt started to broaden his knowledge about the aircraft technology and Bill Boeing took lessons about flying.

Later on Herb Munter, a flyer who was also trying to build a plane, joined them.

After labouring in the project, the day arrived when the B & Ws endowed with a 125-horse power machine was ready to fly. While the aircraft was rising into the air with its dark varnish shining to the sun, their faces beamed with pride.

Steadily the novel Company was growing, in importance. New personnel was hired for the design department.

By that time the United States had entered World War I. During the relatively short time that the nation was at war the Boeing Company enjoyed a period of prosperity.

But the factory almost sank when the peace was sealed.

These were trying times for those whose business was linked with the nation's war efforts.

As almost all the employees were skilled carpenters, in order to survive, the company began to manufacture household furniture, school benches, etc.

But this was only one of the ups and downs the Boeing Company would endure in its ascension to begin to be known as one of the most importat factory of planes in the world.

During World War II the company experienced an enormous expansion as it mass-production was the famous B-17 "Flying Fortress" whose name was coined in battle in December 1941.

One week after the attack of Pearl Harbor, a B-17 started a memorable duel. Attacked by eighteen Japanese Zeros, who swiftly moving around dispatched their deadly weapons. The B-17 valiantly fought back eliminating four of its enemies. The Zeros circled around its victim inflicting serious damages in some virtual points of the aircraft. It seemed impossible to resist their furious attacks, when unexpectedly the remaining Zeros disappeared into the fog, having exhausted their ammunition.

While the factory was engaged in this task, Bill Boeing didn't stop thinking how to open new horizons for his flying machine. Thus, he put into action his idea to transport fast mail by air.

Thus, at the beginning of 1919 he established a mail service between Vancouver, British Columbia, and Seattle, creating the first Air Mail Service.

As the government was by that time the best customer of airlines, he carried to the Post Office Department, his ideas and designs for a mail carrying plane.

As he dreamed with the time when ordinary people would dare to fly, and to enjoy this fascinating experience, he started to add a few passengers seats in his mail carrying planes. He also operated this Model through a subsidiary company which years later would be known as the United Airlines.

It was a heroic feat. Nobody could have given credit to his eyes, when the crippled bird commanded by Weeless managed to reach the Philippines. From there on, the reliable bomber B-17 was deserving its new acquired name of "Flying Fortress".

The war was continuing its course and the Allies were in a very difficult position; at the same time, and surrounded with utmost secrecy and only known by a few persons, a mysterious and powerful weapon was being developed.

On the other hand, the Boeing Company was investing all its efforts for a new plane the Army needed, that would be faster and mightier than the Flying Fortress, for which 3 billions dollars had been assigned.

The secret fate of such a plane was to drop the atom bomb in Hiroshima and Nagasaki, to bring the war to an end.

Next came the B-47, a medium bomber, that initiated the stratojet era. Boeing continued to expand supplying bombers and jet stratolankers for the U.S. Strategic Command and later concentrated on jet planes for commercial service and the development of missiles. Next came the 707, the first air-ready plane of the transport line; a hydroplane that caused the admiration of a crowd of thousands of onlookers who witnessed for the first time the machine rising from the shores of Lake Washington, soared into the sky, rolling in the air.

The days of war were left behind in the misty days of a historical past and Bill Boeing was facing a new era of world airspace and travel. The perspectives couldn't be better according to Bill Boeing's calculations: half of the American population had never flown and many were eager to try a faster means of transportation and to enjoy visiting and knowing beautiful distant lands by a way that reduced weeks to hours.

Then the Boeing Co. produced the big Boeing 747, built for mass transportation. It was a mammoth plane; its towering tail surpassed a five storey building in height.

Its first flight caused commotion in Seattle, throngs of people came from everywhere to see openmouthed the gigantic airplane majestically rising on the air.

Everybody was exultant with pride at the sight of such a conqueror of the space that would shed honor on Seattle's air industry.

Space seems to be Boeing's domain. Ever since the first airplane built of wood and cloth, shining with dark varnish rising in the air, thousands of its progeny, every time more perfect, have flown the world's skies.

The Boeing Co symbolyzes the spirit of enterprise and progress that characterized the Seattleites since its pioneering days.

Seattle's Symphony Orchestra

It has been commented that the ambition for a well developed cultural life in Seattle was awakened as if it were by the sting of a wasp when Sir Thomas, who for a time conducted the Seattle Symphony, before leaving the country alerted a group of ladies about the need to give

economical support to the Symphony, "unless Seattle wanted to be regarded as a cultural dustbin".

The acidity of the blunt remark greatly outraged the Seattleites and determined them to erase from their consciusness this detestable comment.

Nowadays, Seattle's cultured life has flourished in several aspects. A very successful Seattle Symphony has a wide range of activities that may satisfy different kinds of musical lovers; from Symphony to Chambers and from baroque to contemporary americans.

Following a tradition, the Musical Director Gerard Schwarz has enhansed the Symphony's repertoire inviting stars of first magnitude in the musical world to conduct the orchestra.

The Pacific Northern Ballet means another source of national pride. Its brilliant performances are artistically and technically superb, exhibiting a vitality and delicacy that makes the delight of the audience.

The Pacific Northern Ballet's reputation is mainly due to the Company's Artistic Director Kent Stowell and Francis Rusell who in 1977 took over the company which at that time was constituted by 18 dancers. Now 45 dancers are beautifully performing not only in Seattle and in the most important cities of the United States, but also in many cities between Canada and Taiwan.

Seattle's cultural life received a great uplift soon after the close of the 21st Century World Fair, when the site in which the fair stood became the Seattle Center, a spacious and modern complex, in which many important cultural activities were reunited.

The Center has a number of museums, theaters and other attractions including the Opera House, the home of the city's Symphony Orchestra and one of the most beautiful buildings in the county.

The Center's most striking features are the Pacific Science Center, which is not only important because it aims at the promotion of scientific knowledge at a popular level but, also, because it is housed in the most exquisite enviroment; it is really an architectural jewel with its lacy towers and refreshing whirling pools, which impart a sedative atmosphere in the midst of a bustling city.

The Seatlle Center Playhouse supported by the University of Washington is a famous repertoire theater company.

A city's cultural ambition can be measured by its libraries. Remarkable attractive, the Seattle Public Library is located since 1960 with an annual circulation of about 4 millions books. It is connected with many branch libraries and a library for the blind with more than 20.000 talking books.

A library is a living organism in constant evolution satisfying the needs generated by progress in every field of human interest.

Next to it was the Space Needle, a real feat of engineering ability, which has become the Symbol of the Century 21st Exhibition.

It is a source of pride for the Seattleites who are eager to show it to the visitors. The Space Needle is crowned by an attractive glass enclosed restaurant with an observation platform on top of it.

The restaurant can accomodate two hundred and sixty people. They can enjoy a panoramic view of indescribable beauty while tasting the most delicious food from the Puget Sound for which the restaurant is famous. They would realize from time to time that the panorama has completely changed for another of captivating charm. This is due to the fact that the whole restaurant is a kind of merry-go-round, almost imperceptible day and nigth a full 360 degrees an hour, offering to the onlooker a complete survey of Seattle's awe inspiring surroundings.

The circular restaurant and the observation deck are sustained by three steel legs which has

a graceful design. The trip to the restaurant from the ground level is made by two high speed elevators operating on the outside of the central support. The elevators ride at the speed of eight hundred feet per minute, reaching the top in forty seconds.

Undoubtedly, the Space Needle was one of the main attractions for the crowds who attended the opening of the fair. To this event attended Vice President Lyndon B. Johnson, representing the White House, because President John F. Kennedy was at that time facing Russia in a critical situation regarding the Cuban crisis.

Lyndon B. Johnson was on that ocasion accompanied by John Glenn, the pionner astronaut considered a national hero. The Space Needle seemed to celebrate the entering of humanity in a new era in the space conquest. A wave of excitement vibrated in the multitude at Glenn's presence. Everybody wished to demonstrate in one way or another the admiration for the astronaut who symbolized man's highest aspiration, to extend his dominium into the outer world.

Museum Building At Volunteer Park

In 1991 the Museum Building at Volunteer Park was closed for restoration; at the same time a new building assigned by Pritzker Prize winner, Architect Robert Venturi, was inaugurated in the heart of downtown and it has evolved in a world class institution, unmatched in the region, with an ample scope in its artistic perspective.

The Seattle Art Museum collection amounts to about 21.000 art objects from ancient Egiptian relics to contemporary America.

They are outstanding in the following areas, African, Northwest Coast, Native American, Modern Art and Decorative Art. It also includes some fine examples of Asian Art in its collection.

In 1994 the Volunteer Park Museum reopened its doors, this time under the name of Seattle Asian Art Museum. It is the only Museum dedicated to Asian Art in the Northwest.

Already famous for its Japanese Art and Chinese Jades it is also enriched for an evergrowing collection of Korean Art of exceptional beauty.

Dr. Richard E. Fuller

Dr. Richard E. Fuller's love for art has been of upmost importance in the development of Seattle's cultural life.

