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Cytogenetic Study of Otosclerosis

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Otosclerosis is a localized disease of bone in which the pathological changes are limited to the bony otic capsule and to the middle ear ossicles, the most common localization being the area between the border of the stapedial footplate and the anterior part of the oval window.

This study concerns a cytogenetic evaluation of 62 otosclerosis patients: from Kluyskens' series of over a 1 000 otosclerosis patients, 642 entered the hospital for stapes-surgery, among whom during a certain period of time 62 were selected for chromosomal evaluation (Tab. 1).

Histology

M. Weber (1961) finds in normal cases cartilaginous rests and connective tissue in the bone and labyrinthic capsule. According to Bast & Anson (1949) new cartilage can develop from this connective tissue during postfetal life. The otosclerotic process starts with lacunar resorption of preexisting bone or cartilage by giant cells especially around the bloodvessels; there after a pathological osseous neoformation takes place (Mannassé, 1922; Guild, 1944; Nylen, 1949; Ruedi, 1957; Altmann, 1960; Shuknecht, 1960; Weber, 1961).

Frost (1960) finds in otosclerotic bone a structural pattern similar to that in fibrous bone in human skeletal diseases such as healing fractures and osteomyelitis. Normally fibrous bone is replaced relatively quickly by lamellar bone and is therefore usually in a state of incomplete mineralization. The otosclerotic footplates are however normally mineralized which indicates that they are present in the patients' ears for years. There exists also a resemblance between otosclerotic bone and the bone in osteogenesis imperfecta: i.e. a similar number of lacunae per unit volume, a peculiar basophilia of the bone matrix and a dense, irregular cement line (Frost, 1960).

In all our otosclerotic patients where histologic examination (Fig. 1) is done postoperatively, otosclerotic foci are found (marked + in Tab. 1). In one case (V. J.) an osteoma is found at the upper border of the oval window and at the round window. This is a rare phenomenon and indicates a pronounced form of otosclerosis.

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Tab. 1. Data on 62 patients, selected chronologically during a period of time upon their admission to the hospital for stapessurgery from Kluyskens' series of 642 operated otosclerosis patients

Name	Sex	Age of onset	Age at operat.		Laterality		Familial incidence	Diagnosis			Cytogen. eval.
			R.	L.	Uni.	Bil.		Audio.	Surg.	Hist.	
A. R.	♂	15 y	43 y	46 y		+	None	+	+		+
B.	♂♂	?	50 y			+	None	+	+		+
B. L.	♂♀	24 y	39 y	40 y		+	Father and paternal aunt	+	+	+	+
B. N.	♂♂	Puberty	38			+	Father	+	+		+
B. J.	♂♂	6-7	43	43		+	Unknown	+	+		+
C. J.	♂♂	25		33		+	None	+	+		+
C. M.	♂♀	20	55	56		+	Father	+	+		—
C. F.	♂♂	38		51	+		Unknown	+	+		+
C. Fr.	♂♂	20	34			+	Father	+	+		+
C. L.	♂♂	39	43		+		None	+	+		+
C. S.	♂	45	55			+	One brother, one uncle unknown paternal or maternal	+	+		—
C. E.	♀	25	26			+	3 paternal uncles, 2 paternal aunts	+	+	+	—
C. A.	♂♂	26		39		+	Mother	+	+		+
C. P.	♂♀	47	49			+	Mother and maternal aunts	+	+	++	+
D. B. P.	♂♂	27	34			+	None	+	+		—
D. B. M.	♂♀	38		42	+		Father and sister	+	+		—
D. B. M.	♂♀	36	45			+	Unknown	+	+		+
D. C. D.	♂♀	14	37			+	Mother and maternal uncle	+	+		+
D. K. J.	♂♂	?	54	55		+	Unknown	+	+		—
D. L. M.	♀♀	23	32			+	None	+	+		+
D. M. F.	♂♀	31	35			+	Unknown	+	+		—
D. P. A.	♀♀	30	40			+	Mother	+	+	+	+
D. S. Y.	♀♀	25	35	35		+	None	+	+		—
D. W. L.	♀♀	?	36			+	None	+	+		+
D. P.	♀	Puberty	44			+	Uncle and greater part of the family after age of 60 y. Unknown whether paternal of maternal	+	+		+
E. G.	♂♂	20	30			+	Brother and mother	+	+	+	+
F. L.	♂♂	18		50		+	One sister	+	+		+
G. A.	♀	12	44			+	One maternal aunt	+	+		+

Tab. 1. continued

G. D.	♀♀	21	38		+	Unknown	+	+		+
H. M.	♀♀	16	47		+	Mother, maternal grandmother, aunt and cousins	+	+		+
H. I.	♀♂	26		36	+	Paternal aunt	+	+		+
J. J.	♂♂	28		43	+	Unknown	+	+		+
K. F.	♂♂	16	42		+	None	+	+		+
L. L.	♂♂	23	33		+	Unknown	+	+		+
L. M.	♂♂	Puberty	38		+	Unknown	+	+	+	+
L. A.	♂♀	21		35	+	None	+	+		+
M. E.	♀♀	Puberty	47		+	Mother and maternal cousin: unil. deafness	+	+		—
M. A.	♂	49	50		+	Unknown	+	+		+
M. I.	♂♀	18	51		+	None	+	+	+	+
M. Y.	♀♀	Puberty	30	32	+	Unknown	+	+	+	+
N. A.	♀♀	20	43		+	2 maternal greataunts	+	?		+
N. M.	♀♀	30	49		+	None	+	+		—
N. M.	♀♂	20	70		+	Positive, no details known	+	+		+
P. J.	♀♂	22	25		+	None	+	+		+
P. A.	♂♂	36	43		+	None	+	+		+
P. E.	♂♂	44	64		+	None	+	+		+
P. J. M.	♀♀	Childhood		16	+	Maternal grandmother and aunt	+	+		+
S. Y.	♀♀	Adolesc.	45		+	None	+	+		+
S. D.	♂♂	Puberty	18		+	Unknown	+	+		+
S. R.	♂♂	21	33		+	Unknown	+	+		—
S. L.	♂♀	13	33		+	Unknown	+	+	+	—
V. D. R.	♂♀	21	39	42	+	Mother	+	+		+
V. L.	♂♀	25	37		+	Mother	+	+		+
V. S. A.	♂♀	44	57	59	+	Unknown	+	+		+
V. D. Y.	♀♀	20	49		+	Unknown	+	+	+	—
V. I. S.	♀♀	30	50		+	None	+	+		—
V. K. J.	♂♂	24	26		+	Father and paternal aunt and uncle	+	+		+
V. L. F.	♂♂	45	55		+	Brother bil. since age of 20 y.	+	+		+
V. M. O.	♂♂	?	44	46	+	None	+	+		+
V. S.	♂♀	2-3	13		+	Paternal grandfather & mother	+	+		+
V. J.	♀♂	Puberty	43	34	+	Unknown	+	osteoma		—
W. G.	♂	49	59		+	Unknown	+	+		—

Main clinical signs

The first clinical signs can appear around puberty. According to Shambaugh (1956) the average age of onset is around 20 years. At the age of 50 the clinical manifestations are pronounced. The histologic evolution of otosclerosis corresponds with the clinical history of the patient. The beginning destruction of the normal bone in the congestive phase is characterized by *tinnitus*. The consecutive resorption of the osseous tissue corresponds with *progressive hearing loss*. The formation of anarchic sclerous bone in the last phase leads in a considerable number of cases to anky-

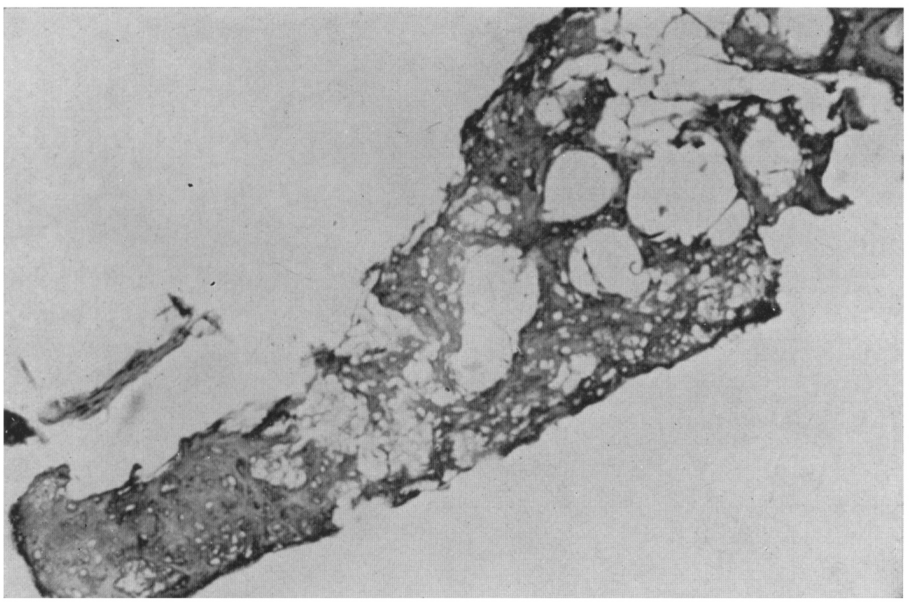

Fig. 1. Vertical section through the footplate. Active otosclerosis bone. Vascular fibrous tissue and irregular bone trabeculae (*pers. obs.*)

losis of the stapedial footplate, which results in conductive deafness. It is thought that as long as the disease is limited to the oval window, the *deafness* remains *conductive*, but that extension to the cochlea causes signs of *perceptive or nerve deafness* which occurs in 30-40% of the cases.

Other important clinical signs are Willis' *paracusis* or the ability to hear better in noise, and slight postural *dizziness*.

In nearly every case of otosclerosis the tympanic membrane appears somewhat translucent and with a pinkish tinge (*Schwarze's spot*) due to transmitted colour from changes on the inner tympanic wall.

The *functional hearing tests* such as pure tone audiometry and vocal audiometry do not show typical characteristics and vary in the various phases of the disease. The " dip " in the bone conduction curve at 2 000 C.S. is often termed Carhart's notch and is characteristic for deafness caused by stapes fixation.

In our patients the diagnosis is made on clinical and audiological grounds and is confirmed by surgery.

The disease usually develops *bilaterally* as can be seen from Tab. 1. Among the 62 cases there are 59 bilateral and only 32 unilateral ones. The latter may in time of course also develop the disease on the other side.

Classification and differential diagnosis

1. Chronic conductive deafness as *sequelae of otitis media* can make the differential diagnosis somewhat difficult. Familial anamnesis can help elucidate the differential diagnosis.

2. Otosclerosis can be classified among the *hereditary bone diseases* which are characterized by hearing impairment. In otosclerosis the latter is the result of a localized osteodystrophy.

There exist several skeletal diseases in which there is conductive hearing loss, but where other clinical signs lead easily to the correct diagnosis. The hearing impairment can either be the consequence of malformation in the middle ear, the inner or external auditory canal or the base of the skull, or due to a thesaurismosis with central lesions.

A. Some generalized osteodystrophies show audiological characteristics which are more or less identical to the ones found in otosclerosis. This is the case in the following conditions:

1. *Albright's disease* (osseous fibrous dysplasia, cutaneous pigment spots and precocious somatic and sexual development in women) – Narrowing of the auditory canals, ankylosis of the stapes footplate in the oval window (Pellegrini, 1962) can cause conductive deafness; narrowing of the inner acoustic meatus can cause perceptive deafness.

2. *Osteopoikilia* (osteitis condensans disseminata: round or streaky bone condensations usually found fortuitously on X-ray examination) – Perceptive deafness occurs in families where osteopoikilia exists and conductive deafness has been observed in patients affected with osteopoikilia (Bonduelle, 1944).

3. *Albers-Schönberg's disease* (osteopetrosis: thickening of the cartilages, disappearance of the bone-marrow which is replaced by calcified tissue, osseous fragility, frequent fractures, with extensive callus formation and anemia) – Conductive and perceptive deafness can be due to stenosis of the outer or inner auditory canal.

4. *Paget's disease* (osteitis deformans: hyperplasia and osteoporosis of the bones of the cranium and face, the diaphyses of the bones of the limbs, the pelvis and the

spine) – The three forms of deafness may occur: the conductive type due to lesions of the external auditory canal, middle ear and stapes' footplate; the perceptive type due to compression of N. VIII in the inner auditory canal or to extension to the cochlea, or to a deformity at the base of the skull with compression of the internal auditive artery and consecutive sensorial degeneration; the mixed type due to ankylosis of the footplate and consecutive cochlear degeneration. Some authors, such as Nager & Mayer (1932), consider otosclerosis as a localized form of Paget's disease.

5. *Hurler's lipochondrodystrophy* (*Gargoylism*: craniofacial malformations with large head, scaphocephaly, saddle-nose, prominent cheeks, flattened orbits, hypertelorism, thick tongue; dwarfism; polyepiphyseal osteochondral dystrophy especially of the carpal and metacarpal bones with deformation of the joints and limited movements; mental retardation; hepatosplenomegaly, and corneal dystrophy in 75% of the patients) — There is auditive impairment in 23% of the cases: conductive deafness due to a tubocatarrhal lesion or to deformation or ankylosis of the ossicles; perceptive deafness, due to degenerative lesions of the ganglia or central nuclei.

6. *Morquio's Chondro-osteodystrophy* (same type of skeletal alterations but exceptional corneal dystrophy, absence of neurologic signs, mental retardation or visceral syndrome) – Very frequent usually mild hearing loss of the mixed type is found.

7. *Generalized cortical hyperostosis* (cortical thickening in the ribs, claviculae, diaphyses of the long bones, the skull and its base and the mandibula; osteophytes) – Deafness is due to narrowing of the inner acoustic meatus.

8. *Cockayne's syndrome* (disproportioned dwarfism due to skeletal anomalies, pigmentary degeneration of the retina, progeroid facies, lightsensitivity of the skin, neurologic signs) – Deafness and deafmutism can be found in the severe cases.

9. *Van de Hoeve's syndrome*, *Lobstein's osteopsathyrosis*, *Osteogenesis imperfecta* (blue sclerae, osseous fragility, fractures, especially subperiostal, occurring spontaneously or after a minor traumatism, nanism, exostoses, joint-laxity) – There is no agreement yet as to the exact type of deafness in this disease. Ogilvie & Hall (1962) connect otosclerosis with this syndrome. According to Ruttin (1922), Clerc & Deumier (1958) and Fowler (1949) conductive deafness would be due to otosclerosis. Fowler (1949) finds in otosclerotic patients a high percentage (69%) of blue sclerotics, although these patients are not affected by osseous fragility. Nager (1921), however, detects an anatomopathologic difference between classical otosclerosis and osteogenesis imperfecta with deafness. In other cases of the syndrome conductive deafness has been ascribed to a laxity of the ossicular chain, an abnormal inclination of the tympanic membrane due to cranial deformation (Apert, 1928); Le Mee, *cit. by* Clerc, 1965), fractures of the ossicles, thinning of the tympanic membrane (Funch & Rosenbauw, *cit. by* Clerc, 1965), etc. The perceptive deafness has been ascribed to fractures of the temporal bone, haemorrhage in the labyrinth, microtraumata (Gillain, *cit. by* Clerc, 1965), or to otosclerosis of the cochlea. The mixed type of deafness can be due to the association of above mentioned factors.

