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An Experimental Study of the Effect of Periosteal Stripping on Limb Length in Rabbits

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Abstract

Introduction: Various methods have been tried in the past for lengthening the shorter limb, e.g., introduction of foreign material or drilling of the metaphysis, obliteration of nutrient foramen, use of short-wave diathermy, lumber sympathectomy, creation of an artificial arteriovenous fistula, and epiphyseal distraction. This study was undertaken to find a simple technique for limb lengthening by a minor surgical procedure, particularly where limb discrepancy is short.
Materials and Methods: The experiments were carried out on the right hind leg of 40 rabbits of three months of age in batches of five, each of which was a control. All were anesthetized using Nembutal intra-peritoneally at 40–60 mg/kg of body weight and were then allocated to one of the five experimental groups: A] Control rabbits – anesthesia given but no operation done; B] Vertical periosteal division; C] Vertical periosteal division with periosteal stripping; D] Circular periosteal division; E] Circular periosteal division with periosteal stripping. Before proceeding to operate on the rabbits, the measurements of right and left tibiae of each rabbit were recorded by a vernier caliper from the highest point of tibial tuberosity to the tip of the medial malleolus. After a total duration of 8 weeks from the date of operation, the animals were sacrificed and the tibial lengths were compared from the upper margin of the medial condyle (as this was now the most prominent point) to the tip of the medial malleolus again for comparison.
Results: The maximum increase was obtained in the group with circular division along with periosteal stripping, while in the group with vertical division, the minimum increase was obtained.
Conclusion: Circumferential periosteal division with stripping is an ideal procedure for minor length discrepancies or for those with limb inequality undergoing other procedures. It is simple and safe and would not require more than a short period in the hospital.

Key words: *Periosteum, animal experimentation, rabbits, animals, bone development*

Introduction

That growth of the bone can be controlled and has been known for centuries. This is supported by the fact that various bone deformities have been produced by artificial means in the past, e.g., act of foot binding to stop the growth of the foot as a custom in China [1].

In the present age, the knowledge that limb growth can be altered is being utilized in cases of limb discrepancies. The problem of limb discrepancy is quite common in orthopedic practice. Daily we see a large number of patients having unequal limb lengths of various extents, whether due to poliomyelitis, septic arthritis of the hip, after fractures, after old osteomyelitis, and various congenital causes. Limb discrepancy is associated with the following problems: a patient becomes incapacitated as his normal locomotion is hampered. Gait becomes unsightly and may create social problems. A difference in limb lengths can lead to changes in the spine, opposite hip, and in the same extremity in the process of adaptation, which later on may become permanent, e.g., equinus at the ankle joint of the short extremity and scoliosis [2].

Various methods have been tried for equalizing the limb lengths. Broadly these can be divided into: A) Shortening the longer limb: Epiphyseal arrest, Epiphyseodesis and Bony resection. B) Lengthening the shorter limb: Insertion of dissimilar metals widely separated in the electromotive series and fused together to form a small battery has been tried in an experimental study. This produced a chronic irritation which stimulates a foreign-body reaction, leading to an increase in length of the long bone [3]. Drilling of the metaphysis, which enhances the vascularity in the growth area and thereby increases the limb length. Obliteration of nutrient foramen of the bone is known to produce hyperemia of both epiphysis and the growth plate [4]. Use of short-wave diathermy. Lumber sympathectomy causes an increase in the average turnover rate of muscle and bone flow, as shown in an experimental study in dogs [5]. Creation of an artificial arteriovenous fistula. Removal of long segment of bone, boiling it, and reinserting the dead bone as a stimulus to increase the blood supply. Epiphyseal distraction, wherein Kirchner wires were placed on either side of the distal femoral epiphysis along with constant tension devised to produce a distracting force across the plate in rabbits. A growth increase was found to be 150% greater than the normal growth between two control (undistracted) wires on the left side [6].

This study was undertaken to find a simple technique for limb lengthening by a minor surgical procedure, particularly where limb discrepancy is short.

