

The Department of Orthopaedic Surgery, University of Lund, Sweden.

EFFECT OF VENOUS STASIS ON LONGITUDINAL BONE GROWTH IN THE RABBIT

LARS INGVAR HANSSON, ANDERS STENSTRÖM & KARL-GÖRAN THORNGREN

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The effect of so-called growth-stimulating operations on the increase in length of bones has been the subject of many clinical and experimental investigations (Goff 1960, Taillard & Morscher 1965, Hansson 1967, Sundén 1967). These operations have been performed on the bone, on the vascular system, or on the nervous system.

Since the latter part of the 19th century, various investigations have been made to find out the effect of venous stasis on bone growth. Venous stasis has usually been induced by ligation or by external compression by a tourniquet. Judging from the experimental investigations, the growth-stimulating effect of venous stasis is questionable. Stimulation of longitudinal bone growth has been described by some authors (Borel 1922, Bergmann 1931, Kishikawa 1936, Servelle 1948, Hutchison & Burdeaux 1954, Colt & Iger 1963), whereas others have been unable to demonstrate any such effect with certainty (Wu & Miltner 1937, Dickinson 1953, Keck & Kelly 1965). Venous stasis has also been described as accelerating the healing of fractures (Pearse & Morton 1930) and the periosteal bone formation (Lilly & Kelly 1970).

The discrepancies between the various investigations are probably due to several factors. Different sorts of experimental animals have been used, and venous stasis has been produced in different ways and at different levels. Moreover, the methods used for measuring longitudinal bone growth have been uncertain, probably having serious inherent sources of error which have not been taken into account.

Therefore the effect of venous ligation on longitudinal bone growth was studied. The femoral vein was chosen because it is readily accessible, and because ligation of this vein has been described as having a growth-stimulating effect (Borel 1922, Kishikawa 1936, Servelle 1948). The longitudinal bone growth was measured with the tetracycline tech-

nique, making it possible to determine the growth per day with good accuracy (Hansson 1967).

MATERIAL AND METHODS

131 white rabbits of both sexes, aged 35–40 days at the time of the operation were used. The rabbits were treated as previously described (Hansson 1967).

During the operation, the animals were given an injection of mebumal sodium intraperitoneally and then anaesthetized with ether. All operations were performed on the left side. In the first group of animals (59 animals, Group I), the femoral vein, the circumflexal veins, and the deep femoral vein were ligated with silk. In the other group of animals (72 animals, Group II), the sciatic vein and the above-mentioned veins were ligated.

Of these animals, five in each group were only used to register the venous pressure and for phlebography. The venous pressure was measured about 10 mm distal to the place of ligation in the femoral vein by means of a Scalp Vein Set including a 23 Gauge Needle (Portex®). Phlebograms were made by injection of a contrast medium (Isopaque®) by means of a small polyethylene catheter (P.E. 160) in the femoral and/or the sciatic vein.

The longitudinal bone growth was determined during the day before operation and at different postoperative time intervals up to 40 days after operation in both Groups (see Tables 1 and 2). Each animal received two or four intravenous injections of 1.0 mg oxytetracycline (Terramycin®) per kg body weight with an interval of 24 hours between consecutive injections. The animals were killed about 10 minutes after the last injection by intravenous injection of mebumal sodium.

The rate of longitudinal bone growth was determined in the following regions: bilaterally in the proximal tibiae and distal tibio-fibulae, and on the right side in the distal radius. These regions were fixed, dehydrated, sectioned manually, and examined in a fluorescence microscope as previously described (Hansson 1964, 1967).

FINDINGS

At the operation, the venous system distal to the ligatures was usually dilated, but no signs of postoperative oedema distal to the ligatures were seen with certainty during the experimental period. Autopsy invariably showed that the surgical wound had healed without complications and that the ligatures were intact.

