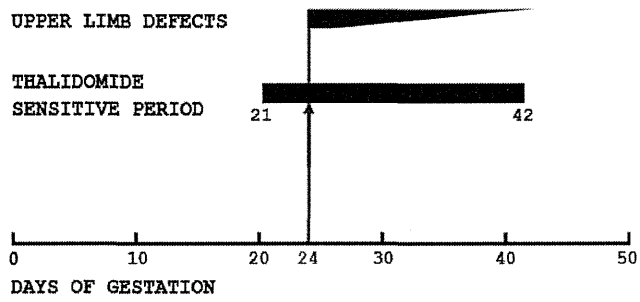
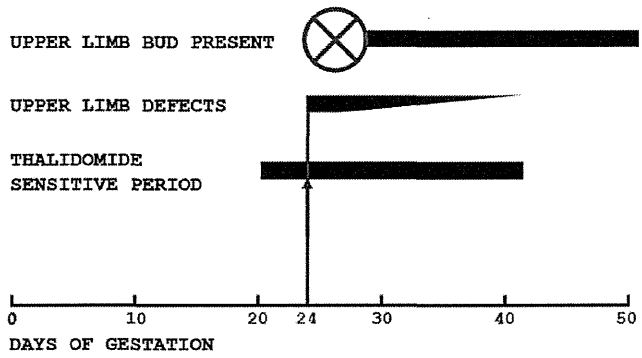
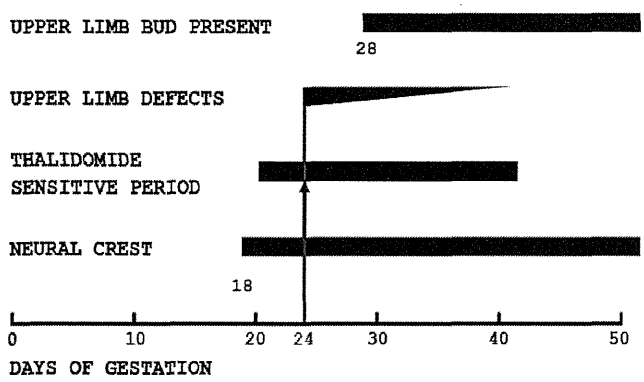



**2.The "sensitive period"** was established by Lenz as 21 to 42 days gestation (11). It possibly extends longer, but its commencement is not debated. Lenz emphasised that upper limb defects resulted from exposure from day 24 onwards, day 24 being the earliest date of exposure recorded with arm defects (arrow).

All embryology textbooks state that the upper limb bud does not appear until day 28.




Therefore thalidomide cannot act within limb buds - because they do not exist for another four days!
It follows that any hypotheses that are set within limb buds are refuted by these facts. Molecular and vascular hypotheses fall over because neither limb buds nor forelimb arteries exist when the drug attacks.




Neural crest, however, is present from day 18 of gestation (13) and is highly active during the thalidomide-sensitive period, dividing, migrating and sprouting axons (14). These activities make neural crest cells highly vulnerable to injury at this time.

## 3. Cranio-caudal sequence of thalidomide damage.

Lenz recognised that a wave of sensitivity swept down the embryo from head to tail between 21 and 42 days of life. Particular defects followed thalidomide exposure on particular days (12):

- between days 21 and 24 resulted in eye, ear, cranial nerve and other head and neck defects;
- exposure from day 24 resulted in arm defects;
- exposure from day 27 caused leg defects as well.

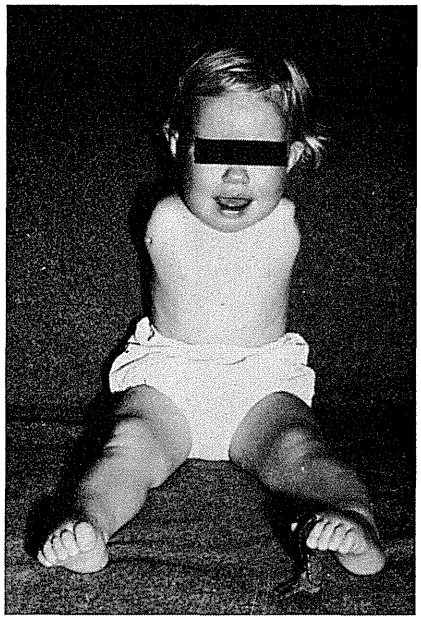
Nowack and Lenz concluded that "the morphological type of the birth defect was essentially a function of the TIME of exposure, not the dose" (12).

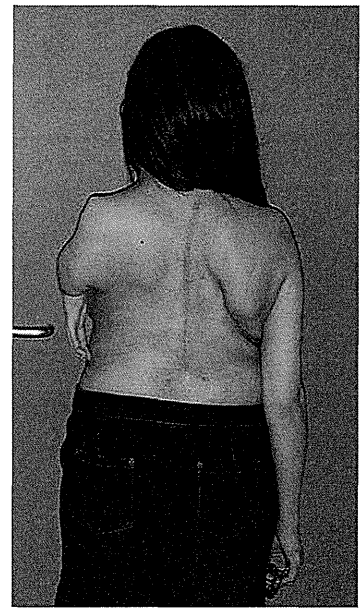
This data from humans is replicated in laboratory primates. Neubert's laboratory in Berlin found the same cranio-caudal sequence of occurrence in primates exposed to thalidomide. Upper limb defects resulted from exposure before the limb bud existed (15).

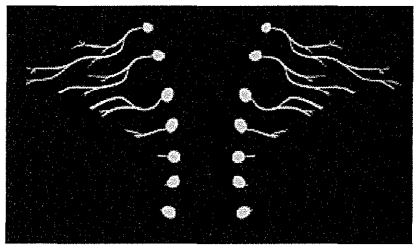
Neubert et al concluded that thalidomide must act within the torso of the embryo, deep to the site of the future limb bud. This is exactly where neural crest axons are infiltrating undifferentiated mesenchyme - axons of naked cytoplasm, unprotected and highly vulnerable.

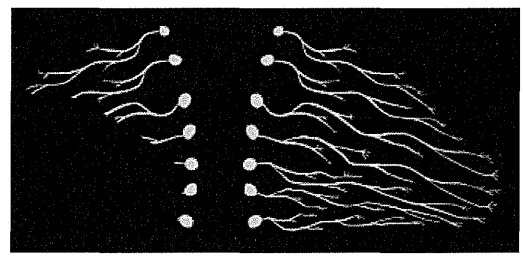
## 4. Symmetry versus asymmetry:

Publication of large series of thalidomide cases in the aftermath of the epidemic proved that the majority were bilaterally symmetrical (16). But all series had 15 - 25% of cases with asymmetrical or even unilateral deformities.