10. *Pyle's disease or familial metaphyseal dysplasia* (thick metaphyses with thinning of the cortex and absence of the trabecular structure of the bone, frequent fractures)

– Deafness can be of the conductive type when caused by stapedial ankylosis in the oval window or by obtrusion of the round window. It can be of the perceptive type, when caused by hyperostotic compression of N. VIII in the inner ear meatus.

B. Several cranial deformities are known where the different types of deafness can be found. The hearing impairment is, however, not as much the result of deformities at the base of the skull, but is rather due to a developmental hypoplasia of the ear.

1. *Apert's syndrome.* Although deafness is not frequent, stapedovestibular ankylosis and narrowing of the inner acoustic meatus have been reported.

2. *Crouzon's syndrome* (precocious craniostenosis with brachycephaly, scaphocephaly, acrocephaly or trigonocephaly, hypoplasia of the superior maxilla, shortening of the orbital cavities, hypertelorism, inferior prognathism, arched palate and short upper lip, the nose resembling a parrot's beak, exophthalmos, sometimes subluxation of the globe, divergent strabismus, optic atrophy and sometimes nystagmus; hydrocephalus, epilepsy and mental retardation are not exceptional) – Otologic defects are frequent: middle-ear deafness due to malformation of the ossicles and sometimes of the auditory meatus (Nager, 1339; Nager & De Reynier, 1948). Kluyskens (1965) found in a case of Crouzon's disease ankylosis of the stapes' footplate caused by an osseous callus, which histologically is found to be otosclerosis.

C. Affections of the spine.

1. *Bechterew's disease* (rhizomelic spondylosis, ankylosing sacroiliac arthritis followed by spondylarthritis, frequent iridocyclitis) – In three cases of Bechterew's disease, Kluyskens (1965) found otosclerosis surgically and anatomopathologically.

2. *The Klippel-Feil syndrome* (synostosis of the cervical vertebrae with a short, slightly mobile neck and forward inclined head) – There may be deafness and deafmutism.

D. Syndromes of the first and second Gill-arch can present different forms of deafness. Their etiology is however distinct from the one in otosclerosis and will not be discussed here.

3. Other associations

Keiser (1952) observes a case of typical otosclerosis associated with oligophrenia and general hypotrophia.

Van Leeuwen (1955) describes otosclerosis, mental retardation, lipodystrophy, nanism and osseous cysts in three sisters born from consanguineous parents.

Frequency, sex and racial distribution

The disease is found at least in its histological manifestation in 10% of the population of the Western world (Engström, 1940; Guild, 1944; Weber, 1935).

The reported frequencies vary according to the different authors: in adults 6% (Guild, 1944, 1965), 10% (Fowler, 1954).

Sercer (1961) finds 15 times signs of manifest otosclerosis (1.2%) on macroscopic examination of 1 200 skulls and concludes that otosclerosis is a bilateral and symmetrical disease.

Histologic otosclerosis is 10 times more frequent than clinical otosclerosis. It can be found at necropsy in normal hearing persons. Only in 10 to 15% of histological otosclerosis cases the osteodystrophy foci are located in such areas of the labyrinth that the transmission of sound from the ear drum to the inner ear is prevented (Guild, 1944). Guild considers the ankylosis of the stapes to be fortuitous. *As the presence of otosclerosis is not clinically observable until the stapes has been ankylosed, one should not attach too much importance to a negative family case history.* An otosclerotic focus which is only 0.1 mm from the footplate of the stapes gives no symptoms whatever.

Larsson (1960) calculates that the degree of manifestation of clinical otosclerosis varies between 15 and 35%. In determining the degree of manifestation the person's age must be taken into account.

Joseph and Fraser (1964) find otosclerosis to be 2.1 times as common in Caucasians as in Japanese. Guild (1950) finds the incidence of histological otosclerosis to be 8.3% in 518 whites (12.3% in women and 6.5% in men) and 1% in 482 negroes (1.1% in men and 0.7% in women). Clinical otosclerosis is much less common in the yellow than in the white race.

Different authors (Bauer & Stein, 1925; Cawthorne, 1955; Davenport *et al.*, 1933; Nager, 1939; Shambaugh, 1956) stress the fact that clinical otosclerosis is more frequent in women. Histological otosclerosis on the other hand, is about as common or even more frequent in men than in women (Weber, 1936; Engström, 1940; Guild, 1944; Schambaugh, 1956). The statistics of Kluyskens (1965) based on clinical surgical material of 271 patients indicate 51.5% women and 48.5% men. A comparison of the age at which the two sexes undergo operation, shows that otosclerosis causes a social deafness at a younger age in women than in men. As can be seen from Tab. 1 in our series of 62 patients selected chronologically for chromosomal evaluation upon their admission for surgery, there are 32 men and 30 women, the mean age of onset of clinical disturbance being 27 in the men and 21 in the women, the mean age at operation of the first ear being 43 for the men and 38 for the women.

The frequency of otosclerotic manifestation is probably the same in men and women when investigated at an older age, i.e. at the end of the risk period, but in women, under influence of intenser hormonal and metabolic conditions (puberty, pregnancies), the disease may start earlier and evoluate faster than in men.

Etiology

The etiology of otosclerosis remains obscure. Many theories have been suggested, of which the following are the most important.

1. *Infection* (Manasse, 1922; Fraser, 1932) would cause changes in the labyrinthic capsule. This theory is no longer accepted.

2. *Venous stasis* (Wittmaack, 1919) in the labyrinthic capsule. Narrowing of the small channels through which pass all vessels entering or leaving the labyrinthic capsule, interferes with venous drainage, so that osteophyle hormones, although their level in the blood serum is normal, can intervene, as they are in relative abundance in the region of the circumscript venous stasis. Because the bony mass of the labyrinthic capsule is not more than 2 g a slight quantity of hormone can produce localized characteristic changes (Sercer, 1961). This theory does not elucidate the primary cause of the disease.

3. *Mechanical or anthropological theory.* Brühl (1926) and Mayer (1922) advocate the angulation of the base of the skull, which is the consequence of the erect human posture, as the cause of a mechanic stress on the labyrinthic capsule by pressure of the temporo-mandibular articulation on the lateral side of the temporal bone.

The influence of the mechanic forces on the labyrinth would manifest itself first in a physical sense: compression, deformation and microfractures (described by Mayer, 1922, Uffenorde, 1922; Lange, 1929; Nager, 1932, M. Meyer, 1932; Eschweiler, 1933; Greifenstein, 1935; Ullrich, 1939). If the bone is resistant the mechanic force would act in a biological sense: i. e. activation of osteoblasts and metaplastic changes in the bone, often seen in the periostal layer. If the periostal layer is not sufficiently isolating, the enchondral layer is also submitted to the metaplastic changes. Mechanic factors acting on long bones can produce analogous changes (M. Meyer, 1939). A mechanically caused bone transformation area is, however, not identical with otosclerosis. In the former the metaplastic areas are limited to the field which is influenced directly by mechanic forces. The otosclerotic foci, on the other hand, have a tendency to spread and to persist for a longer time. The first effect of compression of a long bone, and of the labyrinthic capsule is identical, i.e. an active congestion and consecutive bone metaplasia. Sercer (1961) points out that in the region of the pyramids, due to a progressively stronger angulation, the channels through which the veins of the labyrinthic capsule leave the inner ear are narrowed, which results in a chronic venous stasis in the region of the windows, where hormones can play an important role in producing otosclerotic osteodystrophy. The difference between a simple bone transformation area and an otosclerotic focus is the fact that in otosclerosis the hormonal influence due to the venous stasis is superimposed on the mechanic action. In the same way authors, who are in favor of this theory, explain the otosclerotic foci in the ossicular chain. The veins of this region leave the bone through the fissure of Glasser, narrowing of which would produce venous stasis in the head of the hammer and the body of the incus, the rest of the process being analogous to the one in the labyrinthic capsule. This theory would explain that the disease appears only in postfoetal life, that it is specific for the human race, that the process is localized in the labyrinthic capsule, that the rest of the skeleton remains normal and that the process develops bilaterally and simultaneously on the corresponding spots, terminating in old age, when the angulation of the base of the skull becomes fixed. It also would explain that otosclerosis occurs less frequently in negroes and Japanese.

4. *A modification of the foregoing theory* considers the pulsations of the carotid artery, the vibration of the footplate and the tension of the basilar membrane as the essential cause of otosclerosis. These mechanical factors would cause electro chemical and consecutive electrolytic phenomena in the labyrinthic capsule (Leiri, 1951).

5. Portmann (1952) and Kobrak (1950) consider an abnormal *endo-labyrinthic tension* and local hyperhaemia as etiologic factors.

6. *Hormonal influences* are considered by Escat (1922), Leicher (1925), Voss (1927), Alexander (1927), Seiffert (1938), Borghesan (1946) to explain the high erincidence of otosclerosis in women.

7. Factors concerning the *bone metabolism* are studied by Leicher (1925), Berberick (1928), Weber (1936), Tobeck (1955), Fowler (1956) without any definite result. De Jorge *et al.* (1965) find normal blood values for sodium, potassium, chlorine, uric acid, total cholesterol, and copper metabolism in 20 white otosclerosis patients. Inorganic phosphorus is low, inorganic sulphur high and there is a tendency for a high calcium and a low alkaline phosphatase activity and magnesium concentrations in the blood serum. Histochemical analysis of the otosclerotic focus reveals an important histoenzymatic disturbance of the metabolism of the glucids, the phospholipids and the sulphur compounds (Ardouin, 1961). The production of otosclerotic bone in the oval window can be the result of some abnormal enzyme activity.

Maggio (1959) finds in otosclerotic patients an inhibition of 24% of cholinesterase activity in the serum. He also establishes a concordance between the diminution of this enzyme and the state of sympathic hypertonia, characteristic of the otosclerotic process. Maurer (1960) finds decreased alkaline phosphatase in blood.

8. Bast (1929), Anson (1933) and G. Wilson (1933) ascribe otosclerosis to *embryologic cartilagenous rests* remaining in the labyrinthic capsular bone, when it develops out of the primordial cartilage. These remnants show an extreme power of growth as soon as some unphysiological forces penetrate the isolating layer.

9. The connection between otosclerosis and some general diseases such as osteogenesis imperfecta has drawn attention on the possibility of a general *mesenchymal disease* (Adir-Dighton, 1912; Van der Hoeve, 1918; Towler, 1949). The histological findings in otosclerosis correspond to those in traumatic osteodystrophy (Sercer, 1961). Nager (1951) considers otosclerosis as osteodystrophy observed under a time lens, and Weber (1961) points out that the histological bone changes in otosclerosis are identical with those in localized osteitis fibrosa.

Generalized osteitis fibrosa and osteogenesis imperfecta should however not be considered identical with otosclerosis, because these diseases attack the whole skeleton. Arslan (1960) finds mesenchymal alterations in otosclerotic focus, Beutzen (1964), Liveriero & Loggia (1962) mesenchymal hypoplasia in skin biopsies. Shadil (1961) suggests to use the latter as a test for alterations of connective tissue in the healthy members of families with otosclerosis or osteogenesis imperfecta.

10. Weber (1961) states that *two factors* are probably responsible for the development of otosclerosis: a local one, i.e. the anomalies and variations of the old and

new cartilage, and a general one, i.e. a metabolic disorder affecting the labyrinth via the vascular system.

The three layers of the labyrinthic capsule, i.e. the endostal layer consisting of fibrillary bone, the middle enchondral, protecting layer, consisting of strand bone and the periostal, isolating layer consisting of fibrillary bone, strand bone and lamellar bone, have each a different function in protecting the inner ear, from the pathological influences and the mechanical forces from outside.

The trigger to otosclerotic bone formation in the oval window must be released by something. Different stresses, hormonal, mechanic, inflammatory, metabolic, patient's way of life, his psychism, his diet and intestinal and renal conditions can be contributing factors in producing the same bone changes (Ardouin, (1961).

The otic capsule can become sensitized by various hormonal and enzymatic metabolites, which act trough the humeral way at crucial evolutive periods of life and growth of bone (puberty, maturity, pregnancy and old age).

Heredity

Questioning the otosclerotic patients for other familial cases of deafness, the age of their onset, the fact wether any surgery was performed for hearing improvement and if so with which result, usually reveals the occurrence of similar cases among relatives. Familial anamnesis is positive in 52% of the cases (Bezold, *cit. by* Müller, 1961), 58% (Nager, 1939) and 54.4% (Shambaugh, 1935). In our series of 62 patients there are 26 cases (42%) where familial anamnesis is positive for otosclerotic hearing loss. In the 36 others the existence of familial cases was either denied (18 cases) or reported unknown by the proband (18 cases) (Tab. 1).

The fact that in twins otosclerosis is practically almost concordant, bilateral and symmetric, constitutes a strong argument for heredity (Hernandez-Oroszo & Courtney, 1964).

Different interpretations have been given to the mode of inheritance of otosclerosis:

1. *Monofactorial*

Regular dominant monofactorial heredity is described by Körner (1943): 8 cases in 18 in 3 generations; Chumlea (1942): father and children of two wifes; Kabat (1943): 19 cases in 94 in 4 generations; Hustin (1951): 6 cases in 13 and 8 in 18 in 3 generations.

Irregular dominance has been described by Lang and Haïke (1928), Chumlea (1928), Kabat (1943), Pfändler (1949). The latter author stresses the varying degree of manifestation of the disease.

Even when the disease does not manifest itself clinically for several generations, it is not justified to deduce recessive heredity, because histologic lesions may be present without clinical manifestation.

Hernandez-Oroszo & Courtney (1964) reviewed 24 otosclerotic twin pairs

and add one pair of discordant monozygous twins. Twenty-two pairs were concordant for clinical otosclerosis, but nothing is mentioned about the zygosity. In one discordant twin the deaf patient showed blue sclerae. The hearing loss began at the same age in 11 of the 24 pairs of twins. The absence of deafness in one partner twin does not necessarily imply the absence of otosclerosis; he can harbor the otosclerosis, although it may be subclinical for a long time.

Larson (1960) investigated a total of 257 families (1740 persons) starting from 262 affected propositi, chosen independently from the fact whether a positive family history was present or not. Cases with a possible inflammatory cause for the deafness were discarded, so that a few otosclerosis cases may have been lost, but that the material was kept free from cases with uncertain diagnosis. The author concludes that the exact mode of inheritance cannot be established with certainty, but that his results strongly indicate monohybrid autosomal dominance with penetrance of the pathological gene between 25 and 40%. A most important argument in favor of dominant transmission was the occurrence of otosclerosis in several of the families in 3 successive generations and in 2 families in 4 successive generations.