The phlebograms in the normal unoperated leg showed a venous blood flow not only in the femoral vein and sciatic vein but also in several communicating veins to the vertebral and epigastric veins. In the group of animals with the anterior ligations (Group I), the phlebograms showed that the venous blood rapidly passed through the sciatic vein and the communicating veins in the muscles. In the group of animals with both anterior and posterior ligations (Group II), the liga-

Table 1. Longitudinal bone growth in μ per day after ligation of femoral vein, circumflexal veins and deep femoral vein of the left side (Group I).

| Observation day | Number of animals | Proximal tibia | | | Distal tibio-fibula | | | Distal radius Right | | | | |
|----------------------|-------------------|---------------------------------|--------------------------------|---|---------------------------------|--------------------------------|---|---------------------|----|-------|-------|-----|
| | | Right M \pm s _d | Left M \pm s _d | Difference between left and right M \pm s _d | Right M \pm s _d | Left M \pm s _d | Difference between left and right M \pm s _d | | | | | |
| Day before operation | 11 | 516 | 36 | -1 | 448 | 31 | 448 | 30 | 0 | 6(-) | 465 | 32 |
| Day after operation | | | | | | | | | | | | |
| 1st | 6 | 514 | 40 | -3 | 439 | 39 | 442 | 36 | 4 | 11(-) | 459 | 36 |
| 2nd | | | 510 | 32 | 2 | 449 | 34 | 444 | 32 | -5 | 6* | 464 |
| 4th | 6 | 485 | 35 | 0 | 423 | 37 | 417 | 39 | -6 | 27(-) | 430 | 46 |
| 6th | | | 436 | 42 | 0 | 361 | 44 | 364 | 40 | 3 | 10(-) | 412 |
| 8th | 6 | 496 | 32 | -3 | 436 | 37 | 437 | 37 | 1 | 5(-) | 457 | 41 |
| 10th | | | 505 | 64 | -7 | 406 | 74 | 403 | 72 | -3 | 5(-) | 448 |
| 20th | 7 | 372 | 36 | 3 | 283 | 37 | 279 | 34 | -5 | 14(-) | 362 | 27 |
| 30th | | | 356 | 28 | 0 | 255 | 32 | 251 | 35 | -4 | 8(-) | 357 |
| 40th | 6 | 259 | 40 | 2 | 156 | 29 | 157 | 31 | 1 | 2(-) | 273 | 59 |

Statistical analysis (Student's t-test) of the difference.

* 0.01 < P < 0.05; (-) P > 0.05

Table 2. Longitudinal bone growth in μ per day after ligation of femoral vein, circumflexal veins, deep femoral vein and sciatic vein of the left side (Group II).

| Observation day | Number of animals | Proximal tibia | | | Distal tibio-fibula | | | Distal radius |
|----------------------|-------------------|---------------------------------|--------------------------------|---|---------------------------------|--------------------------------|---|---------------|
| | | Right M \pm s _d | Left M \pm s _d | Difference between left and right M \pm s _d | Right M \pm s _d | Left M \pm s _d | Difference between left and right M \pm s _d | |
| Day before operation | | 420 | 418 | -1 | 356 | 351 | -6 | 372 |
| Day after operation | 12 | | | | | | | |
| 1st | | 375 | 382 | 7 | 314 | 318 | 4 | 330 |
| 2nd | | 400 | 406 | 6 | 339 | 344 | 5 | 360 |
| 4th | 12 | 372 | 389 | 17 | 329 | 335 | 5 | 348 |
| 6th | 8 | 400 | 403 | 4 | 325 | 330 | 5 | 360 |
| 8th | 7 | 423 | 434 | 11 | 351 | 346 | -5 | 388 |
| 10th | 9 | 376 | 374 | -1 | 320 | 321 | 1 | 353 |
| 20th | 7 | 421 | 421 | 0 | 333 | 332 | -1 | 387 |
| 30th | 6 | 391 | 402 | 11 | 319 | 313 | -6 | 372 |
| 40th | 6 | 346 | 336 | -10 | 227 | 226 | -2 | 339 |

Statistical analysis (Student's t-test) of the difference.

• $0.01 < P < 0.05$; (-) $P > 0.05$

tures did not permit any passage of the contrast medium through these vessels. Instead, immediately after operation, the contrast medium passed through smaller dilated veins originating from the femoral vein and sciatic vein distal to the ligatures; after a short delay, the sacral veins and the inferior cava vein were filled. Two days after operation, a rich collateral circulation was established especially round the ligature of the sciatic vein permitting the venous blood to re-enter this vein. Simultaneously, all the peripheral parts of the circumflexal veins were filled and took part in a collateral circulation.

