7. OSSIFICATION STUDY

7.1. Experimental study:

As we had some difficulty in conducting the animal experimental study we decided to take up the work of Prof. Ilizarov in this work.

The study of bone tissue regeneration under the influence of tension stress was conducted utilizing the canine hind limb in a series of 4 experiments to evaluate the effect of blood supply preservation and fixation upon the quantity and quality of newly formed bone. In the first three groups, an open transverse osteotomy of the tibial diaphysis, periosteum, and bone marrow was performed.

In the remaining group closed osteoclasis was done and evaluated under conditions of stable fixation, but without local damage of the bone marrow and nutrient artery branches in the region of the osteotomy. Tension stress was created longitudinally. In all experimental groups, distraction started on the 5-7 days after the operation.

Wire Tension in group 1, one pair of crossed Krischner wires in each fragment with relatively marked mobility, in group 2, one pair of crossed wires with securely fixed, with reduced fragment mobility, in group 3 still more rigid fixation achieved by two pairs of wires in each fragment. Each pair of wires was tensioned and fixed to a separate ring, resulting in four-ring configuration. In-group 4, same four-ring construct with closed osteoclasis with out damage to nutrient artery, periosteum and bone marrow was done

Distraction was started after 5-7 days, 0.125mm every 6 hours, and in closed osteoclasis, 1.5 mm per day in 4 equal turns was done [14,21,].

After distraction, there was a period of 35 days neutral fixation lasting for 3-6 weeks; thereafter the fixator was removed. Removal of the fixator was followed by a period of observation lasting up to 6 months.

Within each experimental group, animals were killed immediately after osteotomy and 7 days, 14 days, 21 days, 28 days, 6 weeks, 2 months, 3 months 4, months, and 6 months after osteotomy.

The osteotomized regenerate [40, 41, 42,] and the contralateral limbs were removed from each dog and prepared for histological, biochemical, morphologic and angiographic studies. Viable tissue was obtained from the distraction zone to access lactic dehydrogenase, alkaline phosphotase, ATPase, and other substrates. Routine and special stains of the sections were prepared. Radiographic sections were obtained. Radio-angiographic studies were prepared by injecting some animals with radio opaque contrast materials, while India ink and other substances were used for microangiographic analysis.

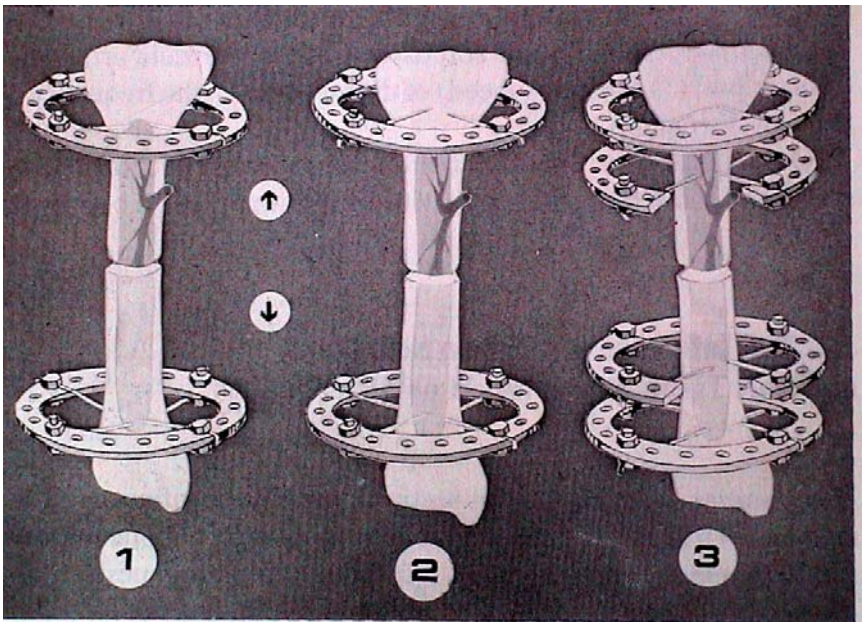

Figure 7.1

1. 2 rings, open osteotomy, loose wires,
2. 2 rings, open osteotomy, tensioned wires,
3. 4 rings, open osteotomy, tensioned wires,