Materials and Methods

The experiments were carried out on the right hind leg of 40 male Soviet chinchilla rabbits of three months of age in batches of five, each of which was a control. Animals were killed 8 weeks later and the results noted. Prior to the operation, the color of the animal was noted, animals were weighed, and an identification mark was put on them using either Acriflavin or Gention violet on their ears, forehead or legs.

All were anesthetized using Nembutal intraperitoneally at 40–60 mg/kg of body weight and were then allocated to one of the five experimental groups, as follows: A) Control rabbits – anesthesia given but no operation done; B) Vertical periosteal division; C) Vertical periosteal division with periosteal stripping; D) Circular periosteal division; E) Circular periosteal division with periosteal stripping.

Before proceeding to operate on the rabbits in groups B, C, D and E, both hind limbs of each rabbit were shaved from above the knee to the foot, and then in the 90 degrees flexion position at knee joints, the measurements of right and left tibiae of each rabbit were recorded by a vernier caliper from the highest point of tibial tuberosity to the tip of the medial malleolus (being the most prominent points) to see if both limbs were equal or not.

In each of the right hind limbs, a 1.5cm-long vertical incision was given in the skin over the anteromedial surface of each tibia, extending above and below the most central point of the bone, and then the procedure which was adopted was as follows: In group B: Two parallel vertical incisions of 1 cm each given over the anteromedial surface of the right tibia with a gap of about 0.5 cm by a sharp blade of a Bard-Parker knife only. In group C: After making two parallel vertical incisions of 1 cm each over the anteromedial surface of the right tibia with a gap of 0.5 cm, the periosteum was stripped in between the two vertical incisions, in a side-to-side direction by the help of a mastoid chisel. In group D: After retracting the skin, the bone was exposed at the most central point of its muscle attachments all around by inserting Lane's tissue forceps, and then a sharp incision was made circumferentially at the central point over the periosteum. In group E: With a similar procedure to D, periosteal stripping was done with the help of a mastoid chisel above and downwards over the circumferential incision to an area of about 0.5 cm.

Wounds were closed in each group of cases with three cutaneous silk sutures, followed by sterile gauze, cotton and leucoplast application, following which: animals were kept in iron cages. All five groups were injected daily with Streptopenicillin. 1 gram was dissolved in 2 ml of distilled water, and then 0.2 ml (i.e., one tenth of a gram) was injected in each animal intramuscularly in the thigh. Stitches were taken out on the 7th post operative day. In cases where the wound was healthy, the antibiotic was discontinued and in unhealthy wounds was continued till the wound was healthy. Animals were exposed to sunlight daily for 2 to 3 hours and inside the room were kept in a warm environment by a room heater.

All animals were subsequently maintained on the following diet: 1. Grams – 50 gm soaked in water. 2. Vegetables – 100 gm daily, which included: spinach, carrots and reddish. 3. Water.

After a total duration of 8 weeks from the date of operation, the animals were sacrificed by decapitation. Muscle mass around the bones was removed after keeping the legs in 2% solutions of formic acid for 24 hours. (With this procedure the soft tissues around the bone were easily removable, without any damage to the bones.) Then the tibial lengths were compared from the upper margin of the medial condyle (as this was now the most prominent point) to the tip of the medial malleolus by vernier calipers.

Results

Survival: Out of 40 rabbits, seven could not survive the duration of 8 weeks. Four of the seven died within 1 week of operation, probably as a result of post-operative infection or poor resistance. Two animals died after 1 week of operation either due to extreme cold or negligence in feeding by the staff of the animal house, following which nutritional factors were neutralized. One animal died on the operation table itself because of asphyxia due to suffocation, while tying for anesthesia by an injection of Nembutal.

Anesthesia used and dose: In each of the animals, Nembutal was used as an anesthetizing agent. 100 mg was dissolved in 5 cc of distilled water and then injected intra-peritoneally at 40–60 mg/kg of body weight. It was seen that in a few of these animals, a higher dose was required to anesthetize them. The mean dose required was 2.74 cc or 54.92 mg (Table 1). It was ob-

Table 1. Showing the mean dose of Nembutal required when anesthetizing the animals. Average dose of Nembutal required = 2.74 cc or 54.92 mg.