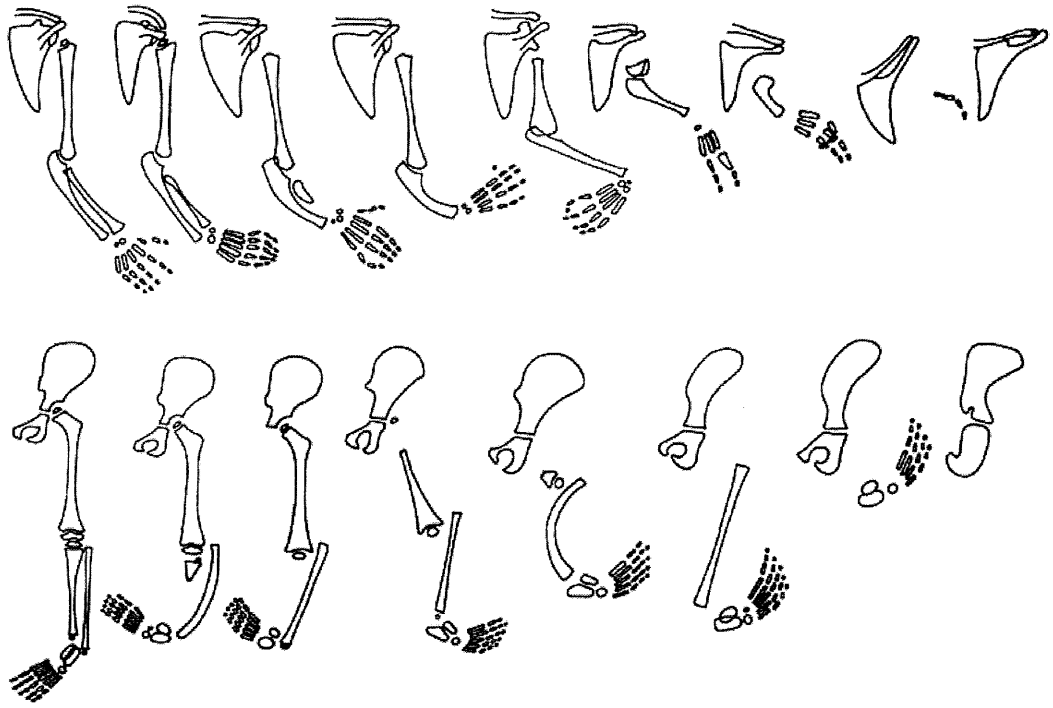
**80%**
**Symmetrical**

**20%**
**Asymmetrical**

I sought advice from Professor Nishimura, who had studied and staged thousands of normal Japanese abortuses. I asked him whether all human embryos develop the left and right sides of the body at the same time, in unison. He said that they do not! Professor Nishimura told me that 80% of human embryos develop symmetrically, but in about 20%, the left and right sides are out of step, by up to 4 embryonic days difference (17). This means that neural crest must develop asymmetrically in such embryos. It follows that 80% of thalidomide defects should be bilateral and symmetrical, and that 20% should be asymmetrical with a few even being unilateral.

## 5. Frequency of upper > lower limb defects.

This master diagram is from Henkel and Willert's landmark paper of 1969 (18), that defined the pattern of dysmelia in over 200 German thalidomide children.



Upper limb defects were most numerous, especially absent radius and thumb. Perhaps this is because nausea of pregnancy is characteristically early, sometimes the presenting symptom, around the time of the missed period. Nausea is usually short in duration, and a woman may take only one or two tablets, sufficient to target the neural crest at about day 24, causing arm rather than leg malformations.

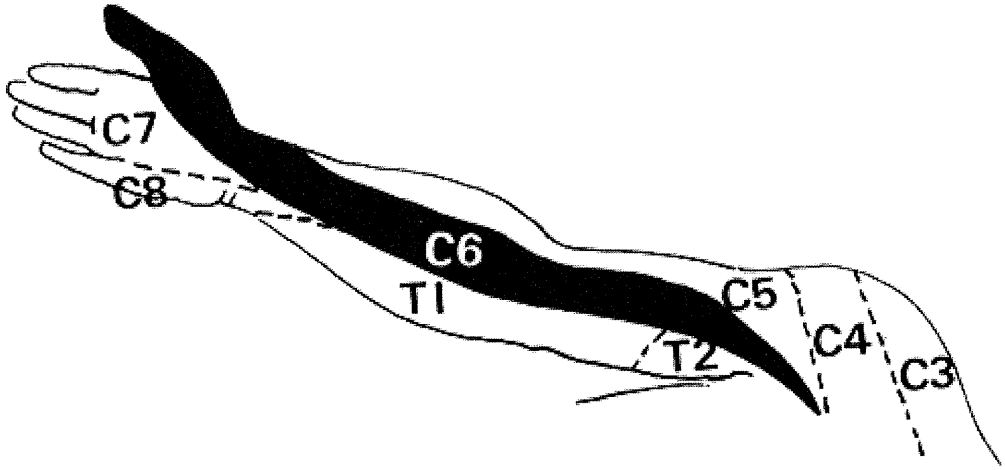
## 6. Sparing of central nervous system.

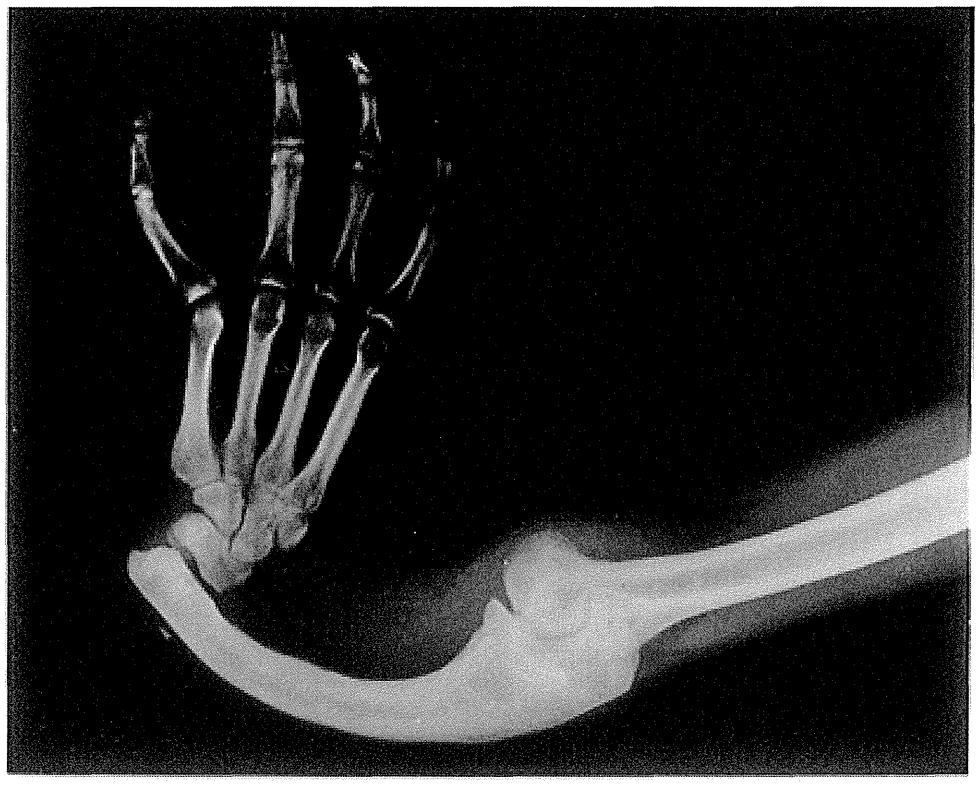
The brain and spinal cord in thalidomide were usually normal, but the peripheral nervous system (outside CNS) was not. The immense network of nerve fibres of peripheral nerves extend to every part of the body. Peripheral nerves have evolved for the purpose of signalling and communication throughout the bodies of all animals, including man. The basic cell in the sensory division of the peripheral nervous system is the bipolar neuron. As shown in the introduction, in human adults, thalidomide attacks this group of sensory neurons (derived from neural crest) not brain cells (derived from neural tube). In human embryos, thalidomide also targets neural crest, not tube. The target is consistent throughout life from embryo to adult.