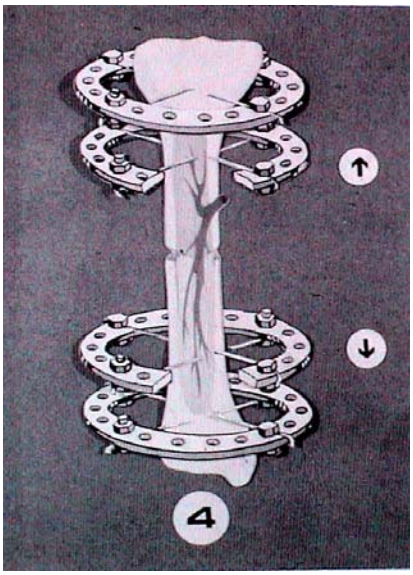

Figure 7.2

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4. 4 rings, closed osteoclasis, tensioned wires.

7.2. Results of experimental study

In Group 1 with considerable mobility between the bone ends demonstrated least osteogenic activity.14 th day, at 0.5 mm distraction per day was filled with poorly differentiated connective tissue containing large islands of cartilage in the distraction zone.28 th day, the distraction ends of the bone were sealed over with thin cone shaped osseous plates which were separated by a zone of fibrous tissue containing islands of cartilage.

In-group 2 with somewhat less motion between the bone ends, within the same time frame, there was substantially more osteogenic activity in the space between the ends. The gap was filled with cone shaped segments of regenerated osseous tissue separated by fibrocartilaginous layer. This tissue within the distraction gap had no definitive orientation. 35^{th} days, formations of cones of bone 1.5-2 mm height as the only osseous tissue within the regenerate zone. The middle part of the gap was occupied by cartilaginous connective tissue, indicating a low level of osteogenesis.

In-group 3 with rigid fixation, by 14^{th} day there was still more osteogenic activity in the space between the bone fragment ends and marrow canals. An even greater proportion of the space was filled with regenerated osseous tissue where osteoid trabeculae were intensively forming. As a result of this high level of osteogenic activity, the regenerate bone had already fused to the cortical plate of the proximal fragment in some animals. The connective tissue fibers, cells, and osseous trabeculae on the bone-forming region had a longitudinal orientation along the tension vector. By 35 days the gap was almost filled with regenerate bone and the formation of a new bone marrow canal within the distraction gap, and a circumferential cortex around it.

In-group 4, with maximum preservation of the medullary and periosteal bone forming elements by means of closed osteoclasis, it was necessary to distract the gap by 1.5-1.6 mm per day to prevent the intense osteogenesis from overtaking distraction and consolidating bone. On the 14 th day the osteotomy gap consolidated in some

animals, testifying to the intensity of the osteogenic process. 30th day of neutral fixation, the formation of a substantial cortical plate, covered with periosteum, surrounding the regenerate zone. Starting at the middle of the regenerate zone, the osseous trabeculae that thinned out in both proximal and distal directions, while the intra trabecular spaces came to be filled with increasing concentration of fatty and haematopoietic marrow cells. The same was observed in groups 3 and 4 on 77th day and 109th day respectively. By 103 days the newly formed cortex of the regenerate zone did not differ substantially from the old bone in either density or thickness. In fact in some areas it was even thicker and no well-defined boundary between the original bone and the newly formed osseous tissue. The same was observed in-group 3 and 4, on 139th and 169th day.

We have given the points for the mineralization according to the histological events i.e., fibroblasts formation, cartilage tissue formation, fibrocartilage, osseous tissue formation, remodeling etc. Accordingly separate graphs were drawn with the mineralization versus period in days.

The graphs clearly indicate that the group 4 has shown very good mineralization (bone formation) compared to other groups. The extent of mineralization is in the order of 4>3>2>1. The closed osteotomy (fixed rigid) experimental group has shown very good osteogenesis compared to other groups. *The mechanism of the bone formation has followed the first order kinetics.* The following mechanism has been proposed for mineralization.