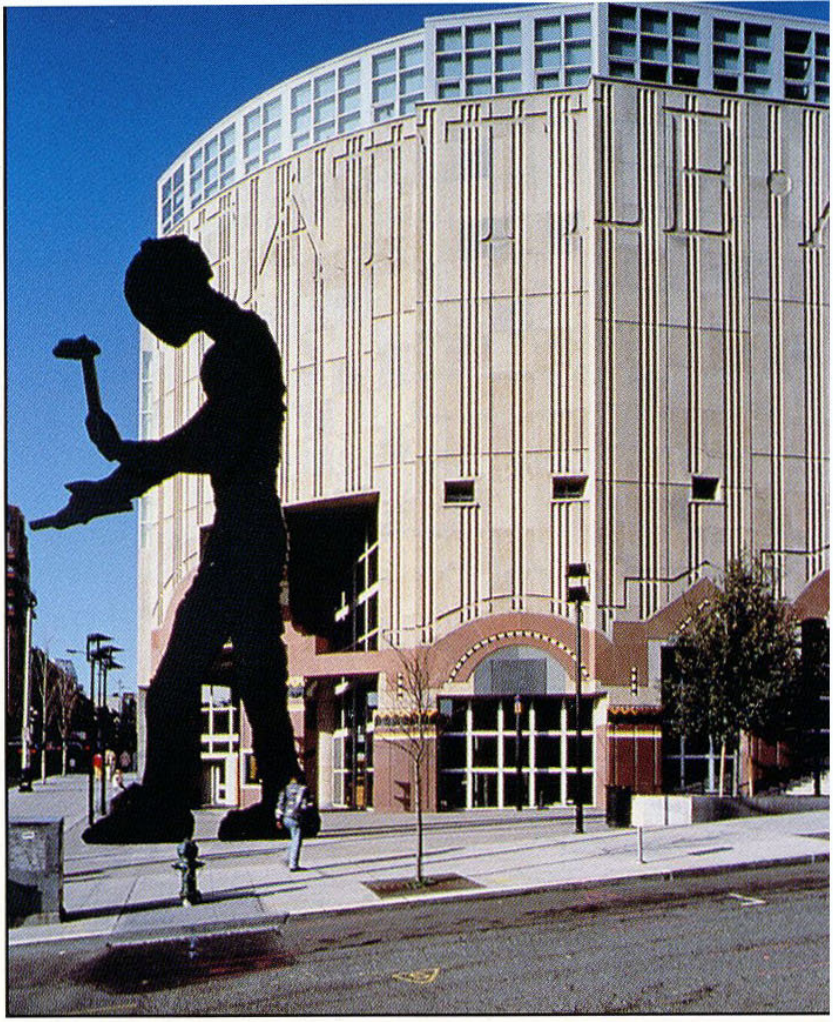
Dr. Fuller belonged to a Bostonian family who after a trip to the Orient became bewitched by the charm of oriental art. Mrs. Fuller began to collect small Chinese antiques and Dr. Fuller was fascinated by the beauty of jade, and the objects gathered, became the beginning of the outstanding famous Seattle Art Museum Jade collection.

Years later, the Fuller family established their quarters in Seattle, and in 1936 Richard Fuller was elected President of the Art Institute of Seattle.

In the midst of the Great Depression, when everybody was panicked because of the economical situation, Dr. Fuller and his mother, Mrs. Eugene Fuller offered to the city of Seattle, $ 250.000 which by that time was a considerable amount of money for the construction of an Art Museum.

The site of election to raise the Seattle Art Museum, was the Pergola at Volunteer Park. This was a very beautiful natural landscape in which was erected a lovely white stone building designed by Seattle architect, Carl Gould. This had two sculptured Ming Dynasty camels flanked the wide entrance steps. Later on the place was enhanced by a fifteen ten granite sculpture. It is Isuma Noguchi Black Sun.

SEATTLE ART MUSEUM



SEATTLE ASIAN ART MUSEUM

The Seattle Art Museum opened its doors on June 21, 1933 with Richard Fuller as his Director. It became famous for its Asian Art Collections, considered one of the best in the county, with works originally from Japan, China and India.

Dr. Fuller exerted an important role in spreading the knowledge about Art and Culture.

As time passed by, the Museum's art collection was enriched by new acquisitions and valuable donations such as the one received from Mrs. Donald Frederick, which included the most significant of Japanese Art in the Museum's collection. It is wortwhile to mention, the early 17 th Century deer Scroll which is a portion of a scroll considered a National Treasure of Japan. The rest is in the collection of the Emperor of Japan.

Soon after the close of the Century 21st World Fair, the Seattle Art Museum established at the Fair site an Art Pavilion which essentially was considered an Arm of the Museum. Later on, when another museum building was place downtown, this was closed.

Dr. Fuller not only had tried to reflect in the museum collection and changing exhibits the whole sequence of mankind artistic genius but he has stimulated the creative power of local artists, acquiring some of their best artistics achievements.

In 1983, celebrating the Seattle Art Museum 40th birthday, the permanent collection was featured in a series of exhibits.

The same year Dr. Fuller retires, after serving for four decades the Museum as President and Director without salary. This is quite an unusual situation among Museum Directors which exalted Dr. Fuller's exceptional personality.

Mark Tobey

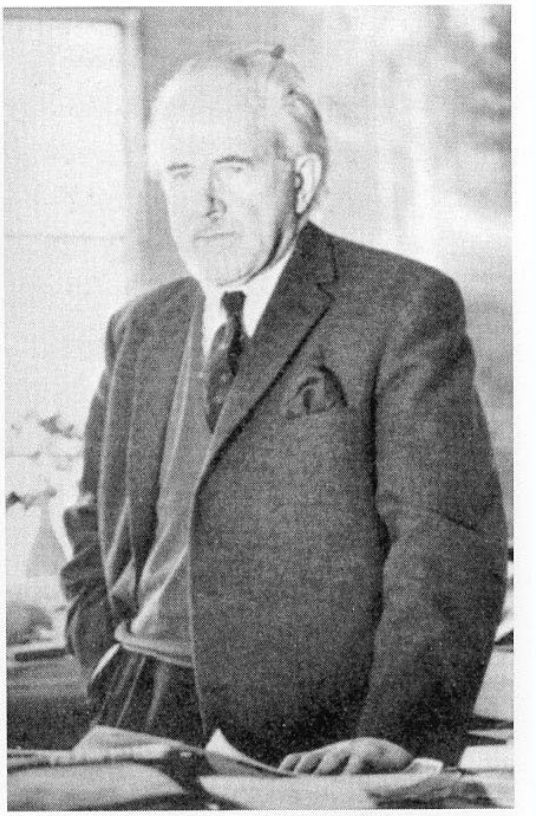
In 1922, Mark Tobey, a young painter arrived at Seattle. He left behind successful career as fashion illustrator for women, wear and portrait painter of outstanding personalities. He also left behind a brief and wrecked marriage. The Seattleites described the newcomer as "a handsome restless stranger with blazing cooper hair and a temperament to match and no money".

Living in a region whose geographic position favoured the Japanese and Chinese population, Tobey fell under the influence of Far Eastern Culture.

Soon after his arrival, Tobey began to study Chinese calligraphy with T'eng Kuei, a student at the University of Washington.

The following years were of a continuous searching for his own true expression in art. For that time, he made a personal discovery of cubism, which he described as "multiple space" adding that: "space was anywhere and everywhere".

After a trip to Europe and the Near East which lasted two years, Tobey began to have interest in the design of the Arabic and Persian scripts. As the years passed by, Tobey continued trying different forms of artistic expressions. Following the orientation of relevant artists



Tobey in the University District studio.

of that epoch, his form at that time was realistic, but also slightly cubistic; the colors used were greens and browns. His most important painting at that time was "The Middle West" (1929), in which the dynamic handling of the space was demostrating his originality.

When Tobey along with Mrs. Edgar Ames founded the "Free and Creative Art School" in Seattle his strong personality and leadership was a great stimulus to young artists to pursue an independent mind in their creative artistic expressions. The school has remained active, until nowadays keeping alive the ideals of its founders.

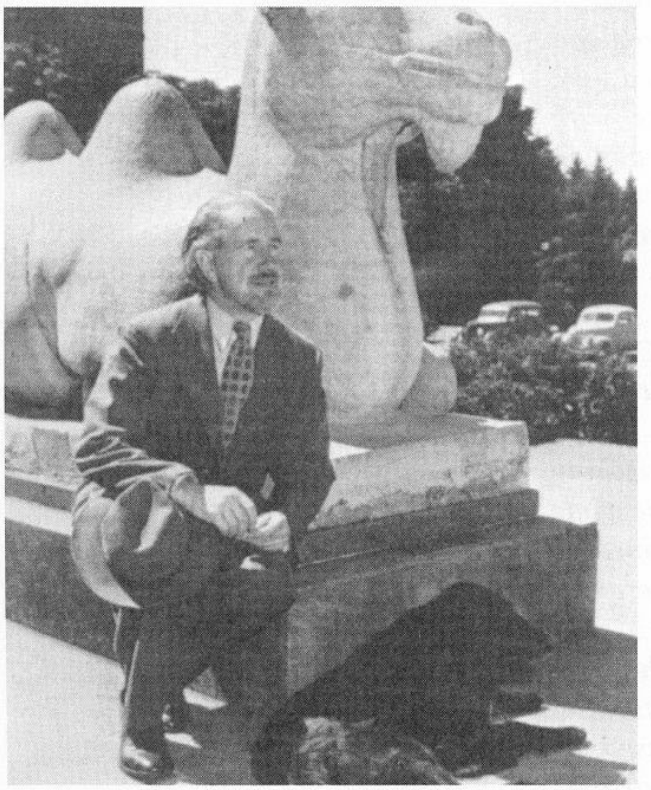
While he was an artist in residence in England (1931-1938) in a progresive school at Dartington Hall, Devonshire, he left the place twice, in 1931 to go to Mexico and to the Far East, in 1934.