## 7. Longitudinal reduction deformities of the limbs.

In the limbs, thalidomide deleted longitudinal bands of bone, and such defects were classified as "longitudinal reduction deformities". This distribution of disease, subdividing bones, is not consistent with bone pathology.
On the other hand, skin is innervated in longitudinal strips called dermatomes, each dermatome supplied by one spinal segmental sensory nerve.

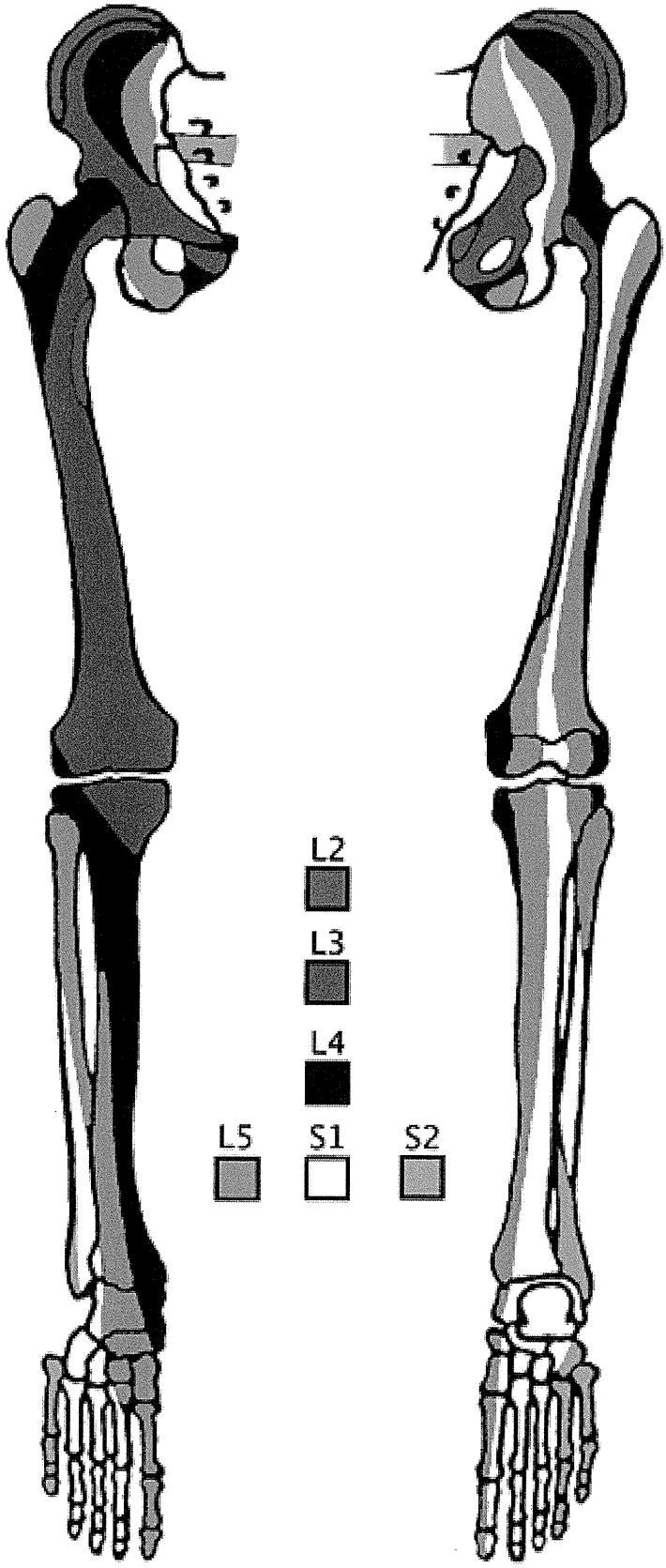
## C6 Dermatome Subtraction



Here the dermatome of the 6th cervical nerve is black. If it is excised together with the underlying bones, radial aplasia and absent thumb would result as shown in this Xray film of the most common upper limb defect in thalidomide embryopathy.

The skeleton has similar (but less well-known) segmental sensory innervation. The strips of segmental nerve supply of bones are called "sclerotomes". Based upon past literature plus their own experiments and clinical observations, Inman and Saunders of San Francisco drafted these maps of the segmental sensory nerve supply of the limb skeleton in 1944 (19).