G is the gap between the distracted ends. M is the mineralization.

$$G \rightarrow M$$

At the beginning of the reaction ($t = 0$) the value of G is g_0 and that of M is zero. If after time t the concentration of M is m, then the value of G is $g_0 - m$. The rate of formation of M is dm/dt, so that for a first order reaction

$$dm/dt = k\,(g_0 - m) \qquad 1.1$$

$$dm/(g_0 - m) = kdt \qquad 1.2$$

on integrating gives

$-ln\ (g_0 - m) = kt + k'$ 1.3

where *k'* is a constant of integration. This constant may be evaluated using the boundary condition that $m = 0$ when $t = 0$

hence $- ln\ g_0 = k'$

and insertion of this into 1.3 leads to

$ln\ (g_0 / (g_0 - m) = kt$ 1.4 $- m = go\ (1-e-kt)$

First order reactions can be tested and constant evaluated using graphical procedure. It follows from 1.4 that a plot of $ln\ (g0 / (g0 - m)$ against *t* will give a straight line if the order is first. The rate constant is slope.

The slopes for the 1st, 2nd, 3rd and 4th groups are 0.0173, 0.0256, 0.0312 and 0.035 respectively. The slope 0.035 for the group is higher than the other groups, indicating the increased rate of mineralization in the gap compared to other groups.

The graph (Figure 7.3) shows the ossification was proportional to the stability of the construct. Even though the construct of 3 and 4 are of equal stability, the ossification is better in group 4 where closed osteoclasis is done. This clearly apart from mechanical stability there is a biological element responsible for bone healing.