Again under the spell of the Orient, Tobey, reassumed in Shangai his studies of Chinese calligraphy with T'eng Kuei. Thus, his spiritual proclivity to the Far Eastern way of thinking was intensified when he afterwards took instructions in brushwork and meditation in a Zen Monastery in Japan. This decisively affected his artistic development which would become the exteriorization of his mystic philosophical maturing as a follower of Baha'i World Faith which recognizes the prophets of all great religions, established the unity of all creation, and declares the equality of all mankind.

While practicing calligraphy in the Far East, Tobey became aware of the difference between volume and the agility of the living line. The volume and contents of the West, with the fluidity of the Eastern calligraphy. Thus, Tobey tried to integrate East and West in different forms of expressions. This became a personal style which would bring him world acclaim for his originality.

While searching for his true expression in Art, Tobey suffered several relapses in his past realistic creations which deserved to be qualified by a critic as "A Stylistic wanderer".

In 1935, and under the influence of Baha'i religion, he painted a mural entitled "Rising Orb" whose metaphoric meaning was expressed in the most pure realism. According to Tobey's own words the man and women in the left represent local time, while the orb with the broken moorings represent solar time.



Tobey beside one of the great T'ang camels at the Seattle Art Museum, probably late 1940's.

This was followed by other works in which the flowing line was of a continuous white calligraphy on a silent background, which became his favorite way of expression, whose meaning was in "Broadway Norm", as "figures caught in light".

During his forties Tobey acquires maturity in this type of painting which according to some critics would make of him "one of the few original contributions to American painting". From now on his personality was firmly established gaining worldwide recognition.

In 1946, Tobey was one of the "Fourteen Americans" who deserved the honor to be selected for the Museum of Modern Art for an especial exhibit. Like many painters, he shared his spiritual creativity with other forms of artistic expressions, like music and poetry. While he was living in Seattle he was fond of

playing the piano and composing music. His flute solo was performed at the University of Washington. Among his musical compositions was a suite for children. His artistic feelings found also expression in poetry. In 1956 he deserved to be elected member of the National Academy of Arts and Letters in New York City. Thus, Tobey, also conferred to poetry his armonious way of thinking. He was already 66 years old when he was elected Member of the Academy. He travelled widely, lived in different cities, at different times, participated in many successful exhibits, and created many valuable art work. His paintings were bought after by the most famous Museums, and private collections. He reaches the climax of his career, when he won the first prize for painting at the Venice Biennale. The only other American ever to win a comparable Venetian Prize, was Mc Neill Whistler. He was internationally acclaimed as one of the most original contributors to art, in which the mystical Oriental thought was coupled with his humanism based on a truly American love for liberty and individuality.

Happily the Seattle Art Museum and galleries own Tobey's finest works of art, which can also be found in many private collections in Seatlle.

At seventy, Tobey moved to Basel, Switzerland to spend the rest of his life in a pleasant and quiet atmosphere. He visited Seattle from time to time, to enjoy the energizing spirit of this city so dear to him.

One year after his arrival to Basel, the Musée des Arts Decoratifs of the Louvre gave a large retrospective exhibit of his work, an honor never before conceded to an American Artist.

He continued to work all his life in spite of the ill health which affected him in his eighties.

All the mystical philosophical feelings that sustained his life was poured into those vibrant flowing white lines symbolizing the light which revealed, according to his explanation: represents the unifying thought which streams accross the compartments of life, and these give strength to the spirit and are constantly renewing their energies so that there is a great understanding of life.

Morris Cole Graves

Morris Cole Graves was a frail child who spent his first years in an isolated background Oregon home.

Maybe living in a remote region imprinted in Morris a delicate sensibility, his unlimited love for birds, small animals, and nature, besides his fondness for solitude.

His family moved to Seattle when he was a boy, and he gave up his studies after two years of High School, to enroll as a seaman in the American Mail Line.

Between 1928 and 1931, the boat took young Graves to Hawaii, the Phillippines, Hong Kong and Tokyo. These short trips to the Orient would exert an everlasting impression on him. He was particularly impacted with Tokyo and its way of living.

He said that he had the feeling that this was the way to do everything. He admired their acceptance of nature instead of resistency to it. In a way it is to recognize that man is a part of nature, and not an alien to it; and like it.

During these trips Morris began to draw local birds and animals; these were the first artistic expressions of a subject he loved so much. Besides he was enraptured with Asian Art and deeply impressed with the Vedanta and Buddhist philosophy.

As the animals were his favorite subject, Morris spent many hours at Los Angeles Zoo. Before returning to Seattle, Graves spent some time in Texas with relatives and finished High School.

Life was stern with Graves; he was an unknown artist, with no money in the worst period of the Depression. This put to test his ability to survive. Somebody remarked that Graves was akin to the birds he loved: so much like them, he was easily disturbed by noise, he was withdrawn, mobile and migratory when regional conditions were adverse.

By that time he painted "Moor Swan" his first important oil which deserved a $100.- acquisition prize by the Seattle Art Museum, which following the policy of its Director, Richard Fuller, tried to stimulate talented local artists.

Unhappily, "Moor Swan" is the only one of his first series of oils, that survived a fire. Two years later (1935) Graves returned to Los Angeles, this time accompanied by Guy Andersen, another painter.

Adjusting to the economical situation both painters were lodged in a Model T, laundry truck.

Then, an important event took place. The Seattle Art Museum displayed his first solo show. As a consequence he was hired as an easel painter by the Federal Art Project of the Work Project Administration, an Institution which helped many Northwestern painters and writers to survive during the worst of the Depression years.

Morris Graves' delicate sensibility was affected by the events occuring in the world by that time and it was reflected in two very queer series of paintings entitled "Night Fall Pieces", which depicted deteriorated European furniture, which has been interpreted as a protest towards the Munich Pact. These impressions were obtained from the outside world; from within emerged a "Purification Series" in which he expressed in terrifying semi-abstract paintings the conflicts that agitated his inner self.

Happily his works gradually evolved towards a more pleasant and ethereal style indicating that "purification" had been attained.

Finding oil and canvas a too heavy medium for his mystical feelings, he turned to tempera, ink and gouache, using rice paper.

Graves' well earned fame derives from the fine quality of his phantasmal creations, of which his first expression was "Bird in the Midst".

His paintings were greatly influenced by the Chinese and Japanese Art and philosophy, mixed with his intense love for nature. Like in a crucible feeling and impressions rended his personal interpretations and grasping of the "ultimate reality".

He found rest refuging himself in his inner world. To paint, to translate to the exterior his visions, were for Graves a painful experience, plagued with anxiety and despair. During the 40's he used to work at nigth; as a symbol of his dramatic state of mind he painted "Little Known Bird of the Inner Eye", a fearful and solitary four legged red bird, caught in a bad red dream, or mental experience.

In 1939, Graves met Tobey with whom he had a great affinity sharing the same devotion for Zen Buddhism, which inspired "Bird Singing in the Moonligth" and his outstanding "Blind Bird Series" wich deserved world wide recognition.