In fact, in simple dominant inheritance, when the parents have passed the whole risk period, there are no affected children, without at least one affected parent, unless the penetrance is reduced, which is the case in otosclerosis.

Guild (1944) indicates that in 10% of the cases otosclerosis is restricted to histological abnormalities. He reports that only 7 out of 44 cases (16%) of histologic otosclerosis found in necropsies had had clinical manifestation. This is a strong argument in favour of dominant heredity. The fact that histologic otosclerosis is exceptionally diagnosed during life, explains why the mode of inheritance has long been a matter of discussion.

Larssons' figures (1960) for morbidity risk and rates of manifestation are compatible with both simple dominance and simple recessivity. His data on the occurrence of *secondary cases among parents, parents' siblings and grandparents*, however, elucidate the question. In recessive inheritance, the affected person is homozygous; consequently, there are other cases among relatives of both parents. In dominant inheritance, on the other hand, there is an accumulation of secondary cases on the side of one of the parents. Larsson (1960) studied 57 families in which more than one case occurred on the side of the father or mother of the propositus, i.e. parents, the parents' siblings and grandparents. In 53 families (93%) he found secondary cases only on the side of one parent, which supports the hypothesis of a simple dominant inheritance. This is confirmed in our own material (Tab. 1): in 10 families where more than one other case of otosclerosis occurred among the relatives, they were found 3 times (B. L., W. K. Y., C. E.) on the paternal side, 6 times (D. C. D., M. E., C. P., H. A., P. Y., N. A.) on the maternal side and only once (V. S.) simultaneously on paternal and maternal side.

Larsson (1960) indicates that the morbidity rate for otosclerosis is higher for siblings and parents of persons with otosclerosis than for the general population. It is higher for siblings in families where one of the parents is affected than in families

where none of the parents are affected. There is no significant difference in the morbidity risk for siblings and parents.

Another important argument in favour of dominant inheritance is the fact that transmission from father to son is frequently seen (Tab. 1).

Recessivity is accepted by Gradenigo (1924) and Tinkle (1933).

2. *Poly-allelism*

Hammerschlag (1904-1934) suggests *two dominant and two recessive factors* (Albrecht, 1932).

Dominance and possibly recessivity is accepted by Albrecht (1923). Hernandez-Orozco and Courtney (1963) studied the last filial generations of 70 families of Mexican patients with clinical otosclerosis. The families were divided into three groups: 1) otosclerotic father and healthy mother; 2) otosclerotic mother and healthy father and 3) healthy parents. The authors concluded that transmission is determined by an interaction of two genes, one autosomal recessive, and another sex-linked dominant.

Dimeric dominance. Davenport *et al.* (1933), Hustin (1951), Körner (1905) suggest an autosomal gene determining the reaction of the osteoklasts and osteoblasts in the mensenchym besides a sex-linked gene, this in order to explain the higher frequency of otosclerosis in women. After comparing the distribution of affected siblings in matings, where both parents are affected, with those in matings, where only one or neither of the parents has otosclerosis, Davenport *et al.* (1933) conclude: " Otosclerosis develops under *external conditions* that favour it whenever the patient has a constitution that combines *2 dominant factors* as follows: a factor which lies in the sex chromosome and also a factor which lies in one of the autosomes ".

Dimeric recessivity. Bauer and Stein (1925) are the first to investigate otosclerosis with genetico-statistical methods. By using a modification of Weinberg's propositi method they find a morbidity risk for siblings in the group unaffected × unaffected parents of 6.06%. In the group affected × unaffected parents the corresponding figure was 25.26%. They interpret this great difference between the morbidity risks as a proof that otosclerosis is hereditary. They exclude dominant heredity and single recessive heredity and conclude that otosclerosis is determined by two recessive hereditary factors (dihybrid recessive heredity).

As Larsson (1960) indicates the two main investigations (Bauer & Stein, 1925; Davenport *et al.*, 1933) are prone to some criticism. Because of the selection of the material, the pedigree studies have only a limited value. Moreover the patients studied by the two groups of authors are not examined by an otologist, which makes the diagnosis doubtful in at least a part of the patients.

Larsson (1960) states: " Polyhybrid inheritance, i.e. interaction between two or more major genes, suggested by Bauer & Stein (1925) and Davenport *et al.* (1933) is difficult to disprove ".

The inheritance of genetically determined quantitatively distributed variables can be multifactorial, i.e. due to the combined action of several additive genes (poly-

genes). In otosclerosis where the clinical degree of manifestation varies strongly, it is theoretically possible that polygenes are active either in the form of a pure multifactorial inheritance or as modifying genes to a major gene. This theoretical hypothesis, cannot, however, be proved on the basis of the existing material.

3. *The penetrance and the expressivity* of the gene vary in the different otosclerosis families. This is probably one of the reasons for the multiple interpretations which have been given by the different authors to the mode of inheritance.

Penetrance influences the frequency of the manifestation. An individual can be genetically affected and carrier of the gene, but because of the gene's weak penetrance, he is phenotypically normal, does not become deaf, but can transmit the pathologic gene to his descendants. In this way a generation can be jumped. Guild (1944) illustrates this with patients who are histologically, thus also genetically, affected, but where the lesions are very benign and do not produce clinical manifestations.

The expressivity of the gene can manifest itself in the sense of quantity (variations of expression) and of quality (variations of specificity).

The relationship which exists with other hereditary diseases, where pleiotropic genes are involved (i.e. genes which are capable of affecting several tissues at the same time), has to be kept in mind; this is the case in Paget's disease, and in osteopsathyrosis, where the same gene acts at the same time on the skeletal tissues (fragilitas ossium), on the sclerotics (blue coloration), and on the ear (deafness).

Peristatic factors can modify the penetrance, expression and specificity of the gene.

In fact, otosclerosis starts at the moment of the fastest *growth rate*, where the skeletal tissues of the adolescent are in full evolution.

Moreover *hormones and enzymes* can also play a role. The predominance of otosclerosis in women, although the disease is not due to a sex-linked gene, can be explained by a mechanism of sex-control, i.e. the intenser hormonal transformation at puberty.

Emotional strains with the correlated hormone and neurovascular disturbances are important factors in the timing, genesis and exacerbations of the otosclerotic bone dyscrasia. In fact they trigger the faulty bone metabolism which is the operating factor in the genesis of otosclerosis in predisposed individuals. Fowler (1358) showed that there exists in twins a close correlation between hyperemotional sensitivity and the priority of onset of puberty on the one hand, and the onset of the deafness on the other hand. In identical twins there exists a consistent relationship to factors affecting the timing, prevention, genesis, or course of the deafness from otosclerosis. Usually the twin, who first develops deafness from otosclerosis, is the twin who matured first, and who was subjected to the more marked emotional disturbances near pubescence (Fowler, 1958).

4. *Cytogenetics*

A. Tato (1963) studied otosclerosis cytogenetically by skin and blood cultures in 10 otosclerotic patients and in 4 normal relatives in all of whom he finds *chromosomal*

mosaicism. Two blood cultures from patients with hearing impairment due to perceptive neural hearing loss and a progressive mixed type of hearing loss were established for differential diagnosis. The mosaicism included cells with 46/47/48 chromosomes, the extra chromosomes being identified as D-chromosomes presumably trisomy and tetrasomy of number 13 (Figs. 2-3). From the study of two families in respectively 3 and 2 generations he concluded to a dominant inheritance of the mosaicism and presumed that the cases of mosaicism in the clinically normal relatives of affected patients might have been due to possible histologic otosclerosis. He postulated that mosaicism might have been present in all cases of histologic otosclerosis and could be used as a test for detection of cases without clinical manifestations and even for differential diagnosis with other forms of hearing impairment. His two control cultures showed a diploid chromosomes number of 46. The author did not report however how many cells were counted in these cultures. He admitted that there was no indication of congenital malformations or increased rate of abortions in the patients' families and suggested that the trisomy D syndrome described by Patau might involve another D-chromosome than the one which was involved in his otosclerotic mosaicism or that the normal phenotype of the otosclerotic patient might be ascribed to the line of normal cells.

He formulated the followings hypotheses:

1. Otosclerosis is due to chromosomal aberrations which produce mensenchymal alterations, especially in the otic capsule, another factor such as a mechanical one being the trigger for focus.

2. Chromosomal alterations and otosclerosis are coexistent and due to some unknown common causal factor.

3. Otosclerosis and chromosomal alterations are independent.

B. Some *syndromes* characterized cytogenetically by chromosomal aberrations show clinically, besides multiple congenital malformations, also deafness. Until 1966 no report of structural abnormalities of temporal bones was known. Schuknecht (1966) observed them first in cases of trisomies 13-15 and 18. The abnormalities are similar to the genetically inherited cochleosaccular degeneration described by Scheibe (1895).

Trisomy 6-12. Jennings & Turner (1961) reported a case of a 14 y. old girl showing multiple malformations: mental retardation, cutaneous folds, hypotonia, synostosis of cervical vertebrae, genital infantilitism, cleft palate and bilateral and pronounced deafness (75 db left, 85 db right). There was monosomy 16 and an additional chromosome in the C-group.

Trisomy 13-15 (microcephalia, mental retardation, arhinencephalia, eye defects ranging from anophthalmia and microphthalmia to colobomata, cleft palate and lip, hyperconvex fingernails, hernias, hemangiomata, abnormal horizontal hand palm creases, poly- and syndactylia, heart defects, visceral anomalies, rocker bottom feet, a.s.o.) – The outer ear is frequently deformed. Deafness occurs but, because of the early death of these children, it is difficult to determine its exact type. The

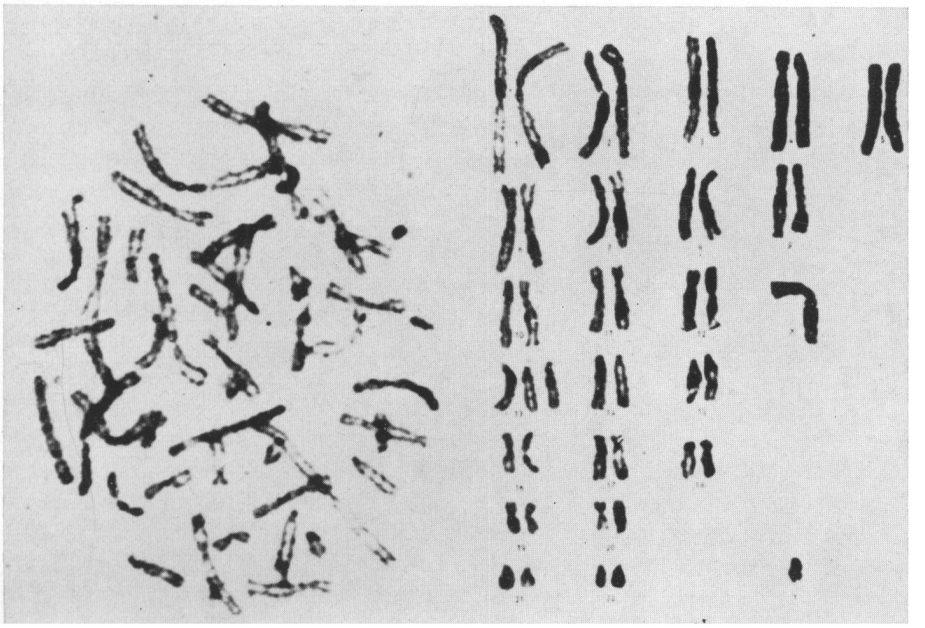

Fig. 2. Trisomy in D group according to Tato *et al.* (1963)

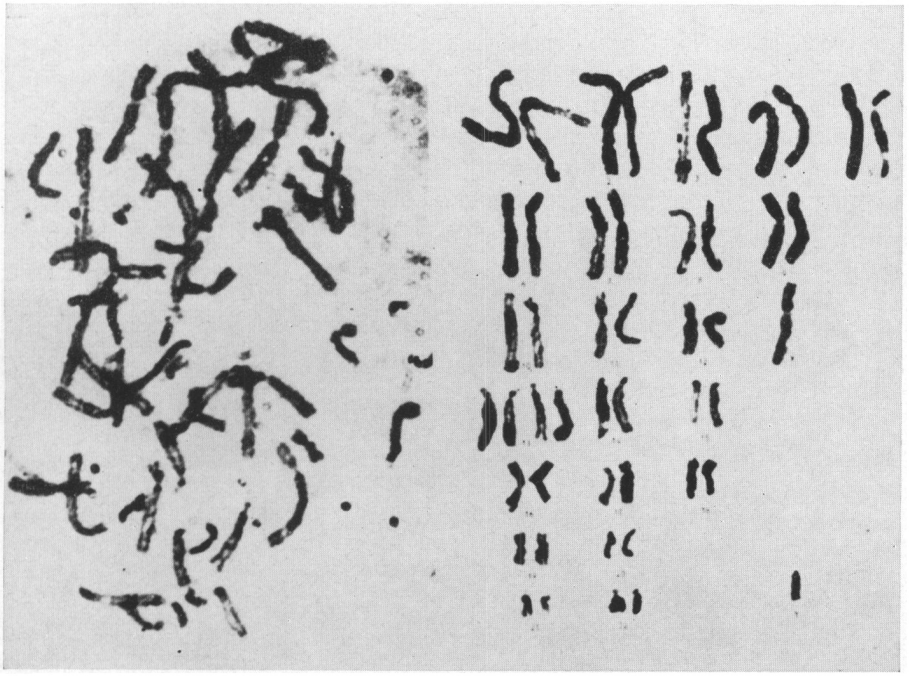

Fig. 3. Tetrasomy in D group according to Tato *et al.* (1963)

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congenital anomalies are presumably the direct result of the presence of the additional chromosome, which is thought to be the same element in all cases, although at present cytogenetic analysis is unable to discriminate among chromosomes 13, 14 or 15 with certainty. The excess chromosomal material appears to be directly responsible for induction of an excess amount of normal (duplications) and abnormal tissues. The excess tissue may provoke an imbalance between it and the surrounding normal tissue, resulting in focal morphologic abnormalities.

In addition, there are malformations (e.g. the skeletal abnormalities of the skull and face) which are better interpreted as the result of secondary anomalies, having their origin in alterations occurring early in embryonic life, i.e. defective formation of the skull blastema, particularly of the ethmoid bone.

Trisomy 18 (mental retardation, hypertonia, flexion of the fingers 2 and 5 on top of 3 and 4, short neck, micrognathia, flat thorax, short sternum, a.s.o.) – External ear deformities and deafness have been described. The early death of these patients often exclude the study of its type. Kluyskens (1965) examined two cases and found total deafness in one and partial deafness with vestibular areflexia in the other.