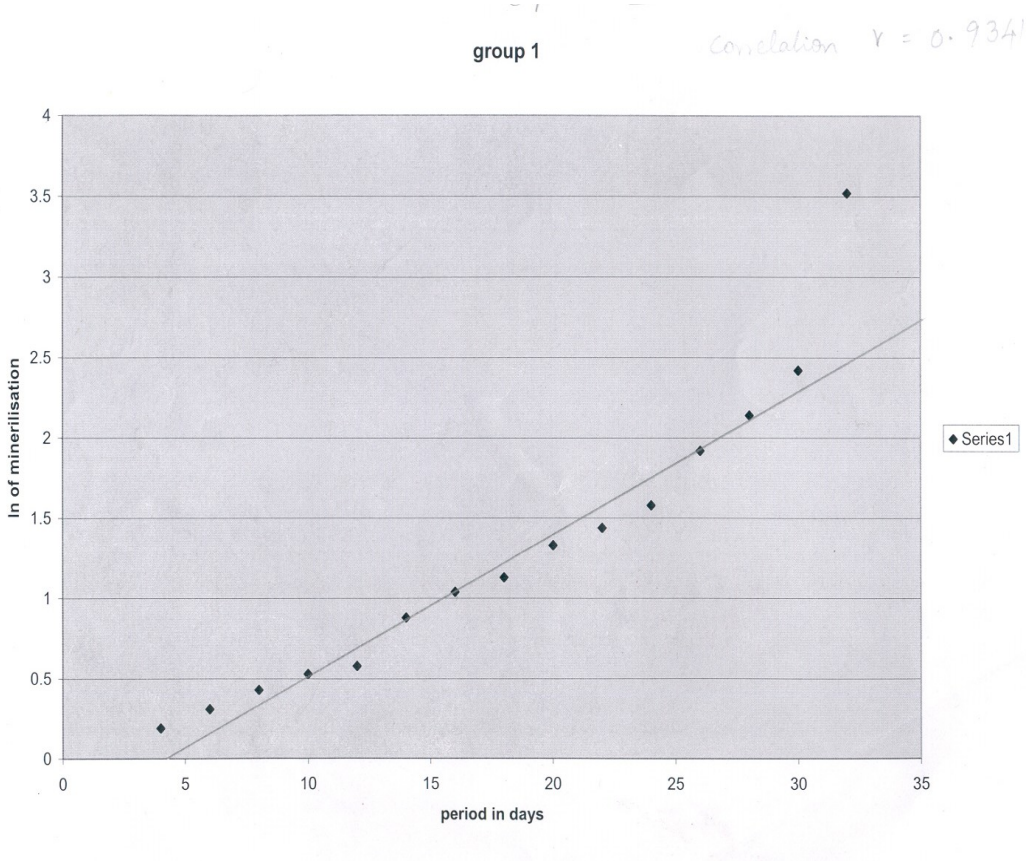

Figure 7.3 - Mineralisation in Group 1

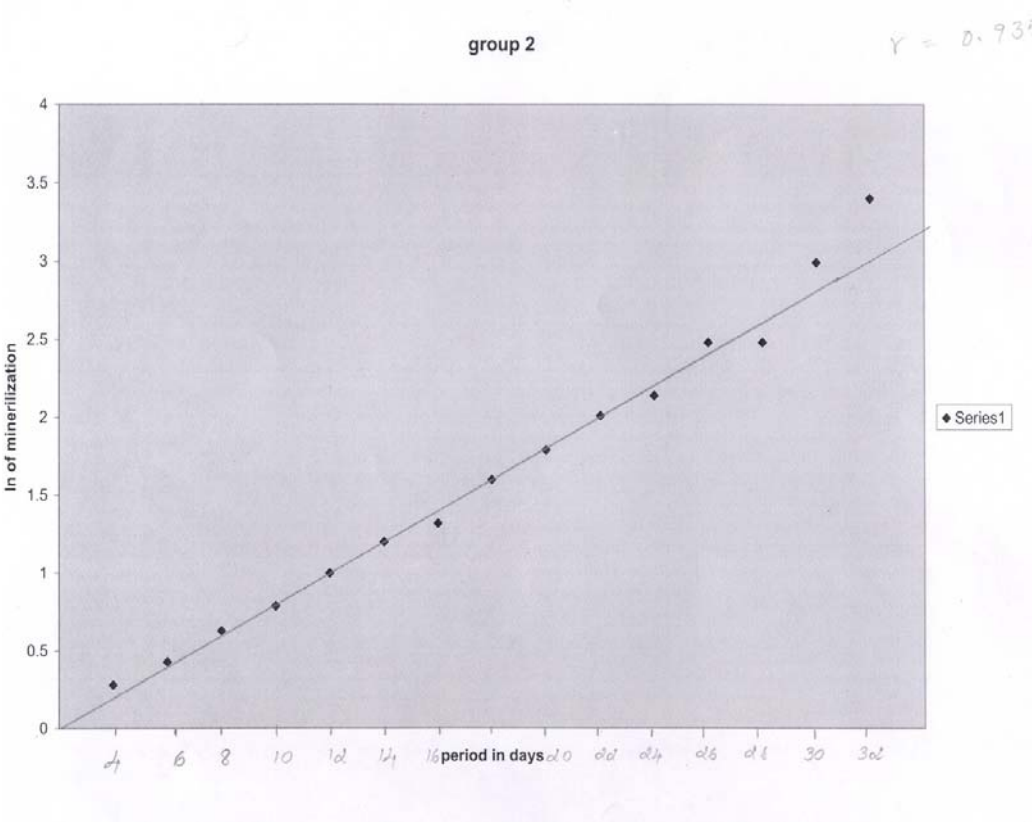