L2
L3
L4
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S1
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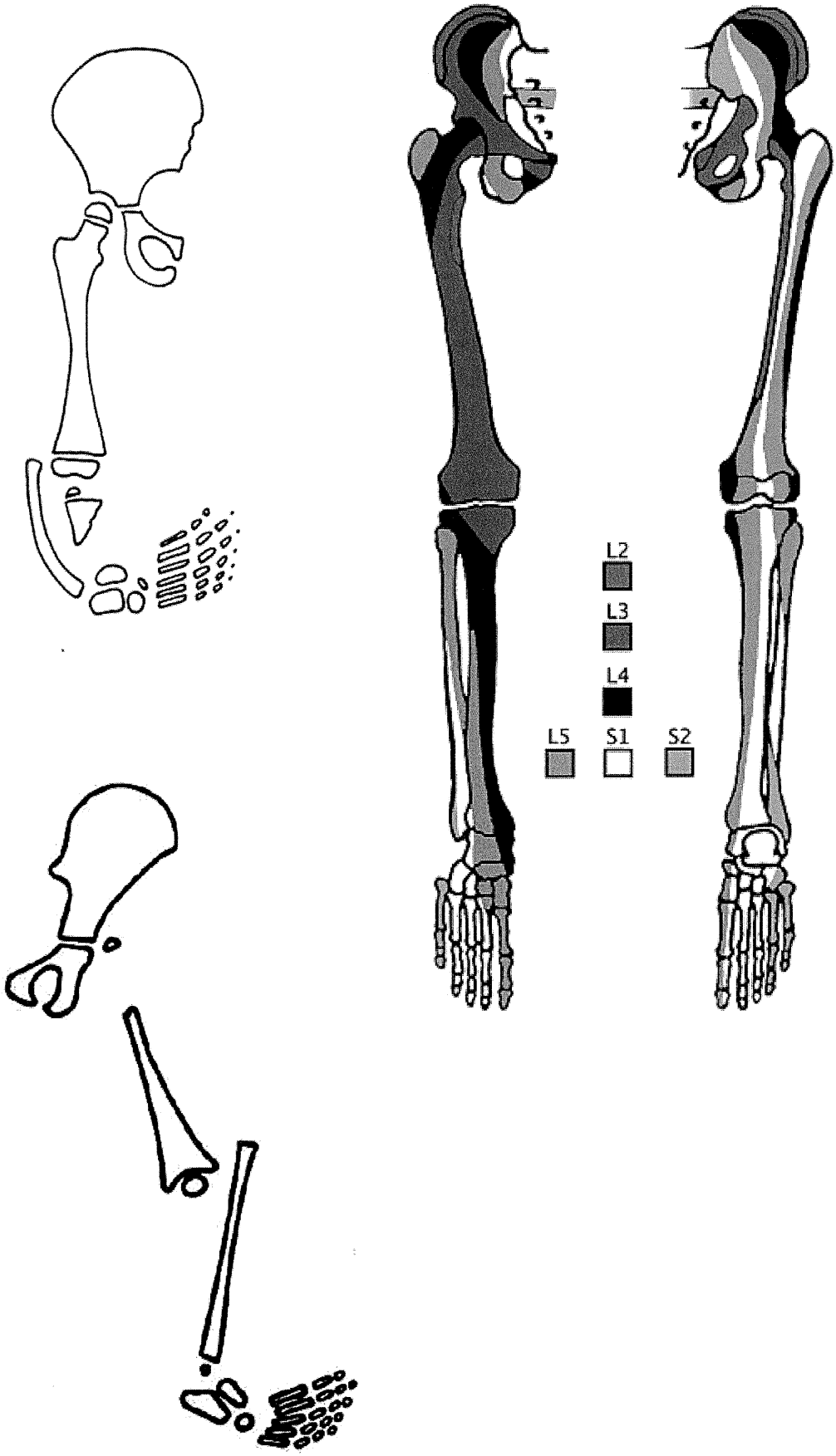
L2
L3
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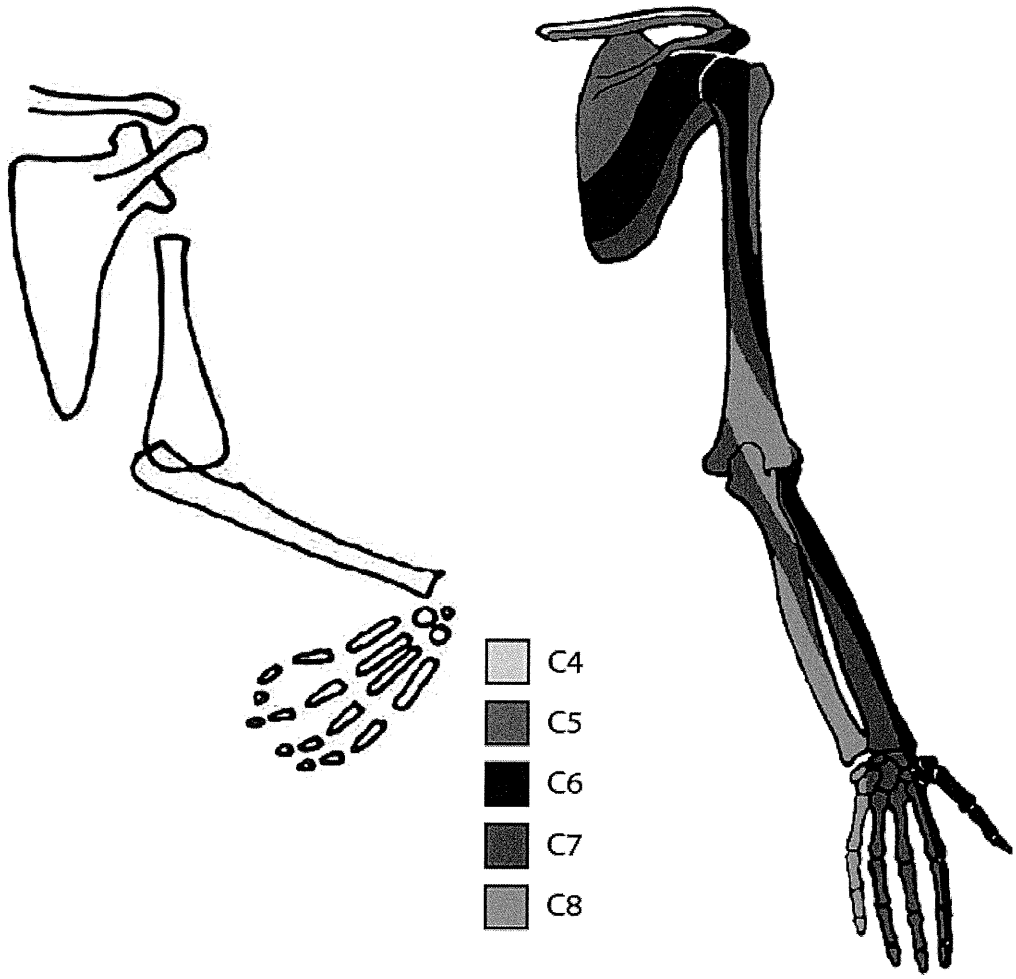
When I applied the sclerotome maps to the radiographs of thalidomide cases, the deformities emerged as failure of formation of sclerotomes (20).




That indicates some connection between sensory nerves and limb growth. Is there such a connection?
There certainly is, as has been proved in regeneration biology (21,22,23).

Sensory nerves possess an ability to make other cells divide. This particular ability is called "neurotrophism": neuro = nerve, trophism = growth.

Regeneration biologists know most about neurotrophism because limbs will not grow without sensory nerves (21,22,23). In studies of amphibian limb regeneration, these biologists have shown that sensory nerves stimulate mitosis of undifferentiated cells (21,22,23). Through neurotrophism, sensory nerves recruit mesenchyme cells by making cells divide, repeatedly, until a heap of undifferentiated cells creates a limb bud. This recruitment through mitosis was shown to begin from the inception of limb formation (22,23,24). The conclusion is that sensory nerves drive mitosis/cell division.