Trisomy 21. Koch & Serra (1962) examined the cochlear-vestibular system in 14 cases. All presented anomalies. Hypoacousia of the conduction type was more pronounced in the children, which presented an important hypertrophia of the lymphoid tissue. The cases with neurological signs had an hypoacousia of the perception or the mixed type. These cases had also a disturbed labyrinthic function (spontaneous nystagmus, vestibular hyperreflexia). The authors concluded that it is not possible to correlate the clinical condition with the degree of the hearing impairment.

Turner's syndrome (lack of one X chromosome, pterygium colli, low implantation of the hair line, dwarfism, cubitus valgus, streak ovaries) – The auditive impairment is always of the same type, with a typical audiometrical curve. This is characterized by a loss of 20 db for the low frequencies, a loss of 50 db for middle frequencies and a loss of 25 db for the high frequencies.

Lindsten (1963) found hypoacousia in 68% of 41 patients, examined audiometrically. Twenty in twenty-eight had perception type of hearing loss, five conduction type and two the mixed type. Kluyskens (1965) examined audiometrically a 12 year old XO patient and found hypoacousia of the perception type, although the patient did not complain of hypoacousia.

C. *Personal findings*

Cytogenetic evaluation was done on all the 62 patients, but only those patients were kept for interpretation of the cytogenetic results, in which the tissue culture succeeded very well, so that chromosomal evaluation could be done in a sufficient number of cells. The patients marked with the negative sign in Tab. 1 represent the patients where the tissue culture either lacked growth or did not produce enough dividing cells for allowing a reliable analysis. Tab. 2 represents the cytogenetic results obtained in 46 patients, where the tissue culture succeeded well. This includes

Tab. 2. Cytogenetic data on 46 otosclerosis patients

Name	Sex	Cell type	Total N. of cells counted	Hypodiploidy: 2n < 46			Diploidy: 2n = 46			Hyperdiploidy: 2n > 46		
				% cells	N. of karyo's	Evaluation	% cells	N. of karyo's	Evaluation	% cells	N. of karyo's	Evaluation
A. R.	♂	leuco's	36	9% < 44	—	In vitro loss	91%	11	Normal	0%	—	—
B.	♂	leuco's	22	9% < 44	—	In vitro loss	82%	8	Normal	0%	—	—
				9% = 45	2	Monosomy E						
B. L.	♂♀	fibroblasts	18	6% < 44	—	In vitro loss	89%	7	Normal	5% > 48	1	2n = 92 endoredupl.
B. N.	♂	leuco's	36	19% < 45	—	In vitro loss	72%	9	Normal	0%	—	—
				9% = 45	3	Inconst. monosomies						
B. J.	♂	fibroblasts	22	50% < 45	—	In vitro loss	45%	6	Normal	5%	1	2n = 47 extra C (trisomy 9?)
C. J.	♂	fibroblasts	66	23% < 45	—	In vitro loss	76%	5	Normal	1%	1	2n = 47 extra E (trisomy 16?)
C. F.	♂	fibroblasts	21	57% < 45	1	In vitro loss	33%	6	Normal	5% > 48	1	2n = 59 - aneuploidy
				5% = 45	1							
C. Fr.	♂	fibroblasts	47	30% < 45	—	In vitro loss	62%	6	Normal	2% > 48	1	2n = 88 - aneuploidy
				6% = 45								
C. L.	♂	fibroblasts	22	23% < 45	—	In vitro loss	73%	7	Normal	0%	—	—
				4% = 45								
C. A.	♂	fibroblasts	55	14% < 45	—	In vitro loss	84%	6	Normal	2%	1	2n = 47 extra C (trisomy 12?)
C. P.	♀	leuco's	32	12% < 44	—	In vitro loss	69%	10	Normal	0%	—	—
				19% = 45	5	Inconst. monosomies						
DB. M.	♀	fibroblasts	19	0%	—	—	100%	7	Normal	0%	—	—
DC. D.	♀	fibroblasts	17	30% < 45	—	In vitro loss	70%	7	Normal	0%	—	—
		leuco's	16	0%	—	—	100%	7	Normal	0%	—	—
DL. M.	♀	leuco's	34	6% < 44	—	In vitro loss	82%	7	6: normal; 1: transl. ?/G	3%	1	2n = 47 extra (F?)
				9% = 45	3	Inconst. monosomies						
DP. A.	♀	fibroblasts	23	0%	—	—	100%	8	7: normal			
									1: deletion C chrom.	0%	—	—
DW. L.	♀	fibroblasts	14	14% < 45	—	In vitro loss	86%	7	Normal	0%	—	—
D. P.	♂♀	fibroblasts	38	16% < 45	—	In vitro loss	84%	8	Normal	0%	—	—
E. G.	♂♀	leuco's	32	9% = 45	1	Monos. C_6 — In vitro loss	91%	10	Normal	0%	—	—
F. L.	♂	fibroblasts	10	10% < 44	—	In vitro loss	90%	6	Normal	0%	—	—
		leuco's	17	0%	—	—	95%	12	9: normal; 1 tr. E/C	5%	1	2n = 47 extra C (Ic = 0.408) tris. C_7?
									2: Ic B chrom.			
G. A.	♀	fibroblasts	30	7% < 45	—	In vitro loss	87%	8	6: normal; 1 monos. C + tr. or del. chrom.; 1: mon. A_2, tris. A_4 mon. C_{12}, trans. D/G	6%	1	2n = 47 extra C (length = C_7; Ic = C_9)
G. D.	♀	leuco's	35	9% < 45	—	In vitro loss	91%	8	Normal	0%	—	—
H. M.	♀	leuco's	26	11% < 44	—	In vitro loss	85%	9	Normal	4%	1	2n = 47 extra C (nr?)
H. I.	♀	leuco's	28	0%	—	—	100%	8	Normal	0%	—	—
J. J.	♂	fibroblasts	25	8% < 45	—	In vitro loss	92%	7	6: normal	0%	—	—
									1: monos. A_1' Tris A_2			
K. F.	♂	leuco's	17	6% = 45	—	In vitro loss	94%	8	7: normal			
									1: Ic B_4 ↗	0%	—	—
L. L.	♂	fibroblasts	9	22% < 45	—	In vitro loss	78%	7	Normal	0%	—	—
L. M.	♂	leuco's	10	20% < 45	—	In vitro loss	80%	5	Normal	0%	—	—
L. A.	♂	leuco's	25	4% < 44	—	In vitro loss	88%	7	6: normal			
				8% = 45	2	Inconst. monosomies			1: Ic A_1 ↗	0%	—	—
M. A.	♂	leuco's	32	3% = 45	1	In vitro loss — monos. E	90%	8	Normal (enlarged satellites			
				6% < 45					in D group)	0%	—	—
M. I.	♀	leuco's	30	0%	—	—	100%	7	Normal (enlarged satell. in			
									D & G groups)	0%	—	—
M. Y.	♀	leuco's	16	6% = 45	—	In vitro loss	94%	7	6: normal			
									1: monos C_7 + transl ?/E	0%	—	—
N. A.	♀	leuco's	30	3% < 45	—	In vitro loss	97%	9	Normal (↗ satellites in D			
									& G groups)	0%	—	—
N. M.	♂	leuco's	33	18% < 44	—	In vitro loss	79%	7	Normal	0%	—	—
				3% = 45	2	Inconst. monos. (D resp. E)						
P. J.	♀	leuco's	30	3% < 44	—	In vitro loss	90%	9	6: normal			
				7% = 45	1	Monosomy C			2: Ic B chromos. ?			
									1: inversion A_2	0%	—	—
P. A.	♂	leuco's	31	3% < 44	—	In vitro loss	94%	8	Normal	0%	—	—
				3% = 45	1	Monosomy E						
P. E.	♂	leuco's	16	0%	—	—	100%	9	Normal	0%	—	—
P. JM.	♂♀	leuco's	26	0%	—	—	100%	6	Normal	0%	—	—
S. Y.	♂♀	leuco's	35	3% < 44	—	In vitro loss	83%	9	8: normal			
				14% = 45	4	2 : monos. C; 2 : monos. E			1: abn. Ic A_2, C_{12}, E_{18}	0%	—	—
VD. R.	♂	fibroblasts	35	14% < 45	—	In vitro loss	80%	7	Normal	3%	1	2n = 47 extra E (trisomy 16?)
				3% = 45	—							
		leuco's	13	10% < 45	—	In vitro loss	79%	7	5: normal			
				11% = 45	—				2: Ic B_4' ↗	0%	—	—
V. L.	♀	fibroblasts	18	6% = 45	—	In vitro loss	89%	8	Normal	5%	1	2n = 66 = aneuploidy
VS. A.	♀	fibroblasts	13	23% < 45	—	In vitro loss	46%	4	Normal	23% (?)	3	1: extra B(nr ?)
				8% = 45	1	Monosomy C						2n=47 1: extra C(nr ?)
												1: extra G(nr ?)
VK. J.	♂	fibroblasts	14	14% < 45	—	In vitro loss	79%	8	Normal	0%	—	—
				7% = 45	—							
VL. F.	♂	fibroblasts	11	36% < 45	—	In vitro loss	46%	5	Normal	0%	—	—
				18% = 45	2	Monosomies D resp. E						
VM. O.	♂	leuco's	18	6% = 45	1	In vitro loss - Monos. C_{11} Ic of B_5 ↗	94%	4	3: normal			
									1: Ic B chrom. ↗	0%	—	—
V. S.	♀	fibroblasts	14	14% < 45	—	In vitro loss	79%	5	3: normal			
				7% = 45	1	Monosomy C			2: Ic B_5 ↗	0%	—	—
V. J.	♀	leuco's	14	0%	—	—	100%	8	Normal	0%	—	—

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fibroblast cultures in 19 patients, blood cultures in 24 patients and both types of cultures in 3 patients, thus a total of 49 cultures. Fibroblast cultures were established from skin and fascia. The skin was obtained from the back of the hand during the surgical procedure of taking a vene graft for interposition in stapes surgery. The fascia was prelevated from the fascia lata. The blood cultures were established from peripheral blood in some patients and from venous blood in others, both being cultured following a modification of Lejeune's micromethod (1964). Chromosomal counts were made in two different ways, of which the results were combined, i.e. on projections with the drawing tube and on photographs. Structural analysis was done in karyotypes made up from enlarged microphotographs and verified when necessary by microscopic observation.

1. *Numerical analysis* (Tab. 2)

a) As can be seen from the *diploidy column*, in each patient the percentage of cells with a normal ($2n = 46$) chromosome number was high enough to conclude to the existence of normal diploidy. It attained 70-79% in 9 cultures, 80-89% in 13 cultures, 90-99% in 13 cultures and even 100% in 8 cultures. The few cases, where the percentage of normal diploid cells was lower than 70%, were:

Five cases with a considerable number of hypodiploid cells, where chromosomes were lost during the in vitro procedures (B. J., C. F., C. P., C. Fr., V. L. F.).

One case (V. S. A.) where the total number of counted cells was very small, so that the percentages could not be accurately calculated and consequently had no meaning. This case was not discarded from the table for reasons which are further exposed.

In no cases was a significant per cent of hyperdiploid cells responsible for a low per cent of normal diploid cells, exception made for the just mentioned case (V. S. A.), where the percentages were not reliable.

b) In the *hypodiploidy column* ($2n < 46$), the countings were subdivided in cells with $2n = 45$ and in cells with $2n < 45$. This was done routinely to detect the presence of a possible constant monosomy. In all cells the hypodiploidy could be ascribed to in vitro loss of chromosomes, a constant monosomy being absent.

c) The *hyperdiploidy column* ($2n > 46$) showed that on the total of 49 cultures there are 36 cultures where no hyperdiploid cells were present, 12 cultures with an extremely low percentage (maximum 6%) of hyperdiploid cells and 1 culture (V. S. A.) where the high percentage of hyperdiploid cells (23%) was not reliable as mentioned before. Amongst the cases with hyperdiploid cells there were 1 case of endoreduplication, $2n = 92$, 3 cases of aneuploidy, $2n > 47$ and 9 cases of trisomy ($2n = 47$).

a) *Endoreduplication* is a doubling of the chromosomes during the resting stage, occurring without any other mitotic events and resulting in a tetraploid cell with 23 pairs of double chromosomes. The nuclear material reproduces, but is retained within a single nucleus. Cytokinesis does not take place. Each chromosome remains in close association with its duplicate, and their chromatids do not separate until the following metaphase. The diplochromosomes take a random position in the

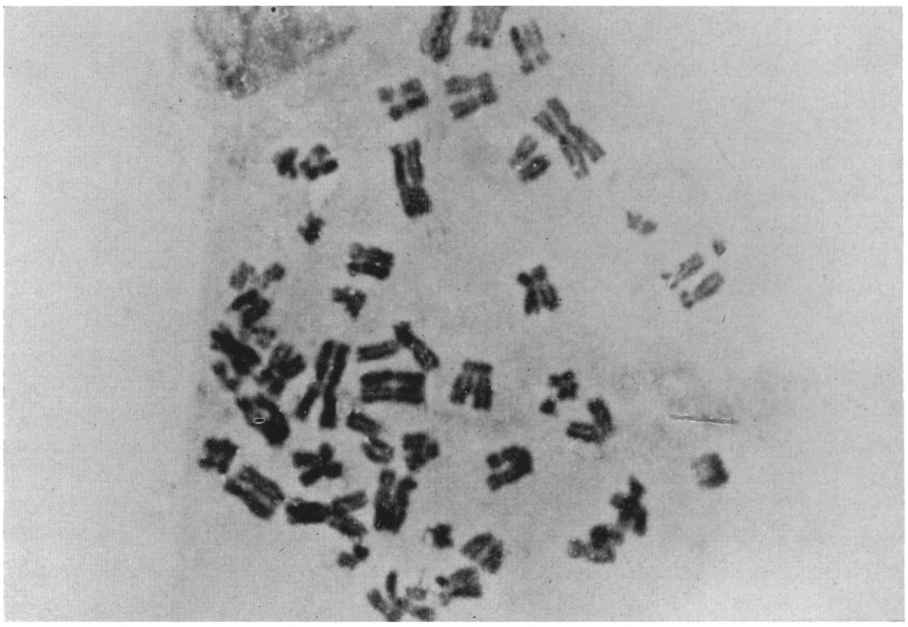

Fig. 4. Endoreduplication (*pers. obs.*)

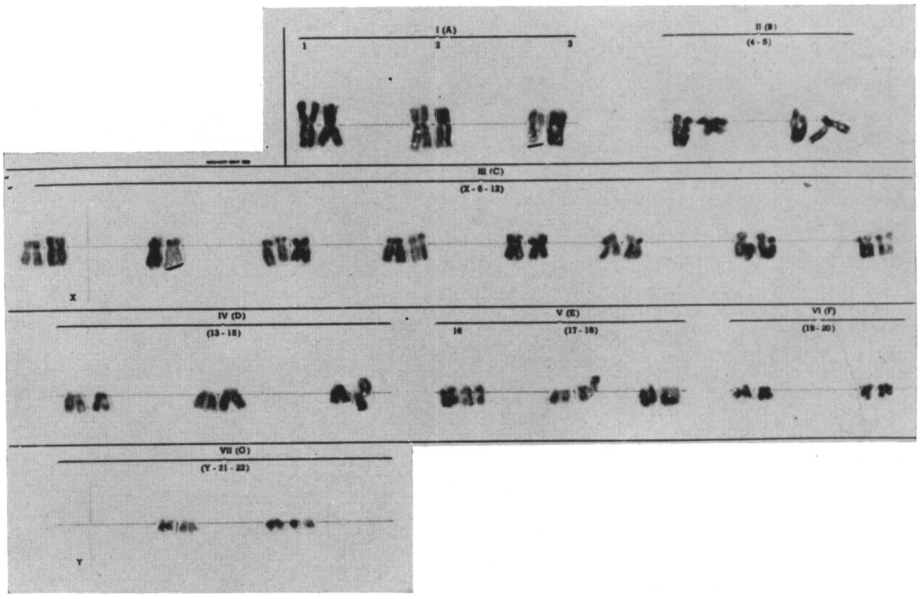

Fig. 5. Karyogram from cell with endoreduplication (*pers. obs.*)

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daughter nuclei. Therefor, tetraploid mitoses with irregular chromosome distribution appear in later generations of mitoses with diplochromosomes. They may also result from other chromosome doubling mechanisms, such as endomitosis in a restricted sense (the chromosomes go through a normal cycle of duplication and condensation and reassembly into an interphase nucleus) or arrested (c-type) mitosis (the nuclear membrane breaks down and the only missing phase of normal mitosis in the anaphase separation of the chromosomes): Geitler (1953), Schwarzacher & Schnedl (1965). It is known that agents that inhibit the mitotic spindle such as colchicine can cause endoreproduction in vitro. Mitoses with diplochromosomes can also occur spontaneously in human cultures, without any specifically known cause (Aspillaga *et al.*, 1964; Bishun *et al.* 1964; Valdmanis & Mann, 1964).