Groups	N	Mean	Std. Deviation
Group A-Control	8	2.63	.16
Group B-Vertical division	8	2.38	.08
Group C-Vertical division with Periosteal Stripping	8	2.86	.12
Group D-Circular division	8	2.81	.11
Group E- Circular division with Periosteal Stripping	8	3.06	.12

Table 2. Showing **A)** Differences in mean group weights in grams after 8 weeks in Groups A, B, C, D and E (each having eight animals), **B)** An average increase in weight in grams in a single rabbit after 8 weeks. Average increase in a single animal = 219.04 gm. Average % increase after 8 weeks in a single rabbit = 43.0%.

	A Control	B Vertical Division	C Vertical Division with Periosteal Stripping	D Circular Division	E Circular Division with Periosteal Stripping
1-Initial mean group weight in gm	531.25 gm. S.D.=44.95	435.71 gm. S.D.=70.79	566.66 gm. S.D.=18.33	530.0 gm. S.D.=47.58	464.28 gm. S.D.=24.92
2-Mean Group weights in gm. after 8 weeks	746.90 gm. S.D.=41.69	642.85 gm. S.D.=71.26	783.33 gm. S.D.=35.06	725.0 gm. S.D.=41.83	725.0 gm. S.D.=27.12
3-Difference (2 minus 1) and % increase in weights after 8 weeks	215.65 gm. 40.4%	207.14 gm. 45.0%	216.67 gm. 38.0%	195.0 gm. 36.7%	260.72 gm. 56.0% (Maximum)