Although the biological principles were established in amphibia, the findings can be translated to human embryos, because the pentadactyl limb that results is similar throughout the vertebrate kingdom (25).

It follows that if a segmental sensory nerve is damaged, its neurotrophic ability fails, and mitosis of mesenchyme will fail downstream. This would cause one or more sclerotomes to drop out of the blueprint of the limb, along with any other structures supplied by the damaged nerve.

I took the sclerotome maps to Göttingen, where Professor Willert and I re-examined his collection of Xray films of over 200 thalidomide children. We wanted to see whether or not the maps of segmental sensory nerve supply could explain the pattern of thalidomide malformations. The sclerotome maps and thalidomide defects coincided

- exactly in 80%,
- fairly well in 15%,
- poorly in 5%

We concluded that (26)

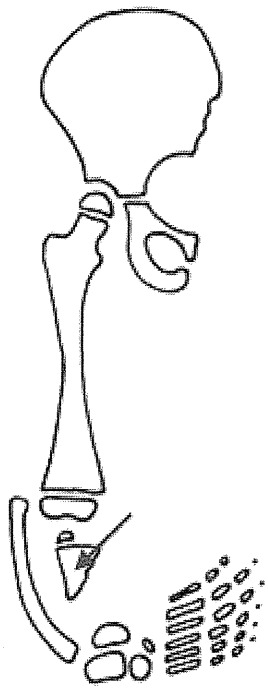
- both patterns are expressions of the underlying sensory segmental innervation of the skeleton, and that
- the sensory nervous system is involved in limb morphogenesis and teratogenesis.

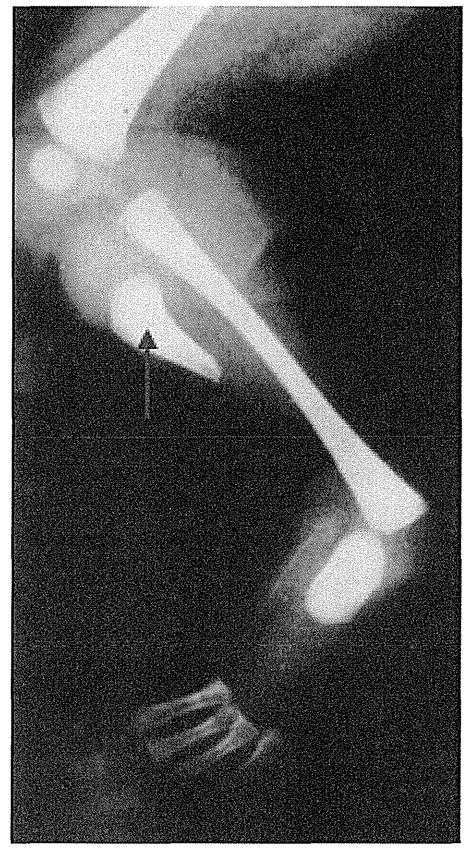
## 8. Triangular residual bone remnants:

These triangular remnants of tibia, femur and humerus were frequent. Only the sclerotome maps can explain them (26).

TIBIA:

The most curious remnant was a triangular piece of upper tibia bearing the tibial articular surface of the knee joint. The maps show that this is the distal end of the 3rd lumbar sclerotome. It lies beside the most vulnerable 4th lumbar sclerotome that is commonly deleted from the leg by thalidomide (26).

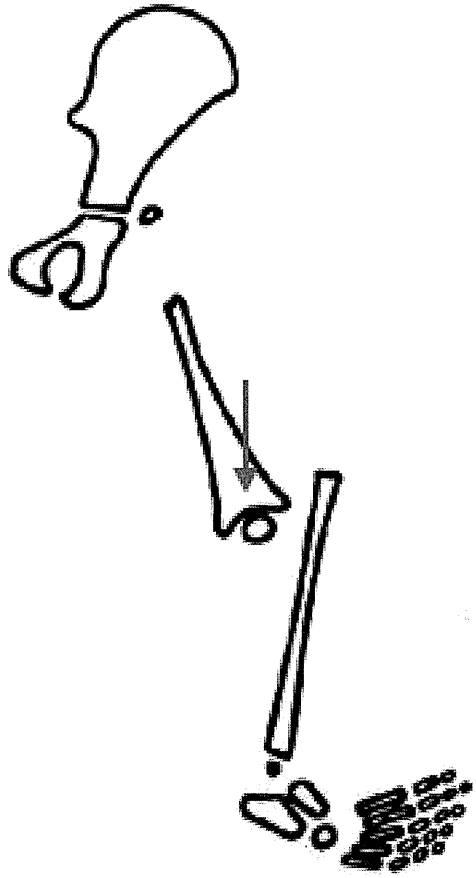


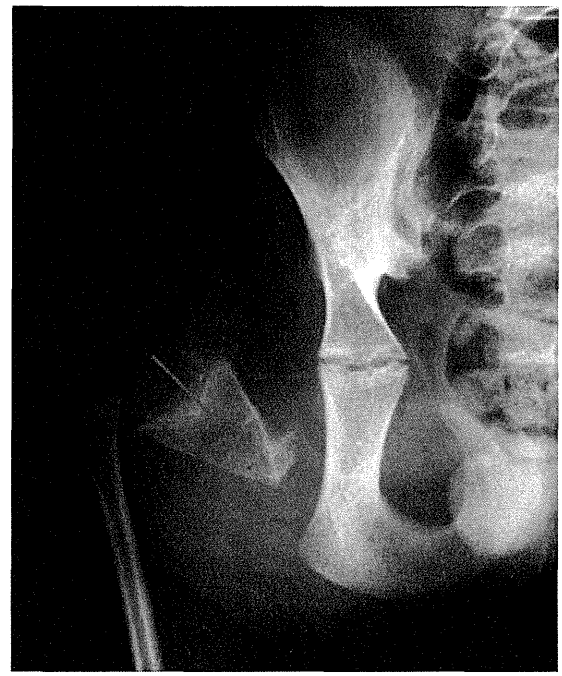


FEMUR:

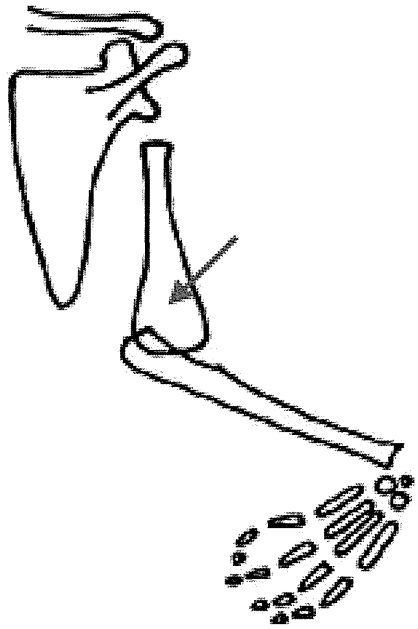
Proximal focal femoral dysplasia was common in thalidomide embryopathy but not confined to it. It occurs in other conditions.