In cultured spleen necropsy material Bain and Gauld (1963) found diplochromosomes in 1 to 8% of the mitoses. These figures were somewhat high, possibly because of the normally high frequency of polyploid cells in the spleen. Schwarzacher (1961) examined 5 fibroblast cultures, from skin or fascia biopsies in normal persons and from the rib cartilage of an apparently normal human embryo. Between 0.1 and 4.1% of the mitoses were found to be tetraploid with the chromosomes arranged as diplochromosomes; 0.5-5.8% of the mitoses were tetraploid with randomly positioned chromosomes.

In cultured blood cells the frequency of mitoses with diplochromosomes, as reported from one culture by Aspillaga *et al.* (1964), was in the same range as in the fibroblast culture of Schwarzacher (1965) with the highest number of polyploid cells. The values given by Valdmanis & Mann (1964) for leukocyte cultures were about the same as those of Schwarzacher (1965). Also Jackson's (1963) finding of four tetraploid cells including one with diplochromosomes among 1 000 mitotic figures was comparable with Schwarzacher's results.

In our material endoreduplication with $2n = 92$, the chromosomes being arranged as diplochromosomes, was seen in one fibroblast culture (B. L.) and only in one cell on the 18 counted in that patient (5%) (Figs. 4-5).

b) The cases C. F., D. Fr. and V. L. had *aneuploid chromosome numbers* $2n > 47$.

C. F.: $2n = 59$ in only one cell on 21 counted (5%). There was trisomy in group A (N. 1, 2, 3), group B (N. 4), group C (N. 6, 7, 9, 10, 12), group E (N. 16,18); tetrasomy in group B (N. 5), group C (N. 8 and 11); normal diploposition in group D (N. 13, 14, 15), E (N. 17) and F (N. 20); monosomy in group F (N. 19), group G (N. 21); nullisomy in group G (N. 22) and a normal XY constitution.

C. Fr: $2n = 88$ in only one cell on 47 counted. There was tetrasomy in group A (N. 1, 2, 3), group B (N. 4, 5), group C (N. 6, 7, 8, 9, 10, 11, 12), group D (N. 13, 14, 15), group E (N. 16, 17, 18), group F (N. 19, 20); trisomy in group G (N. 21, 22) and a normal XY constitution.

V. L.: $2n = 66$. There was tetrasomy in group C (N. 9, 10, 11, 12), group D (N. 16, 17) and group G (N. 22); trisomy in group A (N. 1, 3), group C (N. 7, 8), group E (N. 18), and group G (N. 21); normal diploposition in group A (N. 2), group B (N. 4, 5), group C (N. 6), group F (N. 19, 20) and a normal XX composition.

Figs. 6-11. D group cut out from 96 karyograms (*pers. obs.*)

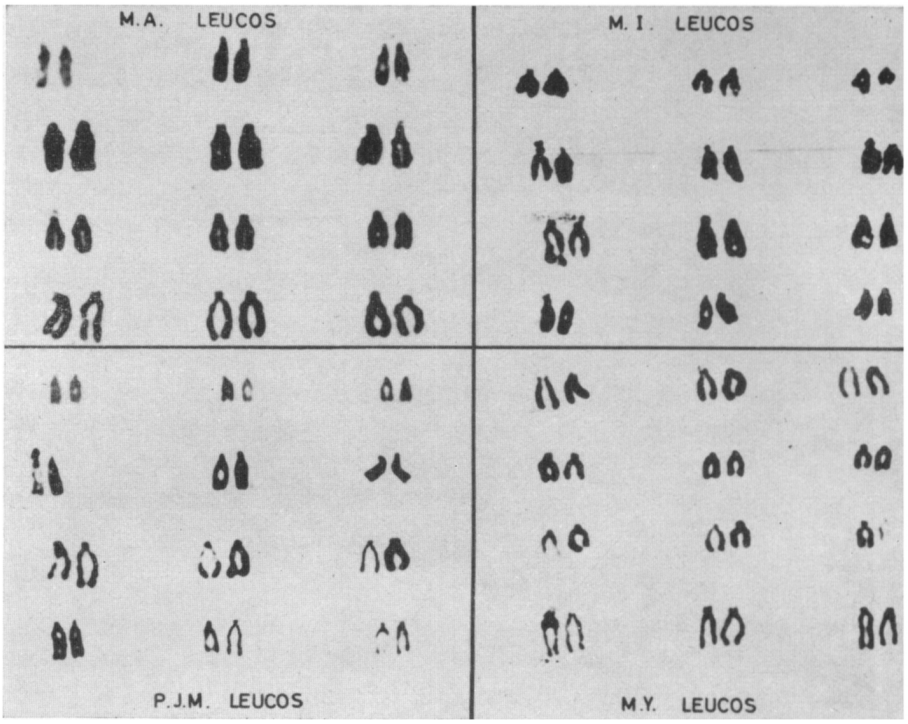

Fig. 6

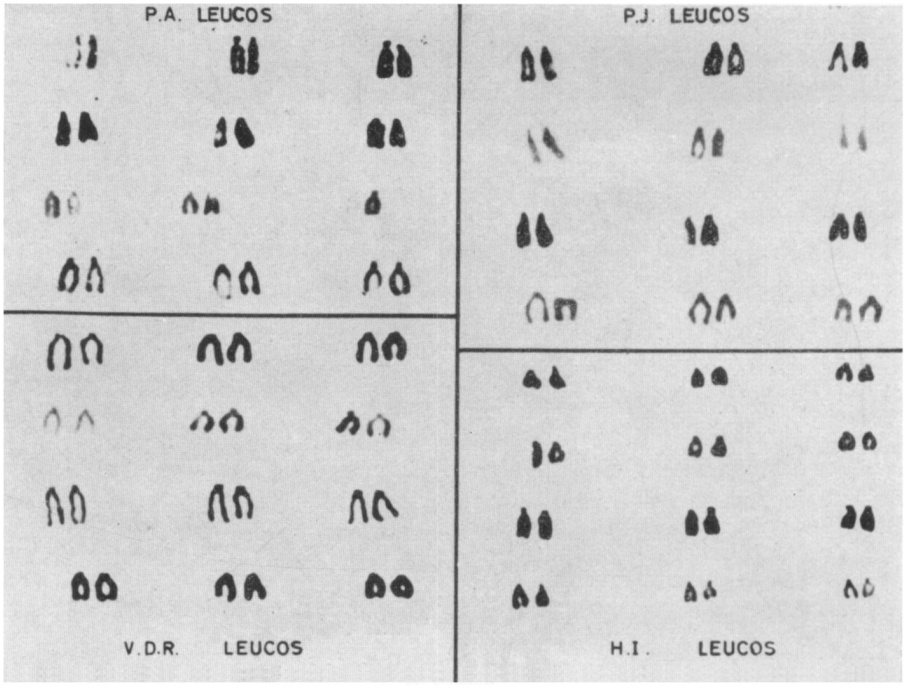

Fig. 7

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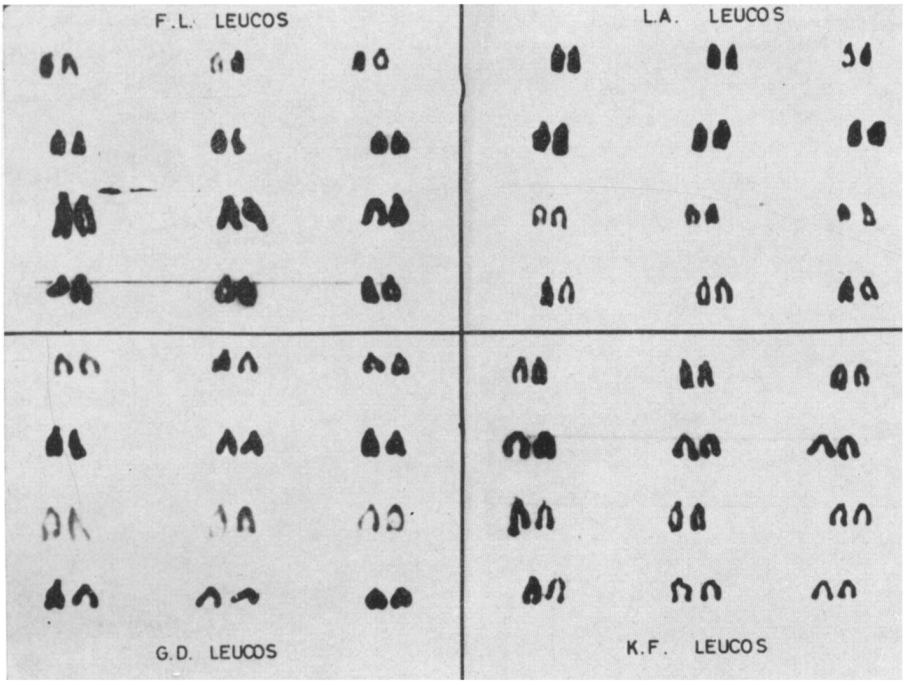

Fig. 8

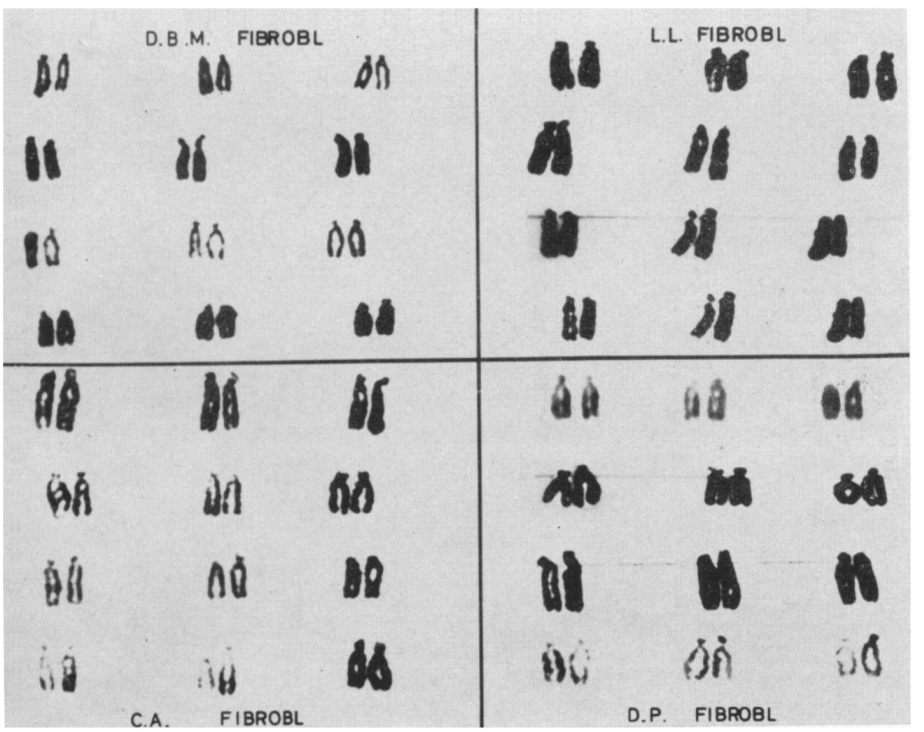

Fig. 9

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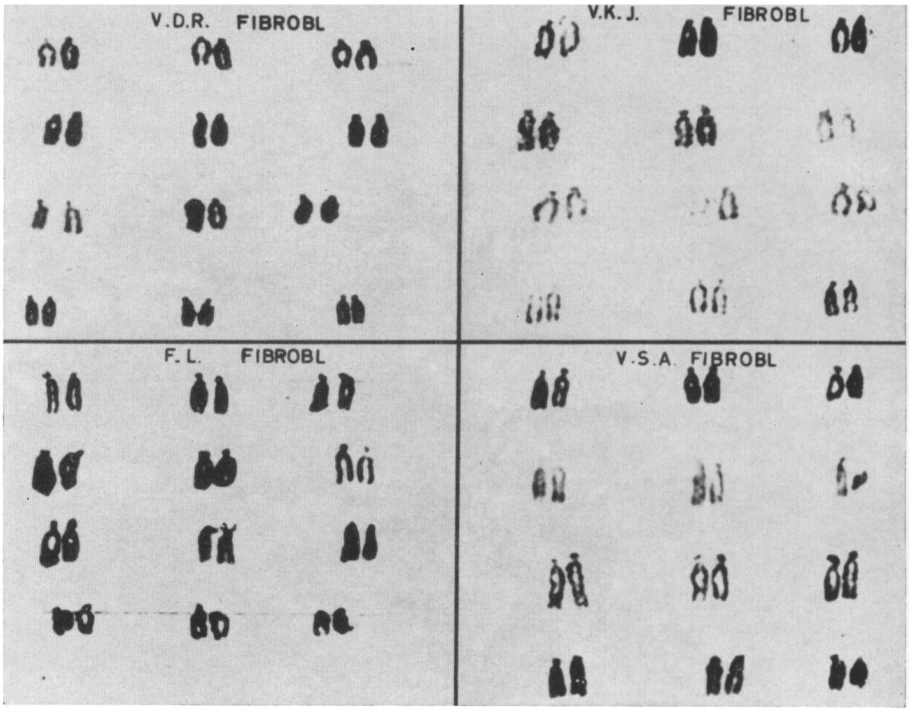