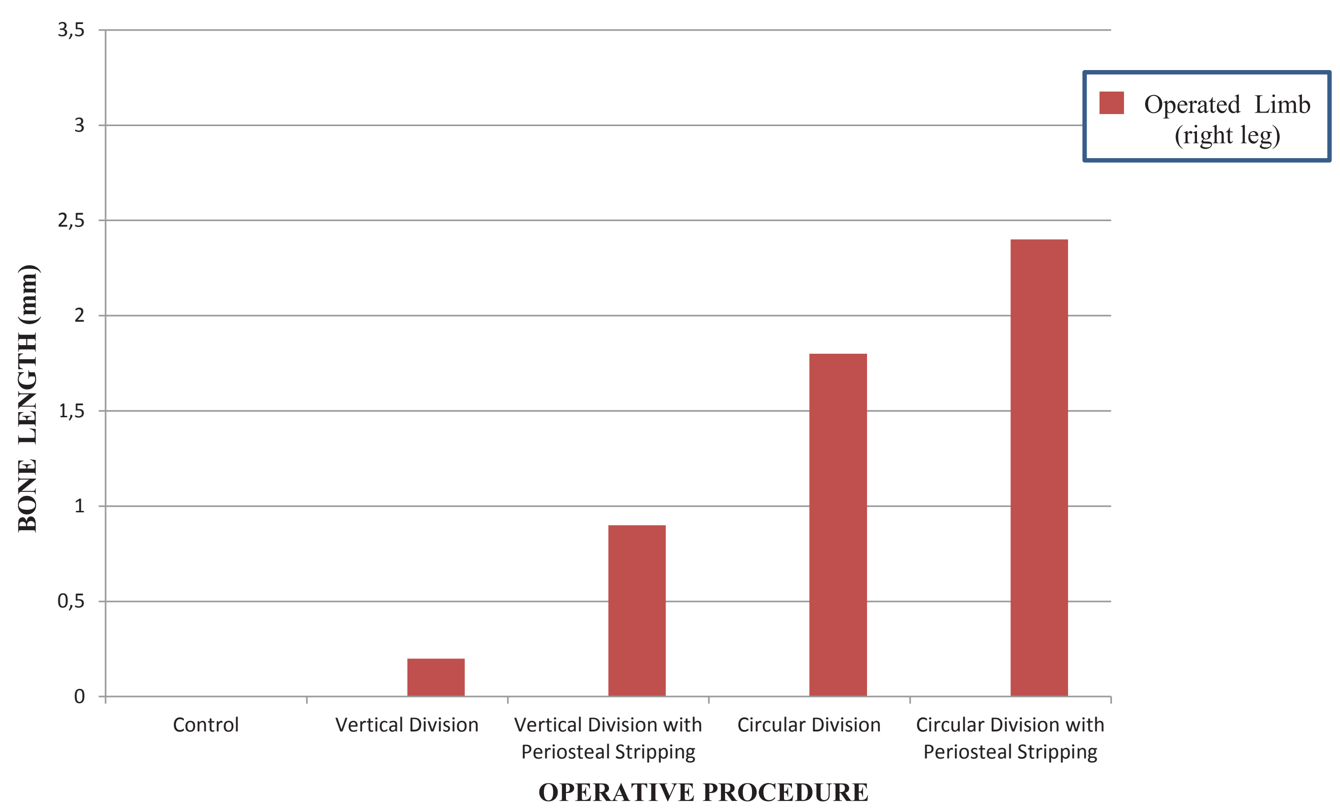

Figure 1. Showing differences in mean leg lengths in Groups A, B, C, D and E.

Table 3a. Comparison of mean difference in leg lengths per 100 grams of initial body weight in mm after 8 weeks.

Group	N	Mean	Std. Deviation	H value	p value
Group A-Control	8	.000	.000	37.72	0.001
Group B-Vertical division	8	.054	.021		
Group C-Vertical division with Periosteal Stripping	8	.171	.043		
Group D-Circular division	8	.345	.050		
Group E- Circular division with Periosteal Stripping	8	.523	.089		

Table 3b. Post-hoc analysis for pairwise comparison using Mann-Whitney U test with Bonferroni correction.

	2	3	4	5
1	0.001	0.001	0.001	0.001
2		0.001	0.001	0.001
3			0.001	0.001
4				0.001

p value < 0.005 is statistically significant.

served that the dose of anesthesia did not have any effect on the increase of their limb lengths. Post-operative behavior: The time taken by the animals to recover from anesthesia was from 20 minutes to 1.5 hours. The average time taken by one animal was 53 minutes. It was seen that after the operation, when animals were kept near the room heater (1,000 watts), the process of recovery was hastened.

Weights: The mean difference in the increase in weights after 8 weeks was calculated in each group and the average increase equaled 219.04 grams. The average percentage increase after 8 weeks was 43% (Table 2).

An artificial correction to allow for variations in body weight was made to express the final leg length as millimeters per unit of body weight to calculate the leg length per 100 grams of body weight. The formula used for this calculation: observed leg length (mm) divided by initial body weight (grams) and the result multiplied by 100. The results were used to recalculate the mean length for each group and it was observed that the mean leg length per unit of body weight does not diminish the significance of the discrepancy between right (operat-

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Table 4a. Comparison of mean difference in leg lengths in cm after 8 weeks.

Group	N	Mean	Std. Deviation	H value	p value
Group A-Control	8	.0000	.0000		
Group B-Vertical division	8	.0218	.0035		
Group C-Vertical division with Periosteal Stripping	8	.0964	.0031	37.76	0.001
Group D-Circular division	8	.1802	.0162		
Group E- Circular division with Periosteal Stripping	8	.2415	.0130		

*Krukall Wallis test.

Table 4b. Post-hoc analysis for pairwise comparison using Mann-Whitney U test with Bonferroni correction.

	2	3	4	5
1	0.001	0.001	0.001	0.001
2		0.001	0.001	0.001
3			0.001	0.001
4				0.001

p value < 0.005 is statistically significant.

ed) and left (un-operated) legs, produced by periosteal division with or without stripping (Table 3).

Limb lengths: The maximum increase was obtained in the group with circular division along with periosteal stripping, while in the group with vertical division, the minimum increase was obtained (Table 4 and Figure 1).