The preoperative venous pressure in the femoral vein was found to be about 8.5 cm H₂O. In the animals with anterior ligation (Group I), the venous pressure immediately after operation increased to about 24.0 cm H₂O. One day later, the pressure had decreased to about 9.5 cm H₂O; two days after operation the venous pressure was equal to the preoperative pressure. In the animals with both anterior and posterior ligation (Group II), the immediate postoperative venous pressure was the same as in the first group of animals, but the decrease was slower. Thus two days postoperatively, the pressure was found to be about 15.0 cm H₂O; after ten days, the venous pressure was slightly above the normal—about 11.0 cm H₂O. Later, the venous pressure was almost equal to the preoperative pressure.

The determination of longitudinal bone growth showed a tendency to general growth retardation immediately after operation. This tendency was especially marked in the group of animals with both anterior and posterior ligations, and then during the first postoperative day.

The comparison of daily longitudinal bone growth between the left operated side and the right control side revealed no significant differences in growth rate (Tables 1 and 2). However, small and variable differences of low significance were registered in both groups of animals, but no general tendency was found.

DISCUSSION

The general retardation of longitudinal bone growth observed during the first postoperative days was probably due to general impairment of the blood flow after the operation. This growth retardation was marked in the second group of animals where the operation was more extensive than in the first group of animals. Similar growth retardation has previously been observed after bone operations (Hansson 1967), which are known to reduce the blood flow (Gelin 1956, Bergentz 1961).

The operative procedure used in this investigation unquestionably results in venous stasis as the venous pressure increased temporarily and there was a delay of the venous blood flow according to the phlebograms. This venous stasis was of the same degree both after anterior ligation of veins and the additional ligation of the sciatic vein, but it was more longstanding in the latter case. As found in a previous investigation (Brookes & Singh 1972), an extensive venous collateral circulation develops after venous ligation, bypassing the obstruction and eliminating the venous stasis.

The present investigation showed no obvious growth stimulating effect after ligation of veins. The differences of low significance found in this investigation were of the same order or slightly above the error of method for determination of the growth rate (Hansson 1967). Moreover, these small differences were variable in time and localization and seem uncertain. The result thus agreed well with the results obtained by some previous investigators (Wu & Miltner 1937, Dickinson 1953, Keck & Kelly 1965). Furthermore, it has previously been found that ligation of veins does not result in active but passive hyperaemia with a slight reduction of the intraosseous blood flow and a varying intramedullary pressure (Keck & Kelly 1965, Sundén 1967).

There are probably several reasons why many investigators (Borel 1922, Bergmann 1931, Kishikawa 1936, Servelle 1948, Hutchison & Burdeaux 1954, Colt & Iger 1963) have found venous stasis to be growth stimulating. In general, the series of animals used in most investigations were small. The most probable explanation for the differing results is the large error of the previously used methods for measuring the rate of longitudinal bone growth. Moreover, the application of a tourniquet to achieve venous stasis probably results in a compression not only of superficial and deep veins but also of other structures. Also, the growth stimulation might not be a result of the venous stasis, but of the general operative trauma to the extremity. Trauma is known to induce active hyperaemia and thereby stimulation of longitudinal bone growth (Hansson 1967).

S U M M A R Y

The effect of ligation of veins on the longitudinal bone growth was studied in growing white rabbits. The daily longitudinal bone growth was determined with the tetracycline technique from the day before up to 40 days after operation. Ligation of the femoral vein, the circum-

flexal veins, and the deep femoral vein resulted in a temporary venous stasis. Additional ligation of the sciatic vein resulted in the same degree of venous stasis but a more longstanding effect. Neither of the operations resulted in growth stimulation or growth retardation compared with the control side. On the contrary, the ligation resulted in a minor temporary general growth retardation during the first days after the operation.

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Correspondence to:

L. I. Hansson, M.D.
Department of Orthopaedic Surgery
Lasarettet
S-221 85 Lund, Sweden