Fig. 10

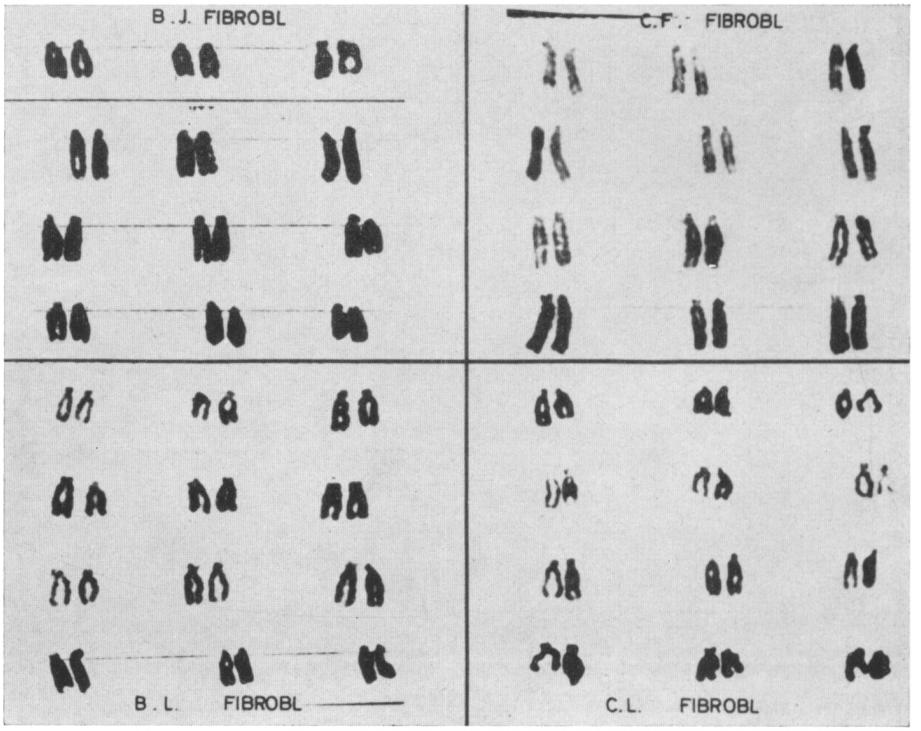

Fig. 11

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This enumeration shows that aneuploid findings in the few cells with $2n > 47$ are not consistent and present in such a low percentage that mosaicism can be excluded. They certainly do not occur constantly in the D group and contradict Tato's finding. In Figs. 6 to 11 the D groups cut out from 96 karyograms, from 24 patients (4 groups per patients) are classified together. Every group has the normal number of 6 chromosomes.

These few aneuploid cells are probably the result of sporadic erroneous mitosis in vitro. These results do certainly not support Tato's findings of a constant mosaicism in the D group. We want to mention that the latter author does not cite the percentage in which the different cell lines were present, so that it is not possible to judge about their significance.

c) In our 9 cases where *one extra chromosome* ($2n = 47$) was found (B. J., C. J., C. A., D. L. M., F. L., G. A., H. M., V. D. R., V. S. A.), this involved different chromosomal groups (B - C - E - F - G) and was never present in a sufficiently high percentage (max. 6%) to conclude to the presence of mosaicism between a normal and a trisomic cell-line.

B. J. : fibroblasts — trisomy C (N. 9?) 5%
C. J. : fibroblasts — trisomy E (N. 16?) 1%
C. A. : fibroblasts — trisomy C (N. 12?) 2%
D. L. M.: leucocytes — trisomy F? (N. ?) 3%
F. L. : leucocytes — trisomy C (N. 7?) 5%
G. A. : fibroblasts — trisomy C (N. 7? or 9?) 6%
H. M. : leucocytes — trisomy C (N. ?) 4%
V. D. R.: fibroblasts — trisomy E (N. 16?) 4%
V. S. A. : fibroblasts — The culture in this case was of poor quallity so that only 13 cells could be counted in total. Amongst those there were 3 cells with $2n = 47$, the extra chromosome having respectively a length of B, C, and G chromosomes.

This case shows that poor quality of a chromosome preparation as well what concerns the total number of cells counted as the morphologic aspect of the chromosomes, can lead to erroneous and non reliable interpretations. We wonder whether this has not been the cause in Tato's interpretation of mosaicism in the D-group (Figs. 2-3).

The numerical analysis of our material allows to conclude to the presence of normal diploidy, since no chromosome(s) is (are) constantly added or absent (Tab. 3).

2. *Structural analysis* (Tab. 2)

The only peculiarities encountered were: enlarged satellites in large and small acrocentrics, sporadically abnormal centromeric indexes and occasionally translocation and deletion chromosomes.

a) *Enlarged satellites* were seen in 3 of our cases (M. A., M. I. and N. A.). Enlargement of the satellite itself or the short arm between the centromere and the secondary constriction close to the satellite, of large and/or small acrocentric chromosomes is known to be compatible with normal development, and not necessarily

to imply an association with developmental disorder in the carriers or their offspring (Ellis & Penrose, 1961; Schmid, 1962; de la Chapelle, 1963). It has been demonstrated in mitoses from leucocyte and skin cultures. Familial occurrence is frequently found (Ellis & Penrose, 1961; Cooper & Hirschhorn, 1962; de la Chapelle, 1963). Some authors think that the enlargement only reflects altered chromosomal behaviour and not a difference in chromatin content. De la Chapelle (1963) postulates however that there really is an additional piece of chromatin which may represent a reciprocal translocation, or a duplication, or possibly a pericentric inversion. If it were a pericentric inversion, as suggested by Gray *et al.*, (1962), a satellite or constriction should be seen in the long arm of the chromosome, which is not the case. De la Chapelle therefor supports the theory of translocation of duplication.

Some reports emphasize an association with other chromosomal aberrations, and the occurrence of developmental diseases, mongoloid traits or repeated abortions in the propositi or their relatives. De la Chapelle *et al.* (1963) studied a family in which a number of subjects showed apparent enlargement of the short arm or satellite region of a small acrocentric. Despite the finding of 2 mentally retarded subjects

Tab. 3. Summary of numerical cytogenetic findings

N. of cultures	43
N. of counted cells	1237
N. of diploid cells	1032 = 83.4%
N. of hypodiploid cells	189 = 15.2%
N. of hyperdiploid cells	16 = 1.2%

with lesions of the central nervous system in the third generation, they suggest that the association with mental retardation and anomalies of the central nervous system in 2 out of 9 members of the large sibship may well have been fortuitous and entirely unconnected with the apparent chromosomal aberration seen in 5 members, one of whom was mentally retarded. Since four of the five affected members of the family were entirely normal, the apparently enlarged satellite region in itself can hardly be the causative agent of the malformation of the central nervous system in the fifth.

It should be remembered that most of the chromosome investigations, so far published, start from abnormal clinical findings in one or several propositi. When a chromosome marker like an apparently enlarged satellite region is detected, it is not surprising that it occurs in a family with clinical disorders. Furthermore, negative results usually are not published. The finding of enlarged satellites in 3 of our cases may be fortuitous. If it would be limited to the etiology of otosclerosis it certainly would have been present in a higher number of patients.

b) Sporadic abnormalities of the *centromeric index* were found in 6 patients. The centromeric index was found too high in a B chromosome: 0.350 and 0.353 respec-

tively in 2 karyo's of F. L., 0.358 in one karyo of K. F., 0.363 in one karyo of V. D. R., 0.342-0.354-0.369-0.383 respectively in 4 karyo's of V. M. O.

The centromeric index was found too low (0.348) in an A_1 chromosome in one karyo of L. A., (0.295) in an A_2 chromosome in one karyo of P. J. These abnormalities can be ascribed to sporadic in vitro deletions or translocations.

c) *Translocation* chromosomes were found occasionally in 5 patients: ?/G in 1 karyo of D. L. M., E/C in one karyo of F. L., D/G and D/C in two karyo's of G. A., ?/E in one karyo of M. J., C/E in one karyo of S. J.

d) A *deletion* of C_9 was seen in one karyo of D. P. A.

All the above mentioned structural peculiarities or abnormalities occurred in only a few patients and moreover only sporadically in the cultures from each patient

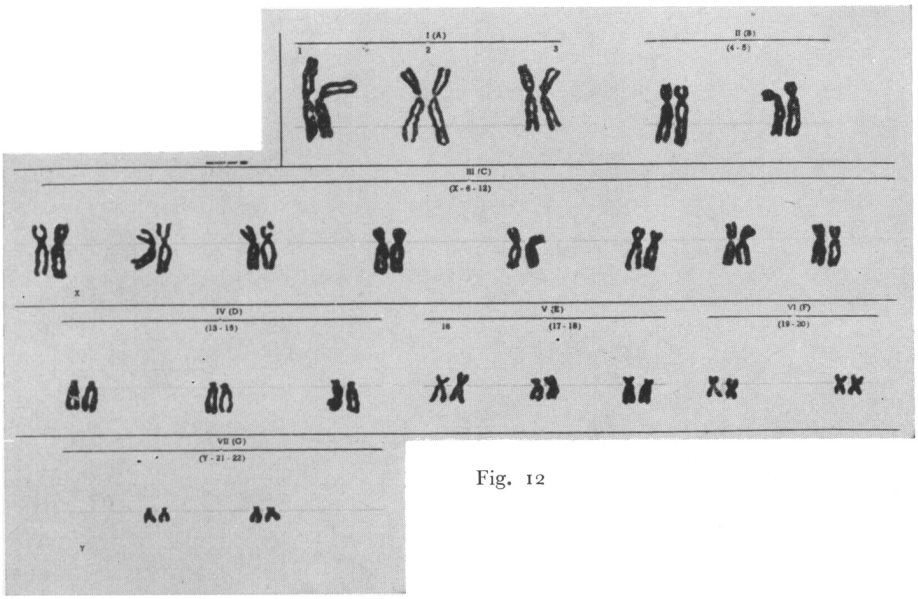

Fig. 12

Figs. 12-14. Karyograms from fibroblast cultures (*pers. obs.*)

concerned. They could certainly not be linked with the otosclerotic phenotype. Figs. 12-14 are examples of our karyo's obtained from fibroblastcultures, Figs. 15-18 of karyo's from blood-cultures.

D. *Related phenotypical conditions with normal karyotype.*

So far no cytogenetic abnormalities are known to be linked with local or generalized osteodystrophies. The karyotype has been studied in some generalized osteodystrophies, and found to be normal.

— Apert's syndrome:		Pfeiffer & Kosenow (1962)
— Pfaundler-Hurler's lipochondrodystrophy:	44A + XY	Pfeiffer & Kosenow (1962)

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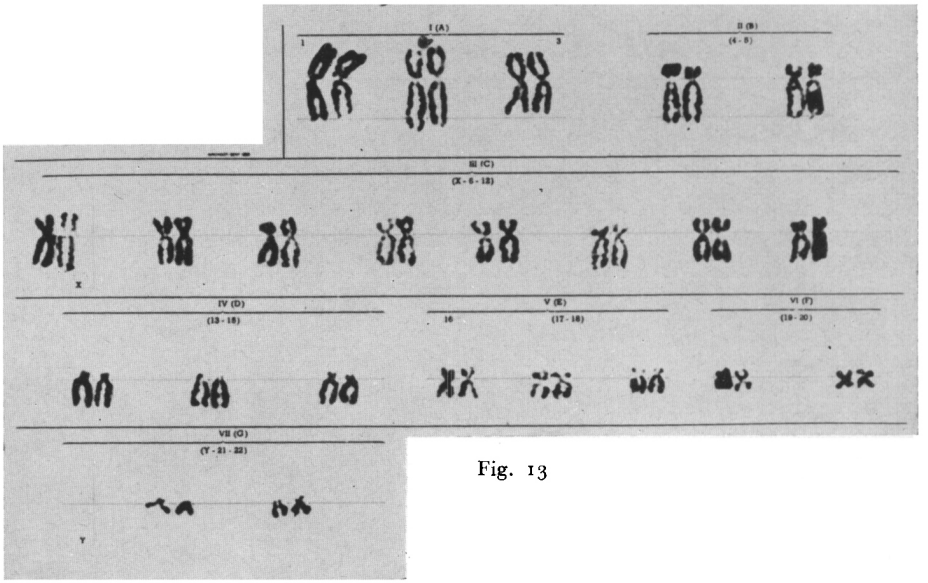

Fig. 13

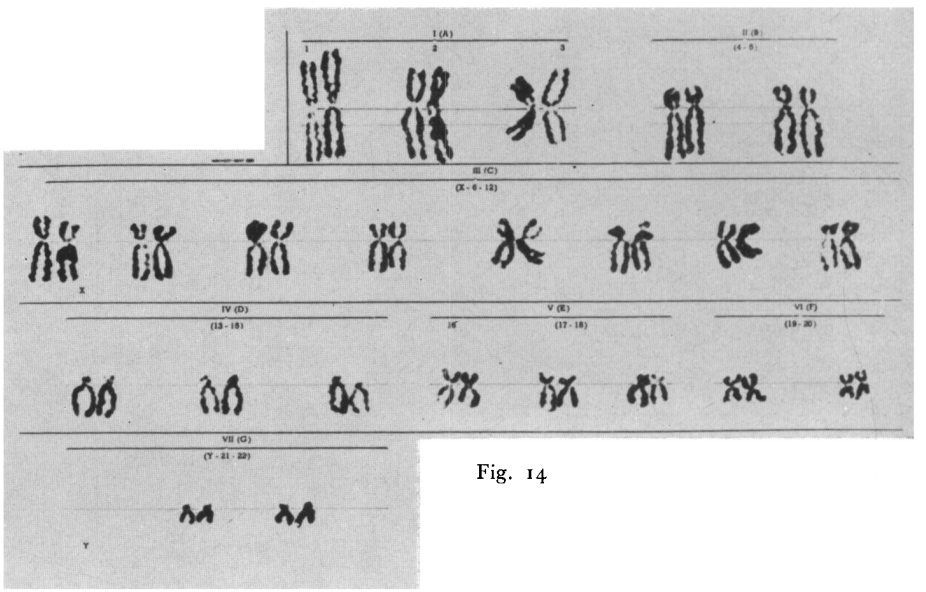

Fig. 14

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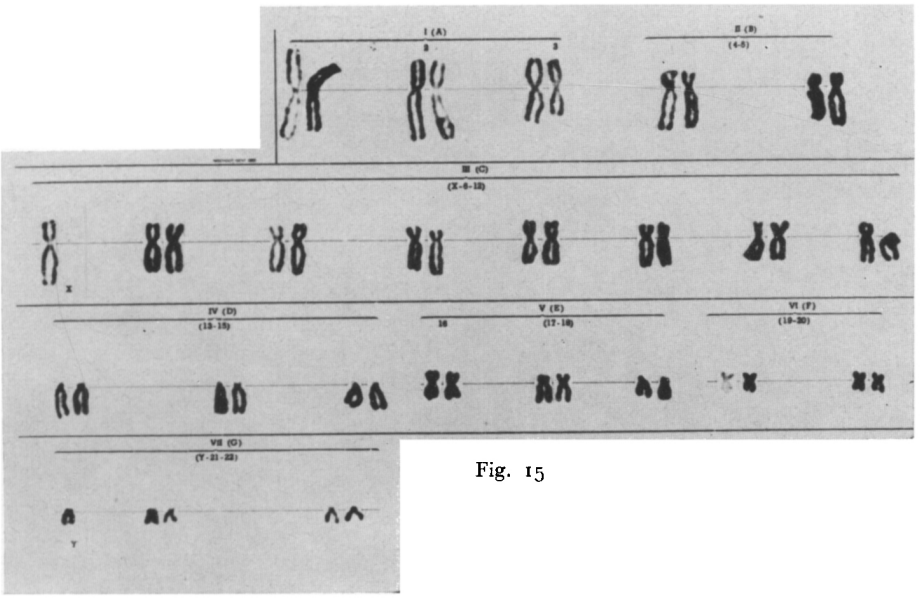