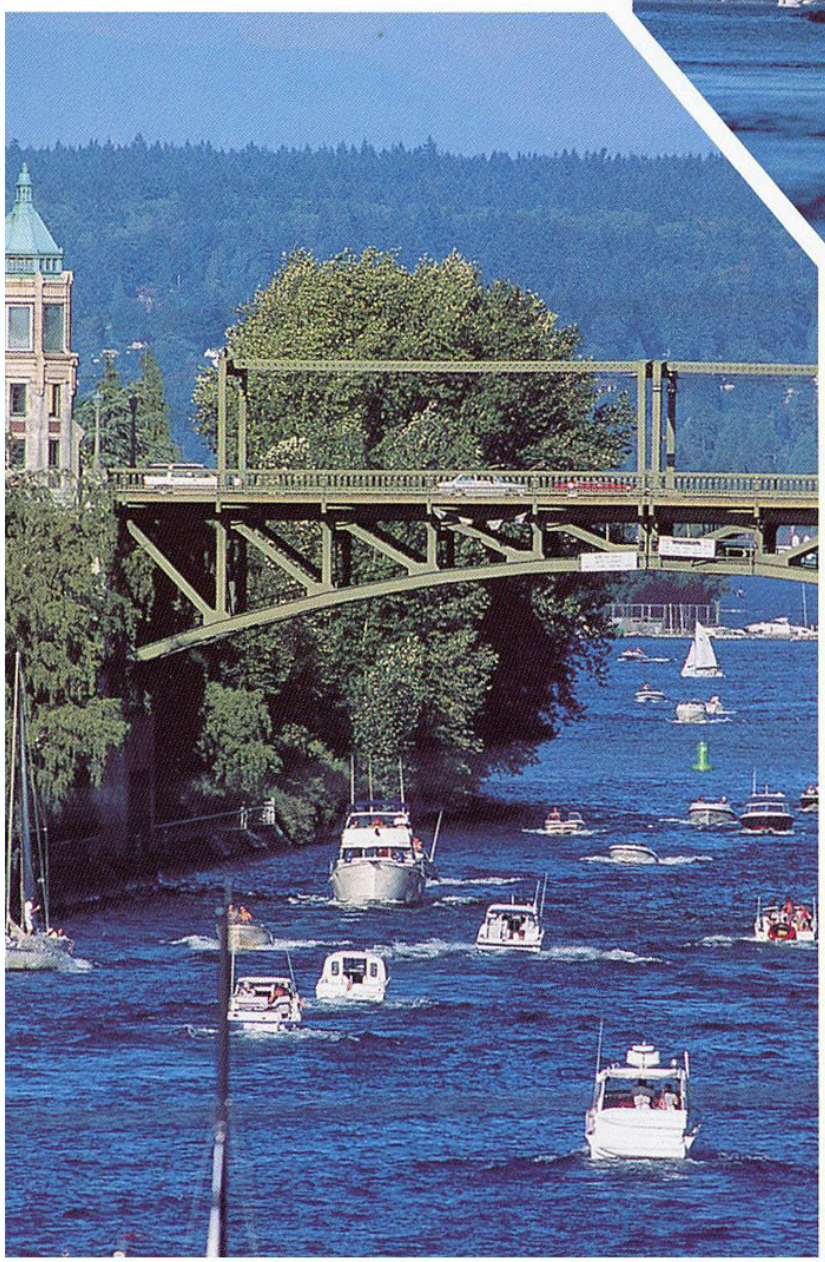
By 1942, a major event occurred in his artistic career when 31 of his paintings were included in the "AMERICANS 1942" an exhibition carried out by the Museum Art of New York.

Under Tobey's influence Graves created his own version of Tobey white writings, imparting to the white brushstrokes the same mysterious feelings presented in his other creations.

By the end of 1942 Graves was drafted, but his military service didn't last long. Being a man of principles, Morris objected for conscience sake to militar service, and in 1943 he was discharged.

This would have an unexpected repercussion in the future. Having in 1946 earned a

Big green and white automobile-carrying Ferries are extensions of the State Highway System, making frequent trips accross Puget Sound.



Boaters in the Montlake Cut with the Montlake Bridge in background.

Guggenheim grant to study painting in Japan, the permission to enter the country was denied to him, by the American authorities, maybe because he was dismissed from the Army.

The time saved from the military duties during the war years were dedicated to paint a very pleasant "Joyous Young Pine Series", to these were added a series of painting undoubtedly influenced by the Japanese and Chinese styles. They have the graceful wave and swirlling movement so characteristic in the Oriental Art. To this series belongs "Sea, Fish and Constellation". Unable to accede to Japan, Graves went to study the Asian Art Collection at the Honolulu Academy of Art, where he painted "Ceremonial Bronze Taking the Form of a Bird", belonging to a series of Chinese Bronzes that were exhibited and praised in New York.

Afterwards Graves decided to build a home on Edmonds, Oregon, which he frequently abandoned to spend much time travelling throughout Europe, Mexico and Japan.

The pleasant atmosphere of his Edmonds, Oregon, was disrupted by the ever increasing noise of urbanization of the NW until it became intolerable for him to remain there, and he abandoned the place.

To express his protest about the invasion of the machinery noise in his private life, Graves painted an acrid series of pictures entitled "Machine Age Noise" and "Spring and the Machine Age Noise" in explosive masses of red and black. They were a bitter cry against the destruction of nature of the "so-called": Progress.

By the end of the 50's and the following two decades, Graves wandered around the world visiting different cities of the settled USA; he travelled to Ireland, India, Japan, Africa and South America.

Motion was beautifully represented in some of his much praised work in the "Fligth of Plover" in which the fluid pattern of living motion was artistically perfect. The same characteristic is found in some of his later works, like in the small mandalas, whose concentric shapes represent little minnows swimming in circles.

When he turned to still life, he seemed to have found the so much needed inner peace, painting glass bottles and flowers, such as "Winter Bouquet". They imparted a sense of serenity, a reasuring atmosphere of homely quietness and solitude; this was the mental state that presided his creations.

Graves' works have been exhibited several times in Seattle and in almost all the important museums and art centers in the United States such as Willard Gal. NYC 1942, 43, 44, 45, 53, 54, 55, 59, 71, 73, 76, 78, 83: Univ. of Minnesota, Minneapolis; Arts Club, Chicago 1943; Detroit Inst. of Arts. 1943: Phillip Gal, Washington D.C., 1943, 54; etc. and abroad in outstanding art museums, such as the Museum of Art of Tokyo, the Institute of Contemporary Art in London, 1957, etc. His paintings are included in the permanent collections of renowned museums such a the Metropolitan Museum of Art and MOMA, NYC, Museum of Art in Cleveland, also in San Francisco; Museum and Sculpture Garden in Washington, D.C., and many others located in important cities of the USA.

There is also an important collection in Seattle, his home town, which saw him to grow as his work exhibited and to become one of the finest artists in American painting.

Seattle -Writers

Even if the schools of writers has not reached the level of its outstanding painters, we will briefly mention among those who have excelled in the literary field: Vernon L. Parrington, who is considered an historian who greatly influenced contemporary historical and literary thought.

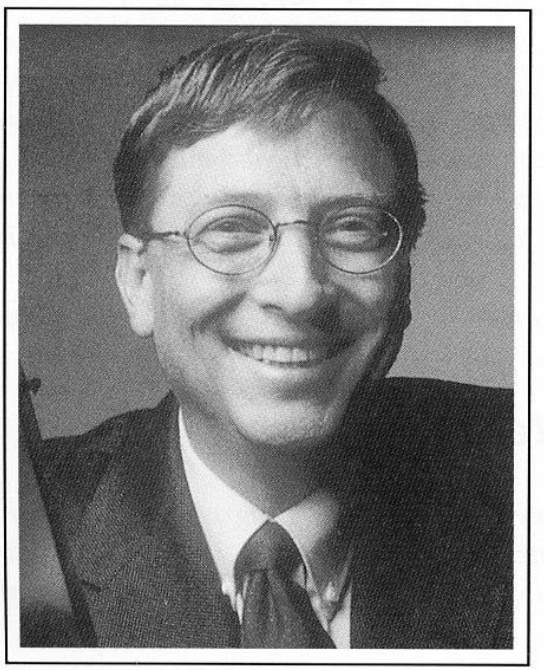
Theodore Roethke, 1908 received the award for THE WAKING in 1956.

Vernon L. Parrington.

He won the Pulitzer Prize for his two volumes about "MAIN CURRENT IN AMERICAN THOUGHT" and Theodore R. Roethke who won the Pulitzer Prize for poetry in 1954 for "THE WAKING".

It is also worthwhile to mention Betty MacDonald, undoubtedly Seattle's best known popular writer, author of the widely distributed book "THE EGG AND I".

Personality



Bill Gates

"An extraordinary life"

In a sun bathed Seattle suburban house overlooking Lake Washington grew up the boy who would become one of the richest men of the United States: Bill Gates.

Endowed with a brilliant intelligence, his technological creativeness and business dexterity has made of him a renowned celebrity in our days.

His father, William Henry Gates II, a huge buoyant lawyer, was married to Mary, a good natured banker's daughter who shined with equal charm at social and business gatherings. Contrasting with them, Bill was a small and timid child, socially withdrawn, who was frequently secluded in his basement bedroom just... thinking.

As a gesture of defiance to a world which overpowered him, and avid to earn self reliance, Bill was at war with his domineering mother. It was a tempestuous relationship. His parents thought to seek the help of an analyst who could tame the little rebel.

Thus, while at sixth grade, Bill was carefully studied by a psychologist who submitted him to a battery of test and advised him to do some readings which made him to be specially aware of Freud's theories.

The interviews lasted for one year, and the anxious mother learned the counselor's verdict. She ought to forget her ambition to rule over her son's life, and try to find a way to adjust to her son's nature.

As time passed by, his parents realized that his son's interests differed in many ways to the children of his age; his curiosity was insatiable and his inquisitive spirit aspired to get to the core of everything that attracted his imagination.

Thus, when he was ready for Junior High School, they decided to elect for him the Lakeside School, a select private school, reserved for highly qualified children.

Housed in a New England style classroom building, it was a noisy teletype computer and Bill and his life long friend, Paul Allen, were assiduous visitors to this place which enticed their imagination and set in motion their creative powers.

Together they went through a manual on Basic Languaje. Soon Bill produced, during his eighth grade, two programs, the first of the long series that would make of him a successful software producer.

One of Bill's most important characteristics was his competitiveness and his will to succeed over his opponent. It is not surprising that after reading Napoleon's strategy he designed a computer version of Risk, a game involving the military tactics of a conclave aiming a world domination.

Over the years this will to succeed has been transferred to the business competition, where Bill is not satisfied with less than total surrender.