Figure 7.4 - Mineralisation in Group 2

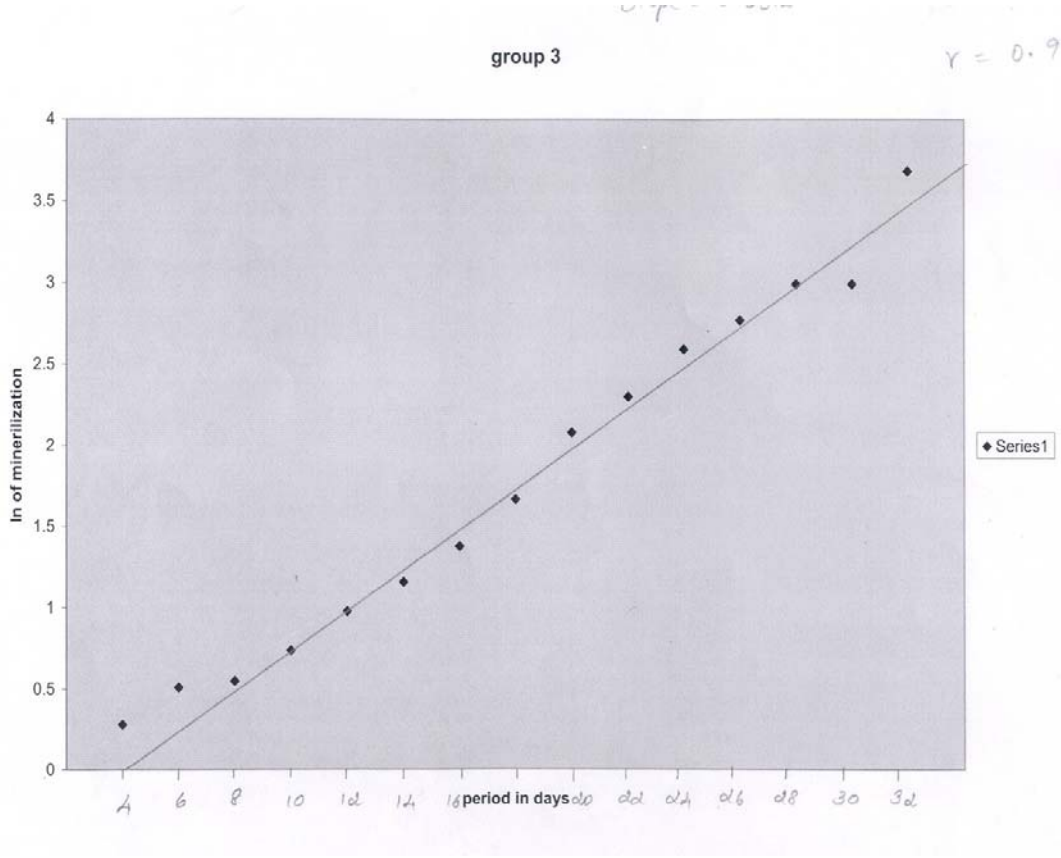

Figure 7.5 - Mineralisation in Group 3

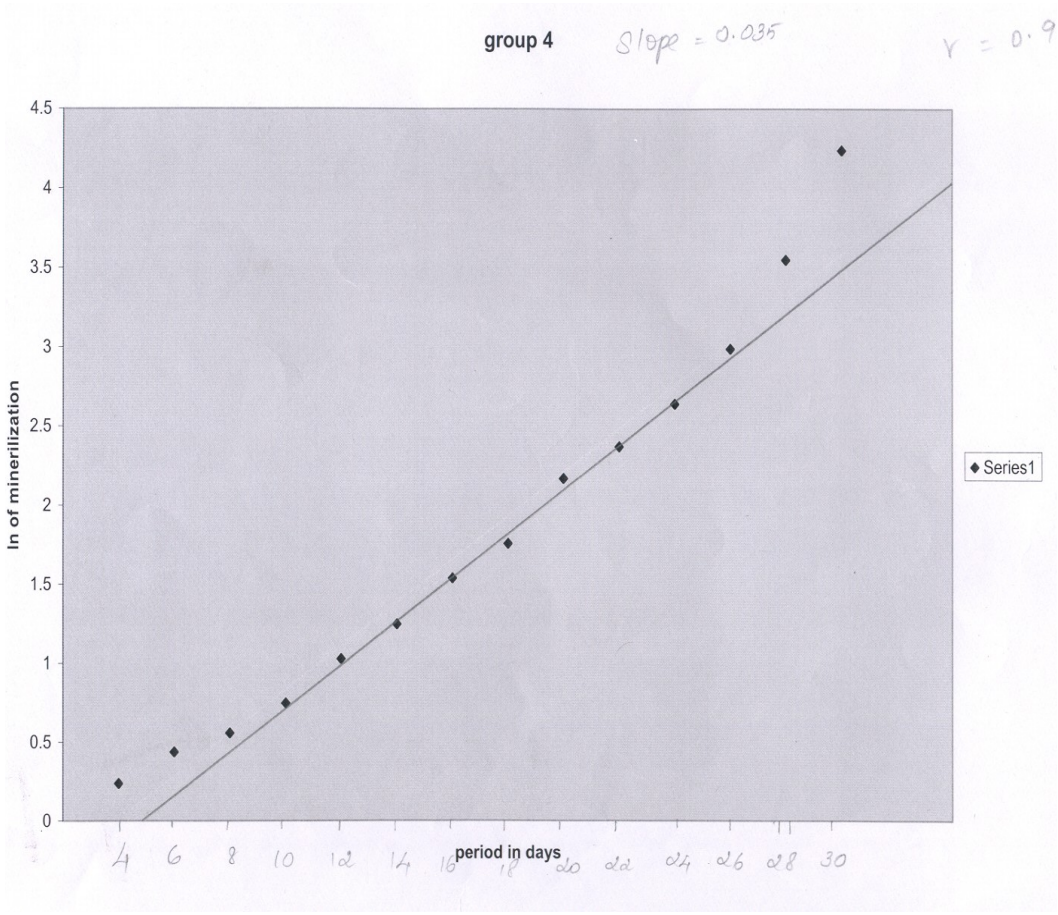