Fig. 15

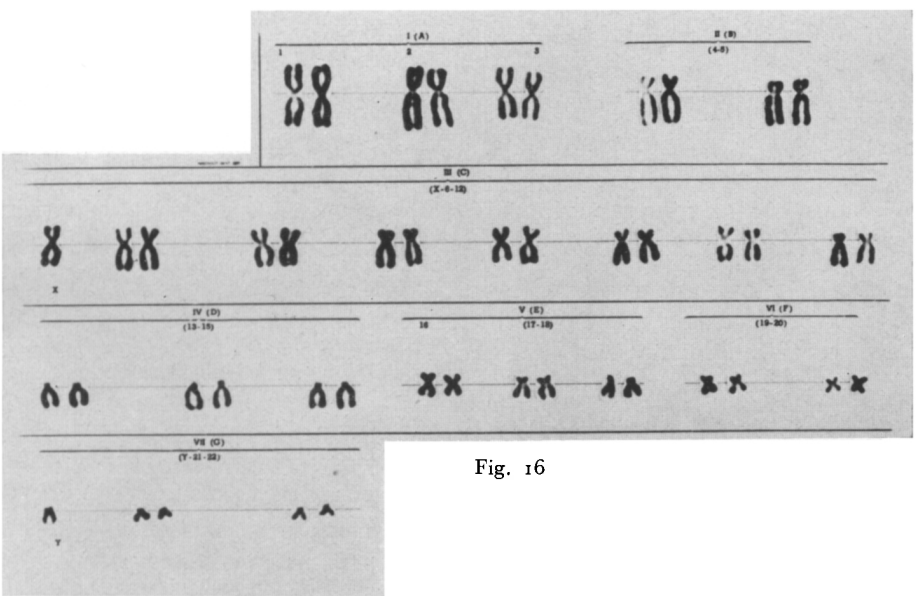

Fig. 16

Figs. 15-18. Karyograms from bloodcultures (*pers. obs.*)

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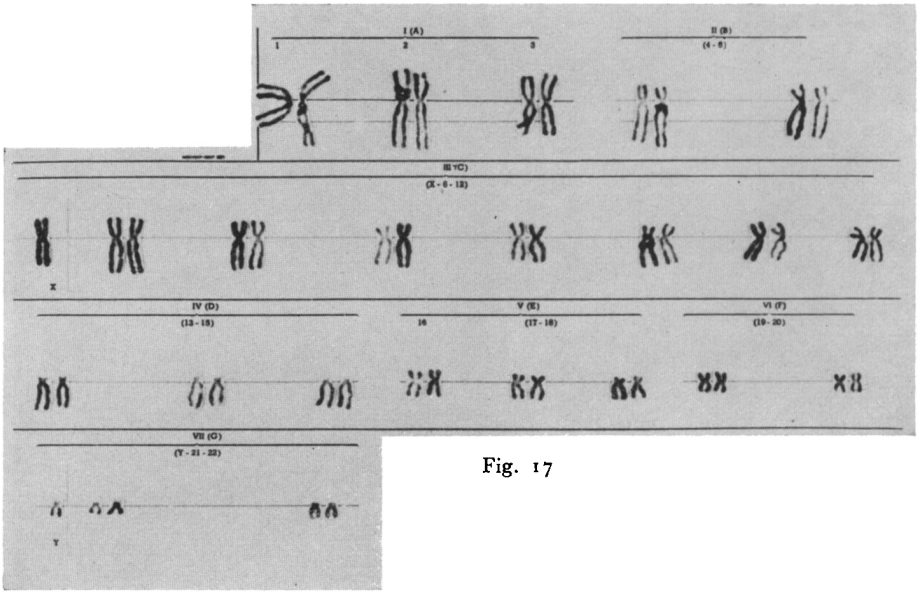

Fig. 17

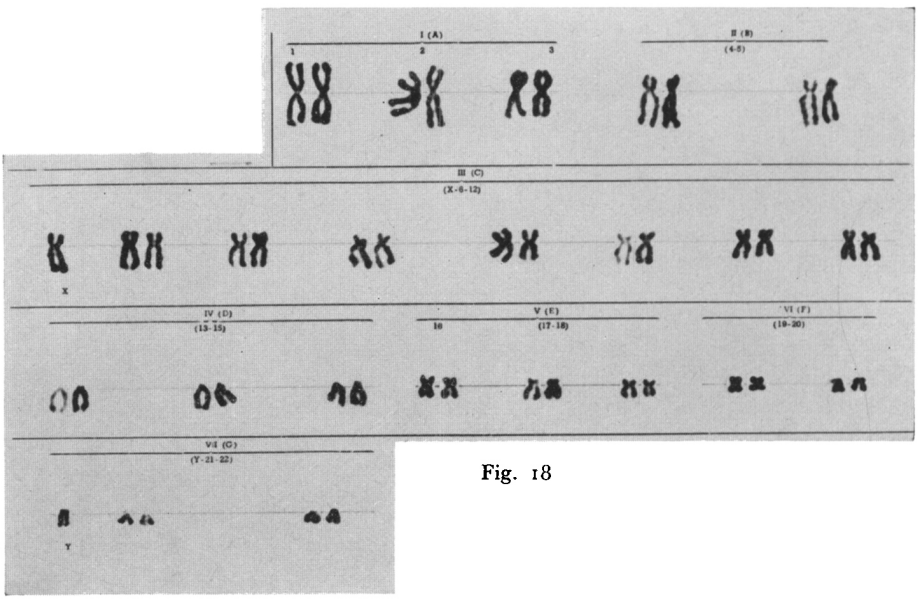

Fig. 18

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— Klippel-Feil's syndrome, Deaf and Dumb:	44A + XX	Giraud *et al.* (1963)
— Chondrodystrophy:	44A + XY	Giraud *et al.* (1963)
— Morquio's chondrodystrophy and arthrogryposis:	44A + XY	Dumars & Gaskill (1964)
— Huler-Hunter's lipochondrodystrophy gargoylism:	44A + XY	Abrisqueta (1966)
— Congenital chondrodystrophy:	44A + XY	Farber *et al.* (1966)
— Osteogenesis imperfecta:	44A + XX	Lozzio (1966)
— Osteogenesis imperfecta:	44A + XY	Lozzio (1966)
— Osteodystrophia with spondyloschisis-genu valgum:	44A + XX	Lozzio (1966)

E. *Conclusion*

Our personal cytogenetic findings certainly do not support Tato's viewpoint of a chromosomal etiology for otosclerosis. Moreover the published reports on chromosomal evaluation of generalized bone diseases all mention a normal karyotype. These results are to be expected. Indeed the hereditary bone diseases concern faulty metabolic conditions, determined by pathologic enzymatic factors, which in their turn can depend from one pathologic gene. Otosclerosis belongs to the group of qualitative genetic diseases, characterized by mendelian inheritance and is not determined by abnormal chromosomal gene quantity. It has to be stressed that before linking a cytogenetic finding to a phenotypical abnormality, one has to be sure that the cytogenetic anomaly is not an in vitro phenomenon, and if not, that it occurs in a sufficient amount of cells to be significant.

Summary

Chromosomal evaluation was done in 62 patients affected with otosclerosis, clinically and surgically proven. Numerical and structural karyotype analysis of leucocyte and fibroblast cultures, showed normal results.

Cytogenetic findings published earlier by other authors in a small series of otosclerosis patients and where mosaicism in the D-group was detected, are contradicted.

The importance of the critical evaluation of the cytogenetic method used and of the necessity to investigate cytogenetically a large enough number of patients, before linking a clinical and phenotypical condition to cytogenetic finding, is stressed.

Bibliography

ABRISQUETA J. A. (1966). Chromosome study of different phenotypical deviations. *Hum. Chrom. Newsletter*, **18**: 4.

ALBRECHT W. (1923). Ueber die Vererbung der konstitutionell sporadischen Taubstummheit und der Otosklerose. *Arch. Ohr. Nas. Kehlheilk.*, **110**: 15.

— (1940). Erbbiologie und Erbpathologie des Ohren und des oberen Luftwage. *Hdb. Erbbiol.*, **4**: 83.

— (1932). Die Otosklerose. *Z. H.N.O.*, **29**: 55.

ALEXANDER G. (1927). Die Ohrenkrankheiten im Kindesalter. Verlag. F.C.W. Vogel, Leipzig, 158.

ALTMAN (1960). Histopathology and etiology of otosclerosis. *Int. Symp. Detroit*, 15.

APERT E. (1928). Les hommes de verre. *Presse Med.*, **36**: 805.

— (1965). Otospongiose et maladie de Lobstein. *In CLERC*: Problèmes Actuels d'O.R.L. Maloine, Paris.

ARDOUIN P. (1961). Les facteurs biologiques dans l'otosclérose. *Rapp. I, 7e Congr. Int. Oto-Rhino-Laryng.*, Paris *Adv. Oto-Rhino-Laryng.*, **8**: 113 (Karger, Basel/New York).

ARSLAN M., RICCI V. (1960). L'otosclerosi può essere considerata come una malattia del collageno? *Otolaring. Ital.*, **29**: 6.

ASPILLAGA M. J. *et al.* (1964). Chromosomal endoreduplication in a case of testicular feminization. *Lancet*, **1**: 937.

BAIN A. D., GAULD I. K. (1964). Chromosome endoreduplication in cultures of necropsy spleen. *Lancet*, **I**: 936.

BAST TH., ANSON B. J. (1949). The Temporal Bone and the Ear. Thomas Ed., Springfield.

BAUER J., STEIN C. (1925). Vererbung und Konstitution bei Ohrenkrankheiten. Beiträge - Klinischen Konstitutionspathologie. *XI Z. Menschl. Vererb. Konstitutionsl.*, **10**: 483.

— — (1936). Konstitutionspathologie in der Ohrenheilkunde. Spinger, Berlin.

BERBERICH J. (1928). Cholesterinstoffwechsel und Gehörorgan. *Deutsch. Med. Wochschr.*, **54**: 514.

BEZOLD (1961). *Cit. by* MÜLLER: Zur Histopathologie der Otosklerose. *Z. Laryng. Rinol.*, **40**: 455.

BORGHESAN (1946). L'insorgenza della sordità nella sindrome di Van der Hoeve. *Atti. Clin. O.R.L.*, Palermo.

BRÜHL (1926). Otosklerose Handbuch. *H.N.O.*, Berlin, **7**: 409.

CAWTHORNE T. (1955). Otosclerosis. *J. Laryng. Otol.*, **69**: 437.

CHUMLEA B. (1942). A pedigree of otosclerosis. *J. Hered.*, **33**: 98.

CLERC (1965). Otospongiose et maladie de Lobstein. Problèmes actuels d'O.R.L. Maloine, Paris, 145.

CONEN, ERKMAN (1963). *Abstr. Am. Assoc. Pathol. Bacteriol.*, Cincinati, **28**: 28.

COOPER H. L., HIRSCHHORN K. (1964). Enlarged satellites as a familial chromosome marker. *Amer. J. Human Genet.*, **14**: 107.

DAVENPORT CH. B. *et al.* (1933). The genetic factors in otosclerosis. Carnegie Institute. Eugenic Record Office. Bull. nº 27, *Arch. Otolaryng.*, **17**: 135, 340, 503.

DE JORGE F. B. *et al.* (1965). Some biochemical findings in otosclerosis. *Ann. Otol. St. Louis*, **74**: 189.

DE LA CHAPELLE A. *et al.* (1963). Enlarged short arm or satellite region. A heritable trait probably unassociated with developmental disorder. *Cytogenetics*, **2**: 129.

DEMYER W. *et al.* (1963). Familiar alobar holoprosencephaly (arrhinencephaly) with median cleft lip and palate. Report of a patient with 46 chromosomes. *Neurology*, **13**: 913.

DEUNIER (1962). Troubles de l'appareil auditif et manifestations ophtalmologiques associées. *Rapp. Soc. Franç. ORL*, 108.

DUMARS K. W., GASKILL C. (1964). Pathological conditions with apparently normal chromosomes. *Hum. Chrom. Newsletter*, **12**: 2.

EDWARDS T. H. (1963). Terminology of trisomic syndromes. *Lancet*, **II**: 197.

ELLIS J. R., PENROSE L. S. (1961). Enlarged satellites and multiple malformations in the same pedigree. *Ann. Hum. Genet.*, **25**: 159.

ENGSTROM H. (1940). Ueber das Vorkommen der Otosklerose nebst experimentellen Studien über chirurgische Behandlung der Krankheit. *Acta Oto-laryng.*, Suppl., **43**: 1.

FARBER P. A. *et al.* (1966). Cytogenetic studies of selected patients. *Hum. Chrom. Newsletter*, **18**: 9.

FOWLER E. (1949). The incidence and degrees of blue sclerae in otosclerosis and other disorders. *Laryngoscope*, **59**: 406.