Discussion

The experiments in this study were done in the right hind leg in rabbits of the same age (3 months) and the results were noted after a duration of 8 weeks. Many authors have undertaken a similar study on the effect of periosteal stripping on the limb lengths:

CK Sola and Silberman [7] have done works on dogs and monkeys. Dogs were between 3–4 months of age when subjected to periosteal stripping and 12 months when results were noted. The ages of monkeys used could not be determined but all of the animals were young at the time of operation. These were sacrificed from 12–18 months after the operation. DH Jenkins and Chang [8] have done a clinical study on the effect of periosteal stripping in 30 children with shortening after poliomyelitis. The age of the boys ranged between 6–14 years (mean age 8.2) and those of girls between 7–15 years (mean age 10.1). The results were noted after a duration of 5 years. Edwards DJ and Bickerstaff DB [9] have done a prospective study on the results of periosteal stripping and division in 10 achondroplastic children. A single limb (femur and tibia) was operated on and the change in actual length of each bone and the percentage change in growth were compared to those of the non-operated limb. Mr. JF Taylor and Mrs. E. Warrell [10] have done a similar work in rats. The experiments were carried out on outbred wistar male rats of 28 days and the results were noted 34 days later. In our experimental study, the animals were of the same age (8 weeks), which was in conformity with the work done by Mr. J.F. Taylor and Mrs. E. Warrell [10]. Animals were taken purposely of the same age, keeping in view that the growth pattern in all animals undergoing the experimental procedure would remain identical during the period of the study.

In the study done by CK Sola and Silberman [7] on dogs and monkeys, all of the animals tolerated the operation well and also there was no post-operative infection. However, there were transverse or oblique spontaneous fractures after periosteal stripping due to which out of 37 dogs and 10 monkeys subjected to the study, 30 dogs and eight monkeys respectively were only fit to undergo measurements for their limb discrepancies. DH Jenkins and Chang [8] performed operations on a total of 30 children. Follow-up after a period of 5 years was possible in all cases. The only complication which was encountered in their study was superficial wound infection only in one case. In our study, however, out of 40 rabbits, seven could not survive the total duration of 8 weeks. Four died as a result of post-operative infection, two because of extreme cold and one because of suffocation while tying the animal to the operation table. We did not encounter any pathological fractures in any of the animals. As such, all of the animals which survived could be subjected to measurements for leg discrepancies though; however, we did have wound infection in four of our cases.