Figure 7.6 - Mineralisation in Group 4

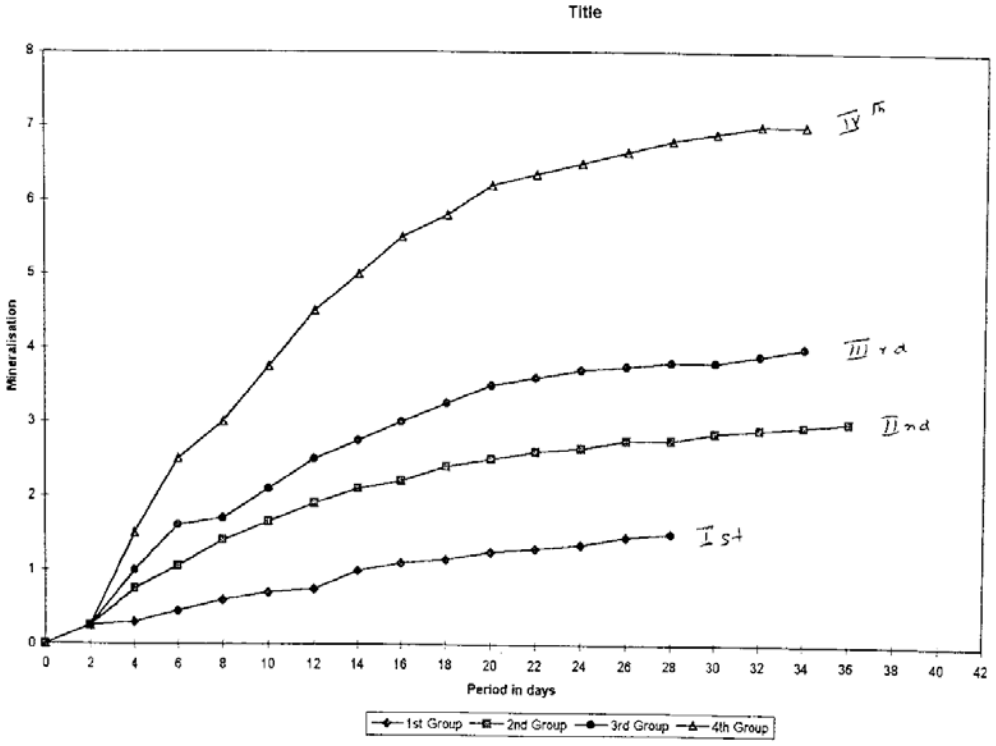

Figure 7.7 - Mineralisation in Group 1, 2, 3 &4