— (1954). Otosclerosis in identical twins. *J. Amer. Med. Ass.*, **154:** 304.
— (1958). Otosclerosis in 10 pairs of identical twins. *Ann. Otol.*, **67:** 889.
Frank O. (1923). Die Leitung des Schalles im Ohr. *S.-B. bayer. Akad. Wiss. Math.-Phys. Kl.*, **53:** 11.
Fraser (1932). Das Klinische Bild der Otosklerose. *II Int. Kongr. O.R.L.*, Madrid **1:** 59.
Frost H. M. (1960). Observations on the fundamental nature of otosclerosis. Otosclerosis, Ed. H. F. Schunknecht, Churchill Ltd., London, 54.
Funch (1965). *Cit. by* Clerc: Problèmes actuels d'O.R.L. *Maloine*, Paris.
Geitler L. (1953). Endomitose und endomitotische Polyploidisierung. Protoplasmatologia VI/C, Springer, Wien.
Gillain (1965). *Cit. by* Clerc: Problèmes actuels d'O.R.L. *Maloine*, Paris.
Giraud P. *et al.* (1963). Clinical conditions with a normal chromosome complement. *Hum. Chrom. Newsletter*, **9:** 5.
Gradenigo (1924).La sordità ereditaria. *Arch. Ital. Psicol.*, **4:** 45.
Gray J. E. *et al.* (1962). Pericentric inversion of chromosome 21. A possible further cytogenetic mechanism in mongolism. *Lancet*, **I:** 21.
Greifenstein A. (1935). Vergleichende Untersuchungen zur Histologie der Otosclerose. *Arch. Ohr.-Nas-Kehlk.-Heilk.*, **139:** 14.
Guild S. R. (1944). Histologic otosclerosis. *Ann. Otol. St. Louis*, **53:** 246.
— (1950). The profession of empaired hearing during childhood. *Laryngoscope*, **60:** 885.
— (1953). Does otosclerosis cause cochlear nerve degeneration? *Trans. Amer. Acad. Ophthal. Otolaryng.*, **57:** 356.
Haïke H. (1928). Zum Erbgang der Otosklerose. *Arch. Rass. Ges. Biol.*, **20:** 155.
Hammerschlag V. (1904). Zur Frage der Vererbbarkeit der Otosklerose. *Wien. Klin. Wschr.*, **1.**
— (1934). Einführung in die Kenntnis einfacherer mendelistischer Vorgänge. Perles, Wien/Leipzig.
Hernandez-Orozso F., Courtney G. T. (1965). Otosclerosis in identical twins. A genetic and clinical study. *Ann. Otol. St. Louis*, **74:** 252.
— (1964). Genetic aspects of clinical otosclerosis. *Ann. Otol. St. Louis*, **73:** 632.
Huizing E. H. *et al.* (1965). Studies of progressive hereditary deafness in a Dutch family of 335 members. *Ned. T. Geneesk.*, **109:** 499.
Hustin A. (1951). L'hérédité en O. R. L. *Acta Oto-Rhino-Laryng. Belg.*, **5:** 387.
Jackson J. F. (1963). Polyploidy and endoreduplication in human leucocyte cultures treated with mercaptoethanol. *Exp. Cell Res.*, **31:** 194.
Jennings A. N., Turner B. (1961). Autosomal chomosome anomalies. *Med. J. Aust.*, **2:** 830.
Joseph R. B., Frazer J. P. (1964). Otosclerosis incidence in Caucasians and Japanese. *Arch. Otolaryng.*, **80:** 256.
Juers A. (1950). Otosclerosis in identical twins. *Ann. Otol. St. Louis*, **59:** 205.
Kabat C. (1943). A family history of deafness. *J. Hered.*, **34:** 377.
Keiser D. (1952). Syndroom van Waardenburg. *Ned. T. Geneeskunde*, **96:** 2541.
Kluyskens P., Geldof (1965). La surdité héréditaire. *Acta O.R.L. Belg.*, **4:** 19.
Kobrak (1950). Physiology and pathology of sound conduction in the ear. *Trans. Am. Acad. O.O.*, **54:** 708.
Koch C., Serra M. (1962). Ipoacusia del mongolismo. *Arch. Ital. Laring, Suppl.*, **70:** 45.
Körner O. (1905). Das Wesen der Otosklerose im Lichte der Vererbungslehre. *Z. Ohrenheilk.*, **50:** 98.
Lange W. (1929). Über die Anfänge der otosklerotischen Herderkrankung. *Z. Hals-Nas.-Ohrenheilk.*, **25:** 1.
Larson (1963). Genetic problems in otosclerosis. *Brown*, Boston.
Larsson A. (1960). Otosclerosis. A genetic and clinical study. *Acta Otolaryng. Suppl.*, 154.
— (1960). Otosclerosis. Detroit, 109.
Leicher H. (1925). Weitere Beiträge zur Serodiagnostik der Otosklerose und ihre Beziehung zur Geschwulstforschung. *Münch. Med. Wschr.*, **72:** 762.
Leiri (1951). Is there an electrochemic factor in the pathogenesis of otosclerosis? *Acta O.L.*, **39:** 395.
Lejeune J. (1964). The 21 trisomy, current stage of chromosomal research. *Progress in Medicinal Genetics.* A. G. Steinberg and A. G. Bearn, **III:** 143, Grune & Stratton, New York, London.
Lennartz K. L., Rudolf G. (1959). Entwicklungsanomalien am glomerulären Apparat der menschlichen Niere. *Z. Anat. Ent. Gesch.* **121:** 38.
Lin H. M., Potter E. L. (1962). Development of the human pancreas. *Arch. Path.*, **74:** 439.

LINDSTEN J. (1963). The nature and origin of X chromosomal aberration in Turner Syndrome. Almkvist, Stockholm-Uppsala.
LIVERIERO E. *et al.* (1962). Fattori ereditari in otorinolaringologia. Tip. Grovamir Capella., 123.
LOZZIO C. B. (1966). Clinical conditions with apparently normal chromosomes. *Hum. Chrom. Newsletter*, **18:** 13.
MAGGIO (1939). L'escrezione urinaria dei 17 chetosteroidi totali nell'otospongiosi. *Arch. Ital. Otol.*, **60:** 37.
MANASSE (1922). Neue Untersuchungen zur Otosklerosefrage. *Z. Ohrenheilk.*, **82:** 76.
MARIE J. *et al.* (1958). Nanisme avec surdi-mutité et rétinite pigmentaire (syndrome de Cockayne). *Arch. Franc. Pediat.*, **15:** 1101.
— *et al.* (1958). Nanisme avec rétinite pigmentaire et surdité (syndrome de Cockayne). *Ann. Pediat.*, **34:** 500.
MARIN-PADILLA M. *et al.* (1964). Anatomic and histopathologic study of two cases of D_1 (13-15) trisomy. *Cytogenetics*, **3:** 258.
MAURER (1960). Otosklerose und Osteopsatyrosis. *Z. Laryngol. Rhin.*, **35:** 192.
MAYER O. (1931). Referat über die patholog-anat- und Pathogenese der Otosklerose. *Acta. Otolar.*, **15:** 35.
— (1922). Der gegenwärtige Stand der Otosklerosefrage. *Int. Zbl. Ohrenheilk.*, **19:** 257.
MEYER M. (1939). Otosklerose. *Mschr. Ohrenheilk.*, **73:** 140.
MORISHIMA A. *et al.* (1962). Asynchronous duplication of human chromosomes and the origin of sex chromatin. *Proc. Natl. Acad. Sci. U.S.*, **48:** 756.
MÜLLER (1961). Zur Histopathologie der Otosklerose. *Z. Laryng. Rhin.*, **40:** 45.
NAGER F. R. (1939). Zur Klinik und pathologischen Anatomie der Otoscklerose. *Acta Oto-laryng.*, **27:** 542.
— (1951). Ein paar Eineiige Zwillinge mit Otosklerose. *Acta Oto-laryng.*, **39:** 300.
— MEYER (1932). Die Erkrankungen des Knochensystems. Karger, Basel.
—, DE REYNIER (1948). Das gehörorgan bei den angeboren Kopf-missbildungen. *Karger*, Basel.
NAGER F. (1921). Otosklerose bei infantiler osteopsathyrosis und Blaufärbung der Skleren. *Schweiz. Med. Wschr.*, **2:** 66.
NYLEN L. (1949). Histopathological investigations on otosclerotic foci. *J. Laryng.*, **63:** 321.
OGILVIE, HALL (1962). On aetiology of otosclerosis. *J. Laryng.*, **76:** 841.
PELLEGRINI G. *et al.* (1962). Syndrome d'ankylose stapedo-vestibulaire au cours d'une maladie d'Albright. *J. Franç. O.R.L.*, **11:** 1113.
PFEIFFER R. A., KOSENOW W. (1962). Further cases with apparently normal pattern. *Hum. Chrom. Newsletter*, **6:** 3.
ROSENBAUW (1965). *Cit. by* CLERC: Problèmes actuels d'O.R.L., Maloine, Paris, 145.
RUEDI L. (1961). Histopathologische Veränderungen im Innenoht bei Otosklerose. Rapp. I. 7e Congr. Int. Oto-Rhino-Laryng., Paris 1961. *Fortschr. Hals.-Nas.-Ohrenheilk.*, **8:** 77 (Karger, Basel/ New York).
—, SPÖNDLIN H. (1957). Die Histologie der otosklerotischen Stapesankylose im Hinblick auf die chirurgische Mobilisation des Steigbügels (Histologisches Atlas). *Fortschr. Hals-Nas-Ohrenheilk.*, **4:** 1.
RUTTIN (1922). Osteopsathyrose-Otosklerose. *Z. Hals.-Nas.-Ohr.*, **13:** 263.
SCHEIBE (1895). A histological contribution to deaf-mutism. *Arch. Otol.*, **24:** 280.
SCHMID W. (1962). A familial chromosome abnormality associated with repeated abortions. *Cytogenetics*, **1:** 199.
SCHUKNECHT (1960). Otosclerosis. *Detroit*, 97.
— (1966). Temporal bone studies in 13-15 and 18 trisomy syndromes. *Arch. Otolaryngol.*, **83:** 5, 439.
SCHWARZACHER H. G., SCHNEDL W. (1965). Endoreduplication in human fibroblast cultures. *Cytogenetics*, **4:** 1.
SCHWARTZE (1893). *Handb. Ohrenheilk.* Leipzig, **2:** 4.
SEIFERT (1938). Ueber die Aetiologie und Pathogenese der Otosklerose. *Arch. Ohr.-Nas.-Kehlk. Heilk.*, **145:** 390.
SERCER A. (1961). Anatomie macroscopique de l'Otosclérose. Rapp. I, 7e Congr. Int. Oto-Rhino-Laryng., Paris 1961. *Progr. Oto-Rhino-Laryng.*, **8:** 24, (Karger, Basel/New York).
SHAMBAUGH J. *et al.* (1956). Otolaryngology. *Hagerstown.*
— (1956). Otosclerosis. *In:* Coates M., Schenk H. P., Miller M. V., Otolaryngology. Hagerstown, Pa.: W. F. Prior CO. Inc. **2:** 2.
— (1960). Otolaryngology, 269.
SIEBENMANN F. (1911). Totaler knöcherner Verschluss beider Labyrinthfenster und Labyrinthitis serosa infolge progressiver Spongiosierung. *Verh. Deutsch. Otol. Ges.*, **267:** 12.

Stadil P. (1961). Examens histologiques de la peau dans le syndrome de Van der Hoeve. *VII Congr. Int. O.R.L.* (Paris).
Stevenson R. E. *et al.* (1966). Possible autosomal isochromosome in a malformed child. *Amer. J. Dis. Child.*, **111**: 330.
Tato J. M. *et al.* (1963). Chromosomal study in otosclerosis. *Acta Oto-Laryng.*, **56**: 263.
Tinkle W. (1933). Deafness as an eugenical problem. *J. Hered.*, **24**: 13.
Tobeck A. (1953). Ergebnisse der Beweglichkeitsprüfung des Steigbügels bei Labyrinthfenstering. *Z. Laryng. Rhinol.*, **32**: 168.
Touraine A. (1955). L'Hérédité en Médecine. 400.
Uffenorde (1922). Die Prüfung des Hörnervenapparates mit der C^5 Stimmgabel. *Deutsch. Med. Wschr.*, **48**: 120.
Valdmanis A., Mann J. D. (1964). Chromosome endoreduplication. *Lancet*, **I**: 1452.
Van der Hoeve (1917). Blauwe sclera, broosheid van het beenstelsel en gehoorstoornissen. *Nederl. T. Geneesk*, **1**: 1003.
Van Leeuwen (1953). *Z. Klin. Med.*, 534.
— (1955). *Diss. Leiden.*
Voss O. (1927). Stoffenwechselstörungen und Ohr. Karger, Berlin.
Weber M. (1936). Zur Frage des Erbganges der Otosklerose. *Erbbl. Hals.-Nas.-Ohrenartz*, **1**: 3.
— (1935). Otosklerose und Umbau der Labyrinthkapsel. Offizin Poescl, & Trepte, Leipzig.
— (1961). Die Histopathologie der Otosklerose. Die Knochenveränderungen. Rapp. I, 7e Congr. Int. Oto-Rhino-Laryng., Paris 1961. *Fortschr. Hals-Nas.-Ohrenheilk.*, **8**: 44.
Wildervanck L. S. (1964). The significance of associated anomalies in deafness. U.S. Goverm. Print. Office, Washington, 632.
Wittmaack K. (1919). Die Otosklerose auf Grund eigener Forschungen. Fischer, Jena,
Yunis J. J. *et al.* (1964). Desoxyribonucleic-acid-replication pattern of trisomy D_1. *Lancet*, **II**: 935.
— Hook E. B. (1965). *In press.*

RIASSUNTO

È stato effettuato l'esame cromosomico di 62 pazienti di otosclerosi dimostrata clinicamente e chirurgicamente. L'analisi di culture di leucotici e fibroblasti è risultata numericamente e strutturalmente normale. I risultati citogenetici pubblicati da altri Autori per piccole serie di otosclerotici che avevano indicato mosaicismo del gruppo D sono controversi. Viene sottolineata l'importanza di una valutazione critica della metodologia impiegata e la necessità di un esame citogenetico condotto su serie numerose di malati, prima di concludere per una relazione fra manifestazione clinica ed eventuale anomalia cromosomica.

RÉSUMÉ

Un examen cytogénétique a été fait chez 62 malades atteints d'otosclérose, démontrée tant au point de vue clinique que chirurgical. L'analyse numérique et structurale des karyotypes, provenant de cultures de leucocytes et de fibroblastes, donne des résultats normaux. Les observations cytogénétiques, faites par d'autres auteurs dans quelques cas d'otosclérose et consistant en l'existence d'un mosaïcisme dans le groupe D, n'ont pas pu être confirmées. Les auteurs soulignent l'importance de l'appréciation critique de la technique cytogénétique utilisée et la nécessité de faire un examen cytogénétique chez un grand nombre de malades avant de conclure à une relation entre une manifestation clinique et phénotypique et une anomalie cytogénétique éventuelle.

ZUSAMMENFASSUNG

Bei 62 P. mit klinisch und chirurgisch festgestellter Otosklerose wurde eine Chromosomenuntersuchung vorgenommen. Das Ergebnis der Kulturen aus Fibroblastenleukozyten war sowohl zahlen- als strukturmässig normal. Die von anderen Verfassern angeführten Ergebnisse mit kleineren Reihen otosklerotischer P., aus denen ein Mosazismus der Gruppe D hervorgeht, widersprechen sich. Es wird daher betont, wie wichtig es sei, die angewandte Methodik kritisch zu bewerten und wie notwendig es ist, eine zytogenetische Untersuchung mit zahlreichen Krankenreihen durchzuführen, bevor man auf eine Beziehung zwischen klinischer Manifestation und evtn. Chromosomenanomalie schliessen kann.