CK Sola and Silberman [7] measured the operated femur and tibiae after their removal with the help of two wooden blocks placed perpendicular to the longitudinal axis of these bones and in contact with the most salient points. A mean increase in length of the operated bone in dogs after the first periosteal stripping reported was 0.16 plus minus 0.03 cm, and after the second was 0.36 plus minus 0.08 cm. In monkeys the mean increase in length of stripped bones was 0.17 plus minus 0.03 cm. In dogs only 63% of stripped bones and in monkeys only 87.5% of stripped bones were reported to demonstrate an increase in longitudinal growth. An increase in lengths obtained by DH Jenkins and Chang [8] in young boys and girls who suffered from poliomyelitis showed a mean overgrowth obtained in femurs equal to 0.70 cm and that in tibiae as 1.0 cm. In the study done by Edwards DJ and Bickerstaff DB [9], the percentage change in growth compared to that of the non-operated limb was measured by a scanogram. The mean absolute increase in growth was small, measuring 3 mm for the femur and 2 mm for the tibia. There was no measurable growth difference after 18 months. This method of increasing limb length in achondroplastic children prior to definitive and extensive lengthening procedures has not been recommended. Lynch MC and Taylor JF [11] have measured the growth at the proximal tibial epiphyseal plate of the rats following three different growth-stimulating procedures. These were proximal periosteal release, distal periosteal release, and full periosteal stripping of the diaphysis. A new radiographic method using a photographic technique made it possible to take accurate measurements of the rate of long-bone growth. From the results they conclude that proximal tibial periosteal division is likely to be the most effective of the three procedures when used to correct leg-length discrepancy in the growing child. Houghton GR and Dekel S [12] have also done an experimental study in rats. Longitudinal growth of immature rat femurs was studied in diffusion chambers after circumferential periosteal division and stripping. After 14 days, significant (P less than 0.02) overgrowth of the periosteally divided femurs was found. Dimitriou CG, Kapetanos GA and Symeonides PP [13] have done an experimental study in 28 rabbits to study the effect of partial periosteal division on growth of the long bones. Rabbits were divided into three groups. A longitudinal periosteum incision was made on the medial upper tibia in rabbits of Group A, and a hemicircumferential periosteum incision was made in Group B. Group C was the control. Certain differences in the development of the right tibia compared to the control side were observed in rabbits of Group B: (1) valgus deformity, 5–10 degrees; (2) overgrowth, 1–2 mm; and (3) an S-shaped tibia deformity. It was concluded that the dynamics of the deformity support the mechanical theory because the direction of periosteum division was an important factor in the appearance of growth disturbances. Limb length equalization was achieved in eight children out of 11 with lower limb length discrepancy by the technique of total circumferential stripping, followed by transverse division of the periosteum by Limpaphayon N and Prasongchin P [14]. They emphasize that this technique should be considered a surgical option for a limb length discrepancy up to 6 cm. This study is in strong support of our experimental study in rabbits. Vernier caliper was used as a measuring tool in our study, which is similar to an experimental study on rats by Mr. J.F. Taylor and Mrs. E. Warrell [10]. The maximum increase was obtained in groups subjected to circular division and periosteal stripping, and minimum in groups subjected to vertical division alone. In comparison with the work done by C.K. Sola and Silberman [7] where all of the operated bones did not show an increase in length, in our experimental study all of the bones (tibiae) which underwent periosteal stripping showed an increase in longitudinal growth. Our findings are in conformity with the reported results of Mr. J.F. Taylor and Mrs. E. Warrell [10] and Limpaphayom N and Prasongchin [14], as we also found a maximum increase in animals subjected to circular division and periosteal stripping.

In our study, though the survival rate in different batches for different animals was different, different colors of animals were used, different marks of identification were utilized, doses of anesthesia required for individual animals were different, and duration of unconsciousness in different batches and groups of animals varied, but these did not have any effect on the limb lengths in these experimental animals; however, it was totally the type of periosteal division and stripping which was solely responsible for achieving limb inequality. An artificial correction to allow for variation in body weight for expressing the final length as millimeter per unit weight by the formula observed, leg length (mm) divided by body weight, and the result multiplied by a hundred has also been done by authors and we have also found that mean leg length per unit weight does not diminish the significance of discrepancy produced by these operations.

Various postulations have been put forward to explain how limb lengthening is produced following periosteal division: hormonal factor-growth stimulating substances released at the fracture site (osteogenin) may be responsible for this overgrowth phenomenon. A corresponding increase in weight in the endocrine glands has been noted [15]. Release of static load on the growth plate or growth plate decompression following periosteal division can also be labeled as the main mechanism. Vasoconstriction or obliteration of the nutrient artery is known to produce hyperemia of both epiphysis and the growth plate [4]. Disturbance of the skeletal electric field may have resulted in growth plate stimulation. It has also been shown that both muscle damage and periosteal stripping procedures produce electric potential in the underlying bone [16,17].

Limitations of study: Antibiotic duration differences can affect bone metabolism as there are several studies reporting the negative effect of antibiotics on bone healing. Radiographic images for the measurements are claimed to be more precise.

Conclusion

Out of all of the different procedures of periosteal division and stripping which were employed in our study, it was noticed that the maximum limb length was achieved in animals subjected to circular division along with periosteal stripping. Therefore, it is suggested that circumferential periosteal division with stripping is an ideal procedure for minor length discrepancies or for those with limb inequality undergoing other procedures. It is simple and safe and would not require more than a short period in the hospital.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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