To achieve this end, Gates maintains a constant control over his competitors' activities; this also works for his potential partners. For him to foresee, is to prevail.

In a Microsoft meeting, Bill obtained full information gathered by employees assigned to follow up a special company wihch may mean danger for the Microsoft's stability. What are they aiming at? Which would be their next step? Everything is analysed and new measures are provided.

Ever since Bill Gates and Paul Allen began in High School to deal with the old teletype computer, this task has captured them and is at the center of their lives. They managed to continue this activity working for a local company who got a big computer and needed help to debug it. They exchanged their services for the right to practice this new science.

With such passion, Gates would surreptitiously open his basement door to spend almost the whole nigth at the computer.

This devotion to work rended its benefits: soon Bill and Paul started a successful company hired by the city to analyse and graph traffic data. By this epoch his feelings of insecurity vanished, he gained self confidence and made peace with his mother.

He excelled in mathematics and in a aptitude survey he was regarded among the ten most conspicuous people in the United States.

Along with his studies, Gates was finding new ways to obtain some profits from his computer addiction. While in the tenth grade he was teaching computers and deviced a program that took care of class scheduling.

Kent Evans, the son of an Unitarian Minister, was one of Bill's closest friends and shared with him the juvenile ambition to conquer the world. Bill, Paul and Kent, together founded the official Lakeside Programers Group, and got some jobs from some business companies.

The group friendly atmosphere, would be sometimes disrupted by furious quarrels specially when one of the group tried to prevail over Gates, something he would not tolerate, and rather would break the bonds of friendship than to be relegated to a second place.

Soon his associates recognized his personal capability and brilliancy, and he settled his rules: to maintain the harmonious relationship he always ought to be the "number one".

His power emanates from an outstanding personality, with the privileged intellectual condition, invaluable ambition and extraordinary capacity for work.

The death of Kent Evans on a climbing mountain accident, left Bill stunned by this tragedy that deprived him of his best friend, and it took some time for him to recover from this blow.

Bill and Paul were many times engaged in endless conversations, and together they learned all the secrets of an artificial intelligence and were avid job seekers. When Gates left for Harvard, the collaboration was not interrupted since Allen would endure long drives to visit his friend and talk to him about start out in business together. This came to reality under the name of MICROSOFT; they were engaged in writing a version of Basic for an important market that was just in its beginnings: The personal computers.

Bill Gates and Paul Allen's fruitful association was frequently shaken by stormy quarrels, and as time passed by, Paul Allen was threatened with Hodgkin's disease, and he decided to leave Microsoft. Gates had few friends but he felt sentimentally attached to them; the same happened with his associates, he had a sense of togetherness, that he regarded his group as a single entity engaged in a common desire, "the will to succeed".

Deeply affected by Allen's desertion, Gates earnestly tried to mend their friendship. Allen was by that time one of the country's most important hightech-investors, being the owner of the Portland Trail Blazers; Gates ougth to have been very convincing because he achieved that Allen accept to return to Microsoft Board.

He would check any kind of expenditure that he would consider unnecessary or out of place. Sometimes even if this did not mean a real danger to Microsoft's economic stability. This generated heated discussions with some of his associates when the same insisted in spending money in some project on which he disagreed. He would cry that he would be plagued with debts even if he had at that time 8 billions dollars in cash.

It was Gates' policy to have enough money in the bank to cover Microsoft for one year even if his entrance were nule. No false steps were allowed in Gates' ascension career.

Gates' ability to handle Microsoft business was put to test when something happened that endagered the Company's future. Microsoft Network began as many other similar companies and as a proprietary of ON-LINE-SYSTEM. A revolution was produced in the market when open standards of Internet settled new rules in the production system.

This caused a turmoil at Microsoft's quarters. The matter was fully discussed. In less than one year Gates introduced the necessary changes and Microsoft not only survived but maintained a leading post in the market.

One of Gates' peculiarities is to rock while thinking, and if the subject's intricacies is demanding more interest, the rocking increases its rhythm. This mannerism seems to be contagious since the Microsoft collaborators have adopted themselves. Thus, when they are discussing some of the many problems that demand their attention, the meeting resembles a "Yeshiva Seminary".

Gates met and married Melinda French, a talented young lady, while she was working at Microsoft. She had taken courses on computer science as an undergraduate at the University where she afterwards also got a graduate degree in business. She made a perfect match with her brilliant husband. She excelled in every aspect of social manifestation aside from being a clever participant in technological and business discussions, and she became the executive in charge of interactive content. After Jennifer, their daughter was born, she left Microsoft to take care of her child and became involved in charitable and other activities at the board of Duke University.

Bill and Melinda started to build a fabulous 40 million home which took more than four years to reach completion, covering an abrupt terrain of about 40.000 sq. feet of space, facing Lake Washigton.

The planning of this strikingly beautiful building has in its form and function the seal of Gates' love for the surprisingly unusual besides of being a clear manifestation of this ingenious, creativeness and extravaganza.

This is perceived by the visitors of his home. Provided with a pin in which the preference of each guest is encoded, the visitor enters the ground floor reception hall and he is confronted with two dozen flat screens covering the whole wall. As he advances the encoded pin will enter into action and in the screens would be displayed his dearest pictures, music or TV Shows. The grandiosity of the spectacle would marvel any person who is facing it.

Next to this is a more sober environment. Under a carved dome is the library. In selecting its content, Gates has consulted a rare book dealer that gathered items that would give delight to any book collector.

Besides them and as an expression of his personal preferences there are books which at different times and occasions have enriched his mind; among them are the books of John Irving, Phillips Roth, David Halbesiteen, Ernest J. Gaines and others.

His office has been described as having a gigantic trampolin in which his rocking habits has been exagerated. A beautiful indoor pool located under the family quarter has its sike waters furtively going to the outside Japanese Bath. Chiseled on the side of the hill stands a 32 car garage in which as a symbol of Gates sentimental remembrances can be seen his parent's old car in which he played many times in his childhood.

From these humble beginnings in driving, Gates has evolved in a daring driver defying speed with the same earnestness he employs in facing any challenge. He raced in many expensive cars ranging among them a 60.000 dollars Carrera Cabriolet 964 to a U$S 380.000 Porshe 959.

One of Gates' main secrets for success consists in being informed, he wishes to be fully aware of what is really happening in the market; which are the rival companies' goals, those who may adversely affect Microsoft, and which of those goals are worthwhile to beat with better ones.

Aside from knowing about competitors, Gates is eager to know about the prospective buyers, opinions and reactions. After contacting them he managed to entice their curiosity in computer goods. After establishing a relationship, he managed so that this may evolve every time closer, until the customer finds worthwhile to enter in a membership which garants him to receive without delay, streams of valuable information.

This is the way how Gates maintains a vigilant eye about everything that is happening that may in the pros and cons affect Microsoft's interests. The computers industry is in a fact a moving field and Gates' wishes are to be ahead of time in every aspect of it.

The research groups engaged in different projects are stimulated to use their creative power. Like a clock's wheels they are smoothly coordinated marking the pace of progress in the computers industry.

Gates takes special delight in supervising them. It is more than pushing an advancing industry. It is a mixture of science and art employed in a visionary effort to achieve perfection. Gates stimulates discussions, so that he could reach the heart of the matter.

He chose his collaborators among those who even lacking experience demonstrate a rapid grasp of the problem, and resourcefulness in finding the correct solution.

This endeavor is permeated by a feeling of adventure in chasing the future. Because Gates lives and creates in the future, many years ahead of our times.

Following Nature's way of evolution, he integrates new possibilities into older ones, picking them in the minimum of space and facilitating the user's efforts to handle it.

This industrial evolution has changed the social environment. Man does not need psicodelic drugs to expand his mind.

Internet has started a big revolution and Gates had to turn quickly the direction of his company's strategy to regain the leadership in the PC Market. Internet opened a variety of possibilities that immediately were capted by a creative genius.

Constantly facing challenges and overcoming them Gates had added more flexibility, versatility to his software.

This is an exciting voyage through the times, that will never end, it is like trying to reach the horizon. This voyage is full of surprises, whether it may be as Gates envisioned, better video compression, security technologies, personalization servers, 3-D browsing, server development or site tracking.

Nothing is created by man, that has not been created before. Man is a plagiarist of Nature. Going in this direction, Gates' dreams to put in a machine, voices recognition.

Once Bill expressed that there is nothing extraordinary concerning human intelligence and he assumed that there will come a day in which it will be possible to replicate in a machine the way in which the neurons work in the brain creating perception and emotions.

After all he explains, life on earth is carbon oxide based and operates in a binary way, and we can parallel them with the artificial intelligence that operates in a machine which is silicon based. Carried by his indomitable imagination Gates seems to be playing a god saying that someday it could be possible to sequence the human genoma and mimic the way nature made intelligence and confined it in a machine.

At this point he placed a bit of humor and treated to soften this so shocking statement arguing that it would be like reversing engineering someone else produced and to reach to the quid of the problem.

In every way that man moves he is imitating nature. In spite of this mechanic way to understand nature,Gates can't hide his perplexity in front of the human soul.

Reluctantly admitting, may be there is something divine in it...