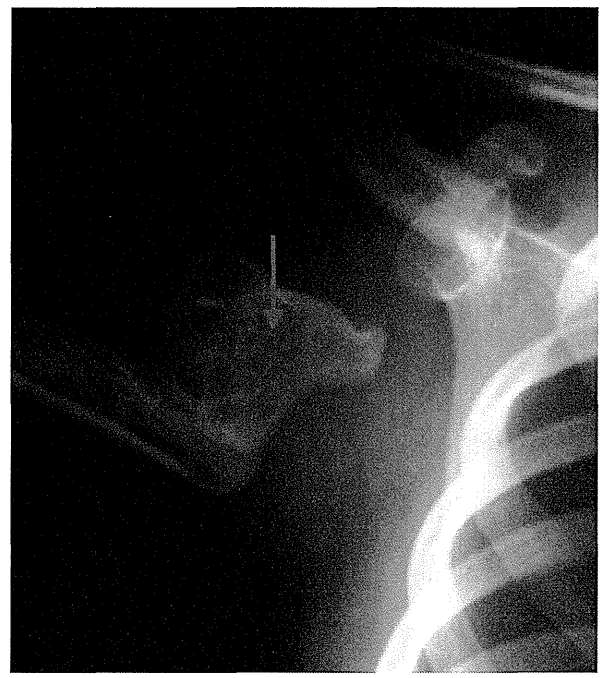
Loss of proximal femur (vulnerable 4th lumbar nerve) leaves an isosceles triangle of femur based at the knee. It matches the residual 3rd lumbar sclerotome of distal femur. So lumbar nerve 3 appears to resist thalidomide while lumbar nerve 4 is affected (26).

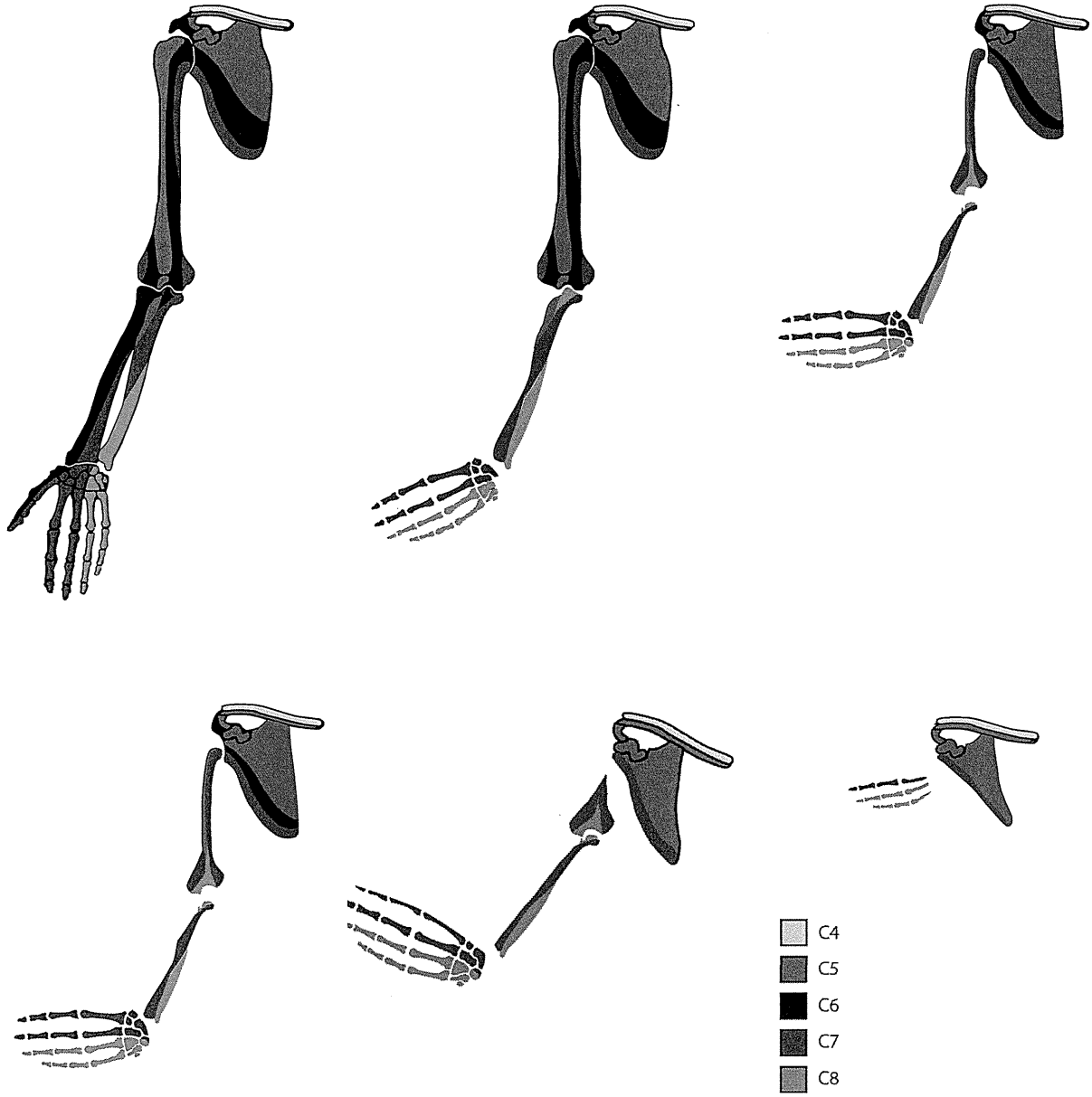




HUMERUS:
Phocomelic arms present variants of this particular complex.
The humerus is reduced to a distal triangle based at the elbow. The upper humerus has failed to form.
This happens when thalidomide deletes both 5th and 6th cervical nerves. What remains of the arm is supplied by the 7th and 8th cervical nerves.
The humerus is so reduced in length that the forearm and hand move up towards the shoulder.
This is phocomelia. It has different degrees of reduction depending on the amount of a sclerotome removed (26).
The external shape of the shoulder is pointed because of absence of musculo-skeletal tissue at shoulder and upper arm.



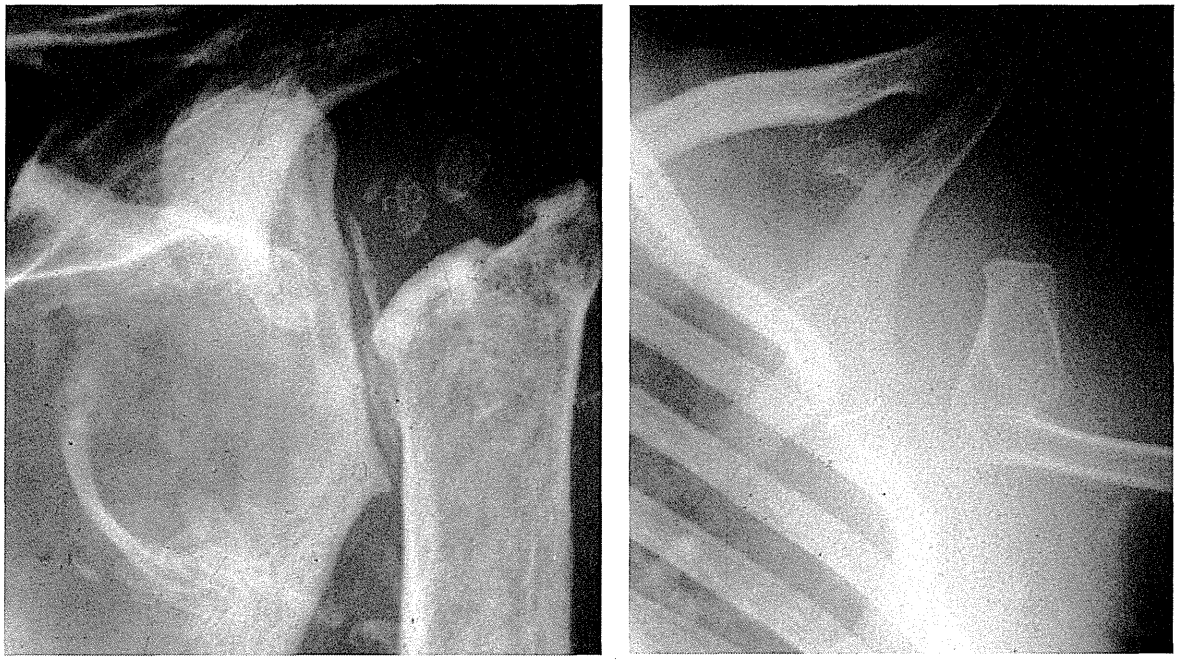





Progressive subtraction of sclerotomes in the arm by thalidomide to form phocomelia.

## 9. Dislocated joints

These were common in thalidomide embryopathy, and they had characteristic signs of neuropathic joints in their radiographs.



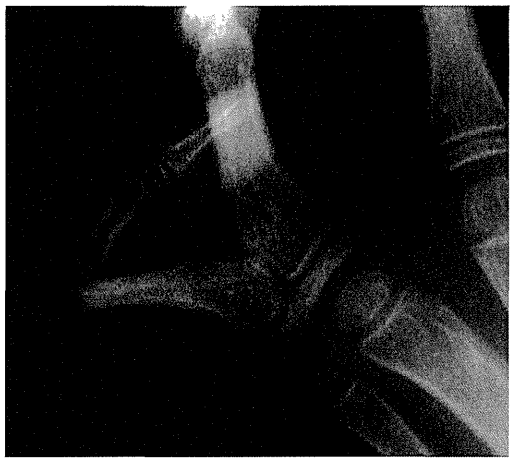
On the right is the shoulder joint of a thalidomide child. On the left is that of a woman with syringomyelia. Both show

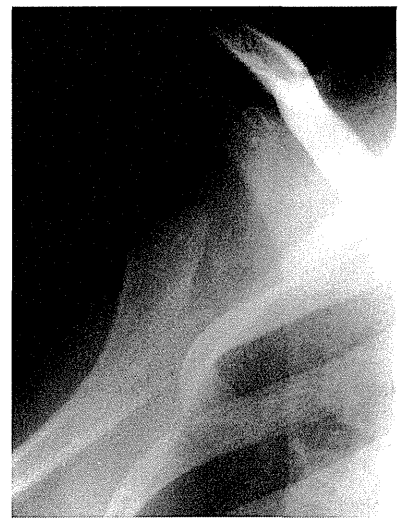
- flattened joint surfaces on both sides of the joint
- with no loss of bone density and
- painless dislocation

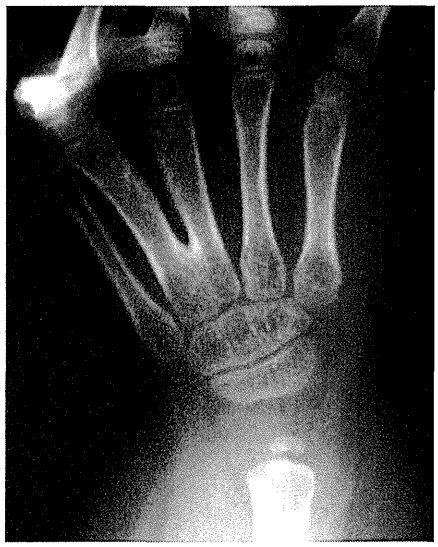
Adult neuropathic joints occur in long-standing sensory peripheral neuropathy (due to diabetes, syphilis, leprosy, syringomyelia and other conditions). That neuropathic joints appear in thalidomide embryopathy (27) is further evidence that sensory nerve damage occurred in the early embryo.

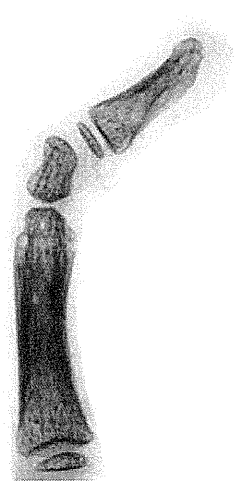
## 10. Synostoses:

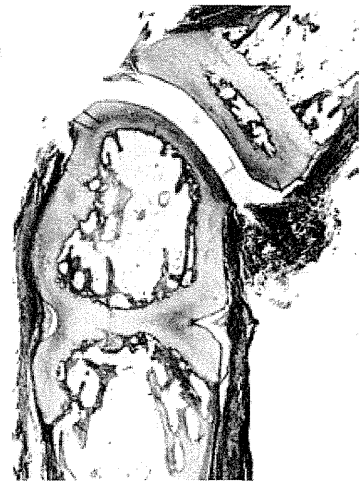
Fusion between bones was common in thalidomide embryopathy (28). Failure of neurotrophism in damaged nerves reduces the number of mitoses downstream.