Prof. Dr. Victor Soriano

Affect Regulation and the Origin of the Self The Neurobiology of Emotional Development

Author Allan N. Schoroe - Book of 669 pages

Publishers: Lawrence Erlbaum Associates, Inc. - 365 Broadway Hillsdale, New Jersey 07642

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Part V: Clinical Issues. 29. The Neurobiology of Insecure Attachment. 30. The Clinical Psychiatry of Affect Dysregulation. 31. The Developmental Psychopathology of Personality Disorders. 32. Vulnerability to Psychosomatic disease. 33. Psychotherapy of Developmental Disorders.

Part VI: Integrations. 34. Right Hemispherio Language and Self-Regulation. 35. The Dialogical Self and the Emergence of Consciousness. 36. Further Directions of Multidisciplinary Study. 37. A Proposed Rapprochement Between Psychoanalysis and Neurobiology.

This is very important book with many explicit figures. It is a rich tapestry of intrincate interdisciplinary threads. This is a pioneering work that holds considerable promise for everyone in the behavioral sciences. It fundamentally alters our traditional fundamentalistic, cycloplan psychodynamic way of viewing infants and patiens and dramatically informs a newer and much needed interdisciplinary perspective.

The intention in writing this volume is to demonstrate that a deeper understanding of affect regulation and dysregulation can offer penetrating insights into a number of affect - driven phenomena - from the motive force that underlies human attachment to the proximal causes of psychiatric disturbances and psychosomatic disorders, and indeed to the origin of the self.

The author of the book Allan N. Schore express appreciation to the personalities that stimulates the work of the book, Dr. James Grotstein, Dr. Henry Krystal, Judith Amsel, Amy Pierce, Sandra Guideman, Professors Rolf Dermietzel, Harry B. Uylings, Neburo Mizuno, Andries Kalsbeak, Ernest Martin, Susan Sesack and Dean Falk, special references to his wife Judith.

Development essentially represents a number of sequential mutually driven infant - caregiver processes that occur in a continuing dialectic between the maturing organism and

the changing environment. It now appears that affect is what is actually transacted within the mother-infant dyad, and this highly efficient system of emotional communication is essentially non verbal human, development, including its internal neurochemical and neurobiological mechanics, cannot be understood apart from this affect-transacting relationship. The study of development must include more than just a documentation of changing functions.

The problem of the maturation of structures responsible for the onset of new functional capacities must also be simultaneously addressed. In fact, development can only be understood in terms of a progression of structure function relationships, since structure, by definition, is continually organizing, desorganizing and reorganizing in infancy. Changes in the child's behavior (studied by developmental psychology) or in the child's internal world (studied by developmental psychoanalysis) can only be understood in terms of the appearance of more complex structure that performs emergent functions. At this stage of our scientific knowledge, any discipline that theorizes about structure needs to evaluate its models against what is now known about the veritable characteristics of biological structure as it exists in nature. This brings psychology back to biology and emphasizes the importance of developmental neuroscience.

The primary social object who mediates the physical environment to the infant is the mother. Her essential role as the psychobiological regulator of the child's inmature psychophysiological systems directly influences the child's biochemical growth processes which support the genesis of new structure. Over the course of development it becomes increasingly self regulated: The elucidation of the psychobiological mechanisms that underlie the experience dependent maturation of a structural system. General Principles of Growth of the Developing Brain- Principle 1: The Growth of the Brain Occurs in Critical Periods and is influenced by the Social Environment.- Principle 2: The Infant Brain Develops in Stages and Becomes Hierarchically Organized- Principle 3: Genetic Systems that Program Brain Development are activated and Influenced by the Postnatal Environment - Principle 4: The social Environment changes over the Stages of Infancy and induces the Reorganization o Brain Structures.-

The experience-dependent neurodevelopmental processes of synaptic overproduction, parcellation and programmed cell death and the evolutionary biology concept of ontogenetic adaptation point to a number of important axioms concerning the growth of the developing brain. It is now thought that the study of development requires both a theory of transition which account for transient structures that are only adaptive for a restricted phase of development and at the same time an explanation of how earlier functions are transformed into more mature functions (Hopkins & Butterworth 1990).- The facts that the infant brain contains from 15% to 85% more neurons that the adult brain (Joseph, 1982) and that a large number of these neurons die (Hamburger & Oppenheim, 1982) and/or their processes are retracted in the early years of life (Cowan, Fawcett, O'Leary, &Stanfield, 1979) must be included into any conception of the genesis of biological structure. Early functional development cannot be understood without reference to epigenetic structural maturation, and pre-and postnatal brain development are both characterized as a process of organization, disorganization, and reorganization.- The continuity of development does not imply a simple progressive pattern of increments but changes in organization, Scott(1979 b), who defines development as change in the organization of Living systems which allows for increasing complexity, stability and adaptivity asserts the general principle that there can be no reorganization without disorganizations: These precepts apply equally well to psychoanalytic conceptions of the development of psychic structure.-

The developmental stage which straddles early and late infancy is a critical period for the development of socioemotional functioning. Evidence that suggests that at the end of the first year increased and more efficient attachment functioning between

mother and child is associated with the appearance of the high levels of positive affect that characterize the early practicing period. These events in turn directly influence the growth of connections between cortical and limbic structures in the infant's developing brain that are associated with attachment function.- A significant change in dyadic affective transactions occurring in the late practicing period accounts for a further maturation of these structures. The interval between 10-12 to 16-18 months is a critical period for the final maturation of a system in the prefrontal cortex that is essential to the regulation of affect over the rest of the lifespan.-

For shorthand purposes, in this book the developmental span marked by these temporal bounderies will be descriptively referred to as the practicing period, without any acceptance or rejection of Mahler's, Bowlby's, or Piaget's interpretations of the underlying mechanism that are operative during this period.

The aim of psychoanalytic phenomenology is to elucidate not only the functional significance, but also the developmental origins of subjective representational configurations.- This is an imposign task indeed, for it involves the formation of a developmental Psychology of the representational world which requires a comprehensive knowledge of both emotional and cognitive development.-

The most fundamental finding of self psychology is that the emergence of the self requires, more than the inborn tendency to organize experience - Also required is the presence of others, technically described as objects, who provide certain types of experiences that will evoke the emergence and maintenance of the self.-

The interconnections between the newer psychoanalytic and neuroscientific concepts are essential to an understanding of the changes in structure-function relationships that characterize early development.- The unique contribution of contemporary psychoanalysis to the study of development lies in its emphasis upon and elucidation of the critical "hidden" self object regulatory functions that are embedded within diadic affect transacting object relational processes, since these characterize the form and dynamics of the "social forces" that influence the maturation of regulatory structure within the infant brain in the first 2 years of life.- Psychoanalysis also articulates the nature of and emphasizes the importance of internal representations to the individual's adaptive object relational (socioemotional) functioning.-

Biologicaly primitive emotions are thought to be evolutionary old, appear early in an individual's development, arise quickly and automatically, are expressed in universally recognizable configurations of facial movements, are correlated with differentiable autonomic system activity may show subcortical conditioning, may be disposed to certain stimuly and serve fundamental motivational functions within the individual and communication functions within a social group.

An important resource for stimulations which are an essential aid to the infant's still inadequate inhibitory capacities.- At birth, the human organism is remarkably well equipped to cope with the variations and excitations of its new enviroment.- It is a subcortical creature, which is in danger of going into shock through overreacting to powerful or unexpected stimuli because it lacks the means for modulation of behavior which is made possible by the development of cortical control. The role of the higher structures is played by the mother: she is the child's auxiliary cortex.- Optimal levels of stimulation during a critical period thus are required to support the expansion of the circuity of the biological hardware of arousal, that is, of the bioaminergic "reticular" systems (both medullary and pontine-mesencephalic) which are in an intense state of active grow at this time and whose neurochemical activity is responsible for arousal Central catecholaminergic neurons undergo an accelerated development in mammalian infancy.- These periods are also a time when regional catecholaminergic receptors are amplified. The experience-dependent expansion and contraction of particular monoaminergic neuronal systems in

specific brain regions is proposed to be a central force which drives brain development and consequent ontogenetic transformations of function. The functional consequence of this process in the orbitofrontal cortex could account for the observation that the infant's ability to tolerate higher levels of arousal and stimulation increases during infancy.-

This entire volume is fundamentally an account of the experience-dependent development of the corticolimbic system and the emotional cognitive and behavioral advances that result from its maturation. A particular focus is on its role in the ontogenetic expression of psychobiological states and primitive affects.-

This work also highlights and emphasizes the essential contribution of the experience dependent maturation of the nondominant right hemisphere to the origins of socioemotional functioning. This hemisphere, although after viewed as more primitive than the linguistic left, is actually more essentially involved in the fundamental capacities that define a human self, show that the development of capacity to self- regulate psychobiological state transitions parallels the primary caregiver influenced maturation of the right cortex, and that the understanding of its developing structure-function relationships in the first 2 years of human life can offer penetrating insights into a host of psychobiological affect-driven human phenomena, from the motive force that drives human attachment to the proximal causes of later forming psychiatric disturbance and psychosomatic disorders, and indeed to the origin of the self.-