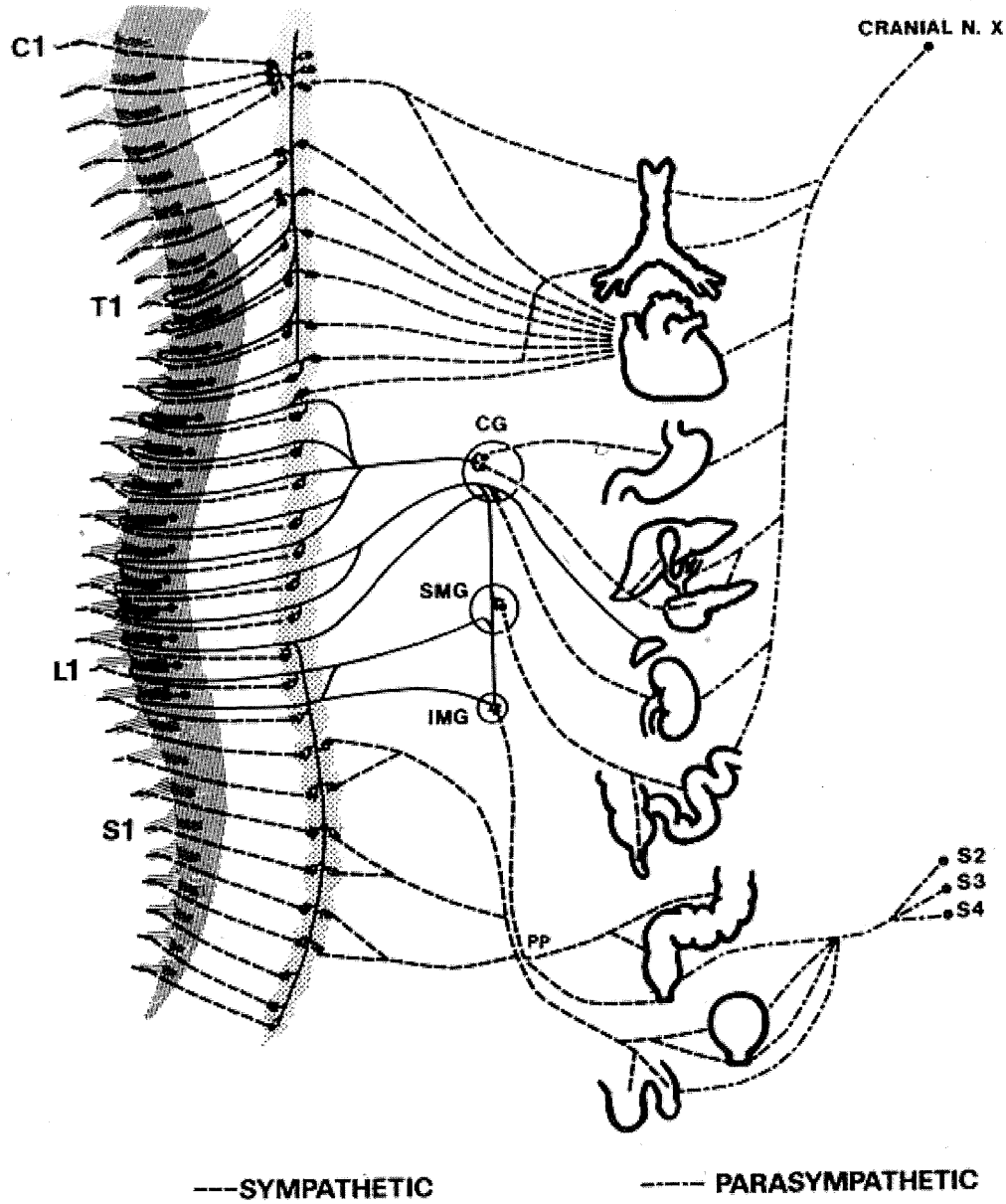


There is reduction in the final number of cells that are destined to differentiate into bone, so the final formation of the bone is reduced in mass in the area supplied by the damaged nerve. Incomplete formation leaves some bones unable to separate. This results in

- transverse fusion between paired bones (eg radio-ulnar, inter-phalangeal, carpal coalition), and
- longitudinal fusion between serial bones (eg humero-ulnar, interphalangeal).

## 11. Associated internal defects:

This large complex subject could easily fill a whole lecture. Here the autonomic division of neural crest comes into play, often associated with sensory lesions at the same spinal level.



One toxic assault upon early neural crest could injure both sensory and autonomic branches, with damage to internal organs as well as to limbs that share their nerve supply. This indeed occurred.

Evidence of such associations emerged in data published by the British Ministry of Health (29) in 1964. Over 90% of children with congenital heart disease also had upper limb phocomelia of major of moderate degree, indicating damage to cervical sympathetic as well as peripheral sensory nerves at the cervical level.

Similarly, leg reductions were associated with ano-rectal and genito-urinary defects, sharing lumbo-sacral nerve supply.

## 12. Pathology:

a. Thalidomide children:

Specimens of bones and joints from autopsies and amputations in thalidomide children were examined by Professor Willert and colleagues in pathology, searching for histopathology in bones and joints. However, no abnormal cells could be found in the skeletal tissues. The architecture of the skeleton was clearly abnormal, but no disease was found in bone or cartilage cells (30).

This is an important negative finding. The conclusion is that the primary pathology is not in the skeleton, but in some tissue outside it, with secondary effects upon the skeleton, consistent with primary nerve damage resulting in disordered skeletal architecture, as proposed by the theory of neural crest injury.

b. Adults with sensory neuropathy caused by thalidomide showed destruction of large diameter axons and abnormal histograms consistent with sensory neuropathology (2) (see introduction).

c. Rabbit fetuses exposed to thalidomide showed similar loss of large diameter axons in sciatic nerves, worst fetuses with absent tibia, and less severe in treated fetuses without skeletal defects (31,32).
North demonstrated that nerve damage preceded skeletal damage in thalidomide embryopathy.

d. The "post-thalidomide syndrome" can be explained by quantitative neuropathology.
All humans possess a generous surplus of axons in peripheral nerves, enough to buffer considerable axon loss without suffering any symptoms. All humans sustain a physiological loss of axons at middle age as part of the normal aging process. But they do not experience symptoms of neuropathy because the surplus axons buffer this physiological reduction.

However, a symptom threshold does exist: a minimum number of axons below which neuropathic symptoms occur - pain and paraesthesia in sensory nerve damage, and weakness, wasting or paralysis of muscles supplied by damaged motor nerves. Normally this threshold is never reached in undamaged nerves.

But the "post-thalidomide syndrome" now besetting middle-aged thalidomide survivors can be explained by quantitative change in axon numbers, with pathophysiology similar to that of "post-polio syndrome". Of course polio affects motor nerves, whereas thalidomide affects sensory nerves. However the quantitative mechanism is the same in each group: two episodes of axon loss in each case. First, there is severe loss of nerve axons in early life (in infancy or embryo) due to a pathological process (polio virus infection