Visual stimulation, embedded in mutual care transactions between caregiver and infant, is an essential component of a growth promoting environment. The mother's emotionally expressive face is the most potent source of visuoaffective information, and in face-to-face interactions it serves as a visual imprinting, stimulus for the infant's developing nervous system.- During visual dialogues the primary caregiver is psychobiologically attuned to the infant's internal state and in the merger experiences she creates and maintains a mutually regulated symbiotic state in the dyad. In mirroring transactions, a dyadic reciprocal stimulating system generates an elevation of regulated sympathetic arousal that supports heighlened levels of interest-excitement and enjoyment-joy. This amplification of positive affect is neurochemically mediated by activation of the ventral tegmental dopaminergic system and the stimulation of engenous , opioids, in reward centers of the infant's brain. The child's capacity to tolerate higher levels of arousal increases over the first year. These phenomena culminate in very high levels of positive affect at the onset of the practising phase at the end of the first year.- The practising period, 10-12 to 16-18 months, spans early and late infancy. Although its onset is marked by the dramatic appearance of upright locomotion, its unique affective characteristics define the essential role of this stage in socioemotional development.- Social referencing transactions visuoaffective communications that efficiently and rapidly amplify infant interest-excitement and enjoyment-joy, begin in this period. This dyadic mechanism generates extremely high levels of positive hedomically toned play behavior in the early practising infant.- As a result, the toddler psychologically experiences a stage-typical, narcissistic state of grandiosity and omnipotence.- This phenomenological state is psychobiologically supported by a hyperarousal of the sympathetic nervous system produced by hyperactivation of the mesocortical component of the ventral tegmental dopaminergic limbic circuit.-

The epigenetic precursors of this stage occur in visuoaffective merger experiences in the preceding symbiotic stage of development. The practising phase in which the infant truly becomes a behaviorally and socially dynamic organism represents a critical period for the formation of enduring attachment bons to the primary caregiver. The nature of the attachment to the mother influences all later socioemotional transactions.

In stable environments in which mothers allow infants to move from them when the infant feels secure enough to do so and in which mothers allow infants to return to them

freely, the infants are able to modulate their arousal levels so as to permit learning of affective responses to the complex physical and environment in which they and their mothers live.

A developmental shift in the orbitofrontal cortex occurs at the end of the first year of human infancy. This shift reflects the onset of a critical period for the postnatal maturation of this cortex. During this period, the infant is most sensitive to high intensity stimulation emanating from the mother's emotionally expressive face. Maternal socioaffective stimulation induces heightened levels of arousal in the infant, and the resultant amplified levels of arousal act as a signal for the imprinting of new circuits in the orbitofrontal cortex. This arousal specifically induces the experience-dependent sprouting of dopamine-reheasing axon variosities in the deep layers of the prefrontal cortex. These axons in the far anterior forebrain represent the terminal of A10 catecholaminergic neurons whose cell bodies lie in the rostral ventral tegmental area of the midbrain. Dopaminergic axons, in a waiting compartment in the subplate zibe immediately beneath the orbitofrontal cortex, are activated by BDNF, a neurotrophic agent that induces axonal growth in mesencephalic dopamin neurons.- The growth of these dopaminergic axons is matched by an intensified synthesis of dopamine trigger a local growth spurt in the blood vessels, neurons, and glia of this prefrontal cortex especially in the early maturing right hemisphere. The neurotrophic actions of this catecholamine is mediate by its biochemical action of degrading glycogen to glucose and of converting glucose to sugars that are specifically used in biosynthetic processes. The expansion of the mesocortical dopamine system into the orbitofrontal cortex represents the maturation of the ventral tegmental forebrain midbrain circuit. Anatomical connections between subcortical limbic regions and frontolimbic cortex. This prefrontal cortical area account for the emergence of the unique functional properties of this frontolimbic cortex. These emergent functions-hyper arousal hyperactivity high levels of exploratory behavior and positive affect are first expressed in the early practising period so far as is present known, the way in which attachment behavior develops in the human infant and becomes focussed on a discriminatory figure is sufficiently like the way in which it develops in other mamals and birds, for it to be included, legitimately, under the heading of imprinting, socioaffective stimulation at the end of the first year induces permanent morphological changes in each of the cellular components of the orbitofrontal cortex.- The heightened levels of arousal associate with imprinting experiences lead to rapid increases in regional blood flow and the establishment of a local blood-brain barrier. The vascular delivery of vital nutrients and oxygen sustains the increased energy demands of biosynthetic processes in the growing brain tissue. Dopamine, in its role of regulation vascular permeability, elevates blood flow and metabolism in the developing prefrontal cortex. The maturation of the microvasculature is associated with a critical period shift from anaerobic to aerobic regional metabolism, and this transition makes possible the rapid and exuberant growth and differentiation of orbitofrontal dendrites.- These dendrites can receive axonal in from various subcortical and cortical areas and it is these varied interconnections that underdo the unique functional capacities of this prefrontal cortex.-

The growth of frontolimbic cholinergic axons back down the nevraxix towards the dopaminergic neurons in the midbrain represents the final maturation of the ventral tegmental forebrain-midbrain circuit.-

A consequence of the infant's attachment to the mother is what the infant develops a finely articulated schema for this mother, specially her face. It is assumed that the infant acquires a mental image of the person who is the object of attachment.-

At the end of the first year the anatomical maturation of the orbitofrontal cortex allows for a developmental advance in cognitive avid memory functions. The increased functional efficiency that results from such experience-dependent structural development also enables this cortex to generate abstract mental images of faces. As a result this prefrontal system can

now generate interactive representations, or internal working models for the infant's affective interactions with the primary attachment figure such internal representations the infant's psychological-affective responses to the emotionally expressive face of the attachment figure.- They can be accessed for regulatory purposes, even in the mother's absence.-

Those models are anatomically expressed in dynamic cortical-subcortical neuronal circuits that are imprinted in high arousal socioaffective face-to-face transactions.

The growth of dopaminergic axon collateral in the orbitofrontal cortex represents an expansion of the ventral tegmental forebrain-midbrain circuit, so that it now includes the frontolimbic cortex. The processes of synaptic facilitation produces linkages between the components of this circuit at different levels, thereby forming a stabilize. H ebbian network. This allows for a reverberating, self-excitational circuit. Components of this circuit, at different levels of the nervous system contains neurons that responds to faces. The orbitofrontal cortex is anatomically situated at the hierarchical apex of this circuit, and as such it categorizes, abstracts, stores, and ultimately modulates the intas emotional responses to the face of the mother.

The excitatory neurotransmitter, L glutamate, plays an important role in the development and operation of this circuit. Its actions at sites of connections between descending orbitofrontal axons and ventral tegmental dopaminergic dendrites mediates a mechanism by which the cerebral cortex regulates affect.

The incipient core relations shame transactions that occur throughout the late practising period are stored in interactive representations imprinted with shame affect.

These internalized visuoaffective images can be accessed, even in the mother's absence in order to automodulate impulsive behavior. Such an advance represents an ontogenetic adaptation, as it mediates further socioemotional development. Socialization shame transactions induce a stress state, and the concomitant elevation of corticosteoid levels and parasympathetic vagal activity specifically facilitates further maturation of the orbitofrontal cortex.

The maturation of the orbitofrontal cortex begins in the early practicing period and concludes in the late practicing period. Dyadic shame transactions involve the infant's interoceptive inhibitory responses to the exteroceptive input of the mother's disgusted fase. These stored interactive representations are distributed along the lateral tegmental limbic circuit. By the middle of the second year these neuropsychobiological advances enable the automodulation of hyperactive behaviors and hyperaroused states. The orbital frontolimbic structural system is intimately involved in the socialization induced diminution of oral activity and appearance of bladden and bowel regulation that are seen at this time.

The later maturing dorsolateral prefrontal cortex contains a set of subcortical connections that are distinct from the orbitofrontal cortex. This accounts for functional operation and capacities that are dissociable from the orbital and medial prefrontal regions. The orbital prefrontal cortex performs an executive control function in the right hemisphere, while the dorsolateral cortex performs such a role in the left hemisphere. The unique anatomical and functional properties of the two prefrontal systems account for the hemispheric differences in the lateralization of emotions.

The child's excited response a wide variety of stimuli will inevitably generate behavior that will be seen by parents, other adults, or children as inappropriate or wrong.

From the child's vantage point, she is not really indecorous, she is merely indulging in natural self-expression, when she is shamed. Children's great capacity for excitement and joy and the activity that these emotions generate greatly increases the range of possibilities for censurable behavior. The importance of a positive and non punitive socialization of shame is evident.

The child's socioemotional environment changes from the early to the late practicing period. The socialization experiences of the second year induce a parcellation, a selective loss and redistribution of connections in the orbitofrontal cortex. Critical period inceptive shame transactions trigger an onset of parasympathetic and offset of sympathetic activities ultimately producing a competitive elimination of excess mesocortical dopaminergic ascons. This prefrontal regressive change is responsible for the loss of the ontogenetic adaptations of the early practicing period. Shame transaction facilitate the expansion of the lateral tegmental limbic circuit in this cortex. Shame regulating transactions and continuing attuned mirroring processes preserve the ventral tegmental limbic wiring of the fronto limbic cortex.

In both phases of this critical period of orbitofrontal maturation, the active expression of genetic systems that program prefrontal anatomical development is directly influenced by the social environment. The environmental challenge of socialization, mediated by maternal stress and stress regulating transactions induces an adaptive change in the toddler. Maternal object relations thus act as a selection pressure that critically shapes the activity dependent Darwinian process of the selective stabilization and elimination of specific dual subcortical oatecholaminergic connections with the anatomically maturing orbitofrontal cortex. The resultant transformation from a single to a dual limbic circuit system defines the final maturation of this frontolimbic cortex. This structural reorganization is responsible for the emergence of new more complex functions at the end of the critical period.

The hypothalamus plays a particularly important role in coordinating the endocriautonomic, and behavioral responses that assure survival of the individual (homeostasis) as well as survival of species (reproduction). And since stress (or a stressor) is usually regarded as any condition or factor that disturbs the dynamic equilibrium (homeostasis) of the body, it has seemed reasonable to assume that hypothalamic circuity mediates a number of adaptive responses initiated by such factors.

The emotional dominance of the right hemisphere is not restricted to the regulation of subjective moods and emotional expression, but can also be found in the metacontrol of fundamental physiological and endocrinological functions whose primary control centers are located in subcortical regions of the brain.

The mother initially provides an external regulating mechanism for many of the physiological mechanisms that the infant possesses but does not regulate itself. These effects are mediated by affects of the mother on the infant's neurobiological processes. At some point in development the infant becomes self regulating through the development of internal regulatory mechanisms entrained to the stimuli that the mother provides.

In the fields of etiology and psycopathology can be used to frame specific hypotheses which relate different family experiences to different forms of psychiatric disorder and also possibly, to the neurophysiological changes that accompany them.

In general the inner experience of affect is congruent with the behavioral expression communicated to others, but one of the most interesting questions confronting psychoanalysts has to do explaining clinical examples of the various ways in which affect regulation fails to develop normally or becomes dissociated from adaptive behavior, from inner experience, and from communicative behavior.

Synoptically, the borderline patient can be understood as one who is readily disposed to symptoms of primary dysregulation on the biopsychosocial continuum and as a consequence, develops secondary symptoms in order forestore a compromised homeostas. The borderline can be seen, furthermore, as a containing "undigested" internal objects which have not been transformable into stouble self or object representations.

Three streams of research are converging on the centrality of mutual connections

between the nervous and immune systems biological studies of the reciprocal interactions between the cellular components of these systems, developmental studies of the effects of early experience on cater immuno competent capacities, and clinical studies of the effects of psychological factors on the outcomes of particular diseases. The developing child's inceptive experiences with the socioaffective environment, embedded in the emotional relationship with the mother, induce both short-term physiological alterations and long-term structural modifications in his developing immune system. These primordial experiences imprint the cortical-subcortical neurophysiological mechanisms that mediates all later psychosocial influences on immunologic reactions. In regulating the infant's autonomic functions the caregiver influences the imprinting of the structures responsible for the communication between the neuroendocrine and immune systems. The hypothalamus which releases its neuropetides into the circulation share an identical set of signal molecules.

Connections between hypothalamic limbic areas and cortical frontolimbic areas of the right hemisphere allow for an integrated circuit that can modulate and be modulated by peripheral immune activity. The patterning of these connections is affected by the infant's practicing period affective transactions with the psychobiologically regulating mother. A relationship characterized by extensive misattunement and regulatory failures engenders an incomplete structural maturation of a dual circuit orbitofrontal cortex. The developmental failure of this system is responsible for future vulnerabilities to psychosomatic disease.

The mortification suffered thirty years ago operates, after having gained access to the unconscious sources of affect, during all these thirty years as though it were a recent experience. Whenever its memory is touched, it revives, and shows itself to be cathected with excitation which procures a motor discharge for itself in an attack. It is precisely here that psychotherapy must intervene, its task being to ensure that the unconscious processes are settled and forgotten.

During the first year and more intensely during the second year developmental process accounts for the formation of the child's self regulatory capability. Through identification with the mother, her regulatory interventions and the attitudes governing them are internalized and become part of the child's own regulatory functions. Emergence of an active self in the middle of the second year of life constitutes a central milestone and transition in the child's development.

The regulatory systems are found on all levels of the organism's functioning, right from the genome up to psychological behaviors; thus they appear to be among the most general characteristics of the organism. Self regulations seem to constitute at the same time of the most universal characteristics of life and the most general mechanism to be found in both organic and cognitive behavior; thus they appear to be among the most general characteristics of the organism. Self-regulations seem to constitute at the same time one of the most universal characteristics of life and the most general mechanism to be found in both organic and cognitive behaviors.

Any psychological theory must, in addition to meeting the demands made by natural science, fullfill another major obligation. It must explain to us the thing that we know, in the most puzzling fashion, through our "consciousness"; and since this consciousness knows nothing of quantities of energy and neurones.

Prof. Dr. Víctor Soriano

Brain Damage in the Preterm Infant

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Contents

This book will be informative to anyone with some understanding of neurobiology who wants to know more about the structural disturbances of the brain that tend to affect preterm newborn infants. This includes, but is not limited to neurologists, pediatricians (and not just neonatologists) obstetricians (and not just perinatologists) and a wide variety of neuroscientists.

Those responsible for the care and rehabilitation of children and adolescents with neurologic disabilities will gain an understanding of why their patients and clients who were born preterm have disabilities. With increasing evidence that some term-born people who have cerebral palsy probably had a leukoencephalopathy, this book will also be of interest to those whose patients and clients were born near-term.

The best use of this book will depend on the needs and interests of the reader. For some, the pictures will be the heart of the book where as others might consider the text more important. Some readers might benefit from reading the last chapter first.

The data reported in this book are almost entirely derived from 82 autopsied infants, especially the 75 who received ultrasound scanning in life. Though constituting only a small minority of the 1105 study subjects they play a crucial role in understanding brain injury.

Scans were initially reviewed by the five board-certified radiologists with training or special interest in neonatal cranial ultrasound studies who were based at the three participants hospitals. The radiologists were given no clinical information about the infant except the birthweight which was needed to judge the size of structures such as the choroid plexus. The scans were then reviewed by either of the two consultants who were unaware of the initial interpretation or by another of the participating radiologists but not from the same hospital.

Both readers recorded ultrasound data on a form that separately requested observation (i.e. ultrasonographic densities and lucaancies by location) and interpretations. Divergent interpretations were resolved by submission of scans to a third review from among the pool of seven participating reviewers. Two raports on interobserver reliability in this diagnostic process have been published (Pinto et al. 1988b, Pinto-Martin et al. 1992)

Germinal matrix hemorrhage (GMH) is often a multifocal form of bleeding originating in several supratentorial and one infratentorial matrix sites. The dominant region for bleeding is the caudothalamic zone, and bleeding in this region is responsible for most intraventricular extension of hemorrhage. More extensive involvement of germinal matrix sites was associated with larger amounts of intraventricular bleeding. The multifocality of GMH argues against the central importance of local anatomic predisposition, such as the anatomic characteristics of the terminal vein, as a major determinant of such hemorrhages.

Damage to white matter underlies much of the neurologic disability experienced by preterm infants. The major form of this damage, described many times by many authors is commonly referred to as periventricular leukomalacia. Its name notwithstanding, this lesion is not necessarily periventricular in location, and its typical focal areas of leukomalacia need not be present. Nonetheless, a general consensus has emerged on the essential neurocytologic features of this lesion in infants who have lived long enough for them to have developed. These features consist of coagulation necrosis, axonal damage, astrocytic and microglial proliferation, deposition of lipid in macrophages and later cavitation.

Several authors have described cellular changes which may represent earlier manifestations of white matter damage than PUT, but no consensus has emerged as to the exact features of these earlier lesions.

What is the relationship of white matter damage to the arterial blood supply to the brain? What discriminates preterm infants with and without white matter damage? There is little question but that better understanding of white matter damage underlies all attempts at the prevention of neurologic disability in preterm infants.

It has been argued that the use of high-frequency transducers increases the precision with which parenchymal lesions can be diagnosed by means of ultrasonography (Grant and Schellinger 1985). In our study, as in most previous studies, 7.5 MHz transducers were not universally used. It is possible that the most up-to-date ultrasound technology may be able to provide better correlations with pathologic findings than has thus far been possible. However, ventricle size, which can be assessed without high-frequency transducers, appears to provide useful information on white matter damage.

A protocol calling for more frequent studies later in life may provide better correlation between abnormal parenchymal echogenicity and white matter damage. These limitations notwithstanding, we conclude that ultrasonography is a valuable tool for the diagnosis of white matter disorders in life.

A highly specific, though somewhat insensitive, sonographic diagnosis of SAH can be made from the appearance of the subarachnoid cisterns on midline sagitral and semicoronal sonograms, obtained through the anterior fontanelle in LBW infants.

The correct sonographic diagnosis of SAH may predate the ultrasound diagnosis of IVH, and may alert the neonatalogist to the need for follow-up sonograms under such circumstances. Accurate sonographic detection of SAH may also facilitate the future evaluation of its effect on the neonatal brain.

A large number of studies now document that ultrasonographic abnormalities which reflect white matter damage are the most potent predictors of CP in VLBW infants.

Prof. Dr. Víctor Soriano